Gαq signals via Trio RhoGEF to modulate synaptic transmission in a model serotonin motor circuit in C. elegans

Pravat Dhakal, Sana Chaudhry, and Kevin M. Collins*

Department of Biology, University of Miami, Coral Gables, FL, United States of America

*To whom correspondence should be addressed. Email: kevin.collins@miami.edu

57 pages with 6 Figures, 2 Tables and 3 Movies
Abstract

Activated Gαq signals through Phospholipase-Cβ (PLCβ) and Trio, a Rho GTPase exchange factor (RhoGEF), but how these two effector pathways promote synaptic transmission remains poorly understood. We used the egg-laying behavior circuit of C. elegans to determine whether PLCβ and Trio mediate serotonin and Gαq signaling through independent or related biochemical pathways. Using genetic rescue experiments, we find that PLCβ functions in neurons while Trio functions in both neurons and the postsynaptic vulval muscles. While Gαq, PLCβ, and Trio RhoGEF mutants all fail to lay eggs in response to serotonin, optogenetic stimulation of the serotonin releasing HSN command neurons restores egg laying only in PLCβ mutants. Vulval muscle Ca²⁺ activity remained in PLCβ mutants but was eliminated in strong Gαq and Trio RhoGEF mutants. Exogenous treatment with Phorbol esters that mimic Diacylglycerol (DAG), a product of PIP₂ hydrolysis, rescued egg-laying circuit activity and behavior defects of Gαq signaling mutants, suggesting both Phospholipase C and Rho signaling promote synaptic transmission and egg-laying via DAG production. DAG has been proposed to activate effectors including UNC-13, however, we find that phorbol esters, but not serotonin, stimulate egg laying in unc-13 mutants. Together, these results show that serotonin signaling through Gαq and PLCβ modulates UNC-13 activity to promote neurotransmitter release. Serotonin also signals through Gαq, Trio RhoGEF, and an unidentified PMA-responsive effector to promote postsynaptic muscle excitability. Thus, the same neuromodulator serotonin can signal in distinct cells and effector pathways to activate a motor behavior circuit.
Introduction

Neurons communicate in circuits via synaptic transmission to initiate, sustain, and terminate behaviors. During neurotransmission, both synaptic vesicles and dense-core vesicles fuse with the presynaptic membrane, releasing neurotransmitters and neuropeptides that activate postsynaptic ion channels and G protein coupled receptors (GPCRs) (Betke et al., 2012; Geppetti et al., 2015). While much has been learned about neurotransmitter signaling pathways through ionotrophic receptors, the diversity of GPCRs and their signaling pathways has complicated our understanding of how their signaling exerts changes on cell excitability and behavior. The G protein, Gαq, is one of the major G proteins expressed in all excitable cells (Offermanns, 2001; Simon et al., 1991; Wilkie et al., 1992). Activated Gαq signals through PIP2-specific phospholipase-C β (PLCβ) to generate the second messengers, inositol 1,4,5 trisphosphate (IP3) and diacylglycerol (DAG). IP3 activates the IP3 receptor to release Ca2+ from intracellular stores and activate downstream kinases, lipases, and ion channels (Berridge et al., 2000; Huang, 1989; Li et al., 2014; Mujica and Gonzalez, 2011). DAG has been shown to recruit and activate numerous effector proteins including UNC-13 and Protein Kinase C (Ananthanarayanan et al., 2003; Brose and Rosenmund, 2002; Lou et al., 2008; Maruyama and Brenner, 1991; Rozengurt et al., 1997; Silinsky and Searl, 2003; Thore et al., 2005), but whether these or other identified DAG targets function to transduce all forms of Gαq signaling in vivo remains an open question.

Genetic studies in the nematode worm C. elegans show that Gαq signaling through both PLCβ and Trio, a Rho GTPase Exchange Factor (RhoGEF), promotes synaptic transmission. Whether Trio signaling through Rho similarly promotes DAG and IP3 production, or instead acts through an independent downstream pathway, is not clear. In worms, Gαq knockouts are lethal while PLCβ or Trio RhoGEF single knockouts show strong defects in neurotransmission that disrupt locomotion and egg-laying behaviors, resembling strong Gαq loss-of-function mutants.
Worms missing both PLCβ and Trio RhoGEF phenocopy the larval arrest phenotype of Gαq null mutants, consistent with these two effectors relaying most or all of the relevant Gαq signaling (Williams et al., 2007). Genetic and biochemical studies showed that Gαq activation of Trio and Rho is conserved (Chhatriwala et al., 2007; Rojas et al., 2007), however, it remains unclear how PLCβ and Rho signaling promotes neurotransmitter and neuropeptide release in vivo. The larval lethality of Gαq null mutants or PLCβ / Trio RhoGEF double mutants can be rescued by the DAG-mimetic phorbol ester, PMA (Reynolds et al., 2005; Steven et al., 2005; Williams et al., 2007), suggesting Gαq signaling through both PLCβ and Trio may ultimately converge to regulate DAG levels and the activation of downstream effectors. Both PLCβ and Trio RhoGEF promote acetylcholine (ACh) release from motor neurons that control locomotion (Lackner et al., 1999; Miller et al., 1999; Williams et al., 2007), although mutations in Trio RhoGEF cause behavior defects more aligned with a function in dense core vesicle release (Hu et al., 2011). PLCβ is expressed in the nervous system and intestine, while Gαq and Trio show additional postsynaptic expression in muscles (Bastiani et al., 2003; Lackner et al., 1999; Steven et al., 1998; Steven et al., 2005). While re-expression of PLCβ or Trio in neurons rescue the locomotion behavior defects of their mutants (Williams et al., 2007), whether these proteins exclusively function in neurons to regulate egg laying, is an open question.

Using genetics, optogenetics, pharmacology, and calcium imaging techniques we have investigated how Gαq signaling through its two effector pathways regulate egg-laying circuit activity and behavior. We find that Gαq signals through PLCβ in the presynaptic neurons while Gαq signals through Trio in both neurons and muscles to promote egg laying. These results clarify that despite Gαq signaling through independent PLCβ and Trio pathways, these effectors may ultimately converge to potentiate DAG levels which promote egg-laying behavior.
MATERIALS AND METHODS

Strains

C. elegans worms were maintained at 20°C on Nematode Growth Medium (NGM) agar plates with E. coli OP50 as a source of food as described previously (Brenner, 1974). All behavior assays and fluorescence imaging experiments were performed with age-matched adult hermaphrodites aged 20 – 36 h after the late L4 stage. All strains used in this study are listed in Table 1.

Molecular biology and transgenes

Vulval muscle GCaMP5 strains

Vulval muscle Ca^{2+} activity was recorded using GCaMP5G (Akerboom et al., 2013) which was expressed along with mCherry from the unc-103e promoter (Collins and Koelle, 2013), as previously described (Collins et al., 2016; Ravi et al., 2018a). The wild-type reporter strain, LX1918 vsIs164 [unc-103e::GCaMP5::unc-54 3’UTR + unc-103e::mCherry::unc-54 3’UTR + lin-15(+) ] lite-1(ce314) lin-15(n765ts) X was used as described previously (Collins et al., 2016).

LX1918 males were crossed separately into DA823 egl-30(ad805) I, MT1434 egl-30(n686) I, JT47 egl-8(sa47) V, MT1083 egl-8(n488) V, KG1278 unc-73(ce362) I, LX1226 eat-16(tm761) I, CG21 egl-30(tg26) I, him-5(e1490) V, or KP1097 dgk-1(nu62) X hermaphrodites to generate MIA140 egl-30(ad805) I; vsIs164 lite-1(ce314) lin-15(n765ts) X, MIA139 egl-30(n686) I; vsIs164 lite-1(ce314) lin-15(n765ts) X, MIA109 egl-8(sa47) V; vsIs164 lite-1(ce314) lin-15(n765ts) X, MIA288 egl-8(n488) V; vsIs164 lite-1(ce314) lin-15(n765ts) X, MIA141 unc-73(ce362) I; vsIs164 lite-1(ce314) lin-15(n765ts) X, MIA287 egl-16(tm761) I; vsIs164 lite-1(ce314) lin-15(n765ts) X, MIA286 egl-30(tg26) I; vsIs164 lite-1(ce314) lin-15(n765ts) X, and MIA296 dgk-1(nu62) vsIs164 lite-1(ce314) lin-15(n765ts) X, respectively. The corresponding gene mutation was confirmed by phenotype, genotype, or both. Presence of vsIs164 was confirmed observing the mCherry
marker, and lite-1(ce314) X was confirmed with PCR genotyping. Oligo sequences used for genotyping the corresponding mutations are shown in Table 2.

### Trio RhoGEF-E transgenes

**Pan-neuronal expression:**

The rab-3 promoter was used to drive expression of GFP alone or with Trio-RhoGEF-E. Briefly, plasmids KG#68 (rab-3p::GFP; 15 ng/µl) alone or with KG#281 (rab-3p::unc-73e; 50 ng/µL) were injected into KG1278 unc-73(ce362) I (Williams et al., 2007). For behavior experiments, five independent GFP-expressing transgenic lines were used, from which a single transgenic line from each was kept: MIA374 unc-73(ce362) I; keyEx66 (expressing GFP alone) and MIA375 unc-73(ce362) I; keyEx67 (expressing GFP and Trio RhoGEF-E). Plasmids KG#281(rab-3p::unc-73e) and KG#68(rab-3p::GFP) were kind gifts from Dr. Kenneth Miller.

**Pan-muscle expression**

Plasmid pKMC33 (rgs-1p::mCherry) was digested with Nhel/KpnI and ligated with similarly digested pPD96.52 (Fire lab C. elegans Vector Kit 1999; 1608: L2534, Addgene) to generate pKMC166 (myo-3p::mCherry). Plasmid KG#281 (rab-3p::unc-73e) was digested with Nhel and KpnI, and the insert was ligated into similarly digested pKMC166 to generate pPD3 (myo-3p::unc-73e). pKMC166 (15 ng/µL) alone or with pPD3 (50 ng/µL) was injected into KG1278 unc-73(ce362) I mutants. Five independent mCherry-expressing transgenic lines were used for behavior experiments from which a single transgenic line from each was kept: MIA376 unc-73(ce362) I; keyEx68 (expressing mCherry alone) and MIA377 unc-73(ce362) I; keyEx69 (expressing mCherry + Trio RhoGEF-E).

**Neuron and muscle co-expression**
Plasmids KG#68 (15 ng/µl; pan-neuronal GFP) or pKMC166 (15 ng/µl; pan-muscle mCherry) alone or with KG#281 (50ng/µl; pan-neuronal unc-73e) and pPD3 (50ng/µL; pan-muscle unc-73e) were injected into KG1278 unc-73(ce362) I, generating five independent mCherry(+), GFP(+) transgenic lines for behavior experiments from which a single transgenic line from each was kept: MIA372 unc-73(ce362); keyEx64 expressing mCherry (muscles) and GFP (neurons) only and MIA373 unc-73(ce362); keyEx65 expressing GFP (neurons), mCherry (muscles) and TrioRhoGEF-E (both neurons and muscles).

**PLCβ Transgenes**

To generate a control plasmid expressing GFP in neurons, GFP coding sequences were amplified from pJM60 (Moresco and Koelle, 2004) using oligonucleotides RE-GFP-FWD / -REV, digested with NheI / KpnI, and ligated into similarly digested pGP3 bearing the rgs-1 promoter (Dong et al., 2000), generating pKMC78. An egl-8 cDNA was used to generate and express a functional GFP fusion protein in neurons. Briefly, oligonucleotides egl-8-cDNA-fwd / -rev were used to amplified egl-8 coding sequences from a plasmid bearing an egl-8 cDNA provided by Dr. Kenneth Miller (pKP309). This amplicon was digested with NheI / Ncol and ligated into a similarly digested pPD49.26 plasmid, generating pKMC193. Quickchange mutagenesis with oligonucleotides egl-8-Cterm-Notl-fwd / -rev were used to insert an in-frame Notl site near the 3’ end of the egl-8 cDNA in a divergent region of the coding sequence, generating plasmid pKMC194. Coding sequences for egl-8 bearing this Notl site were then moved to pKMC78 by digestion of pKMC194 with NheI/Ncol followed by ligation into a similarly digested pKMC78, generating pKMC195. Oligonucleotides Notl-GFP-FWD / -REV were used to amplified GFP coding sequences from pKMC78, digested with Notl, and ligated into a similarly digested pKMC195, generating pKMC196. A strain bearing the egl-8(sa47) mutation was generously provided by Dr. Joshua Kaplan and backcrossed four times to N2 wild-type animals to generate
LX1225 egl-8(sa47) V. MT8189 lin-15(n765ts) males were mated to LX1225 to generate LX1287
egl-8(sa47) V; lin-15(n765ts) X hermaphrodites that were kept at 15°C prior to injection.
Plasmids expressing GFP alone (pKMC78; 5 ng/µl) or egl-8 CDNA fused to GFP (pKMC196; 5
ng/µl) were injected along with pL15EK (50ng/µl) into LX1287 hermaphrodites. For behavior
experiments, five independent GFP-expressing lines were used from which a single transgenic
line (vsEx679 [GFP] and vsEx680 [EGL-8::GFP], respectively) was kept.

Vulval muscle Channelrhodopsin-2 strains
N2 males were crossed into MIA229 keyls48 [ceh-24p::ChR2::unc-54 3'UTR + lin-15(+)], lite-
1(ce314), lin-15(n765ts) X (Kopchock et al., 2021) to produce F1 heterozygous males, which
then were crossed separately into MIA211 unc-73(ce362) I; lite-1(ce314) lin-15(n765ts) X,
MIA299 egl-30(ad805) I; lite-1(ce314) lin-15(n765ts) X, MIA303 egl-8(n488) V; lite-1(ce314) lin-
15(n765ts) X, or MIA307 egl-8(sa47) V; lite-1(ce314) lin-15(n765ts) X hermaphrodites to
generate vulval muscle specific Channelrhodopsin-2 (ChR2) expressing transgenic lines
MIA248 unc-73(ce362) I; keyls48; lite-1(ce314) X, MIA301 egl-30(ad805) I; keyls48; lite-
1(ce314) X, MIA305 egl-8(n488) V; keyls48; lite-1(ce314) X, and MIA309 egl-8(sa47) V;
keyls48; lite-1(ce314) X. The presence of lite-1(ce362) was confirmed by genotyping as above,
and the presence of the ChR2 transgene was confirmed by rescue of the lin-15(n765ts) multi-
vulva (Muv) phenotype.

HSN Channelrhodopsin strains
ChR2 was expressed in the HSNs from the egl-6 promoter via an integrated transgene (Emtage
et al., 2012). This transgene was crossed into Gαq signaling mutants as follows. N2 males were
crossed into LX1836 wzIs30 IV; lite-1(ce314) lin-15(n765ts) X to generate heterozygous F1
males, which were then crossed separately into MIA211 unc-73(ce362) I; lite-1(ce314) lin-
15(n765ts) X, MIA299 egl-30(ad805) I; lite-1(ce314) lin-15(n765ts) X, MIA303 egl-8(n488) V; 
lite-1(ce314) lin-15(n765ts) X, or MIA307 egl-8(sa47) V; lite-1(ce314) lin-15(n765ts) X 
hermaphrodites to generate MIA247 unc-73(ce362) I; wzIs30 IV; lite-1(ce314) X, MIA300 egl-
30(ad805) I; wzIs30 IV; lite-1(ce314) X, MIA304 wzIs30 IV; egl-8(n488)V; lite-1(ce314) X, and 
MIA308 wzIs30 IV; egl-8(sa47) V; lite-1(ce314) X. The presence of lite-1(ce362) was confirmed 
by PCR genotyping, and the wzIs30 transgene was confirmed by rescue of the lin-15(n765ts) 
Muv phenotype.

Behavior assays
Quantification of egg accumulation was performed as described (Chase et al., 2004). Staged 
adults were obtained by picking late L4 animals and culturing them 24-30hr at 20°C. Each animal 
was placed in 7 μL of 20% hypochlorite (bleach) solution and eggs were counted after animals 
had dissolved. Numbers of eggs and any internally hatched L1 animals were combined.

Pharmacological assays
Egg laying in response to exogenous serotonin was performed as described (Banerjee et al., 
2017). Individual staged adult animals were placed in 100 μl of either M9 buffer alone, or M9 
containing 7.5 mg/ml serotonin (creatinine sulfate monohydrate salt, Sigma-Aldrich #H7752) or 
M9 containing 10 μM PMA (Phorbol-12-myristate-13-acetate, Calbiochem #524400) in a 96-well 
microtiter dish. After 1 hour, the number of released eggs and L1 larvae in each well were 
counted. Since egg-laying defective animals sometimes release one or two progeny in response 
to mechanical stimulation when they are first picked into the well, animals were only were 
recorded as responding if they laid 3 or more progeny. For calcium imaging, NGM plates 
containing PMA or control (equivolume ethanol solvent) were prepared as described (Reynolds 
et al., 2005). Age-matched adult worms (n>10) from each genotype were placed on separate
PMA or control NGM plates at room temperature for 2 h, and subsequently transferred onto an agar chunk which was placed between the glass slide and a glass cover slip for Ca$^{2+}$ activity recording (Ravi et al., 2018b). The unused plates were kept at 4 °C for future use.

**Optogenetic assay**

All-trans retinal (Sigma Aldrich, R2500) was resuspended in ethanol (100%) to make 100 mM solution and added to a warmed culture of OP50 bacteria grown in B Broth media to a final concentration of 0.4 mM. Individual NGM agar plates were seeded with 200 μl of freshly prepared +ATR food, and were grown in the dark for ~24 hours prior to use. In all photo-stimulation experiments, control animals were grown in the absence of ATR. Animals were exposed to blue light using a Leica M165FC stereomicroscope equipped with an EL6000 metal halide light source generating 8–16 mW/cm$^2$ (depending on the magnification used) of ~470 ± 20 nm blue light through a GFP filter. The 30 s on/off sequence was programmed and controlled using the Optogenetics TTL Pulse Generator (OTPG-4, Version 3.3) triggering a SHB1 series shutter controller (ThorLabs; 170712-1).

**Microscopy**

**Ratiometric Ca$^{2+}$ imaging**

Vulval muscle Ca$^{2+}$ activity was performed in freely behaving adult animals at 24-30 h past the late L4 larval stage, as described previously (Collins and Koelle 2013; Collins et al. 2016; Ravi et al. 2018a). Worms co-expressing GCaMP5G and mCherry under the unc-103e promoter transgene vsIs164 were mounted beneath the chunk of agar over the glass slide. Ca$^{2+}$ activity was recorded through an 20X Apochromatic objective (0.8 NA) mounted on an inverted Zeiss Axio Observer.Z1. A Colibri.2 LED illumination system was used to excite GCaMP5 at 470 nm and mCherry at 590 nm for 10 msec every 50 msec. GFP and mCherry fluorescence emission...
channels were separated using a Hamamatsu W-VIEW Gemini image splitter and recorded simultaneously for 10 min with an ORCA-Flash 4.0 V2 sCMOS camera at 256 / 256-pixel resolution (4x4 binning) at 16-bit depth. The motorized stage was manually controlled using a joystick to maintain the freely behaving animal in the field of view. Image sequences were exported to Volocity software (Quorum Technologies Inc.) for segmentation and ratiometric analysis. Ca\(^{2+}\) transient peaks from ratio traces were detected using a custom MATLAB script, as described (Ravi et al. 2018a).

**Experimental design and statistical analysis**

Sample sizes for behavioral assays followed previous studies (Chase et al., 2004; Collins et al., 2016). Statistical analysis was performed using Prism 8 (GraphPad). Ca\(^{2+}\) transient peak amplitudes, widths, and inter-transient intervals were pooled from multiple animals (typically ≥ 10 animals per genotype). All statistical tests were corrected for multiple comparisons (Bonferroni for one-way ANOVA or Fisher’s exact; Dunn for Kruskal–Wallis). Each figure legend indicates individual p-values.
Results

Trio RhoGEF acts both in neurons and muscle to drive egg-laying behavior

Prior work has shown that \( \Gamma_\alpha_q \) signaling (Fig. 1A) through PLC\( \beta \) and Trio RhoGEF promotes neurotransmitter release and locomotion (Brundage et al., 1996; Miller et al., 1999; Williams et al., 2007), however, whether \( \Gamma_\alpha_q \) and its effectors similarly regulate other \( C. \) elegans behavior circuits is less well established. To address this uncertainty, we chose to examine the neural circuit driving egg-laying behavior (Fig. 1B). Egg-laying behavior in \( C. \) elegans is regulated by small motor circuit with defined neurons and muscle connectivity (Cook et al., 2019; White et al., 1986). The HSNs are serotonergic command motor neurons (Fig. 1B) that initiate the egg-laying active state and promote the excitability of the vm1 and vm2 egg-laying vulval muscles (Waggoner et al. 1998; Emtage et al. 2012; Collins et al. 2016) while innervating ventral cord neurons release acetylcholine to regulate muscle contraction (Kim et al., 2001; Kopchock et al., 2021; Waggoner et al., 2000a). We first examined the steady-state accumulation of eggs in the uterus as a proxy for changes in egg-laying circuit activity and behavior. As previously shown, animals bearing mutations in the EAT-16 RGS protein, which inhibits \( \Gamma_\alpha_q \) signaling (Hajdu-Cronin et al., 1999), or gain-of-function mutations in \( \Gamma_\alpha_q \) itself, (Doi and Iwasaki, 2002) showed a significant increase in egg laying resulting in a significant reduction in egg accumulation compared to the \( \sim 12 \pm 3 \) embryos retained in wild-type animals (Fig. 1C-E; Fig. 1I). Conversely, animals bearing mutations which reduce \( \Gamma_\alpha_q \) signaling through its effectors showed the opposite phenotype. Animals bearing an early nonsense mutation predicted to be a PLC\( \beta \) null mutant, [egl-8(sa47)], (Lackner et al., 1999; Williams et al., 2007) accumulated an average of 21 eggs, a significant increase (Fig. 1G). Animals bearing a missense mutation in the RhoGEF domain of Trio, unc-73(ce362) (Williams et al., 2007), showed an even stronger increase in egg accumulation, accumulating 31 eggs and closely resembling animals bearing loss-of-function
mutations in G\(_{\alpha q}\) itself (Fig. 1F-1I). Together, these results confirm that G\(_{\alpha q}\) and its effectors PLC\(\beta\) and Trio RhoGEF are required for egg-laying behavior in \textit{C. elegans} and that loss of the Trio RhoGEF branch causes a stronger behavior impairment compared to loss of PLC\(\beta\).

Previous work has shown that PLC\(\beta\) is expressed in neurons and in the intestine while G\(_{\alpha q}\) and Trio are expressed in nearly all excitable cells including neurons and muscles (Bastiani et al., 2003; Brundage et al., 1996; Lackner et al., 1999; Miller et al., 1999; Steven et al., 1998). To understand where G\(_{\alpha q}\) and its effectors function to regulate egg laying, we used cell-specific promoters to express cDNAs encoding PLC\(\beta\) or Trio RhoGEF in either all neurons, in the body wall and egg-laying vulval muscles, or in both neurons and muscles. We found that transgenic expression of PLC\(\beta\) from a neuron-specific promoter in PLC\(\beta\) null mutants was sufficient to rescue their defects in egg laying (Fig. 1J) and acetylcholine (ACh) release as measured by restoration of sensitivity to aldicarb, a cholinesterase inhibitor (data not shown). Previous work has indicated the presence of eight transcript variants of Trio (A, B, C1, C2, D1, D2, E, and F), which are differentially expressed in \textit{C. elegans} (Steven et al., 1998; Steven et al., 2005). Transgenic expression of Trio RhoGEF-E in neurons is fully sufficient to rescue the locomotion defects of Trio RhoGEF mutants (Williams et al., 2007). To explore whether Trio RhoGEF acts similarly in neurons for egg laying, we used a pan-neuronal promoter to express Trio RhoGEF-E and measured egg accumulation in these animals. We observed a partial, but significant reduction in the number of eggs retained in Trio RhoGEF mutants (~36 eggs) compared to control Trio mutant animals (~42 eggs; Fig. 1K). In contrast, transgenic expression of Trio RhoGEF-E in the egg-laying vulval muscles from a muscle-specific promoter showed a much greater rescue of egg accumulation (~25 eggs), and this rescue of egg laying was improved to nearly wild-type levels when Trio RhoGEF was expressed in both neurons and muscles (~19 eggs; Fig. 1K). Together, these results confirm that G\(_{\alpha q}\) signals through both PLC\(\beta\) and Trio.
RhoGEF function in neurons to regulate egg-laying behavior. Our results also suggest the Gαq effector Trio also functions in the postsynaptic vulval muscles for proper regulation of egg laying, a finding consistent with previously results regarding Gαq (Bastiani et al., 2003).

Serotonin signals through Gαq, Trio, and PLCβ to promote egg laying.

Previous studies have shown that serotonin released from the HSNs signals through G-protein coupled serotonin receptors expressed on the vulval muscles (Bastiani et al., 2003; Dempsey et al., 2005; Fernandez et al., 2020; Tanis et al., 2008; Xiao et al., 2006). The vulval muscles are also innervated by cholinergic ventral cord motor neurons (Cook et al., 2019; White et al., 1986) whose release of ACh is regulated by serotonin and G protein signaling (Nurrish et al., 1999).

To test whether serotonin promotes egg laying by activating receptors that couple to Gαq, we measured how serotonin signaling from the HSNs drives egg laying in Gαq signaling mutants. Serotonin promotes egg laying in hypertonic M9 buffer, a condition that normally inhibits egg laying in both wild-type and HSNs-deficient egl-1(dm) mutants, which developmentally lack the HSNs (Fig. 2B). Consistent with the previous results (Bastiani et al., 2003; Brundage et al., 1996; Trent et al., 1983), nearly 78% of wild-type and 70% of HSN-deficient egl-1(dm) animals laid eggs in response to serotonin compared to only 13% of mutant animals lacking Gαq (Fig. 2B).

Serotonin response was similarly and significantly reduced to 23% and 3% in PLCβ and Trio RhoGEF mutant animals, respectively (Fig. 2B). The distinct responses of animals without HSNs and animals lacking Gαq signaling indicate that Gαq, PLCβ and Trio act at least in part outside of HSNs to promote egg laying in response to exogenous serotonin. To determine where Trio RhoGEF deficiency caused serotonin insensitivity, we measured egg laying in animals expressing Trio RhoGEF in either neurons, muscles, or both. Transgenic expression of Trio RhoGEF in neurons failed to rescue egg laying in response to serotonin (Fig. 2C), but expression
of Trio RhoGEF in muscles, or in both neurons and muscles, restored egg laying to of animals (Fig. 2C), suggesting Trio mediates vulval muscle serotonin signaling. Together, these results indicate that Gαq, PLCβ and Trio RhoGEF function outside of the HSNs to drive egg laying in response to serotonin. The fact that PLCβ mutants are resistant to serotonin but unlike Trio mutants, show hyperactive egg-laying behavior upon pan-neuronal overexpression, suggests PLCβ acts presynaptically, either downstream or parallel to the HSNs, possibly to regulate ACh release, while Trio RhoGEF functions downstream of HSN, likely functioning postsynaptically in the egg-laying vulval muscles.

**Optogenetic stimulation of the HSNs and vulval muscles suggests cellular specificity of Gαq effectors for egg-laying.**

Optogenetic stimulation of Channelrhodopsin-2 (ChR2) expressed in either the HSNs (Emtage et al., 2012) or vulval muscles (Kopchock et al., 2021) can drive egg laying. To test whether and how Gαq and its effectors signal downstream to mediate this response, we expressed ChR2 in HSNs in Gαq and effector mutants and measured egg laying during 30s of exposure to blue light. Blue light stimulation of the HSNs drove the release of ~3 eggs in wild-type animals, which was reduced to essentially zero in Gαq [egl-30(ad805)] and Trio RhoGEF [unc-73(ce362)] mutants (Fig. 3A), consistent to our previous results showing Trio RhoGEF acts downstream of the HSNs in the postsynaptic vulval muscles. In contrast, stimulation of the HSNs in PLCβ null mutants [egl-8(sa47) and egl-8(nu488)] drove egg release, releasing ~4 and ~6 embryos respectively in 30 s (Fig. 3A). To test whether the failure of egg laying in Gαq and Trio RhoGEF mutants was caused by developmental defects in the circuit, rather than excitability deficits, we expressed and stimulated ChR2 in the vulval muscles. Blue light exposure drove the release of ~4 eggs in 30 s in wild-type animals. Both PLCβ [egl-8(sa47) and egl-8(nu488)] and Trio RhoGEF mutants
laid a similar number of eggs as wild-type control animals after blue light stimulation (Fig. 3B). The egl-30(ad805) Gαq mutant laid slightly fewer eggs (~3) but this was not significantly different than wild type. Thus, the failure of Gαq and Trio mutants to lay eggs in response to exogenous serotonin or optogenetic stimulation of the HSNs does not arise from some intrinsic defect in vulval muscle contractility, but rather a specific deficiency in muscle electrical excitability. These results support the conclusion from our rescue experiments (Fig. 1J-1K) that Gαq signals through PLCβ and Trio RhoGEF in distinct cells and through unique mechanisms to promote egg-laying circuit activity and behavior.

**Gαq and Trio are required for vulval muscle Ca²⁺ activity**

Egg laying in *C. elegans* is a two-state behavior where ~20 minute inactive states are punctuated by ~2-minute active states with high levels of Ca²⁺ activity in the egg-laying circuit driving release of 3-5 eggs (Collins et al., 2016; Waggoner et al., 1998). Loss of Gαq signaling in egl-30(n686) animals causes a significant reduction in spontaneous and serotonin-induced vulval muscle Ca²⁺ transients in immobilized animals (Shyn et al., 2003). We therefore tested whether these Gαq dependent Ca²⁺ activity defects were shared in PLCβ and Trio RhoGEF mutants. We expressed the genetically encoded Ca²⁺ reporter, GCaMP5, along with mCherry in the vulval muscles of animals with either increased or decreased Gαq signaling and performed ratiometric imaging in freely behaving animals. As shown in Figure 4A, the normal two-state pattern of vulval muscle Ca²⁺ activity is lost in animals bearing strong loss-of-function Gαq or Trio RhoGEF mutations. In both the egl-30(ad805) Gαq and unc-73(ce362) Trio mutants, we failed to observe either the strong egg-laying Ca²⁺ transients where both the vm1 and vm2 muscles contract or even the smaller amplitude rhythmic ‘twitch’ Ca²⁺ transients that are localized to the vm1 muscles (Fig. 4A), strongly reducing the observed Ca²⁺ transient frequency (Fig. 4B). In contrast, the
weaker egl-30(n686) Gαq mutant and two different PLCβ null mutants showed grossly normal vulval muscle Ca^{2+} activity during both egg-laying and twitch transients (Fig. 4A-C). The elevated pattern of vulval muscle Ca^{2+} activity in weak egl-30(n686) Gαq and PLCβ mutants we observed resembled egl-1(dm) animals lacking the HSNs (Collins et al., 2016). These animals still enter and leave infrequent egg-laying active states, possibly driven by the accumulation of eggs in the uterus (Ravi et al., 2018a). DAG Kinase-θ (DGK-θ) is thought to reduce DAG signaling through conversion to phosphatidic acid (Fig. 1A). Loss of the DGK-θ/DGK-1 increases neurotransmitter release and egg laying, likely through elevation of DAG levels and activation of downstream effectors (Jose and Koelle, 2005; Miller et al., 1996; Nurrish et al., 1999). To test if dgk-1 mutants have increased egg-laying muscle activity, we performed Ca^{2+} imaging in dgk-1(nu62) mutants. Somewhat surprisingly, DAG Kinase mutant animals did not show a significant increase in vulval muscle Ca^{2+} activity (Fig. 4A-C). Like PLCβ, DGK-1 is expressed in neurons (Nurrish et al., 1999), suggesting alterations of IP_3 and/or DAG levels in neurons may affect the frequency of egg-laying active states without altering the overall pattern or strength of vulval muscle Ca^{2+} activity within those active states. Conversely, animals with increased Gαq signaling either from a gain-of-function egl-30(tg26) mutations or eat-16(tm761) null mutations showed a significant increase in the amplitude of vulval muscle Ca^{2+} transients (Fig. 4B and 4C). There was also an increase in the frequency of vulval muscle Ca^{2+} transients in egl-30(tg26) mutants, but this effect did not rise to the level of significance in eat-16(tm761) mutants. Together, these results indicate Gαq signaling through Trio RhoGEF, but not PLCβ, promotes vulval muscle activity that drives twitching and egg-laying Ca^{2+} transients during egg-laying active states.

DAG mimetics restore muscle activity and egg laying to Gαq signaling mutants
How does serotonin signaling through $\alpha_q$ promote vulval muscle activity? Previous results have shown that DAG mimetic Phorbol esters restore locomotion and animal viability to $\alpha_q$ null mutants and to PLC$\beta$, Trio RhoGEF double mutants (Williams et al., 2007). However, whether phorbol esters rescued egg-laying behavior in $\alpha_q$ or effector single mutants was not reported, raising questions as to whether $\alpha_q$ rescue by phorbols esters was a general feature or was restricted to specific effector signaling pathways or behaviors (Fig. 5A). We measured the egg-laying responses of wild-type animals and $\alpha_q$ signaling mutants to Phorbol 12-myristate 13-acetate (PMA). As shown in Figure 5B, PMA treatment strongly stimulated egg laying in wild-type animals and all mutants with reduced $\alpha_q$ signaling ($\geq$80% animals laying eggs). Like serotonin (Fig. 2B), PMA also rescued egg laying in egl-1(dm) mutant animals lacking the HSNs (Fig. 4B), but only PMA rescued egg laying in $\alpha_q$ and effector signaling mutants. These results are consistent with PMA acting downstream of both serotonin release from the HSNs and its subsequent signaling through $\alpha_q$. We next imaged how exogenous PMA affected vulval muscle $\text{Ca}^{2+}$ activity. We performed 10-minute GCaMP5 recordings of wild-type or $\alpha_q$ signaling mutants treated for two hours with 10 µM PMA (Movies 1-3). Quantitation of $\text{Ca}^{2+}$ transients showed that exogenous PMA increased vulval muscle $\text{Ca}^{2+}$ activity in wild-type animals, as shown by the more frequent rhythmic twitch $\text{Ca}^{2+}$ transients (Movie 1). PMA also restored both rhythmic twitch and egg-laying $\text{Ca}^{2+}$ transients to strong $\alpha_q$ and Trio RhoGEF signaling mutants, as measured by both an increase in $\text{Ca}^{2+}$ transient peak amplitude and frequency (Fig. 5C-5E). While PMA treatment did not restore vulval muscle $\text{Ca}^{2+}$ transient frequency in $\alpha_q$ and Trio mutants to wild-type levels, $\text{Ca}^{2+}$ transient frequency was significantly improved in $\alpha_q$ mutant animals (Fig. 5C-5D). We did record more $\text{Ca}^{2+}$ transients in Trio mutants after treatment with PMA, but there was no significant difference to the vehicle control. We interpret differences observed in PMA rescue of egg laying (Fig. 5B) and vulval muscle $\text{Ca}^{2+}$ (Fig. 5C-D) as being a
result of the shortness of the 10-minute Ca\textsuperscript{2+} recording period that followed PMA treatment. Egg-laying active states typically occur every ~20 minutes in wild-type animals (Waggoner et al., 1998) and every 12-15 minutes in hyperactive egg-laying behavior mutants that resemble the effects of PMA treatment (Ravi et al., 2020; Waggoner et al., 2000b). The M9 buffer assays allow egg-laying events to accumulate over one hour without having to capture specific events. While we did observe nearly all Gα\textsubscript{q} and Trio mutant animals as now being able to lay eggs, this was not always captured during the Ca\textsuperscript{2+} recording. Together, these studies show that DAG-mimetic phorbol esters restore muscle contractility defects of Gα\textsubscript{q} and Trio RhoGEF mutants, suggesting DAG production is a major and necessary consequence of Gα\textsubscript{q} signaling.

**DAG promotes egg laying independent of UNC-13 and Protein Kinase C**

How do Phorbol esters such as PMA rescue vulval muscle Ca\textsuperscript{2+} activity and egg laying? Previous results have shown that DAG and PMA binds to C1 domain-containing proteins such as mUNC-13/UNC-13 and Protein Kinase C (PKC) to regulate their activity (Betz et al., 1998; Huang, 1989; König et al., 1985; Newton, 2001; Silinsky and Searl, 2003). To test if DAG regulates egg-laying behavior through activation of UNC-13 or PKC, we tested how PMA and serotonin affect egg laying in mutants lacking these proteins (Fig. 6). Mutants that eliminate axonal and synaptic UNC-13 show reduced egg laying, accumulating an average of 22 eggs compared to 15 seen in wild-type animals (Fig. 6A). Null unc-13(s69) and loss of function unc-13(e51) mutants have much less severe egg-laying defects compared to Gα\textsubscript{q} signaling mutants, accumulating ~22 and ~19 eggs (Fig. 6A) compared to >30 eggs (Fig. 1C-I). These results suggest that Gα\textsubscript{q} promotes synaptic transmission and egg-laying through an UNC-13 independent pathway. If PMA activation of vulval muscle Ca\textsuperscript{2+} and egg laying were mediated by UNC-13, then mutations that eliminate this protein should reduce that response. However, unc-13 mutants still laid eggs in
response to PMA (Fig. 6B). Because DAG production may be a conserved biochemical consequence of Gαq signaling through both PLCβ and Trio RhoGEF pathways, and both effector pathways have functions in neurons, we hypothesized that serotonin might promote egg laying via modulation of UNC-13 activity. In support of this model, unc-13 mutants failed to lay eggs in response to serotonin (Fig. 6B). To determine whether Gαq promotes egg laying through Protein Kinase C, we analyzed egg accumulation in animals bearing predicted null mutations in different PKC isoforms (Edwards et al., 2012; Hyde et al., 2011; Okochi et al., 2005; Tabuse, 2002; Tabuse et al., 1989). PKC-1 has previously been shown to promote neuropeptide transmission (Sieburth et al., 2007). While neuropeptides signal to promote egg laying (Avery et al., 1993; Brewer et al., 2019; Jacob and Kaplan, 2003; Kass et al., 2001), PKC-1 (nPKC-ε) null mutants showed a grossly normal egg accumulation of 14–17 eggs (Fig. 6C). Animals bearing predicted null mutants of novel and conventional PKCs such as nPKCδ/θ (TPA-1) and cPKCα/β (PKC-2) orthologs also show no significant differences in egg accumulation (Fig. 6C), suggesting that disruption of PKC signaling does not strongly affect egg-laying behavior. Like UNC-13, If PMA activation of vulval muscle Ca2+ and egg laying were mediated by PKCs, then mutations that eliminate PKCs, should reduce that response. However, PKC mutant animals still laid eggs in response to PMA (Fig. 6C) suggesting PKCs do not mediate the egg-laying response to PMA. Together, these results show that serotonin signals through Gαq via UNC-13 to promote neurotransmitter release, however, there are additional targets of DAG that function in parallel to UNC-13 in neurons and/or downstream of UNC-13 in the vulval muscles to promote egg laying.
Discussion

In this study we demonstrated the cellular specificity of Gαq signaling as it regulates egg-laying circuit activity and behavior using molecular genetics, optogenetics, pharmacology, and calcium imaging techniques. We found that Gαq effectors PLCβ and Trio RhoGEF differentially act in neurons and muscles to promote synaptic transmission and egg-laying behavior. PLCβ signals presynaptically in neurons while Trio signals both presynaptically in neurons and postsynaptically in muscles. Although Gαq, PLCβ, and Trio mutants all fail to lay eggs in response to serotonin, optogenetic stimulation of HSNs fully rescued egg laying in PLCβ but not Gαq or Trio RhoGEF mutants, suggesting hyperactivation of the HSNs may be able to bypass PLCβ signaling in some cases. Recent work has shown that the HSNs releases NLP-3 neuropeptides which can promote egg laying even in tph-1 mutants lacking serotonin (Brewer et al., 2019). Because pan-neuronal expression of PLCβ was able to rescue both egg-laying and cholinergic transmission defects of PLCβ null mutants, we propose that Gαq signals through PLCβ to promote acetylcholine release from the VA, VB, and VC neurons, which also innervate the vulval muscles alongside the HSNs (Cook et al., 2019; White et al., 1986). Consistent with this model, we have recently shown that VC synaptic transmission is important for egg laying in response to serotonin (Kopchock et al., 2021). Previous studies have shown that Trio acts in the presynaptic motor neurons to regulate locomotion behavior (Hu et al., 2011; Steven et al., 2005; Williams et al., 2007). Our studies show that Trio overexpression in either neurons or muscles alone is insufficient to restore wild-type level of egg-laying behavior, but expression in both is sufficient. These results further indicate that Gαq signaling through PLCβ and Trio RhoGEF fulfill distinct functions during locomotion and egg-laying behaviors.
How do Gαq, PLCβ and Trio-RhoGEF signaling promote egg-laying behavior? Earlier studies have suggested that Rho ortholog RHO-1 in *C. elegans* regulates synaptic activity in a mechanism that involves the G12 family protein GPA-12 (Hiley et al., 2006; Lutz et al., 2005). Activated RHO-1 also directly binds to and inhibits the DGK-1 diacylglycerol kinase expressed in neurons that signals to reduce DAG available to bind effectors (Hiley et al., 2006; McMullan et al., 2006). Our data demonstrate that Gαq signaling regulates postsynaptic vulval muscle activity mainly through Gαq-Trio pathway as Gαq, and Trio mutants show a similarly strong reduction in vulval muscle Ca2+ activity. Because muscle activity defects in Gαq and Trio mutants can be restored by the DAG-mimetic PMA, we suggest that insufficient levels of DAG are responsible for the circuit activity and behavior defects of Gαq and Trio RhoGEF mutants. In the absence of PLCβ, how would parallel Gαq signaling through Trio RhoGEF and RHO-1 generate DAG? Besides PLCβ (EGL-8), *C. elegans* expresses four other PLC orthologs: PLCε (PLC-1), PLC-2, PLCγ (PLC-3), and PLCδ (PLC-4) (Vázquez-Manrique et al., 2008). *In vitro* studies with cultured mammalian cells show that small G proteins like Rho can bind to and activate PLCε (Seifert et al., 2008; Wing et al., 2003). Genetic evidence in *C. elegans* suggests a model that both PLCε and PLCγ likely function downstream of Gαq to promote cell activity (Kunitomo et al., 2013; Yu et al., 2013), but whether Trio activation of Rho ultimately acts through these PLCs to produce DAG is not clear. One approach to test if these other PLCs mediate Rho signaling would be to perform genetic epistasis experiments. However, loss of Rho-1 causes lethality (Jantsch-Plunger et al., 2000; McMullan and Nurrish, 2011) and loss of PLCε and PLCγ cause sterility defects, limiting our ability to measure differences in egg laying. Imaging or biochemical experiments documenting changes in PIP2 (Stauffer et al., 1998) and/or DAG (Ohno et al., 2017; Tewson et al., 2012) could allow for direct tests of whether Gαq signaling through Trio and Rho changes PIP2 and DAG levels in vivo.
Previous studies have shown that the DAG mimetic phorbol esters promote both synaptic vesicle and dense core vesicle release from neurons and neurosecretory cells (Silinsky and Searl, 2003). DAG and phorbol esters activate many effectors including mUNC-13 in the brain and Protein Kinase C (PKC) in nearly all cells (Betz et al., 1998; Huang, 1989). In C. elegans, treatment of exogenous PMA promotes hypersensitivity to the paralytic effects of aldicarb (Sieburth et al., 2007). Double mutants of both PKC-1 null and UNC-13 (H17K) show a greater magnitude of resistivity to phorbol esters compared to either mutant alone suggesting that PMA acts in part through these effectors to regulate ACh release (Sieburth et al., 2007; Silinsky and Searl, 2003). Our data show that animals lacking either UNC-13 or single PKC isoforms still show a robust egg-laying response to PMA (Fig. 6), suggesting DAG and PMA promote muscle activity via other targets. In vitro studies show ROCK (Rho-associated coiled-coil kinase) activation in PMA-induced apoptosis and macrophage differentiation (Chang et al., 2006; Yang et al., 2017). ROCK has a predicted C1 domain that mediates protein interaction with DAG and might bind and be similarly activated by PMA (Xiao et al., 2009). In C. elegans, RHO-1 signals through LET-502/ROCK to phosphorylate non-muscle myosin light chain (Shimizu et al., 2018). 

\(G_{\alpha_q}\) also promotes neurotransmitter release via additional kinase targets including SEK-1 Mitogen-Activated Protein Kinase Kinase in the p38 MAPK pathway and KSR-1 in the ERK MAPK pathway (Coleman et al., 2018; Hoyt et al., 2017). KSR-1 is particularly interesting in that its N-terminus shares sequence similarly with C1 domains that might mediate regulation by DAG. Loss of KSR-1 and other ERK MAPK components also suppress the loopy locomotion defects caused by gain-of-function mutations in Rho-1 (Coleman et al., 2018). Taken together, our work is consistent with a model where additional DAG-sensitive effectors act downstream of \(G_{\alpha_q}\) to promote muscle contractility during egg laying.

Apart from direct activation of C1 domain containing effectors, emerging evidence indicates DAG can regulate neural circuit activity via endocannabinoid-mediated retrograde...
signaling. In pyramidal cells of the mouse hippocampus, postsynaptic calcium influx through voltage-gated calcium channels activates PLC\(\beta\)1 to generate DAG (Hashimotodani et al., 2006; Hashimotodani et al., 2007; Soltesz et al., 2015). Diacylglycerol lipase-\(\alpha\) (DAGL\(\alpha\)) can convert DAG to Arachidonoylglycerol (2-AG), which signals from dendrites in a retrograde manner through presynaptic endocannabinoid receptors to inhibit neurotransmitter release (Hashimotodani et al., 2013; Hashimotodani et al., 2005; Soltesz et al., 2015; Tanimura et al., 2010; Wettchureck et al., 2006). In C. elegans, 2-AG activates the NPR-19 endocannabinoid receptor ortholog that couples to \(G_{\alpha}\) to modulate serotonin transmission, pharyngeal, feeding, and locomotory behaviors (Oakes et al., 2019; Oakes et al., 2017; Pastuhov et al., 2016). We have recently shown that feedback of egg accumulation alters vulval muscle \(Ca^{2+}\) activity, which subsequently signals to regulate bursting in the HSNs (Ravi et al., 2018b; Ravi et al., 2020). These results support a model where stretch-dependent feedback of egg accumulation stimulates postsynaptic vulval muscle \(Ca^{2+}\) signaling. This \(Ca^{2+}\) would then activate PLCs to generate DAG and 2-AG which signal to modulate HSN activity, serotonin release, and egg laying. The genetic and experimental accessibility of the C. elegans egg-laying circuit should allow us to determine if conserved \(G\) proteins like \(G_{\alpha}\) act generally to drive neural circuit activity via changes in DAG, subsequent activation of effectors, and retrograde messengers like 2-AG.
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A Neuromodulator

GPCR

RGS (EAT-16)

Gαs (EGL-30)

Trio RhoGEF (UNC-73)

DAG + IP₃

PA

DGK-6 (DGK-1)

C elegans

um1/2

Gαo, lof mutant

15 ± 1.4 eggs

egl-30(ad805)

31 ± 1.8 eggs

eat-16(m761)

4 ± 0.7 eggs

PLCβ null mutant

21 ± 1.6 eggs

egl-8(sa47)

unc-73(ce362)

3 ± 0.4 eggs

Trio RhoGEF lof mutant

31 ± 1.6 eggs

I Unhatched eggs per animal

Wild type
eat-16(m761)
egl-30(bg26)
egl-30(ad806)
egl-8(sa47)
ung-73(ce362)

J Unhatched eggs per animal

Pan-neuronal expression: GFP only

PLCβ + GFP

genoctype: wild type egl-8(sa47) PLCβ null mutant

K Unhatched eggs per worm

Wild type unc-73(ce362) Trio RhoGEF mutant

Transgene promoter: Neurons (GFP) Muscles (mCherry) Neurons + Muscles

UNC-73 expression: − UNC-73E − UNC-73E − UNC-73E

n.s. ns
Figure 1: Trio RhoGEF is required in both neurons and muscles to regulate egg-laying behavior (A) Schematics of excitatory (black) and inhibitory (red) Gαq signaling pathway. C. elegans gene names are beneath the protein they encode. (B) Cartoon of the C. elegans egg-laying behavior circuit from a lateral view. Only the left side of the bilaterally symmetric circuit is shown. HSNL, Hermaphrodite Specific Neuron (left); VC4 and VC5 Ventral C neurons; vm1 and vm2 vulval muscles, um1 and um2 uterine muscles; uv1 uterine-vulval neuroendocrine cells. (C-H) Bright field images of worms of the indicated genotypes; arrowheads indicate accumulated eggs. Mean number of accumulated eggs ± 95% confidence intervals is also indicated. Position of the vulva is shown with asterisk (*). (I) Scatterplot of egg accumulation in wild-type, eat-16(tm761), egl-30(tg26), egl-30(ad805), egl-8(sa47), and unc-73(ce362) mutant animals. Line indicates mean eggs ± 95% confidence intervals. Asterisks indicate p ≤ 0.0001 (One way ANOVA with Bonferroni’s correction; wild type n=49; eat-16(tm761) n=36; egl-30(tg26) n=47; egl-30(ad805) n=44; egl-8(sa47) n=65; unc-73(ce362) n=38). (J) Transgenic rescue of egl-8 PLCβ egg-laying defects. Scatterplot of egg accumulation in transgenic animals expressing GFP only or EGL-8/PLCβ fused to GFP from the rgs-1 promoter in egl-8(sa47) null mutants (n=50) compared to wild-type (n=30) and egl-8(sa47) mutant animals (n=30). Bar indicates mean eggs ±95% confidence intervals. Asterisks indicate p≤0.0001 (One-way ANOVA with Bonferroni’s correction) (K) Transgenic rescue of unc-73 Trio RhoGEF egg-laying defects. Scatterplot of egg accumulation in wild-type (n=60), unc-73(ce362) mutants (n=72), and transgenic animals expressing a fluorescent protein with or without Trio in neurons (n≥32) or in muscles (n≥69), or in both neurons and muscles (n≥41) in unc-73(ce362) mutants. Horizontal line indicates mean accumulated eggs ±95% confidence intervals. Asterisks indicate p≤0.0145; n.s. = not significant (p>0.05; One-way ANOVA with Bonferroni’s correction for multiple comparisons).
Figure 2: Serotonin signals through $\mathrm{Ga}_q$, Trio, and PLC$\beta$ to promote egg-laying.

(A) Model of serotonergic and acetylcholine (ACh) signaling in the egg-laying circuit. (B) Bar plots showing the percentage of animals laying eggs in M9 buffer alone (grey) or M9 +7.5 mg/mL serotonin (green) in 1 hr. Bar indicates mean percent ±95% confidence intervals. Asterisks indicate p<0.0007; n.s. = not significant (p>0.05, Fisher’s exact test with Bonferroni’s correction for multiple comparisons; n>30 animals). (C) Bar plot showing percent of animals laying eggs in M9 buffer or M9 +7.5 mg/ml serotonin in wild-type or Trio RhoGEF mutant animals expressing nothing or Trio RhoGEF in neurons, muscles, or both. Bar indicates mean percent ±95% confidence intervals. Asterisks indicate p<0.0007; n.s. = not significant (p>0.05, Fisher’s exact test with Bonferroni’s correction for multiple comparisons; n>30).
Figure 3: Optogenetic stimulation of the HSNs or vulval muscles indicates cellular specificity of Gαq effectors for egg laying. (A) On left, cartoon of the egg-laying circuit and experiment showing blue light activation of HSN for 30 seconds. On right, scatterplot showing eggs laid per worm in presence (+) or absence (-) of all-trans retinal (ATR) cofactor during the
blue light activation of ChR2 expressed in HSNs of wild-type (grey), *egl-30(ad805) Gαq* strong loss-of-function mutants (red), *egl-8(n488) and egl-8(sa47)* PLCβ mutants (orange), and *unc-73(ce362)* Trio mutant (purple) animals. Line indicates mean eggs laid ±95% confidence intervals. Asterisks indicate *p<0.0001*; n.s. = not significant (*p>0.05*, One-way ANOVA with Bonferroni’s correction for multiple comparisons). (B) On the left, cartoon of the egg-laying circuit and experiment showing blue light activation of vulval muscles for 30 seconds(left). On the right, scatter plots of eggs laid per worm in presence (+) or absence (-) of ATR during blue light activation of ChR2 expressed in the vulval muscles of wild type, *egl-30(ad805) Gαq* strong loss-of-function mutants (red), *egl-8(n488) and egl-8(sa47)* PLCβ mutants (orange), and *unc-73(ce362)* Trio mutant (purple) animals. Line indicates mean eggs laid ±95% confidence intervals. Asterisk indicates *p≤0.0255*; n.s. = not significant, *p>0.05* (One-way ANOVA with Bonferroni’s correction for multiple comparisons).
Figure 4: G\(\alpha_q\) and Trio signaling promotes vulval muscle activity. (A) Representative GCaMP5::mCherry (\(\Delta R/R\)) ratio traces showing vulval muscle Ca\(^{2+}\) activity in freely behaving wild-type (black), egl-30(n686) G\(\alpha_q\) weak loss of function mutant (red), egl-30(ad805) G\(\alpha_q\) strong loss-of-function mutant (red), unc-73(ce362) Trio strong loss-of-function mutant (purple), egl-8(n488) PLC\(\beta\) null mutant (light blue), egl-8(sa47) PLC\(\beta\) null mutant (orange), eat-16(tm761) G\(\alpha_q\) RGS protein null mutant (grey), egl-30(tg26) strong G\(\alpha_q\) gain-of-function mutant (magenta), and dgk-1(nu62) DAG Kinase null mutant (dark blue) animals during active (green solid bar) and
inactive (dotted grey line) egg-laying behavior states. Arrowheads indicate egg-laying events; n>10 animals recorded per genotype. (B) Scatterplots of Ca$^{2+}$ transient frequency for indicated genotypes. Line indicates mean eggs laid ±95% confidence intervals; asterisks indicate p≤0.0340; n.s. indicates not significant (p>0.05, One-way ANOVA with Bonferroni’s correction for multiple comparisons). (C) Scatterplots of Ca$^{2+}$ peak amplitudes for the indicated genotypes during twitch (closed square) and egg-laying transients (open circles). Asterisks indicate p<0.0001, n.s. indicates not significant (p>0.05, Kruskal Wallis test with Dunn’s correction for multiple comparisons).
A

\[ \text{G}_{\alpha_\text{q}} \quad (\text{EGL-30}) \]

\[ \text{PLC}\beta \quad (\text{EGL-9}) \]

\[ \text{Trio RhoGEF} \quad (\text{UNC-73}) \]

\[ \text{Rho} \quad (\text{RHO-1}) \]

\[ \text{PI(4,5)P}_2 \]

\[ \text{DAG} + \text{IP}_3 \]

\[ \text{Phorbol Ester, PMA} \quad (\text{DAG mimetic}) \]

\[ \text{UNC-13?} \]

\[ \text{Protein Kinase C?} \]

\[ \text{ROCK?} \]

\[ \text{Synaptic Transmission} \]

\[ \text{Muscle Contraction} \]

\[ \text{& Egg Release} \]

B

![Graph showing animals laying eggs (%)]

C

**Vulval muscle Ca\textsuperscript{2+} activity**

- **ethanol control**
- **+ 10 \textmu M PMA**

**Wild type**

**G\textsubscript{\alpha_q} loss-of-function mutant**

- **egl-30(ad805)**

**Trio RhoGEF mutant**

- **unc-73(ce362)**

**PLC\beta null**

- **egl-8(n488)**

- **egl-8(sa47)**

D

![Images showing wild type, ethanol control, and + 10 \textmu M PMA conditions for different genotypes]

- **egl-30(ad805)**
- **unc-73(ce362)**
- **egl-8(n488)**
- **egl-8(sa47)**

E

![Graph showing vulval muscle Ca\textsuperscript{2+} transient frequency (min\textsuperscript{-1})]
Figure 5: DAG mimetic PMA rescues egg-laying circuit activity and behavior defects of Gαq signaling mutants. (A) Diagrams showing working model of Gαq and DAG signaling pathway during egg-laying behavior. (B) Bar plots showing the percentage of animals showing egg laying in M9 buffer (open grey bars) or M9 buffer +10 μM PMA (filled green bars). Error bars indicate 95% confidence intervals for the proportion. Asterisks indicate p<0.0006 (Fisher’s exact test with Bonferroni’s correction for multiple comparisons). (C) Representative GCaMP5::mCherry (ΔR/R) ratio traces showing vulval muscle Ca^{2+} activity in wild-type or the indicated Gαq signaling mutant animals in the absence (left) or presence (right) of 10 μM PMA. Arrowheads indicate egg-laying events. Number of animals recorded for each genotype and condition, n>10. (D) Heat map showing intensity modulated color spectrum of GCaMP5::mCherry (ΔR/R) ratio of vulval muscle Ca^{2+} activity ranging from blue (low Ca^{2+}) to red (high Ca^{2+}) of 10 animals. (E) Scatterplots of Ca^{2+} transient frequency in the absence (-) and presence (+) of 10 μM PMA for the indicated genotypes. Line indicates mean eggs laid ±95% confidence intervals. Asterisk indicates p≤0.0275; n.s.= not significant (p>0.05, One-way ANOVA with Bonferroni’s correction for multiple comparisons).
Figure 6: DAG promotes egg laying independent of UNC-13 or Protein Kinase C. (A) Scatterplot of egg accumulation in wild-type, unc-13(e51) loss-of-function mutant, and unc-13(s69) null mutant animals. Line indicates mean eggs laid ±95% confidence intervals. Asterisk indicates p ≤0.0021 (One-way ANOVA with Bonferroni’s correction for multiple comparisons). (B) Bar plots showing the percentage of wild-type, unc-13(e51), or unc-13(s69) mutant animals laying eggs in M9 buffer (grey open boxes), 7.5 mg/ml serotonin (filled blue boxes), or 10 μM PMA (filled green boxes). Bar indicates mean eggs ±95% confidence interval for the proportion. Asterisks indicate p<0.0006; n. s = not significant (p>0.05, Fisher’s exact test with Bonferroni’s correction for multiple comparisons). (C) Scatterplot of egg accumulation in wild-type and indicated Protein Kinase C mutant animals. Line indicates average eggs accumulated ±95% confidence intervals. n.s. = not significant (p>0.05, One-way ANOVA with Bonferroni’s correction
for multiple comparisons). (D) Bar plots showing the percentage of wild-type and Protein Kinase C mutant animals showing egg laying in M9 buffer (open bars) or 10 μM PMA (filled green bars). Bar indicates mean eggs ±95% confidence intervals for the proportion. Asterisks indicate p<0.0007; n.s.= not significant (p>0.05, Fisher’s exact test with Bonferroni correction for multiple comparisons).
Movie 1: GCaMP5:mCherry fluorescence ratio in the vulval muscles of wild-type animals in the absence or presence of 10 µM DAG analogue, PMA. Blue indicates low Ca\(^{2+}\) and red indicates elevated Ca\(^{2+}\).

Movie 2: GCaMP5:mCherry fluorescence ratio in the vulval muscles of strong loss of function Ga\(_q\) mutant, egl-30(ad805), in the absence or presence of 10 µM DAG analogue, PMA. Blue indicates low Ca\(^{2+}\) and red indicates elevated Ca\(^{2+}\).

Movie 3: GCaMP5:mCherry fluorescence ratio in the vulval muscles of strong loss of function Trio mutant, unc-73(ce362), animals in the absence or presence of PMA. Blue indicates low Ca\(^{2+}\) and red indicates elevated Ca\(^{2+}\).

Table 1: Lists of C. elegans strains used in this study

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<td>Wild type</td>
<td>Bristol wild-type strain</td>
<td>1,2,5</td>
<td>(Brenner, 1974)</td>
</tr>
<tr>
<td>MT1434</td>
<td>egl-30(n686) I</td>
<td>G(_a)q loss-of-function mutant, Egg-laying defective</td>
<td>1,2,5</td>
<td>(Trent et al., 1983)</td>
</tr>
<tr>
<td>DA823</td>
<td>egl-30(ad805) I</td>
<td>G(_a)q strong loss-of-function mutant, Egg-laying defective</td>
<td>1,2,5</td>
<td>(Brundage et al., 1996; Mendel et al., 1995)</td>
</tr>
<tr>
<td>KG1278</td>
<td>unc-73(ce362) I</td>
<td>Trio RhoGEF loss-of-function mutant, Egg-laying defective</td>
<td>1,2</td>
<td>(Williams et al., 2007)</td>
</tr>
<tr>
<td>Strain Code</td>
<td>Genotype</td>
<td>Phenotype</td>
<td>Gene Manipulation</td>
<td>Reference</td>
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<tr>
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</tr>
<tr>
<td>KG1397</td>
<td><em>unc-73(ev802)</em> I</td>
<td>Trio RhoGEF deletion mutant, Egg-laying defective</td>
<td></td>
<td>(Williams et al., 2007)</td>
</tr>
<tr>
<td>JT47</td>
<td><em>egl-8(sa47)</em> V</td>
<td>PLCβ null, Egg-laying defective</td>
<td></td>
<td>(Thomas, 1990)</td>
</tr>
<tr>
<td>MT1083</td>
<td><em>egl-8(n488)</em> V</td>
<td>PLCβ null, Egg-laying defective</td>
<td></td>
<td>(Trent et al., 1983)</td>
</tr>
<tr>
<td>LX1226</td>
<td><em>eat-16(tm761)</em> I</td>
<td>Gαq RGS null, Hyperactive egg-laying</td>
<td></td>
<td>(Porter and Koelle, 2010)</td>
</tr>
<tr>
<td>CG21</td>
<td><em>egl-30(tg26)</em> I; <em>him-5(e1490)</em> V</td>
<td>Gαq gain-of-function mutant, Hyperactive egg-laying</td>
<td></td>
<td>(Garcia et al., 2001)</td>
</tr>
<tr>
<td>LX1832</td>
<td><em>lite-1(ce314)</em> lin-15(n765ts) X</td>
<td>strain used for transgenic line creation</td>
<td></td>
<td>(Gürel et al., 2012)</td>
</tr>
<tr>
<td>LX1286</td>
<td><em>egl-8(sa47)</em> I; <em>lin-15(n765ts)</em> X</td>
<td>PLCβ null mutant, Egg-laying defective; multi-vulva</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>LX1674</td>
<td><em>egl-8(sa47)</em> I; <em>lin-15(n765ts)</em> X; vsEx679</td>
<td>PLCβ null, Egg-laying defective; non-Muv, expresses GFP in neurons from <em>rgs-1</em> promoter</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>LX1675</td>
<td><em>egl-8(sa47)</em> I; <em>lin-15(n765ts)</em> X; vsEx680</td>
<td>PLCβ null, Egg laying defective; non-Muv, expresses PLCβ and GFP in neurons from <em>rgs-1</em> promoter</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>MIA26</td>
<td><em>egl-1(n986dm)</em> V</td>
<td>Lacks HSNs</td>
<td></td>
<td>(Ravi et al., 2018a)</td>
</tr>
<tr>
<td>LX1918</td>
<td>vsIs164 <em>lite-1(ce314)</em> lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles</td>
<td></td>
<td>(Li et al., 2013)</td>
</tr>
<tr>
<td>MIA109</td>
<td>egl-8(sa47) V; vsIs164 lite-1(ce314), lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles of egl-8(sa47) mutant</td>
<td>4,5</td>
<td>This study</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
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</tr>
<tr>
<td>MIA139</td>
<td>egl-30(n686) I; vsIs164 lite-1(ce314) lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles of egl-30(n686)</td>
<td>4,5</td>
<td>This study</td>
</tr>
<tr>
<td>MIA140</td>
<td>egl-30(ad805) I; vsIs164 lite-1(ce314), lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles of egl-30(ad805)</td>
<td>4,5</td>
<td>This study</td>
</tr>
<tr>
<td>MIA141</td>
<td>unc-73(ce362) I; vsIs164, lite-1(ce314) lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles of unc-73(ce362)</td>
<td>4,5</td>
<td>This study</td>
</tr>
<tr>
<td>MIA286</td>
<td>egl-30(tg26) I; vsIs164, lite-1(ce314) lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles of egl-30(tg26)</td>
<td>4</td>
<td>This study</td>
</tr>
<tr>
<td>MIA287</td>
<td>eat-16(tm761) I; vsIs164 lite-1(ce314) lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles of eat-16(tm761)</td>
<td>4</td>
<td>This study</td>
</tr>
<tr>
<td>MIA288</td>
<td>egl-8(n488) V; vsIs164 lite-1 (ce314) lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles of egl-8(n488)</td>
<td>4,5</td>
<td>This study</td>
</tr>
<tr>
<td>-------</td>
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</tr>
<tr>
<td>MIA296</td>
<td>dgk-1(nu62) lite-1(ce314) lin-15(n765ts) X</td>
<td>Expresses GCaMP5 and mCherry in the vulval muscles of dgk-1(nu62)</td>
<td>4</td>
<td>This study</td>
</tr>
<tr>
<td>MIA372</td>
<td>unc-73(ce362) I; keyEx64</td>
<td>Expresses GFP in the neurons and mCherry in the muscles of unc-73(ce362)</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>MIA373</td>
<td>unc-73(ce362) I; keyEx65</td>
<td>Expresses GFP and Trio RhoGEF-E cDNA in the neurons, and mCherry and Trio RhoGEF-E cDNA in the muscles of unc-73(ce362)</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>MIA374</td>
<td>unc-73(ce362) I; keyEx66</td>
<td>Expresses GFP in neurons</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>MIA375</td>
<td>unc-73(ce362) I; keyEx67</td>
<td>Expresses GFP and Trio RhoGEF-E cDNA in neurons</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>MIA376</td>
<td>unc-73(ce362) I; keyEx68</td>
<td>Expresses mCherry in muscles</td>
<td>1,2</td>
<td>This study</td>
</tr>
<tr>
<td>MIA377</td>
<td>unc-73(ce362) I; keyEx69</td>
<td>Expresses mCherry and Trio RhoGEF-E cDNA in muscles</td>
<td>1,2</td>
<td>This study</td>
</tr>
<tr>
<td>Lineage</td>
<td>genotype</td>
<td>expression details</td>
<td>replicates</td>
<td>reference</td>
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</tr>
<tr>
<td>LX1836</td>
<td>wzIs30 IV; lite-1(ce314) lin-15(n765ts) X</td>
<td>Expresses Channelrhodopsin-2 (ChR2) in HSNs from the egl-6 promoter</td>
<td>3</td>
<td>(Collins et al., 2016)</td>
</tr>
<tr>
<td>MIA229</td>
<td>keyIs48; lite-1(ce314) lin-15(n765ts) X</td>
<td>Expresses ChR2 in vulval muscles from the ceh-24 promoter</td>
<td>3</td>
<td>(Kopchock et al., 2020)</td>
</tr>
<tr>
<td>MIA300</td>
<td>egl-30(ad805) I; wzIs30 IV; lite-1(ce314) X</td>
<td>Expresses ChR2 in HSN of egl-30(ad805) mutant</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>MIA301</td>
<td>egl-30(ad805) I; keyIs48; lite-1(ce314) X</td>
<td>Expresses ChR2 in vulval muscles of egl-30(ad805) mutant</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>MIA304</td>
<td>wzIs30 IV; egl-8(n488) V; lite-1(ce314) X</td>
<td>Expresses ChR2 in HSNs of egl-8(n488) mutant</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>MIA305</td>
<td>egl-8(n488) V; keyIs48; lite-1(ce314) X</td>
<td>Expresses ChR2 in vulval muscles of egl-8(n488) mutant</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>MIA308</td>
<td>wzIs30 IV; egl-8(sa47) V; lite-1(ce314) X</td>
<td>Express ChR2 in HSNs of egl-8(sa47) mutant</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>MIA309</td>
<td>egl-8(sa47) V; keyIs48; lite-1(ce314) X</td>
<td>Expresses ChR2 in vulval muscles of egl-8(sa47) mutant</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>Strain</td>
<td>Strain Type</td>
<td>Trait Description</td>
<td>L</td>
<td>Reference</td>
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<tr>
<td>---------</td>
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<td>--------------------------------------------------------</td>
<td>---</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>MIA247</td>
<td><strong>unc-73(ce362) I; wzl30 IV; lite-1(ce314) X</strong></td>
<td>Expresses ChR2 in HSN of <strong>unc-73(ce362)</strong> mutant</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>MIA248</td>
<td><strong>unc-73(ce362) I; keyl348; lite-1(ce314) X</strong></td>
<td>Expresses ChR2 in vulval muscles of <strong>unc-73(ce362)</strong> mutant</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>MT7929</td>
<td><strong>unc-13(e51) I</strong></td>
<td>UNC-13 loss-of-function</td>
<td>6</td>
<td>(Brenner, 1974)</td>
</tr>
<tr>
<td>EG9631</td>
<td><strong>unc-13(s69) I</strong></td>
<td>UNC-13 null</td>
<td>6</td>
<td>(Rose and Baillie, 1980)</td>
</tr>
<tr>
<td>IK105</td>
<td><strong>pkc-1(nj1) V</strong></td>
<td>nPKCε null</td>
<td>6</td>
<td>(Okochi et al., 2005)</td>
</tr>
<tr>
<td>IK130</td>
<td><strong>pkc-1(nj3) V</strong></td>
<td>nPKCε null</td>
<td>6</td>
<td>(Okochi et al., 2005)</td>
</tr>
<tr>
<td>RB781</td>
<td><strong>pkc-1(ok563) V</strong></td>
<td>nPKCε null</td>
<td>6</td>
<td>(Consortium, 2012)</td>
</tr>
<tr>
<td>VC127</td>
<td><strong>pkc-2(ok328) X</strong></td>
<td>cPKCα/β null</td>
<td>6</td>
<td>(Consortium, 2012)</td>
</tr>
<tr>
<td>MJ500</td>
<td><strong>tpa-1(k501) IV</strong></td>
<td>nPKCδ/θ null</td>
<td>6</td>
<td>(Tabuse et al., 1989)</td>
</tr>
<tr>
<td>MJ563</td>
<td><strong>tpa-1(k530) IV</strong></td>
<td>nPKCδ/θ null</td>
<td>6</td>
<td>(Tabuse et al., 1989)</td>
</tr>
</tbody>
</table>
**Table 2: Lists of oligonucleotide sequences used in this study**

<table>
<thead>
<tr>
<th>Oligo Name</th>
<th>Sequence (5’&gt;3’)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>lite-1(ce314)-fwd</td>
<td>ACGGGAGACGAAGAGCTAAATAGG</td>
<td>Genotyping of lite-1(ce314)</td>
</tr>
<tr>
<td>lite-1(ce314)-rev</td>
<td>CTAAGTTGCCGGTTCCTTAGAAC</td>
<td>Genotyping of lite-1(ce314)</td>
</tr>
<tr>
<td>egl-30(n686)-F</td>
<td>GCCAACCAGAGCAGGACATTCTCGG</td>
<td>Genotyping of egl-30(n686)</td>
</tr>
<tr>
<td>egl-30(n686)-R</td>
<td>CGGGAAAGTAGTCAAGGAGATGCC</td>
<td>Genotyping of egl-30(n686)</td>
</tr>
<tr>
<td>egl-30(ad805)-F</td>
<td>GCCAGGGCTGTCCCATTACGG</td>
<td>Genotyping of egl-30(ad805)</td>
</tr>
<tr>
<td>egl-30(ad805)-R</td>
<td>TCGGAAAGCGCCACCAGGAAC</td>
<td>Genotyping of egl-30(ad805)</td>
</tr>
<tr>
<td>egl-8-cDNA-fwd</td>
<td>CTTGGCTAGCTAGAAGAAAAATGGGCAAGAAGTTCCAGTTC</td>
<td>For amplification of egl-8 coding</td>
</tr>
<tr>
<td>egl-8-cDNA-rev</td>
<td>CGCCCATGTTATCAACGACAAGGGAGTCCAGG</td>
<td>For amplification of egl-8 coding</td>
</tr>
<tr>
<td>egl-8-Cterm-NotI-fwd</td>
<td>GTGGGTACTCCACTGGGGGTGCGGCCGCTGGAGGTCCCTTTCGAACAGAGGCCGCTGGAGTTCAAGAC</td>
<td>Quick change mutagenesis of egl-8 cDNA to insert in-frame NotI enzyme for GFP insertion</td>
</tr>
<tr>
<td>egl-8-Cterm-NotI-rev</td>
<td>ACCGGTGTCAAGGACCTCCAAGGGAGTCCAGG</td>
<td>Quick-change mutagenesis of egl-8 cDNA to insert in-frame NotI enzyme for GFP insertion</td>
</tr>
<tr>
<td>primers</td>
<td>sequence</td>
<td>description</td>
</tr>
<tr>
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<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>NotI-GFP-FWD</td>
<td>GGTGC GGCGCTGGGAAGTAAA GGAGAAGAACTTTTC</td>
<td>For amplification of GFP with flanking, in-frame NotI enzyme sites for insertion into egl-8 cDNA</td>
</tr>
<tr>
<td>NotI-GFP-REV</td>
<td>TCCAGCGGCGCTCTCTTTTGAT AGTTCATCCATGCC</td>
<td>For amplification of GFP with flanking, in-frame NotI enzyme sites for insertion into egl-8 cDNA</td>
</tr>
<tr>
<td>RE-GFP-FWD-new</td>
<td>GCGTCTAGAACC GGCTGCTAGC GTAGAAAAATGTCAGTAAAG GGAAGAAGAACTTTTC</td>
<td>For amplification of sequences encoding GFP for cloning into pGP3 rgs-1 promoter containing plasmid</td>
</tr>
<tr>
<td>RE-GFP-REV</td>
<td>TACGAATTCGGTACCTCAGATT TATTGTATAGTCCATCCATG</td>
<td>For amplification of sequences encoding GFP for cloning into pGP3 rgs-1 promoter containing plasmid</td>
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</tbody>
</table>
References:


Wing, M.R., Snyder, J.T., Sondek, J., and Harden, T.K. (2003). Direct activation of phospholipase C-

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(MPZ-1) interact in vulval muscle to facilitate serotonin-stimulated egg-laying. Dev Biol 298, 
379-391.

prostate cancer cells via p21Cip1 up-regulation and JNK. J Biol Chem 284, 29365-29375.


Systematic profiling of Caenorhabditis elegans locomotive behaviors reveals additional 