The SEK-1 p38 MAP Kinase Pathway Modulates Gq Signaling in Caenorhabditis elegans

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ABSTRACT Gq is a heterotrimeric G protein that is widely expressed in neurons and regulates neuronal activity. To identify pathways regulating neuronal Gq signaling, we performed a forward genetic screen in Caenorhabditis elegans for suppressors of activated Gq. One of the suppressors is an allele of sek-1, which encodes a mitogen-activated protein kinase kinase (MAPKK) in the p38 MAPK pathway. Here, we show that sek-1 mutants have a slow locomotion rate and that sek-1 acts in acetylcholine neurons to modulate both locomotion rate and Gq signaling. Furthermore, we find that sek-1 acts in mature neurons to modulate locomotion. Using genetic and behavioral approaches, we demonstrate that other components of the p38 MAPK pathway also play a positive role in modulating locomotion and Gq signaling. Finally, we find that mutants in the SEK-1 p38 MAPK pathway partially suppress an activated mutant of the sodium leak channel, NCA-1/NALCN, a downstream target of Gq signaling. Our results suggest that the SEK-1 p38 MAPK pathway may modulate the output of Gq signaling through NCA-1(unc-77).

Gq is a widely expressed heterotrimeric G protein that regulates a variety of biological processes, ranging from neurotransmission to cardiovascular pathophysiology (Sánchez-Fernández et al. 2014). In the canonical Gq pathway, Gq activates phospholipase Cβ (PLCβ), which cleaves phosphatidylinositol 4,5-bisphosphate into the second messengers diacylglycerol (DAG) and inositol trisphosphate (Rhee 2001). In addition to PLCβ, other Gq effectors have been identified including kinases, such as protein kinase Cε and Bruton’s tyrosine kinase (Btk) (Bence et al. 1997; García-Hoz et al. 2010; Vaqué et al. 2013), and guanine nucleotide exchange factors (GEFs) for the small GTPase Rho, such as Trio (Williams et al. 2007; Vaqué et al. 2013). These noncanonical effectors bridge the activation of Gq to other cellular signaling cascades.

In order to study noncanonical pathways downstream of Gq, we used the nematode Caenorhabditis elegans, which has a single Gq homolog (EGL-30) and conservation of the other components of the Gq signaling pathway (Koelle 2016). In neurons, EGL-30 signals through EGL-8 (PLCβ) (Lackner et al. 1999) and UNC-73 (ortholog of Trio RhoGEF) (Williams et al. 2007). UNC-73 activates RHO-1 (ortholog of RhoA), which has been shown to enhance neurotransmitter release through both diacylglycerol kinase (DGK-1)–dependent and –independent pathways (McMullan et al. 2006).

To identify additional signaling pathways that modulate Gq signaling, we screened for suppressors of the activated Gq mutant, egl-30(tg26) (Doi and Iwashki 2002). egl-30(tg26) mutant animals exhibit hyperactive locomotion and a “loopy” posture, in which worms have exaggerated, deep body bends and loop onto themselves (Bastiani et al. 2003; Topalidou et al. 2017). Here, we identify one of the suppressors as a deletion allele in the gene sek-1, SEK-1 is a mitogen-activated protein kinase kinase (MAPKK), the C. elegans ortholog of mammalian MKK3/6 in the p38 MAPK pathway (Tanaka-Hino et al. 2002). The p38 MAPK pathway has been best characterized as a pathway activated by a variety of cellular stresses and inflammatory cytokines (Kyriakis and Avruch 2012). However, the p38 MAPK pathway has also been shown to be activated downstream of a G protein–coupled receptor in rat neurons (Huang et al. 2004). Btk, a member of the Tec family of tyrosine kinases, has been shown to act downstream of Gq to activate the p38 MAPK pathway (Bence et al. 1997), but C. elegans lacks Btk and other Tec family members (Plowman et al. 1999).

SEK-1 is activated by the MAPKKK NSY-1 (ortholog of ASK1) and activates the p38 MAPKs PMK-1 and PMK-2 (Andrusiak and Jin 2016). The p38 MAPK pathway, consisting of NSY-1, SEK-1, and
PMK-1, is required for innate immunity in *C. elegans* (Kim et al. 2002). NSY-1 and SEK-1 are also required for the specification of the asymmetric AWC olfactory neurons (Sagasti et al. 2001; Tanaka-Hino et al. 2002); the p38 orthologs PMK-1 and PMK-2 function redundantly in AWC specification (Pagano et al. 2015). For both innate immunity and AWC specification, the p38 MAPK pathway acts downstream of the adaptor protein TIR-1 (an ortholog of SARM) (Couliault et al. 2004; Chuang and Bargmann 2005). Here, we show that the pathway consisting of TIR-1, NSY-1, SEK-1, PMK-1, and PMK-2 also acts to modulate locomotion downstream of Gq signaling.

**MATERIALS AND METHODS**

*C. elegans* strains and maintenance

All strains were cultured using standard methods and maintained at 20°C (Brenner 1974). The *sek-1(yak42)* mutant was isolated from an ENU mutagenesis suppressor screen of the activated Gq mutant, *egl-30(tg26)* (Allion et al. 2014). *sek-1(yak42)* was outcrossed away from *egl-30(tg26)* before further analysis. Double mutant strains were constructed using standard methods (Fay 2006), often with linked fluorescence markers (Frokjaer-Jensen et al. 2014) to balance mutations with subtle visible phenotypes. Supplemental Material, Table S1 in File S1 contains all of the strains used in this study.

Mapping

*yak42* was mapped using its slow locomotion phenotype and its *egl-30(tg26)* suppression phenotype. *yak42* was initially mapped to the X chromosome using strains *EG1000* and *EG1020*, which carry visible marker mutations. These experiments showed that *yak42* was linked to *lon-2*, but it was at least several map units (cM) away. *yak42* was further mapped to ~1 cM away from the red fluorescence insertion marker *oxTi668*, which is located at +0.19 cM on the X chromosome.

Whole-genome sequencing

Strain XZ1233 *egl-30(tg26)*; *yak42* was used for whole-genome sequencing to identify candidate *yak42* mutations. XZ1233 was constructed by crossing a *yak42* strain outcrossed two times, back to *egl-30(tg26)*. Thus, in XZ1233, *yak42* has been outcrossed three times from its original isolate. DNA was isolated from XZ1233 and purified according to the Hobert Laboratory protocol (http://hobertlab.org/whole-genome-sequencing/). Ion torrent sequencing was performed at the University of Utah DNA Sequencing Core Facility. The resulting data contained 10,063,209 reads of a mean read length of 144 bases, resulting in ~14x average coverage of the *C. elegans* genome. The sequencing data were uploaded to the Galaxy web platform, and we used the public server at usegalaxy.org to analyze the data (Afgan et al. 2016). We identified and annotated variants with the Unified Genotyper and SnpEff tools, respectively (DePristo et al. 2011; Cingolani et al. 2012). We filtered out variants found in other strains we sequenced, leaving us with 605 homozygous mutations. The X chromosome contained 94 mutations: 55 SNPs and 39 indels. Of these, four SNPs were nonsynonymous mutations in protein-coding genes, but only two were within 5 cM of *oxTi668*. However, we were unable to identify *yak42* from the candidate polymorphisms located near *oxTi668*. Transgenic expression of the most promising candidate *pcy-1* did not rescue *yak42*. Instead, to identify possible deletions, we scrolled through 2 MB of aligned reads on the UCSC Genome Browser, starting at ~4.38 cM and moving toward the middle of the chromosome (0 cM), looking for regions that lacked sequence coverage. We found a 3713-bp deletion that was subsequently confirmed to be the *yak42* causal mutation, affecting the gene *sek-1* located at ~1.14 cM.

**Locomotion assays**

Locomotion assay plates were made by seeding 10 cm nematode growth medium plates with 150 µl of an *Escherichia coli* OP50 stock culture, spread with sterile glass beads to cover the entire plate. Bacterial lawns were grown at room temperature (22.5–24.5°C) for 24 hr, and then stored at 4°C until needed. All locomotion assays were performed on first-day adults at room temperature (22.5–24.5°C). L4 stage larvae were picked the day before the assay and the experimenter was blind to the genotypes of the strains assayed. For experiments on strains carrying extrachromosomal arrays, the *sek-1(km41)* control worms were animals from the same plate that had lost the array.

Body bend assays were performed as described (Miller et al. 1999). A single animal was picked to the assay plate, the plate lid was returned, and the animal was allowed to recover for 30 sec. Body bends were then counted for 1 min, counting each time the worm’s tail reached the minimum or maximum amplitude of the sine wave. All strains in an experiment were assayed on the same assay plate. For experiments with *egl-8, unc-73*, and *rand-1* mutants, worms were allowed a minimal recovery period (until the worms started moving forward; 5 sec maximum) prior to counting body bends.

For the heat-shock experiment, plates of first-day adults were paralimed and heat-shocked in a 34°C water bath for 1 hr. Plates were then unparalimed and incubated at 20°C for 5 hr before performing body bend assays.

Radial locomotion assays were performed by picking animals to the middle of an assay plate. Assay plates were incubated at 20°C for 20 hr and the distances of the worms from the starting point were measured.

Quantitative analysis of the waveform of worm tracks was performed as described (Topalidou et al. 2017). Briefly, worm tracks were photographed and ImageJ was used to measure the period and amplitude. The value for each animal was the average of five period-to-amplitude ratios.

**C. elegans pictures**

Pictures of worms were taken at 60× magnification on a Nikon SMZ18 microscope with the DS-L3 camera control system. The worms were age-matched as first-day adults and each experiment set was photographed on the same locomotion assay plate prepared as described above. The images were processed using ImageJ and were rotated, cropped, and converted to grayscale.

**Molecular biology**

Plasmids were constructed using the Gateway cloning system (Invitrogen). Plasmids and primers used are found in Table S2 in File S1. The *sek-1* cDNA was amplified by RT-PCR from worm RNA and cloned into a Gateway entry vector. To ensure proper expression of *sek-1*, an operon GFP was included in expression constructs with the following template: <promoter>p::sek-1(cDNA)::3bb-2utr::gpd-2 operon::GFP::H2B::cyt-1utr (Frokjaer-Jensen et al. 2012). This resulted in untagged SEK-1, but expression could be monitored by GFP expression.

**Injections**

*C. elegans* strains with extrachromosomal arrays were generated by standard methods (Mello et al. 1991). Injection mixes were made with a final total concentration of 100 ng/µl DNA. Constructs were injected at 5 ng/µl injection markers at 5 ng/µl, and the carrier DNA Litmus 38i at 90 ng/µl. Multiple lines of animals carrying extrachromosomal arrays were isolated and had similar behaviors, as observed by eye. The line with the highest transmittance of the array was assayed.
Statistical analyses
At the beginning of the project, a power study was conducted on pilot body bend assays using wild-type and sek-1(yak42) worms. To achieve a power of 0.95, it was calculated that 17 animals should be assayed per experiment. Data were analyzed to check for a normal distribution (using the D’Agostino–Pearson and Shapiro-Wilk normality tests), and then subjected to the appropriate analysis using GraphPad Prism 5. For data sets with three or more groups, if the data were normal, they were analyzed with a one-way ANOVA; if they were not, they were analyzed with a Kruskal-Wallis test. Post hoc tests were used to compare data sets within an experiment. Reported P-values are corrected. Table S3 in File S1 contains the statistical tests for each experiment. * P < 0.05, ** P < 0.01, *** P < 0.001.

Data availability
Strains and plasmids are shown in Tables S1 and S2 in File S1, and are available from the Caenorhabditis Genetics Center or upon request. The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article and Supplemental Material.

RESULTS
sek-1 suppresses activated Gq
To identify genes acting downstream of Gq, we performed a forward genetic screen for suppressors of the activated Gq mutant, egl-30(tg26) (Doi and Iwashia 2002). egl-30(tg26) worms are hyperactive and have a loopy posture, characterized by an exaggerated waveform (Figure 1, B-E). Thus, we screened for worms that were less hyperactive and less loopy. We isolated a recessive suppressor, yak42, and mapped it to the middle of the X chromosome (see Materials and Methods). Whole-genome sequencing revealed that yak42 carries a large deletion of the sek-1 gene from upstream of the start codon into exon 4 (Figure 1A). yak42 also failed to complement sek-1(km4), a previously published sek-1 deletion allele, for the Gq suppression phenotype (Figure 1A) (Tanaka-Hino et al. 2002).

egl-30(tg26) double mutants with either sek-1(yak42) or sek-1(km4) are not loopy (Figure 1, B-D) and are not hyperactive (Figure 1E and Figure S1A in File S1). sek-1(yak42) was outcrossed from egl-30(tg26) and assayed for locomotion defects. Both the sek-1(yak42) and sek-1(km4) mutants are coordinated but move more slowly than the wild type (Figure 1F). The sek-1(egl1) point mutation (Kim et al. 2002) also causes a similar slow locomotion phenotype (Figure S1B in File S1). To test whether the egl-30(tg26) suppression phenotype might be an indirect effect of the slow locomotion of a sek-1 mutant, we built an egl-30(tg26) double mutant with a mutation in unc-82, a gene required for normal muscle structure. unc-82 mutants are coordinated but move slowly, similar to sek-1 mutants (Hoppe et al. 2010). However, although an egl-30(tg26) unc-82(e1220) double mutant moves more slowly than an egl-30(tg26) mutant (Figure S1C in File S1), it is still loopy (Figure 1, B-D). Thus, sek-1 appears to be a specific suppressor of activated egl-30.

The egl-30(tg26) allele causes an R243Q missense mutation in the Goi switch III region that has been shown to reduce both the intrinsic GTPase activity of the Goi protein and render it insensitive to GTPase-activation by a regulator of G protein signaling (RGS) protein, thus leading to increased Goi protein activation (Natochin and Artemyev 2003). To test whether the suppression of egl-30(tg26) by sek-1 is specific for this egl-30 allele, we built a double mutant between sek-1(km4) and the weaker activating mutation egl-30(js126). egl-30(js126) causes a V180M missense mutation in the Goi switch I region immediately adjacent to one of the key residues required for GTPase catalysis (Hawasi et al. 2004). Thus, the tg26 and js126 alleles activate EGL-30 through different mechanisms. The sek-1(km4) mutant also suppresses the hyperactivity and loopy waveform of egl-30(js126) (Figure 1, G and H), demonstrating that sek-1 suppression of activated egl-30 is not allele specific.

EGL-30/Gq is negatively regulated by GOA-1, the worm Goall ortholog, and the RGS protein EAT-16 (Hadju-Cronin et al. 1999). We tested whether sek-1 also suppresses the goa-1 and eat-16 loss-of-function mutants that cause a hyperactive and loopy phenotype similar to activated egl-30 mutants. sek-1(km4) suppresses the hyperactivity and loopy waveform of goa-1(sa734) (Figure S1, D and E in File S1). However, although sek-1(km4) suppresses the hyperactivity of eat-16(tm775), it did not significantly suppress the loopy waveform (Figure S1, F and G in File S1). One possible downstream effector of GOA-1 is the DAG kinase DGK-1, which inhibits DAG-dependent functions such as synaptic vesicle release (Miller et al. 1999; Nurrish et al. 1999). dgk-1(yx428) animals are hyperactive, but the sek-1 dgk-1 double mutant is uncoordinated and looks like neither sek-1 nor dgk-1 mutants, confounding the interpretation of how sek-1 genetically interacts with dgk-1.

sek-1 acts in mature acetylcholine neurons
egl-30 is widely expressed and acts in neurons to modulate locomotion (Lackner et al. 1999), so it is possible that sek-1 also acts in neurons to modulate Gq signaling. sek-1 is expressed in neurons, intestine, and several other tissues (Tanaka-Hino et al. 2002), and has been shown to function in GABA neurons to promote synaptic transmission (Vashishath et al. 2008).

To identify the cell type responsible for the sek-1 locomotion phenotype, we expressed the wild-type sek-1 CDNA under different cell-specific promoters and tested for transgenic rescue of a sek-1 null mutant. Expression of sek-1 in all neurons (using the unc-119 promoter) or in acetylcholine neurons (unc-17 promoter) was sufficient to rescue the sek-1 mutant slow locomotion phenotype, but expression in GABA neurons (unc-47 promoter) was not sufficient (Figure 2, A and B). These results indicate that sek-1 acts in acetylcholine neurons to modulate locomotion rate.

We next tested whether sek-1 acts in neurons to suppress egl-30 (tg26). Expression of sek-1 under pan-neuronal and acetylcholine neuron promoters reversed the sek-1 suppression of egl-30(tg26). Specifically, egl-30(tg26) sek-1 double mutants expressing wild-type sek-1 in all neurons or acetylcholine neurons resembled the egl-30(tg26) single mutant (Figure 2, C–E). However, expression of sek-1 in GABA neurons did not reverse the suppression phenotype (Figure 2, C–E). Together, these data show that sek-1 acts in acetylcholine and not GABA neurons to modulate both wild-type locomotion rate and Gq signaling.

To narrow down the site of sek-1 action, we expressed sek-1 in head (unc-17H promoter) and motorneuron (unc-17β promoter) acetylcholine neuron subclasses (Topalidou et al. 2017). Expression of sek-1 in acetylcholine motorneurons rescued the sek-1 slow locomotion phenotype (Figure S2A in File S1), suggesting that the slow locomotion of sek-1 mutants is due to a loss of sek-1 in acetylcholine motorneurons. However, expression of sek-1 in either the head acetylcholine neurons or motorneurons partially reversed the sek-1 suppression of egl-30(tg26) hyperactivity (Figure S2B in File S1), suggesting that the hyperactivity of activated Gq mutants may result from excessive Gq signaling in both head acetylcholine neurons and acetylcholine motorneurons; sek-1 may act in Gq signaling in both neuronal cell types. By contrast, expression of sek-1 in head acetylcholine neurons but not motorneurons reversed the sek-1 suppression of the egl-30(tg26) loopy waveform (Figure S2C in File S1), suggesting that the loopy posture of activated
Figure 1 sek-1 acts downstream of Gq to modulate locomotion behavior. (A) Gene structure of sek-1. White boxes depict the 5’ and 3’ untranslated regions, black boxes depict exons, and lines show introns. The positions of the yak42 and km4 deletions are shown. yak42 is a 3713-bp deletion that extends to 1926 bp upstream of the start codon. Drawn with Exon-Intron Graphic Maker (http://www.wormweb.org/exonintron). Bar, 100 bp. (B–D) sek-1(yak42) and sek-1(km4) suppress the loopy waveform of the activated Gq mutant egl-30(tg26). unc-82(e1220) does not suppress egl-30(tg26). (B) Photographs of first-day adult worms. All photos were taken at 60x magnification. (C) Quantification of the waveform phenotype. Error bars = SEM, n = 5. *** P < 0.001; ns, P > 0.05 compared to egl-30(tg26). (D) Photographs of worm tracks. (E) The activated Gq mutant egl-30(tg26) is hyperactive and is suppressed by sek-1(yak42) and sek-1(km4). Error bars = SEM, n = 20. *** P < 0.001. (F) sek-1 mutant worms have slow locomotion. Error bars = SEM, n = 20. *** P < 0.001 compared to wild type. (G) sek-1(km4) suppresses the hyperactive locomotion of the activated Gq mutant egl-30(js126). Error bars = SEM, n = 20. *** P < 0.001 compared to wild type. (H) sek-1(km4) suppresses the loopy waveform of the activated Gq mutant egl-30(js126). Error bars = SEM, n = 5. *** P < 0.001. WT, wild type.
Figure 2  sek-1 acts in mature acetylcholine neurons to modulate locomotion. (A) sek-1 acts in neurons to modulate locomotion rate. The sek-1 wild-type cDNA driven by the unc-119 pan-neuronal promoter [unc-119p::sek-1(+)P] rescues the slow locomotion phenotype of sek-1(km4) worms. Error bars = SEM, n = 20. *** P < 0.001. (B) sek-1 acts in acetylcholine neurons to modulate locomotion rate. sek-1 wild-type cDNA driven by the
Gq mutants may result from excessive Gq signaling in head acetylcholine neurons, and sek-1 may act in those neurons to control body posture.

Because sek-1 acts in the development of the AWC asymmetric neurons, we asked whether sek-1 also has a developmental role in modulating locomotion by testing whether adult-specific sek-1 expression (driven by a heat-shock promoter) is sufficient to rescue the sek-1 mutant. We found that sek-1 expression in adults rescues the sek-1 slow locomotion phenotype (Figure 2F). This result indicates that sek-1 is not required for development of the locomotion circuit, and instead acts in mature neurons to modulate locomotion.

The p38 MAPK pathway is a positive regulator of Gq signaling

SEK-1 is the MAPKK in the p38 MAPK pathway consisting of the adaptor protein TIR-1, NSY-1 (MAPKKK), SEK-1 (MAPKK), and PMK-1 or PMK-2 (MAPKs) (Tanaka-Hino et al. 2002; Andrusiak and Jin 2016). We tested whether the entire p38 MAPK signaling module also modulates locomotion rate and suppression of activated Gq. Both tir-1(tm3036) and nsy-1(ok593) mutant animals have slow locomotion on their own, and also suppress the hyperactivity and loopy waveform of egl-30(tm26) (Figure 3, A–D, G, and H). We also tested single mutants in each of the three worm p38 MAPK genes (pmk-1, pmk-2, and pmk-3) and a pmk-2 pmk-1 double mutant. Although we found that the pmk-2 and pmk-3 single mutants were slightly slow on their own, only the pmk-2 pmk-1 double mutant phenocopied sek-1 and suppressed both the hyperactivity and loopy waveform of egl-30(gf26) (Figure 3, E–H). Thus, pmk-2 and pmk-1 act redundantly downstream of sek-1 to suppress egl-30(gf26). These data suggest that the p38 MAPK pathway modulates locomotion rate in C. elegans and acts genetically downstream of egl-30.

The JNK MAPK pathway, related to the p38 MAPK family, also modulates locomotion in C. elegans. Specifically, the JNK pathway members jkk-1 (JNK MAPKK) and jnk-1 (JNK MAPK) have been shown to act in GABA neurons to modulate locomotion (Kawasaki 1999). We found that the jkk-1 and jnk-1 single mutants had slow locomotion and that the double mutants with p38 MAPK pathway members exhibited an additive slow locomotion phenotype (Figure S3A in File S1). Moreover, neither jkk-1 nor jnk-1 suppressed the loopy phenotype of egl-30(gf26) (Figure S3B in File S1). Thus, the JNK and p38 MAPK pathways modulate locomotion independently, and the JNK pathway is not involved in Gq signaling.

We also tested the involvement of possible p38 MAPK pathway effectors. One of the targets of PMK-1 is the transcription factor ATF-7 (Shivers et al. 2010). Both the atf-7(qd22 qd130) loss-of-function mutant and the atf-7(qd22) gain-of-function mutant moved slowly compared to wild-type animals (Figure S3C in File S1). However, atf-7(qd22 qd130) did not suppress the loopy waveform of egl-30(gf26) (Figure S3B in File S1), suggesting that atf-7 is not a target of this pathway, or else it acts redundantly with other downstream p38 MAPK targets. We also tested gap-2, the closest C. elegans homolog of ASK1-interacting protein (AIP1), which activates ASK1 (the ortholog of C. elegans NSY-1) in mammalian systems (Zhang et al. 2003). A C. elegans gap-2 mutant showed no locomotion defect (Figure S3D in File S1). Finally, we tested VIP-1, a phosphatase for p38 and JNK MAPKs that inhibits p38 MAPK signaling (Kim et al. 2004). However, the vhp-1(sa366) mutant also showed no locomotion defect (Figure S3D in File S1).

egl-30(tm26) animals are loopy and hyperactive, so we tested whether increased activation of the TIR-1/p38 MAPK signaling module causes similar phenotypes. The tir-1(ky648) allele leads to a gain-of-function phenotype in the AWC neuron specification (Chang et al. 2011), but does not cause loopy or hyperactive locomotion (Figure S3, E and F in File S1).

Genetic interactions of sek-1 with pathways acting downstream of Gq

Our forward genetic screen for suppressors of egl-30(tm26) identified mutants that fall into three different categories: mutants in the canonical Gq pathway, such as the PLC egl-8 ([Lackner et al. 1999]; mutants in the RhoGEF Trio pathway, such as unc-73 (Williams et al. 2007); and mutants that affect dense-core vesicle biogenesis and release (Alien et al. 2014; Topalidou et al. 2016).

To test if sek-1 acts in any of these pathways, we built double mutants between sek-1 and members of each pathway. Loss-of-function alleles of egl-8(sa47), unc-73(ea317), and rund-1(tm3622) have slow locomotion (Figure 4, A–C). We found that sek-1 enhances the slow locomotion phenotype of egl-8 and rund-1 single mutants, suggesting that sek-1 does not act in the same pathway as egl-8 or rund-1 (Figure 4, A and B). By contrast, sek-1 does not enhance the slow locomotion phenotype of unc-73 mutants (Figure 4C), suggesting that sek-1 may act in the same genetic pathway as the Trio RhoGEF unc-73.

We next tested whether sek-1 interacts with rho-1, encoding the small G protein Rho that is activated by Trio. Because rho-1 is required for viability (Jantsch-Plunger et al. 2000), we used an integrated transgene overexpressing an activated rho-1 mutant allele specifically in acetylcholine neurons. Animals carrying this activated RHO-1 transgene, referred to here as rho-1(gf), have a loopy posture reminiscent of egl-30(tm26) (McMullan et al. 2006), and a decreased locomotion rate (Figure 4, D–F). Rho-1(gf) sek-1(km4) double mutants had a loopy body posture like rho-1(gf) animals, and even slower locomotion rate (Figure 4, D–F), suggesting that sek-1 and rho-1(gf) mutants have additive locomotion phenotypes. However, both sek-1(km4) and sek-1(yak42) weakly suppress the slow growth rate of the rho-1(gf) mutant (data not shown). Because sek-1 does not enhance unc-73 mutants and suppresses some aspects of the rho-1(gf) mutant, sek-1 may modulate output of the Rho pathway, although it probably is not a direct transducer of Rho signaling.
Figure 3 The p38 MAPK pathway modulates locomotion downstream of egl-30. (A) tir-1(tm3036) mutant animals have slow locomotion. Error bars = SEM, n = 20. *** P < 0.001. (B) tir-1(tm3036) suppresses egl-30[ts26], egl-30[ts26] tir-1 animals move more slowly than the hyperactive egl-30[ts26] animals. Error bars = SEM, n = 20. *** P < 0.001. (C) nsy-1(ok593) mutant animals have slow locomotion. Error bars = SEM, n = 20. *** P < 0.001. (D) nsy-1(ok593) suppresses egl-30[ts26]. egl-30[ts26] nsy-1 animals move more slowly than hyperactive egl-30[ts26] animals. Error bars = SEM, n = 20. *** P < 0.001. (E) pmk-2, pmk-2 pmk-1, and pmk-3 mutant animals have slow locomotion. Error bars = SEM, n = 20. * P < 0.05, *** P < 0.001, and ns, P > 0.05 compared to wild type. (F) A pmk-2 pmk-1 double mutant suppresses the hyperactivity of egl-30[ts26]. Error bars = SEM, n = 20. *** P < 0.001 compared to egl-30[ts26]. (G and H) tir-1(tm3036), nsy-1(ok593), and the pmk-2 pmk-1 double mutant suppress the loopy waveform of egl-30[ts26]. egl-30[ts26] animals with mutations in either pmk-1, pmk-2, or pmk-3 are still loopy. (G) Worm photographs. All photos were taken at 60X magnification. (H) Quantification. Error bars = SEM, n = 5. *** P < 0.001 compared to egl-30[ts26]. WT, wild type.
sek-1 and nsy-1 partially suppress activated NCA

To clarify the relationship of the SEK-1 p38 MAPK pathway to the Rho pathway acting downstream of Gq, we examined interactions with nca-1, a downstream target of the Gq-Rho pathway (Topalidou et al. 2017). NCA-1 and its orthologs are sodium leak channels associated with rhythmic behaviors in several organisms (Nash et al. 2002; Lu et al. 2007; Shi et al. 2016). In C. elegans, NCA-1 potentiates persistent motor circuit activity and sustains locomotion (Gao et al. 2015).

We tested whether sek-1 and nsy-1 mutants suppress the activated NCA-1 mutant ox352, referred to as nca-1(gf). The nca-1(gf) animals are coiled and uncoordinated; thus, it is difficult to measure their locomotion rate by the body bend assay because they do not reliably propagate sinusoidal waves down the entire length of their body. Instead, we used a radial locomotion assay in which we measured the distance animals moved from the center of a plate. nca-1(gf) double mutants with either sek-1(km4) or nsy-1(ok593) uncoiled a bit, but still exhibited uncoordinated locomotion (Figure S4A). In fact, although these double mutants showed more movement in the anterior half of their bodies than nca-1(gf), they propagated body waves to their posterior half even more poorly than the nca-1(gf) mutant. However, both sek-1 and nsy-1 partially suppressed the loopy waveform of the nca-1(gf) mutant (Figure 5A, A and B), and in radial locomotion assays, sek-1 and nsy-1 weakly suppressed the nca-1(gf) locomotion defect (Figure 5C). Additionally, both sek-1 and nsy-1 partially suppressed the small body size of nca-1(gf) (Figure S4A in File S1). Together, these data suggest that mutations in the SEK-1 p38 MAPK pathway suppress some aspects of the nca-1(gf) mutant.

Given that sek-1 acts in acetylcholine neurons to modulate wild-type and egl-30(g26) locomotion, we tested whether sek-1 also acts in these neurons to suppress nca-1(gf). Expression of sek-1 in all neurons or in acetylcholine neurons of nca-1(gf) sek-1(km4) animals restored the nca-1(gf) loopy phenotype (Figure 5, D and E). By contrast, expression of sek-1 in GABA neurons did not affect the loopy posture of the nca-1(gf) sek-1 double mutant (Figure 5, D and E). These data suggest that sek-1 acts in acetylcholine neurons to modulate the body posture of nca-1(gf) as well. However, in radial locomotion assays, expression of sek-1 in any of these neuron classes did not significantly alter the movement of the nca-1(gf) sek-1 double mutant (Figure S4B in File S1), although the weak suppression of nca-1(gf) by sek-1 in this assay makes it difficult to interpret these negative results. To further narrow down the site of action for sek-1 for its NCA suppression phenotypes, we expressed it in subclasses of acetylcholine neurons. Surprisingly, expression of sek-1 in acetylcholine motorneurons but not head acetylcholine neurons was sufficient to restore the loopy posture of the nca-1(gf) mutant (Figure 5E), the opposite of what we found for sek-1 modulation of the loopy posture of the activated Gq mutant, suggesting that the loopy posture of nca-1(gf) mutants may result from excessive NCA-1 activity in acetylcholine motorneurons. Additionally, expression of sek-1 in either the head acetylcholine neurons or the motorneurons restored the nca-1(gf) small body size phenotype (Figure 5C in File S1). We make the tentative conclusion that sek-1 acts in acetylcholine neurons to modulate nca-1(gf) body posture and size, but we are not able to conclusively narrow down its site of action further, possibly due to the uncoordinated phenotype of nca-1(gf) and the weaker suppression of nca-1(gf) by sek-1.

Figure 4 sek-1 acts in the same genetic pathway as unc-73. (A) sek-1 does not act in the same genetic pathway as egl-8. The sek-1(yak42) mutation enhances the slow locomotion of the egl-8(sa47) mutant. Error bars = SEM, n = 20. *** P < 0.001. (B) sek-1 does not act in the same genetic pathway as rund-1. The sek-1(yak42) mutation enhances the slow locomotion of the rund-1(tm3622) mutant. Error bars = SEM, n = 20. *** P < 0.001. (C) sek-1 may act in the same genetic pathway as unc-73. The sek-1(yak42) mutation does not enhance the slow locomotion phenotype of the unc-73(ok317) mutant. Error bars = SEM, n = 20. ns, P > 0.05. (D and E) sek-1(km4) does not suppress the loopy waveform of nzIs29[rho-1(gf)] animals. (D) Worm photographs. Photos were taken at 60x magnification. (E) Quantification. Error bars = SEM, n = 5. ns, P > 0.05. (F) sek-1(km4) does not suppress the slow locomotion of rho-1(gf) animals. Error bars = SEM, n = 20. *** P < 0.001. WT, wild type.
DISCUSSION

The p38 MAPK pathway has been best characterized as a pathway activated by a variety of cellular stresses and inflammatory cytokines (Kyriakis and Avruch 2012), but it has also been implicated in neuronal function, including some forms of mammalian synaptic plasticity (Bolshakov et al. 2000; Rush et al. 2002; Huang et al. 2004). In this study, we identified a new neuronal role for the MAPKK SEK-1 and the p38 MAPK pathway as a positive modulator of locomotion rate and Gq signaling. The physiological importance of this pathway is clear under conditions of elevated Gq signaling but is less obvious during normal wild-type locomotion, consistent with the observation that sek-1 mutations have a relatively weak effect on synaptic transmission in a wild-type background (Vashishshan et al. 2008). Thus, the SEK-1 p38 MAPK pathway may be more important for modulation of Gq signaling and synaptic strength than for synaptic transmission per se.

In addition to SEK-1, we identified other p38 pathway components that modulate Gq signaling. Specifically, we found that tir-1, nsy-1, and pmk-1 pmk-2 mutants exhibit locomotion defects identical to sek-1 and suppress activated Gq, suggesting that they act in a single p38 pathway to modulate signaling downstream of Gq. These results indicate a redundant function for PMK-1 and PMK-2 in modulating locomotion rate and Gq signaling. PMK-1 and PMK-2 also act redundantly for some other neuronal roles of the p38 pathway, such as the development of the asymmetric AWC neurons and to regulate induction of serotonin biosynthesis in the ADF neurons in response to pathogenic bacteria (Shivers et al. 2009; Pagano et al. 2015). By contrast, PMK-1 acts alone in the intestine to regulate innate immunity, and in interneurons to regulate trafficking of the GLR-1 glutamate receptor (Pagano et al. 2015; Park and Rongo 2016).

What are the downstream effectors of the SEK-1 p38 MAPK pathway that modulate locomotion? There are several known downstream effectors of p38 MAPK signaling in C. elegans, including the transcription factor ATF-7 (Shivers et al. 2010). Our data indicate that ATF-7 is not required for the p38 MAPK–dependent modulation of Gq signaling. The p38 MAPK pathway may activate molecules other than transcription factors, or may activate multiple downstream effectors.

How does the SEK-1 p38 pathway modulate the output of Gq signaling? One of the pathways that transduces signals from Gq includes the RhoGEF Trio/UNC-73, the small GTPase Rho, and the cation channel NALCN/NCA-1 (Williams et al. 2007; Topalidou et al. 2017). Compared to other pathways downstream of Gq, mutants in the Rho-Nca pathway are particularly strong suppressors of the loopy waveform phenotype of the activated Gq mutant (Topalidou et al. 2017). Similarly, we found that mutations in the SEK-1 p38 MAPK pathway strongly suppress the loopy waveform of the activated Gq mutant, suggesting that the SEK-1 pathway might modulate Gq signal output through the Rho-Nca branch. Consistent with this, we found that mutations in the SEK-1 p38 MAPK pathway partially suppress an activated NCA-1 mutant. Given the precedence for direct phosphorylation of sodium channels by p38 to regulate channel properties (Wittmack et al. 2005; Hudson et al. 2008), it is possible that motomeurons to modulate the loopy waveform of nca-1(gf) nca-1(ox352) sek-1(km4) worms expressing sek-1 in all neurons [unc-119p::sek-1(+)], acetylcholine neurons [unc-17p::sek-1(+)], or acetylcholine motomeurons [unc-17p::sek-1(+)] are loopy like nca-1(gf) but nca-1(ox352) sek-1(km4) worms expressing sek-1 in GABA neurons [unc-47p::sek-1(+)] or head acetylcholine neurons [unc-17p::sek-1(+)] are similar to nca-1(gf) sek-1. Error bars = SEM, n = 5. ** P < 0.01, *** P < 0.001; ns, P > 0.05. WT, wild type.
PMK-1 and PMK-2 phosphorylate NCA-1 to regulate its expression, localization, or activity. Consistent with the observation that Gq acts in acetylcholine neurons to stimulate synaptic transmission (Lackner et al. 1999), we found that sek-1 acts in acetylcholine neurons to modulate the locomotion rate in both wild-type and activated Gq mutants. sek-1 also acts in acetylcholine neurons to modulate the loopy waveform of both activated Gq and activated nca-1 mutants, and the size of activated nca-1 mutants. However, our data obtained from attempting to narrow down the site of action of sek-1 suggest that it may act in both head acetylcholine neurons and acetylcholine motorneurons, and that the waveform is probably controlled by at least partially distinct neurons from those that control locomotion rate. Further work will be required to identify the specific neurons where Gq, NCA-1, and the SEK-1 pathway act to modulate locomotion rate and waveform, and determine whether they all act together in the same cell.

ACKNOWLEDGMENTS
We thank Dennis Kim and Chiou-Fen Chuang for strains, Pin-An Chen and Erik Jorgensen for the nca-1(1gf) mutant ox352, Chris Johnson for the fine mapping of yak42, Jordan Hoyt for help with Galaxy software to analyze whole-genome sequencing data, and Dana Miller for providing access to her microscope camera. Some strains were provided by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health (NIH) Office of Research Infrastructure Programs (P40 OD010440). J.M.H. was supported in part by a Public Health Service, National Research Service award (T32GM007270) from the National Institute of General Medical Sciences. M.A. is an Ellison Medical Foundation New Scholar. This work was supported by NIH grant R00 MH082109, to M.A.

LITERATURE CITED
Afgan, E., D. Baker, M. van den Beek, D. Blankenberg, D. Bouvier et al., 2016 The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res. 44: W3–W10.
Alion, M., M. Hannemann, S. Dalton, A. Pappas, S. Watanabe et al., 2014 Two Rab2 interactors regulate dense-core vesicle maturation. Neuron 82: 167–180.
Andrusiak, M. G., and Y. Jin, 2016 Context specificity of stress-activated mitogen-activated protein (MAP) kinase signaling: the story as told by Caenorhabditis elegans. J. Biol. Chem. 291: 7796–7804.
Bastiani, C. A., S. Gharib, M. I. Simon, and P. W. Sternberg, 2003 Caenorhabditis elegans Gαq regulates egg-laying behavior via a PLCβ-dependent and serotonin-dependent signaling pathway and likely functions both in the nervous system and in muscle. Genetics 165: 1805–1822.
Bence, K., W. Ma, T. Koaza, and X.-Y. Huang, 1997 Direct stimulation of Brunt’s tyrosine kinase by Gq-protein α-subunit. Nature 389: 296–299.
Bolshakov, V. Y., L. Carboni, M. H. Cobb, S. A. Siegelbaum, and F. Belardetti, 2000 Dual MAP kinase pathways mediate opposing forms of long-term plasticity at CA3→CA1 synapses. Nat. Neurosci. 3: 1107–1112.
Brenner, S., 1974 The genetics of Caenorhabditis elegans. Genetics 77: 71–94.
Chang, C., Y.-W. Hsieh, B. J. Lesch, C. I. Bargmann, and C.-F. Chuang, 2011 Microtubule-based localization of a synaptic calcium-signaling complex is required for left-right neuronal asymmetry in C. elegans. Development 138: 3509–3518.
Chuang, C.-F., and C. I. Bargmann, 2005 A toll-interleukin 1 repeat protein at the synapse specifies asymmetric odorant receptor expression via ASK1 MAPKKK signaling. Genes Dev. 19: 270–281.
Cingolani, P., A. Platts, L. L. Wang, M. Coon, T. Nguyen et al., 2012 A program for annotating and predicting the effects of single nucleotide polymorphisms, SnPElF: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 6: 80–92.
Coullault, C., N. Pujol, J. Reboul, L. Sabatier, J.-F. Guichou et al., 2004 TLR-independent control of innate immunity in Caenorhabditis elegans by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. Nat. Immunol. 5: 488–494.
DePristo, M. A., E. Banks, R. Poplin, K. V. Garimella, J. R. Maguire et al., 2011 A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 43: 491–498.
Doi, M., and K. Iwasaki, 2002 Regulation of retrograde signaling at neuromuscular junctions by the novel C2 domain protein AEX-1. Neuron 33: 249–259.
Fay, D., 2006 Genetic mapping and manipulation: Chapter 7-Making compound mutants (June 14, 2006), WormBook, ed. The C. elegans Research Community WormBook, doi/10.1895/wormbook.1.96.2, http://www.wormbook.org.
Frokjær-Jensen, C., M. W. Davis, M. Ailón, and E. M. Jørgensen, 2012 Improved Mos1-mediated transgenesis in C. elegans. Nat. Methods 9: 117–118.
Frokjær-Jensen, C., M. W. Davis, M. Sarov, J. Taylor, S. Filibotte et al., 2014 Random and targeted transgene insertion in C. elegans using a modified Mos1 transposon. Nat. Methods 11: 529–534.
Gao, S., L. Xie, T. Kawano, M. D. Po, J. K. Pirri et al., 2015 The NCA sodium leak channel is required for persistent motor circuit activity that sustains locomotion. Nat. Commun. 6: 6323.
García-Hoz, C., G. Sánchez-Fernández, M. T. Díaz-Meco, J. Moscat, F. Mayor et al., 2010 Goq acts as an adapter protein in protein kinase Cζ (PKCζ)-mediated ERK5 activation by G protein–coupled receptors (GPCR). J. Biol. Chem. 285: 13480–13489.
Hajdu-Cronin, Y. M., W. J. Chen, G. Patikoglou, M. R. Koelle, and P. W. Sternberg, 1999 Antagonism between Gso and Gq in Caenorhabditis elegans: the RGS protein EAT-16 is necessary for Gqo signaling and regulates Goq activity. Genes Dev. 13: 1780–1793.
Hawasli, A. H., O. Saifee, C. Liu, M. L. Nonet, and C. M. Crowder, 2004 Resistance to volatile anesthetics by mutations enhancing excitatory neurotransmitter release in Caenorhabditis elegans. Genetics 168: 831–843.
Hoppe, P. E., J. Chau, K. A. Flanagan, A. R. Reedy, and L. A. Schriever, 2010 Caenorhabditis elegans unc-82 encodes a serine/threonine kinase important for myosin filament organization in muscle during growth. Genetics 184: 79–90.
Huang, C.-C., J.-L. You, M.-Y. Wu, and K.-S. Hsu, 2004 Rap1-induced p38 mitogen-activated protein kinase activation facilitates AMPA receptor trafficking via the GDI-Rab5 complex. Potential role in (S)-3,5-dihydroxyphenylglycine-induced long term depression. J. Biol. Chem. 279: 12286–12292.
Hudmon, A., J.-S. Choi, L. Tyrrell, J. A. Black, A. M. Rush et al., 2008 Phosphorylation of sodium channel Nav1.8 by p38 mitogen-activated protein kinase increases current density in dorsal root ganglion neurons. J. Neurosci. 28: 3190–3201.
Jantsch-Plunger, V., P. Gönzcz, A. Romano, H. Schnabel, D. Hamill et al., 2000 CYK-4: a Rho family gtpase activating protein (GAP) required for central spindle formation and cytokinesis. J. Cell Biol. 149: 1391–1404.
Kawasaki, M., 1999 A Caenorhabditis elegans JNK signal transduction pathway regulates coordinated movement via type-D GABAergic motor neurons. EMBO J. 18: 3604–3615.
Kim, D. H., R. Feinbaum, G. Allowing, F. E. Emerson, D. A. Garsin et al., 2002 A conserved p38 MAP kinase pathway in Caenorhabditis elegans innate immunity. Science 297: 623–626.
Kim, D. H., N. T. Liberati, T. Mizuno, H. Inoue, N. Hisamoto et al., 2004 Integration of Caenorhabditis elegans MAPK pathways mediating immunity and stress resistance by MEK-1 MAPK kinase and VHP-1 MAPK phosphatase. Proc. Natl. Acad. Sci. USA 101: 10990–10994.
Koelle, M. R., 2016 Neurotransmitter signaling through heterotrimeric G proteins: insights from studies in C. elegans (Early Online, March 3, 2016). WormBook, ed. The C. elegans Research Community WormBook, doi/10.1895/wormbook.1.75.2, http://www.wormbook.org.
Kyrkiaks, J. M., and J. Avruch, 2012 Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. Physiol. Rev. 92: 689–737.
Lackner, M. R., S. I. Nurrish, and J. M. Kaplan, 1999 Facilitation of synaptic transmission by EGL-30 Gpalpha and EGL-8 PLCbeta: DAG binding to UNC-13 is required to stimulate acetylcholine release. Neuron 24: 335–346.

Lu, B., Y. Su, S. Das, J. Liu, J. Xia et al., 2007 The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm. Cell 129: 371–383.

McMullan, R., E. Hiley, P. Morrison, and S. J. Nurrish, 2006 Rho is a presynaptic activator of neurotransmitter release at pre-existing synapses in C. elegans. Genes Dev. 20: 65–76.

Mello, C. C., J. M. Kramer, D. Stinchcomb, and V. Ambros, 1991 Ef gene transfer in C. elegans: extrachromosomal maintenance and integration of transforming sequences. EMBO J. 10: 3959–3970.

Miller, K. G., M. D. Emerson, and J. B. Rand, 1999 Goalpha and diacylglycerol kinase negatively regulate the Gpalpha pathway in C. elegans. Neuron 24: 323–333.

Nash, H. A., R. L. Scott, B. C. Lear, and R. Allada, 2002 An unusual cation channel mediates photic control of locomotion in Drosophila. Curr. Biol. 12: 2152–2158.

Natochin, M., and N. O. Artemyev, 2003 A point mutation uncouples Nurrish, S., L. Ségalat, and J. M. Kaplan, 1999 Serotonin inhibition of synaptic transmission: Gpalpha(0) decreases the abundance of UNC-13 at release sites. Neuron 24: 231–242.

Pagano, D. J., E. R. Kingston, and D. H. Kim, 2015 Tissue expression pattern of PMK-2 p38 MAPK is established by the miR-58 family in C. elegans. PLoS Genet. 11: e1004997.

Park, E. C., and C. Rongo, 2016 The p38 MAP kinase pathway modulates the hypoxia response and glutamate receptor trafficking in aging neurons. Elife 5: e12010.

Plowman, G. D., S. Sudarsanam, J. Bingham, D. Whyte, and T. Hunter, 1999 The protein kinases of Caenorhabditis elegans: a model for signal transduction in multicellular organisms. Proc. Natl. Acad. Sci. USA 96: 13603–13610.

Rhee, S. G., 2001 Regulation of phosphoinositide-specific phospholipase C. Annu. Rev. Biochem. 70: 281–312.

Rush, A. M., J. Wu, M. J. Rowan, and R. Anwyl, 2002 Group I metabotropic glutamate receptor (mGluR)-dependent long-term depression mediated via p38 mitogen-activated protein kinase is inhibited by previous high-frequency stimulation and activation of mGluRs and protein kinase C in the rat dentate gyrus in vitro. J. Neurosci. 22: 6121–6128.

Sagasti, A., N. Hisamoto, J. Hyodo, M. Tanaka-Hino, K. Matsumoto et al., 2001 The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. Cell 105: 221–232.

Sánchez-Fernández, G., S. Cabezudo, C. García-Hoz, C. Benincá, A. M. Aragay et al., 2014 Gq signalling: the new and the old. Cell. Signal. 26: 833–848.

Shi, Y., C. Abe, B. B. Holloway, S. Shu, N. N. Kumar et al., 2016 Nalcn is a “leak” sodium channel that regulates excitability of brainstem chemo-sensory neurons and breathing. J. Neurosci. 36: 8174–8187.

Shivers, R. P., T. Kooistra, S. W. Chu, D. J. Pagano, and D. H. Kim, 2009 Tissue-specific activities of an immune signaling module regulate physiological responses to pathogenic and nutritional bacteria in C. elegans. Cell Host Microbe 6: 321–330.

Shivers, R. P., D. J. Pagano, T. Kooistra, C. E. Richardson, K. C. Reddy et al., 2010 Phosphorylation of the conserved transcription factor ATF-7 by PMK-1 p38 MAPK regulates innate immunity in Caenorhabditis elegans. PLoS Genet. 6: e1000892.

Tanaka-Hino, M., A. Sagasti, N. Hisamoto, M. Kawasaki, S. Nakano et al., 2002 SEK-1 MAPKK mediates Ca2+ signaling to determine neuronal asymmetric development in Caenorhabditis elegans. EMBO Rep. 3: 56–62.

Topalidou, I., J. Cattin-Ortolá, A. L. Pappas, K. Cooper, G. E. Merrihew et al., 2016 The EARP complex and its interactor EIPR-1 are required for cargo sorting to dense-core vesicles. PLoS Genet. 12: e1006074.

Topalidou, I., P.-A. Chen, K. Cooper, S. Watanae, E. M. Jorgensen et al., 2017 The NCA-1 and NCA-2 ion channels function downstream of Gq and Rho to regulate locomotion in Caenorhabditis elegans. Genetics 206: 265–282.

Vaqué, J. P., R. T. Dorsam, X. Feng, R. Iglesias-Bartolome, D. J. Forsthoefel et al., 2013 A genome-wide RNAi screen reveals a Trio-regulated Rho GTPase circuitry transducing mitogenic signals initiated by G protein-coupled receptors. Mol. Cell 49: 94–108.

Vashlishan, A. B., J. M. Madison, M. Dybbs, J. Bai, D. Sieburth et al., 2008 An RNAi screen identifies genes that regulate GABA synapses. Neuron 58: 346–361.

Williams, S. L., S. Lutz, N. K. Charlie, C. Vettel, M. Allison et al., 2007 Trio’s Rho-specific GEF domain is the missing Galpha q effector in C. elegans. Genes Dev. 21: 2731–2746.

Wittmack, E. K., A. M. Rush, A. Hudmon, S. G. Waxman, and S. D. Dib-Hajj, 2005 Voltage-gated sodium channel Nav1.6 Is modulated by p38 mitogen-activated protein kinase. J. Neurosci. 25: 6621–6630.

Zhang, R., X. He, W. Liu, M. Lu, J.-T. Hsieh et al., 2003 AIP1 mediates TNF-α–inducedASK1 activation by facilitating dissociation of ASK1 from its inhibitor 14–3–3. J. Clin. Invest. 111: 1933–1943.

Communicating editor: B. J. Andrews