The ERK MAPK Pathway Modulates Gq-dependent Locomotion in *Caenorhabditis elegans*

Brantley David Coleman

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2018

Reading Committee:

Michael Ailion, chair

James Hurley

Alexey Merz

Program Authorized to Offer Degree:

Biochemistry

University of Washington

Abstract

The ERK MAPK Pathway Modulates Gq-dependent Locomotion in *Caenorhabditis elegans*

Brantley David Coleman

Chair of the Supervisory Committee:

Michael Ailion

Department of Biochemistry

The heterotrimeric G protein Gq regulates neuronal activity through distinct downstream effector pathways. In addition to the canonical Gq effector phospholipase C beta, the small GTPase Rho was recently identified as a conserved effector of Gq. To identify additional molecules important for Gq signaling in neurons, we performed a forward genetic screen in the nematode Caenorhabditis elegans for suppressors of the hyperactivity and exaggerated waveform of an activated Gq mutant. We isolated two mutations affecting the MAP kinase scaffold protein KSR-1 and found that KSR-1 modulates locomotion downstream of or in parallel to the Gq-Rho pathway. Through epistasis experiments, I found that the core ERK MAPK cascade is required for Gq-Rho regulation of locomotion, but that the canonical ERK activator LET-60/Ras may not be required. Through neuron-specific rescue experiments, I found that the ERK pathway functions in acetylcholine neurons to control Gq-dependent locomotion. Additionally, expression of activated LIN-45/Raf in acetylcholine neurons is sufficient to cause an exaggerated waveform phenotype and hypersensitivity to the acetylcholinesterase inhibitor aldicarb, similar to an activated Gq mutant. Taken together, our results suggest that the ERK MAPK pathway modulates the output of Gq-Rho signaling to control locomotion behavior in C. elegans.

Table of Contents

List of Figures

Acknowledgments

My experiences during graduate school would not be possible without the mentoring, support, and friendship. I thank my mentor, Michael Ailion,for his constant support and guidance through graduate school as well as instilling fascination with worms. I especially thank Irini Topalidou, who has supported me in practically all aspects of my life. It was great to sit at our adjacent microscopes and pick worms for hours. My fellow graduate students in the lab are invaluable friends as well as colleagues. I love that all of our friendships are unique in some way. I garden with Jill, play Dungeons and Dragons with Piero, and Jerome and I mutually insult each other.

I am lucky to have met and developed so many wonderful friendships. Cohort members Mark, James, Emily and I have supported each other endlessly through our jokes and personalities. My supportive network of other graduate students kept me going throughout the years of grad school. I especially thank Bruce, Rose, Emily, and Laura for our many conversations of mutual support and encouragement.

I thank my committee members Alex Merz, John Scott, John Cooper, and Jim Hurley for their valuable insight into my project. They helped me to expand my ideas of my project to other models and alternative approaches.

Lastly, my family members are the greatest source of love and support. I owe them everything for getting me to this point in my life. Mom, Carolina, Dad, and all of my grandparents are my inspiration and drive. Half of my Ph.D. should be awarded to them.

Chapter 1

Introduction

Molecular signaling pathways are the master regulators of cellular activities. Decades of research have identified the components and signaling pathways, but our understanding of how pathways control intercellular interactions is far from comprehensive. This dissertation describes the identification and characterization of a novel pathway that controls neuronal activity in the nematode *C. elegans.* Through a forward genetic screen, we have shown that the ERK MAPK signaling pathway is activated downstream of the heterotrimeric G protein Gq and the small GTPase Rho. Analysis by genetic epistasis, locomotor behavior, cellular rescue experiments, and transgenic expression shows that ERK signaling is necessary and sufficient for the regulation of locomotion downstream of Gq and Rho. Through this pathway, acetylcholine neurons in the head ultimately control the contraction of body wall muscles that coordinate locomotion. This work furthers knowledge of how several molecular signaling pathways converge to regulate important neuronal activity and behavior.

C. elegans connectome

The process of synaptic transmission between two neurons is a critical process throughout metazoan biology. Nearly all behaviors are controlled through chemical and electrical synapses between neurons, from muscle contraction in *Caenorhabditis* to consciousness in humans. Complex molecular signaling pathways regulate the activity of neurons through neurotransmitter and neuromodulator release. The complex wiring systems of advanced organisms present challenges to precisely mapping synaptic connections and brain architecture (Plaza *et al.* 2014). Thus, it is difficult to determine how specific neurons in the brain control downstream behaviors. However, organisms that are less complex, such as *Caenorhabiditis elegans*, are attractive for understanding neurobiology. While humans have almost one hundred billion neurons and many more synapses, the hermaphrodite nematode has only 302 neurons (Sulston *et al.* 1975). This dramatic reduction of nervous system complexity creates a tractable model organism for neurobiology.

The seminal work on “the mind of the worm” used serial electron microscopy to recreate the entire neuronal connectome of the worm, mapping the structure of all 302 neurons and more than 7000 synapses between them (White *et al.* 1986). The relative simplicity of the worm allowed such a map to be constructed in the worm decades before other models. Importantly, the neuronal morphology of *C. elegans* is essentially predetermined during development. Uniformity in synaptic morphology has led to a thorough understanding of certain circuits (Chalfie *et al.* 1985; Gray *et al.* 2005; Chalasani *et al.* 2007; Jarrell *et al.* 2012; Collins *et al.* 2016). Despite this, the worm connectome has not yet led to a comprehensive understanding of cellular signaling.

Worm behaviors are governed by the chemical and electrical circuits comprised of predetermined sets of neurons. The circuit regulating forward and backwards locomotion in response to touch is one of the earliest described circuits, and is relevant to my work (Figure 1-1). Premotor interneurons AVA, AVB, AVD, and PVC run down the ventral cord of the worm and innervate motor neurons that synapse onto body wall muscles. Through laser ablation of specific neurons, opposing circuits were found to drive forward (AVB/PVC) and reverse locomotion (AVA/AVD). Reciprocal inhibition between the two sets of premotor interneurons prevents both circuits from acting simultaneously (Chalfie *et al.* 1985; Wicks and Rankin 1995, 1996).

Premotor interneurons regulate locomotion through chemical synapses and gap junctions with A- and B-classes of motor neurons. These motor neurons then innervate the dorsal and ventral body wall muscles (White *et al.* 1976; Richmond and Jorgensen 1999). Excitatory A-type and B-type motorneurons release acetylcholine at the neuromuscular junction (NMJ) to elicit muscle contraction. Simultaneously, A- and B-type neurons also excite inhibitory D-type motorneurons that release GABA, to relax muscles. During forward locomotion, B-type motorneurons

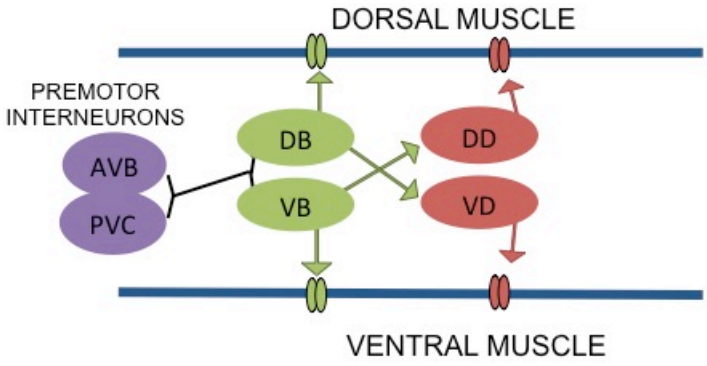

Figure 1-1. The neuronal circuit that regulates forward locomotion. Premotor interneurons form gap junctions with B-type motorneurons. Activation of the DB neuron releases acetylcholine (green) at the NMJ to cause body wall muscle contraction. Cholinergic signals from DB neuron activates the VD inhibitory neuron. Release of GABA (red) by VD causes muscle relaxation.

release acetylcholine at the NMJ to trigger muscle contraction. Reverse locomotion instead occurs through activation of A-type motorneurons. The contraction of body wall muscles from acetylcholine release is balanced by relaxation of opposing body wall muscles. During forward locomotion, dorsal B-type motorneurons activate ventral D-type motorneurons, triggering release of GABA at muscles on the ventral side of the worm. The alternation of contraction and relaxation by acetylcholine and GABA produces undulatory locomotion (Zhen and Samuel 2015).

While a neuronal circuit with binary control of forward/reverse locomotion would be the simplest model, the complex behavior of locomotion is determined by the convergence of upstream activity of sensory and interneurons, allowing worms carefully controlled responses to environmental change. Integration of varying synaptic strengths of upstream neurons regulates forward versus reverse locomotion. Advanced mathematical models more accurately predict complex locomotor behaviors such as velocity, turning, and foraging. However, these models cannot yet comprehensively predict locomotion without further understanding of how the neuronal circuits are regulated (Roberts *et al.* 2016). Researching the molecular signals inside these neurons may refine the model of the locomotor circuit.

C. elegans as a model organism

C. elegans is a premier model animal for the identification of genes and the cellular functions that they control (Brenner 1974). *Caenorhabditis* was the first fully sequenced metazoan genome (Consortium* 1998). While mammalian genomes are much larger and complex, many *C. elegans* genes are highly conserved with their mammalian homologs. Sydney Brenner chose *C. elegans* as a model for its many benefits in genetic studies, simple nervous system, and ease of use. The transparency of worms is amenable to live microscopy. Fluorescent reporter proteins can easily be seen on a compound microscope. Worm genetics is governed by the allelic segregation of hermaphrodites, meaning that the progeny of one animal will produce both homozygotes and heterozygotes. A single hermaphrodite can produce hundreds of eggs in its lifetime, and with a generation time of roughly three days the worm is unrivaled among metazoans in its genetic efficiency. Creation of stable lines expressing transgenic alleles as well as genome modification via CRISPR/Cas9 is efficient and can be done in a matter of days. My work was made possible by these unique characteristics of *C. elegans.*

Forward screens underlie the identification of many signal transduction pathways in worms. Rapid generation time and brood size make worms ideal for forward genetic screens to find suppressors of gene function, as a recessive mutation can become homozygous in the F2 progeny of a mutated worm (Jorgensen and Mango 2002). Many worms are mutated using a mutagen such as UV light or the chemicals EMS or ENU. Worms with altered phenotypes can be identified several days later. A gain of function mutation in a specific gene may cause a phenotype such as hyperactive locomotion, so a forward screen for non-hyper worms allows identification of new alleles that suppress the gain of function phenotype. Suppressors can occur intragenically through a novel mutation that decreases the activity of the original protein or extragenically, due to mutations in different genes that act in parallel or downstream. Protein pathways that regulate developmental and behavioral phenotypes were identified through epistasis analysis of loss of function and gain of function alleles isolated through genetic screens (Sundaram and Han 1995; Sternberg and Han 1998). My work was initiated by using a forward genetic screen to determine novel signaling interactions downstream of Gαq.

G proteins

Regulation of synaptic transmission ultimately occurs at the molecular level. Diverse signal transduction cascades govern development and activity of mature neurons. G proteins are a ubiquitous class of proteins that control diverse functions through interactions with downstream signaling effectors. G proteins are GTPases that act as molecular switches with “on” and “off” states. G proteins exist as either monomeric small GTPases or heterotrimeric G proteins that are controlled by G protein-coupled receptors (GPCRs) in

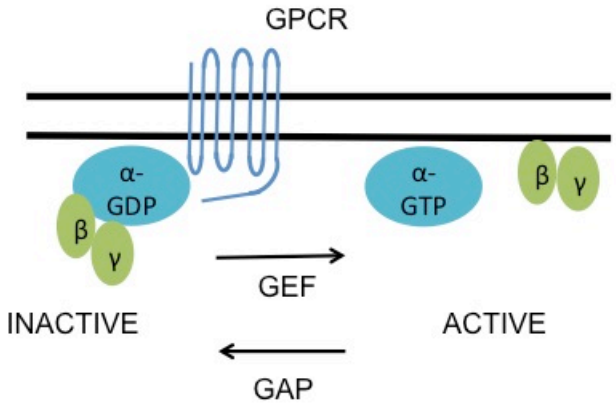

Figure 1-2. Heterotrimeric G protein signaling is regulated through GTP-GDP exchange. GEFs, including GPCRs, trigger the release of GDP by the Gα subunit. Active GTP-bound Gα releases from the Gβγ subunits until GAPs catalyze the hydrolysis of GTP to GDP, completing the cycle of G protein activity.

the membrane. While the two classes are regulated in different ways, they share the same molecular mechanism of action. GTPases form their active conformation when bound to GTP, exposing effector-binding domains for protein interactions that control molecular signals further downstream (Gilman 1987; Sprang 2016). G proteins generally have low native GTPase activity and thus will remain in the active GTP-bound state until stimulated by GTPase activating proteins (GAPs) to hydrolyze GTP to GDP (Siderovski and Willard 2005; Bos *et al.* 2007; Sprang 2016). The GDP-bound G protein undergoes conformational changes that bury the effector-binding switch II and α3 domains, turning the G protein "off." The Gα subunit of the heterotrimeric G proteins, which contains the actual GTPase activity, is reversibly lipid-modified to anchor it to the cell membrane, and associates with GPCRs and the Gβγ subunits to complete the GDP-bound off state (Wedegaertner *et al.* 1995). The G protein remains tightly bound to GDP in its inactive state until the release of GDP is catalyzed by a GEF (guanine nucleotide exchange factor), through both transmembrane GEFs known as GPCRs (G protein-coupled receptors) and receptor-independent GEFs (Tall *et al.* 2003; Siderovski and Willard 2005; Tall 2013; Garcia-Marcos *et al.* 2015). GEFs induce opening of the G protein conformation to allow release of GDP and replacement with cytosolic GTP, thus again activating the protein to complete the on-off cycle (Figure 1-2).

A conserved G protein pathway involves two heterotrimeric G proteins, Gαq and Gαo. In its active conformation, Gαq binds and activates multiple effector proteins, canonically activating phospholipase C in both worms and humans. Activation of PLC leads to hydrolysis of the membrane lipid Phosphatidylinositol 4,5-bisphosphate (PIP_2) into the second messengers diacylglycerol (DAG) and inositol trisphosphate (IP_3) (Rhee 2001). In neurons, increased DAG at the synapse recruits the DAG-binding protein UNC-13 (Munc13 in humans) that ultimately helps to dock neurotransmitter containing-vesicles at the membrane and prime these vesicles to fuse with the active zone (Lackner *et al.* 1999a; Miller *et al.* 1999). Cytosolic IP_3 binds to IP_3 receptors, releasing Ca^{2+} into the cell. Gαo signaling inhibits the activity of the same pathway that Gαq positively regulates. Extracellular signals converge at the Gαq-Gαo pathway through the activation of numerous GPCRs, each regulated by extracellular neurotransmitters and small molecules. The convergence of positive and negative regulation of Go and Gαq allows fine tuning of downstream activation.

In addition to synaptic transmission, Gαq signaling regulates critical cellular processes in worms and mammals. In humans, mutations causing constitutive activation of Gαq and its downstream pathways lead to both developmental defects and cancer. Spontaneous gain of function mutations in GNAQ, the human homolog of Gαq, cause the congenital disorder Sturge-Weber syndrome, characterized by defects in neuronal function and glaucoma (Comi 2015). Overactive GNAQ mutations accrued later in life act as dominant oncogenes causing the development of uveal melanoma (Sisley *et al.* 2011; Huang *et al.* 2015). In worms, Gαq signaling is a major regulator of synaptic activities that govern behaviors. Gαq and Gαo regulate locomotion in opposite ways through the regulated release of acetylcholine at the neuromuscular junction, triggering contraction of body wall muscles (Reynolds *et al.* 2005). As such, mutations affecting Gαq activity are obvious by eye. Gαq hypomorphs move slowly with uncoordinated body bends while Gαq overactivation causes hyperactive locomotion with exaggerated body bends (Brundage *et al.* 1996; Doi and Iwasaki 2002; Bastiani *et al.* 2003). Null mutations of Gαq cause dead worms early in development, highlighting the fundamental roles of Gαq (Brundage *et al.* 1996). Despite PLCβ being a major effector of active Gαq, PLCβ null worms are viable and do not have locomotion phenotypes as dramatic as Gαq mutants, suggesting that other signaling pathways act downstream of Gαq.

Rho acts downstream Gαq

Forward genetic screens for suppressors of activated Gαq hyperactivity identified the RhoGEF Trio as a direct effector of active Gαq (Williams *et al.* 2007), showing that Gαq has diverse mechanisms and protein targets for regulation, in addition to PLCβ. It was specifically shown that active Gαq associates with Rho GEFs that promote activation of the small GTPase RhoA (Lutz *et al.* 2005, 2007; Williams *et al.* 2007). The small GTPase Rho differs from heterotrimeric G proteins in that it is anchored to the plasma membrane without interacting with other G protein subunits or upstream GPCRs. In mature *C. elegans* neurons, the RhoA ortholog RHO-1 regulates synaptic activity through multiple G protein-dependent mechanisms. First, RHO-1 acts downstream of the G_{12}-class G protein GPA-12 by binding to and inhibiting the diacylglycerol kinase DGK-1. Inhibition of DGK-1 allows DAG to accumulate at the synapse, thereby increasing synaptic vesicle priming and release in the same manner as PLCβ activation (McMullan *et al.* 2006; Hiley *et al.* 2006). Gαq -Rho signaling additionally promotes neurotransmitter release by recruiting the sphingosine kinase SPHK-1 to presynaptic terminals where it increases synaptic

activity by phosphorylation of the lipid sphingosine (Chan *et al.* 2012). Lastly, our lab recently described the activation of the voltage-independent NCA/NALCN ion channel by Gαq-Rho signaling, regulating the rhythm of locomotion in worms (Topalidou *et al.* 2017a).

The Rho family of small GTPases are involved not only in synaptic signaling in mature neurons, but also in development and cell polarity. The Rho protein family comprised of Rho, Rac, and CDC42 primarily regulate the dynamics of actin chains, allowing large-scale changes in the cytoskeleton and structure of a cell. The formation of stress fibers and focal adhesions, regulated by different Rho protein families lead to cellular structures such as filopodia and lamellopodia that cause large changes in cellular morphology (Nobes and Hall 1995). Activation of downstream kinases ROCK and PAK promote contraction of actin filaments by phosphorylating myosin, organizing cell movements during wound healing and neuronal development (Abreu-Blanco *et al.* 2014; Stankiewicz and Linseman 2014). During worm and mammalian development, Rho family members control neuronal polarity through the elongation and guidance of axons via actin regulation to shape the growth cone. As such, mutations in Rho family proteins lead to defective neuronal morphology (Zallen *et al.* 2000; Herzog *et al.* 2011). In *C. elegans,* mutants affecting TRIO and RHO-1 activity can disrupt acetylcholine signaling even if neuronal morphology is unaffected (Hu *et al.* 2011). Obviously, developmental defects in neuronal structure can cause significant defects in nervous system function. Importantly, our work uses transgenic strains expressing activated Rho in acetylcholine neurons but neuronal development is not affected (McMullan *et al.* 2006). This suggests that Gαq-Rho signaling regulates synaptic activity post-developmentally.

Ras signaling

Ras MAPK (mitogen activated protein kinase) signaling is one of the well-characterized signaling pathways. The canonical pathway of MAP kinase activation is controlled by an upstream small GTPase in the Ras family of proteins. Ras in its activated conformation binds the kinase Raf as either a Raf-Raf homodimer or a Raf heterodimer with the scaffold protein KSR (Kornfeld *et al.* 1995; Sundaram and Han 1995; Nguyen *et al.* 2002). Active Ras bound to Raf at the membrane triggers a kinase cascade, where the MAPKKK Raf recruits and phosphorylates the MAPKK MEK, and subsequently the MAPK ERK. The now active phospho-ERK then phosphorylates numerous targets in both the cytosol as well as targeting transcription factors in the nucleus to regulate gene expression (Sun *et al.* 2015).

While this basic pathway is relatively simple, the possible complexities of this pathway are endless. Each protein family has multiple isoforms and homologs that regulate different downstream and upstream signals in different cell types and different cellular compartments. Three different kinase families, Raf, MEK, and ERK, are each positively and negatively regulated by phosphatases and kinases. The complexity of this pathway is further increased through crosstalk of multiple signaling events. The critical roles of Ras MAPK proteins are highlighted by their high level of conservation from *C. elegans* to mammals (Han and Sternberg 1990; Reiner and Lundquist 2016), and the dramatic effects of MAPK misregulation.

ERK signaling is essential to nearly all life. MAPKs extensively control development, cellular proliferation, neuronal activity, and cellular transformation in cancer. Ras is the most commonly mutated gene in tumors (Bos 1989) and thus the pathway has been extensively studied to develop potential treatments for Ras-dependent tumors. Two critical domains near the N-terminus are the P-loop domain that interacts with the charged phosphates of GTP, and the switch I domain that changes conformation between its on and off states. These domains are conserved throughout the Ras superfamily (Wittinghofer and Vetter 2011; Wittinghofer 2014). As such, common oncogenic mutations have been found to confer constitutive activation of many Ras family members. Raf kinases are activated by interacting with Ras:GTP via the Ras-binding-domain of Raf, therefore mutations affecting Ras ability to bind GTP can cause misregulation of downstream ERK targets. For example, KRAS mutation G13D causes the protein to be locked in its GTP-bound form. The subsequent overactivation of ERKs downstream is critical for cancer development and progression (De Luca *et al.* 2012; Mandal *et al.* 2016). While Ras mutations are often involved in oncogenesis, mutations in the MAPK pathway can be necessary and sufficient to transform cells *in vitro* that lack all Ras protein (Drosten *et al.* 2010). MAPK signaling regulates transcription of many genes, ultimately ERK activation leads to increased frequency of mitosis and eventually oncogenic transformation (Deschênes-Simard *et al.* 2014). Consequently, efforts to inhibit various proteins in the pathway could result in treatments for Ras-dependent tumors. Despite the importance of Ras in oncogenic transformation, developing drugs that directly target Ras activity has been difficult. Instead, several successful cancer therapies target ERK signaling via small molecules that bind and inhibit BRAF or MEK. These therapies are handicapped because nearly all tumors develop

resistance to these drugs, either through mutation of other proteins in ERK MAPK pathway (Montagut *et al.* 2008) or by enhancing other signaling pathways that act in parallel (Nazarian *et al.* 2010; Takashima and Faller 2013; Drosten *et al.* 2014).

In addition to transformation, mutations in human Ras and MAPKs lead to multiple "RASopathies," developmental disorders leading to craniofacial irregularities caused by aberrant signaling in neurons and other tissues (Zhong 2016). Raf is implicated in neuronal growth as the BRAF constitutively active V600E mutation is shown to increase axonal regeneration in response to damage, while loss of BRAF activity inhibits axonal movement (Zhong *et al.* 2007; O'Donovan *et al.* 2014). In addition to BRAF V600E, some human mutations that cause constitutive activation or inactivation of small GTPases Ras and Rho are functionally conserved in *C. elegans.* These mutations are not only critical for cellular function and disease *in vivo*, but also provided useful tools for transgenic activation or inhibition for the molecular analysis in my work (Coleman *et al.* 2017).

The interactions of the ERK MAPK pathway were identified in *C. elegans* more than thirty years ago through genetic analysis of mutations that control the specification of vulval progenitor cells during development. The *C. elegans* Ras *let-60(n1046)* mutant is a G13E point mutation, homologous to the mutation found frequently in human tumors. Ras overactivation causes a dramatic "multi-vulva" (Muv) phenotype that allowed forward screening for downstream suppressors that lacked the Muv phenotype (Ferguson and Horvitz 1989; Han and Sternberg 1990; Han *et al.* 1993; Kornfeld *et al.* 1995; Sundaram and Han 1995). Dozens of genes were found to suppress Muv, with some instead causing vulva-less or sterile phenotypes. Suppressors of *let-60(n1046)*

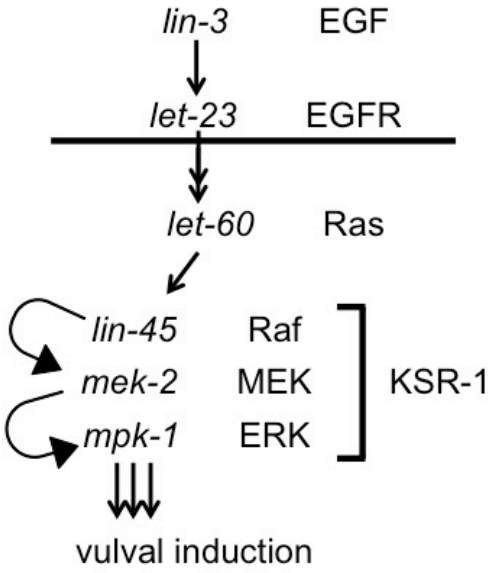

Figure 1-3. Canonical RTK-ERK MAPK signaling in *C. elegans*. Upon growth factor binding, RTKs dimerize and autophosphorylate themselves, recruiting adaptor protein Grb2 and the GEF SOS-1 (not shown). GEFs then activate Ras. Ras-GTP recruits Raf to the membrane where KSR-1, MEK, and ERK coordinate a kinase casade. Active phospho-ERK then phosphorylates many downstream targets, including the transcription factors that regulate vulval induction in *C. elegans.*

were found to be loss of function alleles in the ERK MAPK pathway. Strong loss of function alleles in the MAP kinases downstream Ras could cause vulvaless, sterile, and nonviable phenotypes while weaker loss of function alleles were identified in numerous MAPK regulatory proteins. Rigorous genetic studies helped to identify how MAPK signals are transduced in different cell types (Sundaram 2013), Figure 1-3.

The ERK MAPK pathway is understudied in *C. elegans* neurons compared to other tissues. ERK acts in several types of sensory neurons to control complex behaviors, including locomotion. Chemotaxis to volatile odorants and salts is regulated by ERK activation in the AWC and ASER sensory neurons (Hirotsu *et al.* 2000; Hirotsu and Iino 2005; Tomioka *et al.* 2006; Chen *et al.* 2011; Tomida *et al.* 2012), respectively. ERK signaling regulates foraging behavior by acting in the IL1, OLQ, and RMD neurons (Hamakawa *et al.* 2015). Locomotion is also regulated by the ERK pathway in interneurons to regulate the nose touch response, a mechanosensory behavior (Hyde *et al.* 2011). Our work expands upon the roles of ERK signaling in neurons, specifically acetylcholine neurons that coordinate locomotion.

Multiple projects in our lab originate from a forward genetic screen for suppressors of activated Gαq. Out of many suppressors isolated by our lab, we grouped some alleles that suppress the loopy locomotion and hyperactivity. We identified Rho-pathway genes acting downstream of activated Gαq, suggesting that Gαq-Rho signaling regulates locomotion through unknown mechanisms. At the beginning of my research, I tested if two unidentified suppressors are involved in Gαq/Rho signaling. Simultaneously, our lab identified and characterized similar signaling pathways that regulate Gαq-dependent locomotion (Topalidou *et al.* 2017b). My work outlines how the initial identification of two unknown alleles has lead to an increased understanding of neuronal signaling that controls locomotion.

Chapter 2

The ERK MAPK pathway modulates Gq-dependent locomotion in *Caenorhabditis elegans*

Introduction

The heterotrimeric G protein Gq is a conserved regulator of neurotransmission in metazoans. Gq is highly expressed in neurons in mammals and in the nematode *C. elegans* (Wilkie *et al.* 1991; Lackner *et al.* 1999). In its canonical signaling pathway, Gq activates phospholipase Cb (PLCb) to cleave the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG) (Rhee 2001). An increased DAG concentration at the synapse helps trigger synaptic vesicle release (Miller *et al.* 1999; Lackner *et al.* 1999).

In addition to activating PLCb, Gq directly binds and activates the Rho guanine nucleotide exchange factor (GEF) Trio, which in turn activates the small GTPase Rho (Lutz *et al.* 2005, 2007; Williams *et al.* 2007). In mature *C. elegans* neurons, the Rho ortholog RHO-1 regulates synaptic activity through multiple G protein-dependent mechanisms. First, RHO-1 acts downstream of the G_{12}-class G protein GPA-12 by binding to and inhibiting the diacylglycerol kinase DGK-1. Inhibition of DGK-1 allows DAG to accumulate at the synapse, thereby increasing synaptic vesicle release (McMullan *et al.* 2006; Hiley *et al.* 2006). Second, Gq-Rho signaling promotes neurotransmitter release by recruiting the sphingosine kinase SPHK-1 to presynaptic terminals (Chan *et al.* 2012). Third, Gq-Rho signaling positively regulates the NCA-1/NALCN cation channel to regulate locomotion (Topalidou *et al.* 2017a). Here we identify the extracellular signal-related kinase mitogen-activated protein kinase (ERK MAPK) pathway as a positive regulator of neuronal activity acting downstream of Gq and Rho in acetylcholine neurons.

ERK MAPK signaling acts extensively in animal development, cellular proliferation, and cancer signaling (Yoon and Seger 2006; Karnoub and Weinberg 2008; Sun *et al.* 2015). In *C. elegans*, the ERK pathway is required for multiple developmental events including specification of the vulva (Sternberg 2005; Sundaram 2013). ERKs are highly expressed in mammalian neurons (Boulton *et al.* 1991; Ortiz *et al.* 1995) and act in both the nucleus and at the synapse to regulate synaptic activity and plasticity (Thomas and Huganir 2004; Sweatt 2004; Mao and Wang 2016b). In *C. elegans*, the ERK MAPK

pathway has been shown to act in several types of neurons to control behavior. ERK signaling is activated in response to odorants in the AWC sensory neuron to regulate chemotaxis to volatile odorants and in AIY interneurons to mediate odor adaptation (Hirotsu *et al.* 2000; Hirotsu and Iino 2005; Chen *et al.* 2011; Uozumi *et al.* 2012). ERK is also activated in the ASER sensory neuron to regulate chemotaxis to salt (Tomioka *et al.* 2006; Tomida *et al.* 2012). ERK signaling regulates foraging behavior by acting in the IL1, OLQ, and RMD neurons (Hamakawa *et al.* 2015). Finally, the ERK pathway has been shown to act in interneurons to regulate the nose touch response, a mechanosensory behavior (Hyde *et al.* 2011).

In the canonical ERK MAPK pathway, extracellular ligand binding activates transmembrane receptor tyrosine kinases (RTKs), and adaptor proteins recruit a GEF to activate the small GTPase Ras (LET-60 in *C. elegans*). LIN-45/Raf translocates to the plasma membrane upon Ras activation, forming interactions with Ras and the scaffold protein KSR-1. KSR-1 facilitates the activation of LIN-45/Raf and the subsequent phosphorylation of the MAPK cascade consisting of LIN-45/Raf, MEK-2/MEK, and MPK-1/ERK (Sundaram 2013). In this study, we found that the ERK MAPK pathway consisting of KSR-1, LIN-45/Raf, MEK-2/MEK and MPK-1/ERK modulates Gq-Rho signaling in acetylcholine neurons, but that surprisingly LET-60/Ras may not be required.

Results

KSR-1 regulates locomotion downstream of Gαq

In *C. elegans,* the heterotrimeric G protein Gαq regulates synaptic vesicle release (Hu *et al.* 2015). Gαq is a key regulator of neuromuscular activity, as loss-of-function mutants in *egl-30* are nearly paralyzed (Brundage *et al.* 1996) whereas the gain-of-function mutant *egl-30(tg26)* has hyperactive locomotion with an exaggerated loopy waveform (Doi and Iwasaki 2002; Bastiani *et al.* 2003) (Figure 2-A, C, D). To identify pathways required for Gαq signaling, we performed a forward genetic screen in *C. elegans* for suppressors of the activated Gαq mutant *egl-30*(*tg26)*. Two suppressors identified in this screen, *ox314* and *yak10*, showed similar suppression of the loopy waveform and hyperactivity of *egl-30(tg26)* animals (Figure 2-1D). When crossed away from the *egl-30(tg26*) background, both mutants moved with wild-type waveform (Figure 2-1D), but at a slightly slower rate (not shown). We mapped the *ox314* allele near the center of the X chromosome (see Materials and Methods), and a complementation test showed that *ox314* and *yak10* are mutations in the same gene since they fail to complement in an *egl-30(tg26)* background.

We used whole genome sequencing to identify *ox314* and *yak10* as nonsense mutations in *ksr-1* (Figure 2-1B, see Materials and Methods). KSR-1 is a scaffold protein that facilitates the localization and interactions required for the Ras-mitogen activated protein kinase (MAPK) cascade consisting of Raf, MEK and ERK (Kornfeld *et al.* 1995; Sundaram and Han 1995; Nguyen *et al.* 2002; Zhang *et al.* 2013). The deletion allele *ksr-1(ok786)* also suppressed the loopy waveform of the activated Gαq mutant identically to *ox314* and *yak10.* These results suggest that KSR-1 activity is required for regulation of locomotion rate and waveform by Gαq.

The ERK MAPK cascade acts to promote Gαq signaling

Because the loss of the MAPK scaffold *ksr-1* suppresses the activated Gαq mutant *egl-30(tg26)*, we asked whether other components of the Ras-ERK pathway would also suppress. Since the core components of the Ras-ERK pathway are required for viability, we built double mutants of activated Gαq with reduction-of-function mutations in genes at each step of the ERK cascade. Mutations in Raf (*lin-45(sy96)*), MEK (*mek-2(n1989)*, *mek-2(ku114)*), and ERK *mpk-1(ga117)*, *mpk-1(oz140)*) all suppressed

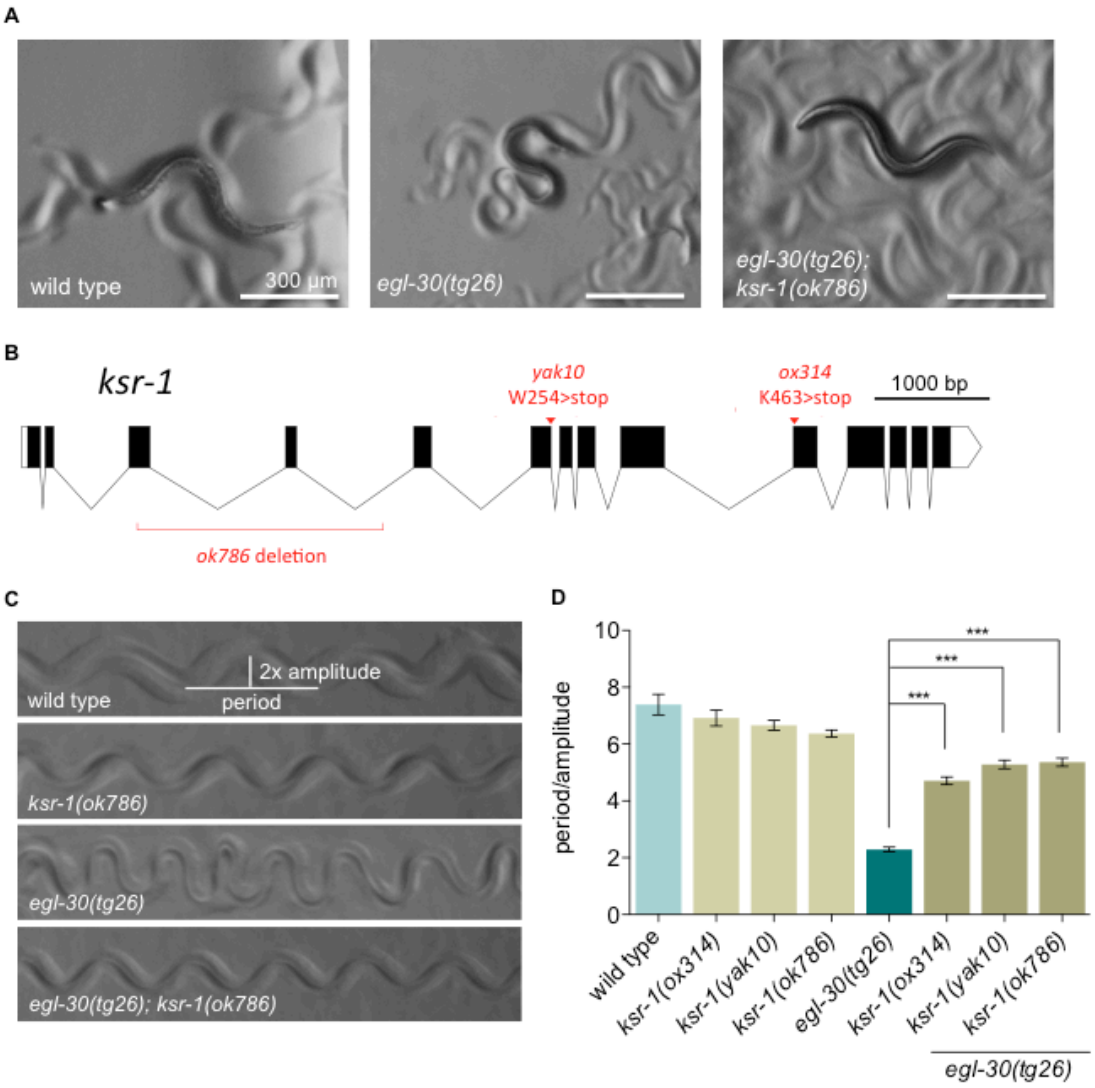

Figure 2-1. *ksr-1* mutations suppress activated Gαq.
(A) A *ksr-1* mutation suppresses the exaggerated body bends of activated Gαq. The activated Gαq mutant *egl-30(tg26)* has small size and deep body bends. *ksr-1(ok786)* suppresses the exaggerated body bends and small size of *egl-30(tg26)* worms

Figure 2-1 (cont.) Gαq. The activated Gαq mutant *egl-30(tg26)* has small size and deep body bends. *ksr-1(ok786)* suppresses the exaggerated body bends and small size of *egl-30(tg26)* worms. (B) Gene structure of *ksr-1* locus. Locations of the *egl-30(tg26)* suppressor alleles *ox314* and *yak10* are indicated, as well as the position of the *ok786* deletion. The gene structure was drawn using Exon-Intron Graphic Maker (www.wormweb.org/exonintron)

the loopy waveform of activated Gαq animals similarly to *ksr-1(ok786)* (Figure 2-2A,B; Figure 2-3A,B). However, mutations in Ras (*let-60(n2021)*) and the upstream pathway activators EGF (*lin- 3(e1417)*) and the EGFR (*let-23(sy12)*) did not suppress activated Gαq (Figure 2-2C). Because *let-60* is required for viability, most *let-60* alleles including *n202*1 are partial loss-of-function (Beitel *et al.* 1990). We also analyzed the dominant negative D119N allele *let-60(sy93)* that disrupts Ras binding to guanine nucleotides and thus prevents Ras activation (Han and Sternberg 1991). We found that *let-60(sy93)* also did not suppress the loopy waveform of activated Gαq (Figure 2-2D), supporting the possibility that ERK activation occurs through a Ras-independent mechanism. Because partial loss-of-function mutations in the ERK MAPK pathway genes downstream of Ras showed clear suppression of activated Gαq, we were surprised to find that partial loss-of-function mutations in Ras did not suppress. If LET-60/Ras is indeed not required, Gαq might instead activate the ERK pathway via other Ras-subfamily proteins. To test this possibility, we made double mutants of activated Gαq with putative null alleles of R-Ras/*ras-1,* M-Ras/*ras-2,* and Rap1/*rap-1* and found that they also did not suppress activated Gαq (Figure 2-4A). To further investigate whether this pathway acts independently ofas, we tested mutations in GEFs that activate Ras. The temperature-sensitive RasGEF mutant *sos-1(cs41)* did not suppress activated Gαq when shifted to the non-permissive temperature (Figure 2-4B). Additionally, a null mutation in the neuronal RasGEF *rgef-1* also did not suppress activated Gαq locomotion Gαq locomotion (Figure 2-4C), supporting the possibility that this pathway is Ras-independent. In genetic screens for vulval induction mutants, additional factors such as the PP2A subunit *sur-6* (Sieburth *et al.* 1999) and ion transporter *sur-7* (Yoder *et al.* 2004) were identified as positive regulators of Ras-ERK activity. However, the *sur-6(sv30)* and *sur-7(ku119)* mutations did not suppress activated Gαq locomotion (data not shown). These data suggest either that only a low level of Ras activity is needed to properly activate ERK signaling downstream of Gαq, or that ERK signaling acts independently of LET-60/Ras and other known *C. elegans* Ras family

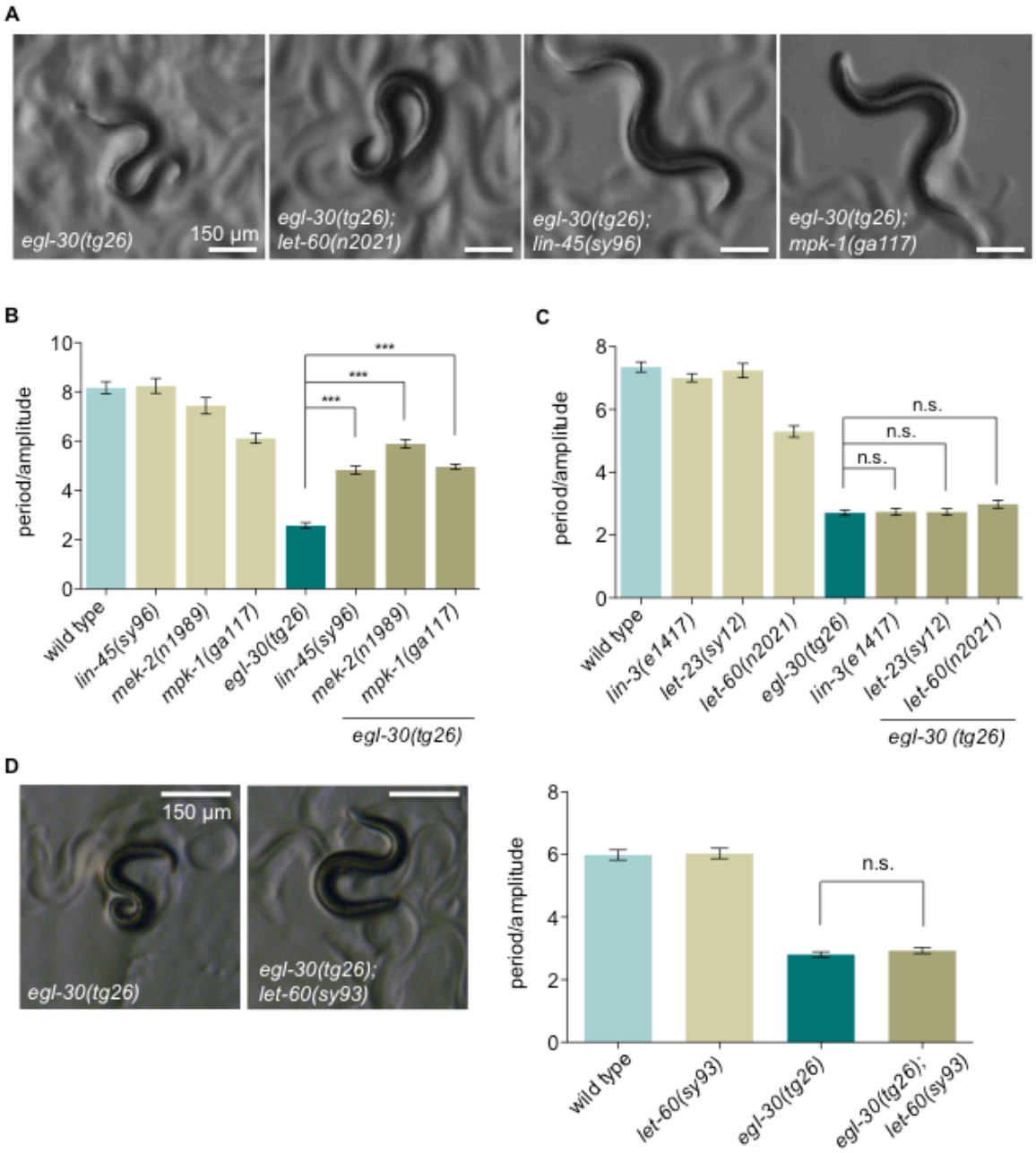

Figure 2-2. Mutations in the ERK MAPK pathway suppress activated Gq.
(A) Mutations in the MAPKKK *lin-45*/Raf, the MAPKK *mek-2*/MEK, and the MAPK *mpk-1*/ERK suppress the exaggerated waveform of *egl-30*(*tg26*) worms. Partial loss of Ras *let-60* activity does not suppress activated Gq waveform.
(B) Waveform quantification of ERK pathway mutants show levels of *egl-30*(*tg26*) suppression similar to *ksr-1* alleles. $N \geq 12$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test. (C) Signaling pathways upstream of the ERK pathway do not suppress activated Gq. Mutations in the EGFR (*let-23*) or the EGF ligand *lin-3* do not affect the exaggerated waveform of *egl-30*(*tg26*) animals. The partial loss-of-function mutation *let-60(n2021)* in the ERK pathway activator Ras does not suppress activated Gq waveform. $N \geq 12$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test.
(D) The *let-60(sy93)* dominant negative mutation in Ras does not suppress activated Gq waveform. $N \geq 13$, n.s., not significant, one-way ANOVA with Bonferroni's *post hoc* test.

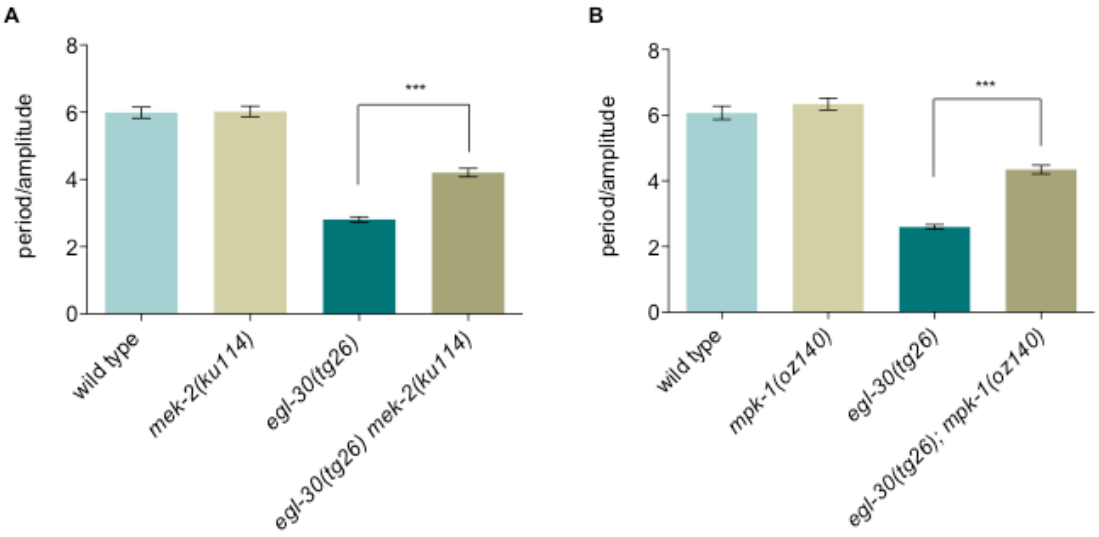

Figure 2-3. Additional mutations in *mek-2* and *mpk-1* suppress activated Gq.
(A) The *mek-2(ku114)* mutation suppresses the exaggerated waveform of *egl-30*(*tg26*) worms. $N \geq 14$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test.
(B) The *mpk-1(oz140)* mutation suppresses the exaggerated waveform of *egl-30*(*tg26*) worms. $N \geq 12$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test.

proteins to regulate locomotion downstream of Gαq.

KSR-1 and the ERK MAPK cascade modulate Rho signaling

Three classes of suppressor mutations were isolated in our forward genetic screen of activated Gαq, as characterized by their molecular role and unique suppression phenotypes (Topalidou *et al.* 2017a; b). We grouped together a class of suppressor mutations including *ox314, yak10*, and the RhoGEF Trio (*unc-73* in *C. elegans*) by their characteristic strong suppression of the loopy waveform of activated Gαq (Topalidou *et al.* 2017a; b), suggesting that *ksr-1* might act in the same pathway as *unc-73*.

We have shown that Gαq regulates locomotion via the small GTPase Rho (RHO-1 in *C. elegans*) (Topalidou *et al.* 2017a). Transgenic expression of an activated RHO-1 mutant (G14V) in acetylcholine neurons (here called "*rho-1(gf)*") causes worms to have a loopy waveform and impaired locomotion (McMullan *et al.* 2006) (Figure 2-5A). To examine whether *ksr-1* acts in the Gαq -Rho pathway we tested whether mutations in *ksr-1* suppress the phenotypes of *rho-1(gf)* worms. We found that the *ksr-1* alleles *ok786, ox314,* and *yak10* all suppressed the loopy waveform of *rho-1(gf)* worms (Figure 2-5A). Because *rho-1(gf)* worms have a slow locomotion rate and loopy waveform, these mutants do not efficiently travel

long distances. We used radial locomotion assays (see Materials and Methods) to quantify the locomotion phenotype of *rho-1(gf)* worms. *rho-1(gf) ksr-1* double mutants had an increased radial distance traveled compared to *rho-1(gf)* alone (Figure 2-5B). These data suggest that KSR-1 acts downstream of or in parallel to the Gαq -Rho pathway to regulate locomotion.

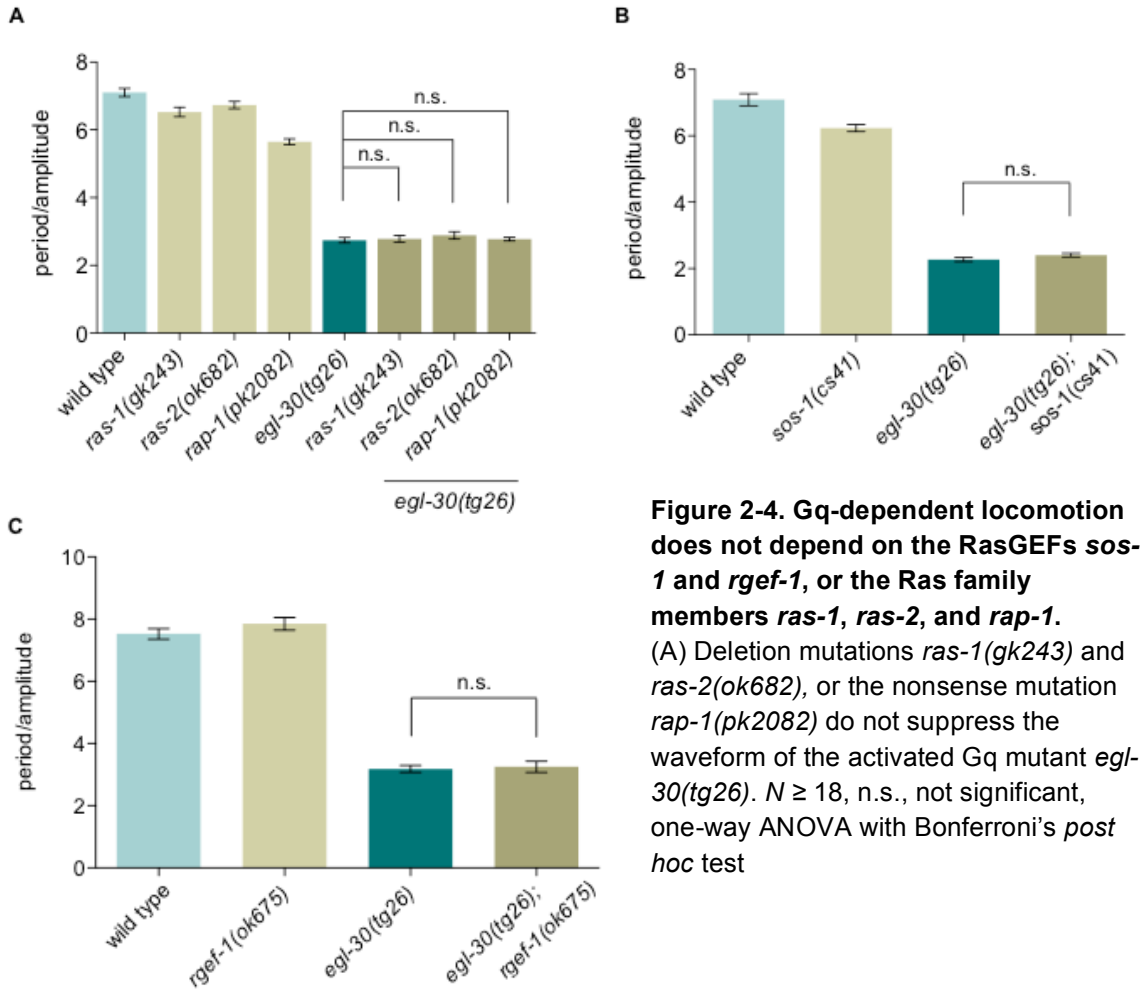

Figure 2-4. Gq-dependent locomotion does not depend on the RasGEFs *sos-1* and *rgef-1*, or the Ras family members *ras-1*, *ras-2*, and *rap-1*. (A) Deletion mutations *ras-1(gk243)* and *ras-2(ok682),* or the nonsense mutation *rap-1(pk2082)* do not suppress the waveform of the activated Gq mutant *egl-30(tg26)*. *N* ≥ 18, n.s., not significant, one-way ANOVA with Bonferroni's *post hoc* test (B) Loss of the RasGEF *sos-1* does not suppress activated Gq waveform. Temperature sensitive *egl-30(tg26); sos-1(cs41)* mutant animals were incubated for 24 hours at the non-permissive temperature of 25° and assayed for their waveform. *N* ≥ 15, n.s., not significant, one-way ANOVA with Tukey's *post hoc* test. (C) The RasGEF deletion mutation *rgef-1(ok675)* does not suppress the exaggerated waveform of activated Gq. *N* = 16, n.s., not significant, one-way ANOVA with Tukey's *post hoc* test.

Since *ksr-1* mutants suppress the exaggerated waveform of both activated Gαq and activated Rho animals, we expected that loss of other ERK pathway components would also suppress activated Rho. We made double mutants of activated Rho (*rho-1(gf)*) with reduction-of-function alleles of the Ras-

ERK pathway and found that mutations in Raf, MEK, and ERK suppressed the *rho-1(gf)* loopy waveform and decreased locomotion phenotypes (Figure 5A,C). However, the *let-60(n2021)* Ras mutation did not

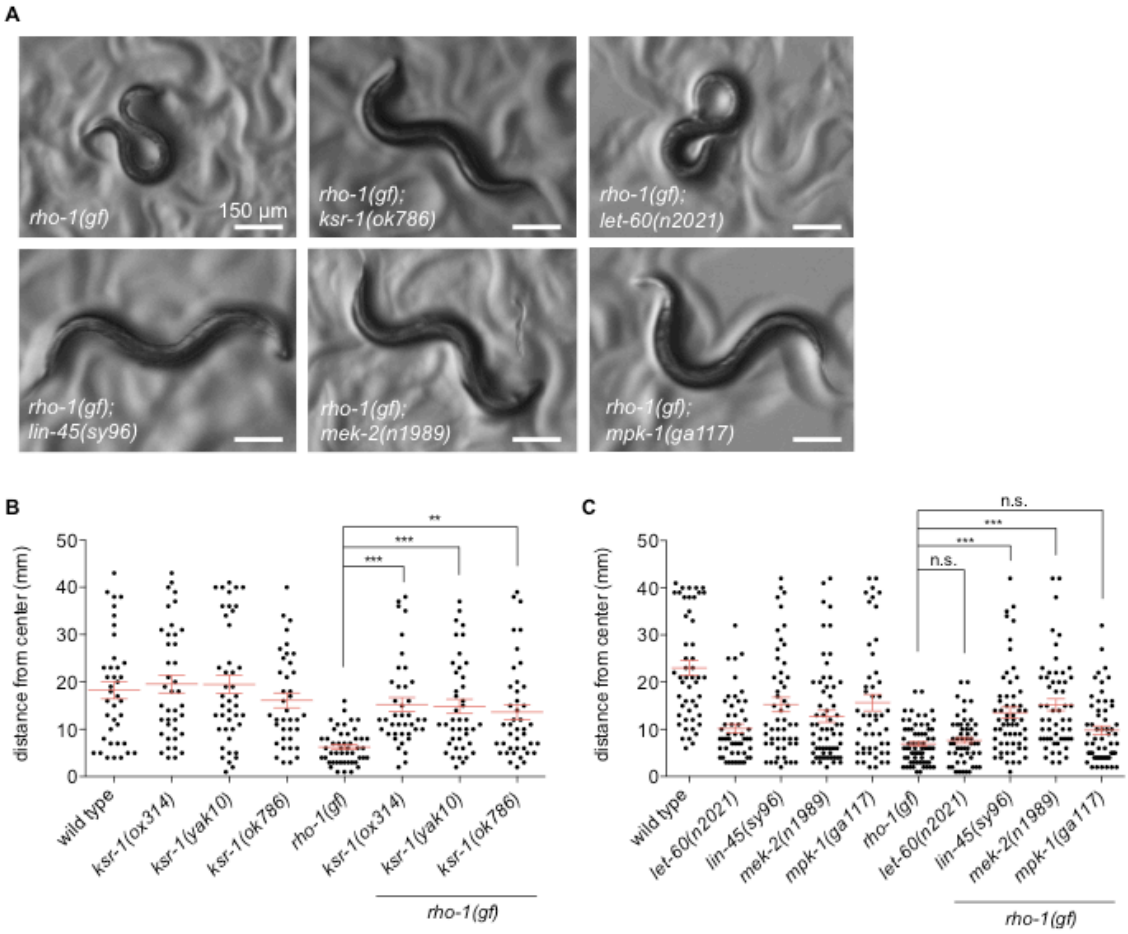

Figure 2-5. Mutations in *ksr-1* and the ERK pathway suppress activated Rho.
(A) Mutations in the ERK pathway visibly suppress the exaggerated body bends of animals expressing an activated Rho mutant (G14V) in acetylcholine neurons (*nzIs29[Punc-17::rho-1(gf)]*). Reduction of LET-60/Ras activity does not suppress the activated Rho waveform.
(B) The *ksr-1*(*ok786*)*, ksr-1*(*ox314*)*,* and *ksr-1*(*yak10*) mutations suppress the locomotion of activated Rho animals as shown by radial locomotion assay. $N \geq 38$ *** $P < 0.001$, ** $P < 0.01$, Kruskal-Wallis test with Dunn's *post hoc* test.
(C) Mutations in *lin-45* and *mek-2* suppresses the locomotion defect of activated Rho animals as shown by radial locomotion assays. The *let-60*(*n2021*) and *mpk-1*(*ga117*) mutations do not significantly suppress activated Rho locomotion. $N \geq 50$ *** $P < 0.001$, Kruskal-Wallis test with Dunn's *post hoc* test.

suppress the loopy waveform or radial locomotion defect of *rho-1(gf)* worms (Figure 2-5A,C). In addition to measuring radial locomotion, we measured the waveform of *rho-1(gf)* and suppressors. The same

pattern as before is repeated in *rho-1(gf)* double mutants; both loss of function and dominant negative alleles of Ras do not suppress *rho-1(gf)* locomotion (Figure 2-6). These data suggest that the ERK pathway acts downstream of or in parallel to the Gαq - Rho pathway to regulate locomotion, possibly in a Ras-independent manner.

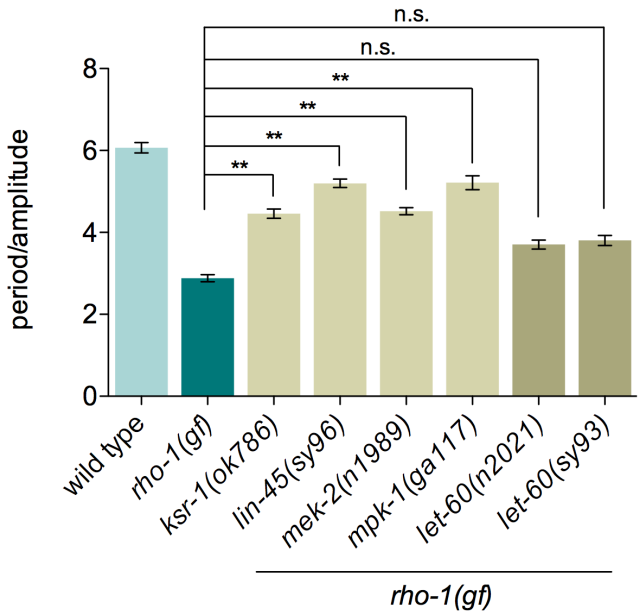

Figure 2-6. Rho-dependent locomotion requires ERK MAPK signaling, but Ras is not required. Mutations in the ERK pathway suppress the coiled locomotion and loopy waveform of *nzIs29[Punc-17::rho-1(gf)].* The reduction of function mutation Ras *let-60(n2021)* as well as dominant negative *let-60(sy93)* D119N fail to suppress Rho waveform. $N \geq 21$ ** $P < 0.01$, n.s., not significant, Kruskal-Wallis test with Dunn's *post hoc* test.

The ERK MAPK cascade acts in acetylcholine neurons to control locomotion

Members of the ERK pathway are expressed in neurons in *C. elegans* (Dent and Han 1998; Hunt-Newbury *et al.* 2007), and Gαq and Rho act in acetylcholine neurons to promote synaptic release and regulate locomotion (Lackner *et al.* 1999b; McMullan *et al.* 2006) To determine whether the ERK pathway also acts in neurons to modulate Gαq signaling, we expressed the *ksr-1* cDNA under promoters driving expression in specific types of neurons. Single-copy transgenic expression of *ksr-1* under an acetylcholine neuron promoter (*Punc-17*) or under a head acetylcholine neuron promoter (*Punc-17H)* fully reversed the *ksr-1* suppression of the loopy waveform of activated Gαq worms (Figure 2-7). *ksr-1* expression in ventral cord acetylcholine motor neurons (*Punc-17β*) or GABA neurons (*Punc-47)* did not significantly reverse the *ksr-1* suppression of activated Gαq (Figure 2-7). This suggests that ERK signaling primarily functions in the acetylcholine interneurons or motor neurons of the head to modulate Gαq -dependent locomotion.

We have shown that the ERK pathway is necessary for Gαq -dependent effects on locomotion. To determine whether ERK signaling is sufficient to modulate locomotion, we expressed an activated form of *lin-45*/Raf specifically in acetylcholine neurons. Raf kinase activity is regulated via conserved phosphorylation events, and phosphomimetic mutations T626E/T629D in the kinase activation loop of *lin-45* are sufficient to confer constitutive Raf activity (Chong *et al.* 2001). We found that expression of

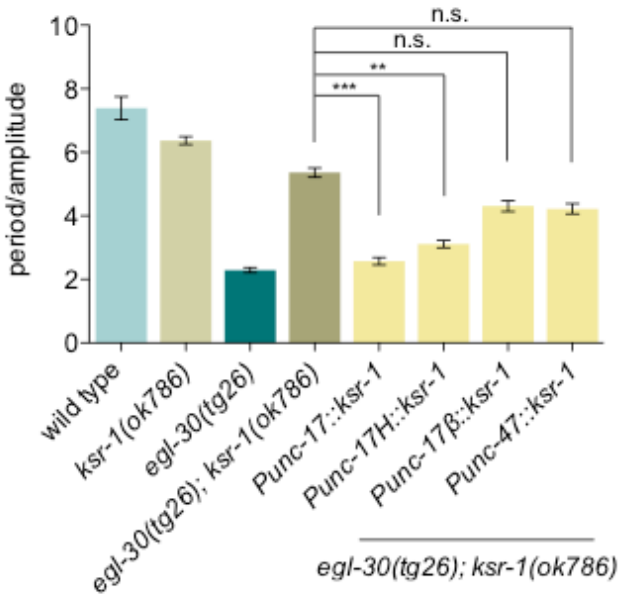

Figure 2-7. KSR-1 acts in acetylcholine neurons to modulate Gαq signaling. Single-copy expression of the *ksr-1* cDNA exclusively in acetylcholine neurons (*Punc-17*, *yakSi26* transgene) or head acetylcholine neurons (*Punc-17H, yakSi27* transgene) is sufficient to reverse the *ksr-1* suppression of the loopy waveform of activated Gαq animals. *ksr-1* expression in ventral cord acetylcholine neurons (*Punc-17β*, *yakSi28* transgene) or GABA neurons (*Punc-47*, *yakSi29* transgene). does not reverse the *ksr-1* suppression of the activated Gαq exaggerated waveform. $N \geq 12$ *** $P < 0.001$, ** $P < 0.01$, n.s., not significant, Kruskal-Wallis test with Dunn's *post hoc* test.

activated Raf in acetylcholine neurons (*Punc-17*) causes a loopy waveform similar to activated Gαq and Rho mutants and similar limited dispersal in radial locomotion assays (Figure 2-8A, B). To determine if Raf activation affects acetylcholine release, we assayed for sensitivity to the acetylcholinesterase inhibitor aldicarb. Mutants with reduced acetylcholine secretion are resistant to aldicarb, whereas mutants with increased acetylcholine secretion are hypersensitive to aldicarb (Mahoney *et al.* 2006).

Activated Gαq and Rho mutants have increased rates of paralysis when exposed to aldicarb (Lackner *et al.* 1999b; McMullan *et al.* 2006) . We found that expression of activated Raf in acetylcholine neurons also led to aldicarb hypersensitivity, similar to activated Gαq and Rho mutants (Figure 2-8C). Because we identified *ksr-1* acting primarily in head acetylcholine neurons to regulate Gαq -dependent locomotion (Figure 2-7), we made transgenic lines to test if Raf activation solely in head interneurons also causes hypersensitivity to aldicarb. Two independent lines expressing activated Raf in head acetylcholine

neurons [*Punc-17H::lin-45(gf)*] share similar sensitivity to aldicarb as [*Punc-17::lin-45(gf)*] lines (Figure 2-9).

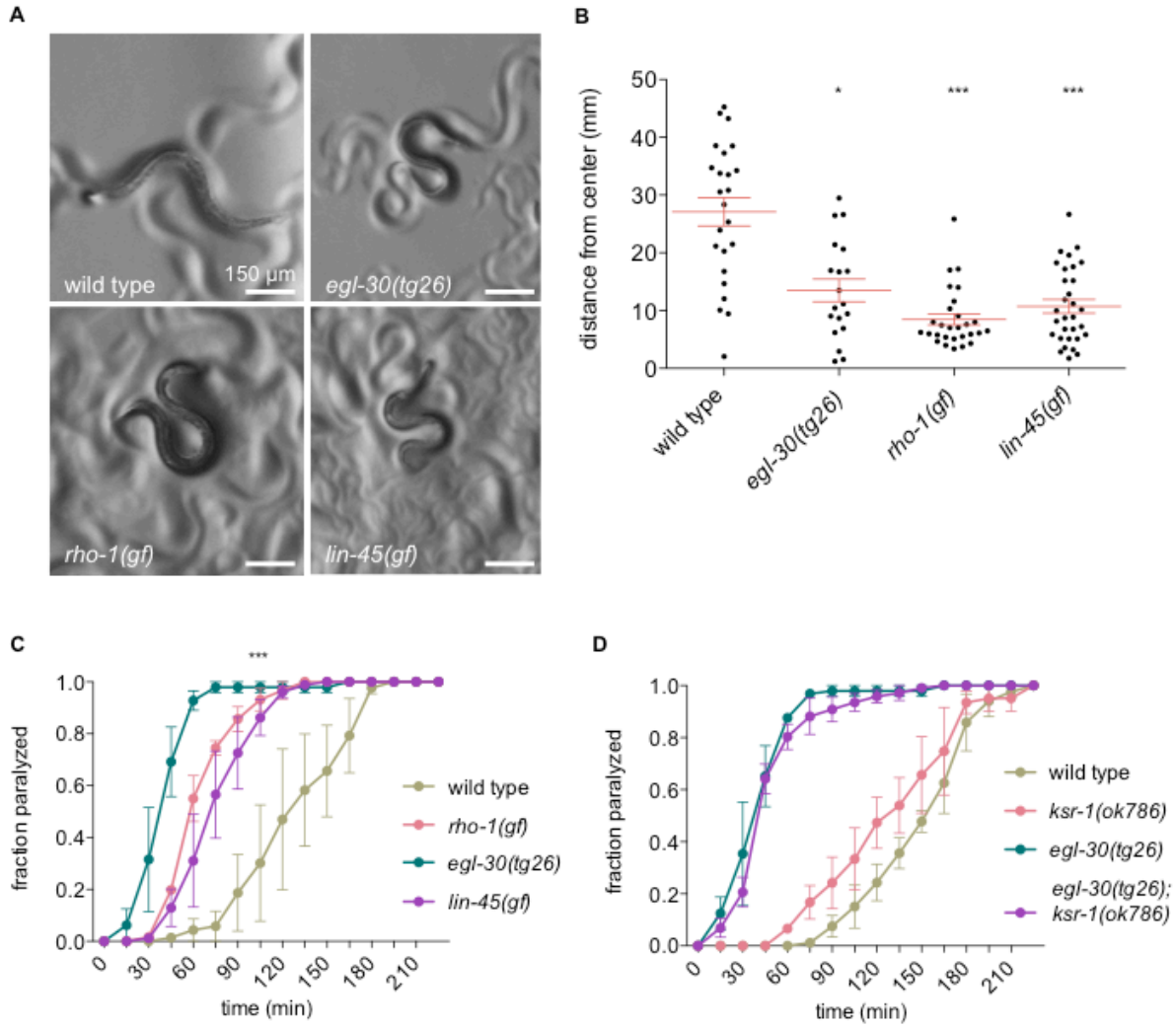

Figure 2-8. Increased ERK signaling in acetylcholine neurons is sufficient to regulate locomotion and increase acetylcholine release.
(A) Transgenic lines expressing an activated form of *lin-45* (T626E/T629D) in acetylcholine neurons (*yakIs34[Punc-17::lin-45(gf)]*) have exaggerated body bends and coiling behavior similar to the activated Gαq mutant *egl-30*(*tg26*) and to animals expressing activated Rho in acetylcholine neurons (*nzIs29[Punc-17::rho-1(gf)]*). The wild type and *egl-30(tg26)* photos are the same as shown in Figure 2-1A, while the *rho-1(gf)* photos is the same as the one in Figure 2-3A.
(B) Expression of activated Rho (*nzIs29[Punc-17::rho-1(gf)]*) or Raf (*yakIs34[Punc-17::lin-45(gf)]*) in acetylcholine neurons impairs coordinated locomotion similarly to activated Gαq (*egl-30*(*tg26*)). $N \geq 19$ * $P < 0.05$, *** $P < 0.001$, Kruskal-Wallis test with Dunn's *post hoc* test.
not suppress the aldicarb hypersensitivity of activated Gαq (*egl-30*(*tg26*)). $N \geq 53$.

Figure 2-8. (Cont.)
(C) Animals expressing activated Gαq, Rho, or Raf are hypersensitive to the acetylcholinesterase inhibitor aldicarb. Activated Gαq (*egl-30*(*tg26*)), activated Rho expressed in acetylcholine neurons (*nzIs29[Punc-17::rho-1(gf)]*), and activated Raf expressed in acetylcholine neurons *yakIs34[Punc-17::lin-45(gf)]*) become paralyzed significantly faster than wild type animals when exposed to 1 mM aldicarb. All strains are significantly different from wild type at t = 60, 75, 90, and 105 minutes. $N \geq 61$ *** $P < 0.001$, two-way ANOVA with Bonferroni's *post hoc* test.
(D) *ksr-1* is not necessary for the aldicarb hypersensitivity of activated Gαq. Paralysis of *ksr-1*(*ok786*) animals on 1 mM aldicarb is not significantly different from wild type. The *ksr-1* deletion *ok786* does not suppress the aldicarb hypersensitivity of activated Gαq (*egl-30*(*tg26*)). $N \geq 53$.

Thus, Raf pathway activation in acetylcholine interneurons is sufficient to increase neurotransmitter release at neuromuscular junctions. However, we found that a *ksr-1* mutation does not suppress the aldicarb hypersensitivity of an activated Gαq mutant, and the *ksr-1* mutant on its own has similar aldicarb sensitivity to wild type (Figure 2-8D). These results suggest that the ERK pathway is not necessary for synaptic transmission, but is sufficient to stimulate synaptic transmission when constitutively activated.

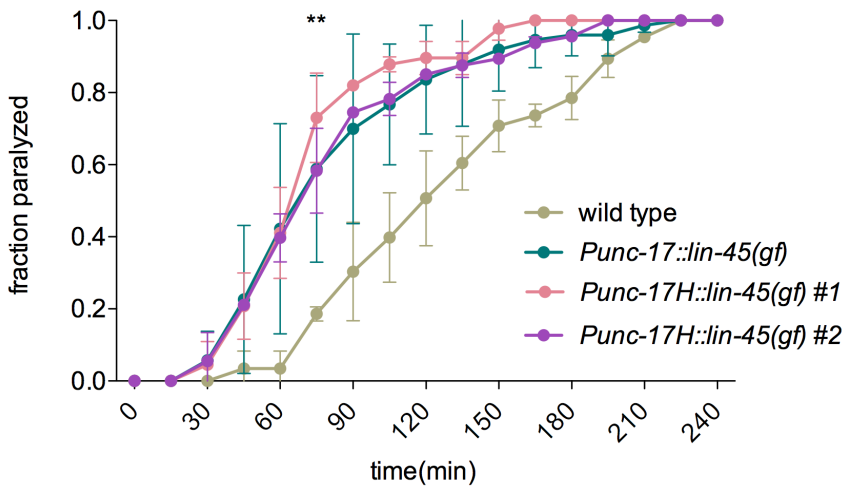

Figure 2-9. Increased ERK signaling in acetylcholine interneurons in the head is sufficient to increase acetylcholine release.
Transgenic animals expressing activated *lin-45* (T626E/T629D) specifically in head acetylcholine neurons *[Punc-17H::lin-45(gf)]* are paralyzed more quickly than wild type when exposed to 1 mM aldicarb. Animals expressing *lin-45(gf)* in head neurons are paralyzed at a similar rate as *[Punc-17::lin-45(gf)]* animals that express in acetylcholine neurons in both the head and body. $N \geq 42$ *** $P < 0.01$, two-way ANOVA with Bonferroni's *post hoc* test.

Chapter 3

Conclusions and Future Directions

My research has revealed that KSR-1 and the ERK MAPK cascade are activated downstream of or in parallel to Gαq-Rho signaling in *C. elegans.* The identification of *ksr-1* as a suppressor of the activated Gαq mutant *egl-30(tg26)* led us to test if other MAPK members interact with Gαq. Further epistasis analysis showed that the ERK MAPK cascade members RAF/LIN-45, MEK/MEK-2, and ERK/MPK-1 also suppress *egl-30(tg26)* locomotion defects. Surprisingly, several reduction-of-function mutations of RAS/LET-60 did not suppress Gαq-dependent locomotion, including dominant negative alleles. We found that ERK signaling acts in cholinergic interneurons to regulate locomotion. Increased Raf/LIN-45 MAPKKK signaling in head acetylcholine interneurons causes locomotion defects and hypersensitivity to the acetylcholinesterase inhibitor aldicarb. Together, these results suggest that ERK activation in head interneurons indirectly increases acetylcholine release at neuromuscular junctions. Our data further indicate that Gαq-Rho signaling may activate the ERK MAPK pathway independently of the small GTPase Ras/LET-60. Overall, we have described a novel signal transduction pathway that regulates neuronal activity in the *C. elegans* motor circuit.

My thesis work advances the field by expanding the array of signaling mechanisms that control locomotion in *C. elegans*. Gαq signaling in *C. elegans* stimulates synaptic release of acetylcholine through the canonical effector PLCβ (Lackner *et al.* 1999). My work shows that Gαq-Rho-ERK mediated signaling acts in parallel to the PLCβ pathway in promoting synaptic activation. Ras-MAPK signaling is involved in neuronal regulation of chemosensory, foraging, and mechanosensory behaviors (Hirotsu *et al.* 2000; Hirotsu and Iino 2005; Tomida *et al.* 2012; Hamakawa *et al.* 2015). My work details a mechanism through which ERK regulates the activation of premotor interneurons in the locomotion circuit. Lastly, previously described roles of ERK signaling are all sensitive to levels of Ras/LET-60 activity. While reduction of Ras function suppresses ERK-dependent vulval induction and chemotaxis (Han and Sternberg 1990, 1991; Tomida *et al.* 2012), Gαq-Rho controlled locomotion is not significantly affected by mutations in Ras/LET-60. This suggests that Gαq-Rho-ERK signaling may involve a Ras-independent mechanism of Raf/LIN-45 activation.

Does Ras regulate Gαq-dependent locomotion? Reduction-of-function mutations in Raf, MEK, and ERK all suppress hyperactive Gαq locomotion, so we were surprised to find that multiple alleles of Ras/LET-60 did not suppress. One possible explanation of these results is that a different Ras family small GTPase activates MAPK signaling. However, we found that loss of Rap1/RAP-1, R-Ras/RAS-1, and M-Ras/RAS-2 similarly had no effect. How might Raf activation occur independently of any Ras family protein? If different Ras proteins act redundantly in this pathway, suppression would only be expected when multiple Ras orthologs were down regulated simultaneously. While technically challenging, knockdown of multiple Ras family proteins in parallel may cause the complete reduction of Ras activity required for suppressing Gαq-dependent locomotion.

Alternatively, this pathway may instead require a small threshold of Ras activity in the locomotor circuit. The strongest dominant negative allele isolated in Ras, *let-60(sy93)* (D119N), did not suppress activated Gαq locomotion. In addition to inhibiting Ras, dominant negative small GTPases inhibit other Ras-family proteins by sequestering RasGEFs, reducing the activity of non-related GTPase signaling pathways. Though dominant negative Ras alleles typically are strong suppressors of the vulval induction pathway (Han and Sternberg 1991), Raf activation might still occur at a low level. Complete loss of Ras causes nonviable larval development (Beitel *et al.* 1990), but loss of Raf does not. Thus dominant negative Ras alleles may have some Raf activation, but the mechanism is unclear (Han and Sternberg 1990). To avoid developmental defects of dominant negative Ras alleles, I made transgenic lines overexpressing dominant negative *let-60* (dn) in neurons. Again, no locomotion phenotypes were seen in wild type or activated Gαq backgrounds, further supporting a Ras-independent role in the Gαq-Rho pathway.

If dominant negative mutations in Ras retain enough activity required to recruit Raf to the membrane and activate Raf, would depletion of Ras protein from the cell more fully reduce Raf activation? The auxin-inducible degron (AID) system would allow temporally controlled degradation of Ras protein (Nishimura *et al.* 2009; Zhang *et al.* 2015). Via CRISPR/Cas9, Ras/LET-60 will be tagged with a degron tag as well as a fluorescent tag. We can then cross degron-Ras with worms expressing the auxin dependent E3 ubiquitin ligase TIR1 in a tissue of interest. When exposed to the small molecule

auxin, the short degron tag triggers degradation of Ras (Nishimura *et al.* 2009). By depleting Ras in cholinergic neurons, we could test whether depleting levels of Ras affect Gαq-dependent locomotion. Modifying the other Ras-family proteins RAS-1, RAS-2, and RAP-1 in parallel would allow us to test if these proteins act redundantly in this pathway.

How might ERK activation occur independently of Ras/LET-60? If Ras is not required in the Gαq-Rho pathway, Raf activation likely occurs through interactions with alternative proteins at the plasma membrane. GTP-bound Ras recruits Raf to the membrane where Raf dimerization stimulates activation. Raf activation can occur independently of Ras when artificially targeted to the membrane (Stokoe *et al.* 1994). One obvious possibility for membrane recruitment is Rho itself. We could test if Rho-KSR-Raf complexes form through co-IP experiments in *C. elegans* or heterologous systems using epitope-tagged proteins. We can also test if these proteins interact by expressing mutants with either activated GTP-bound (G14V) or inactive GDP-bound (T19N) RHO-1 in acetylcholine neurons of interest, then coexpress tagged KSR-1 in the same cells. We would expect activated, but not inactive, Rho to interact with KSR-1 and LIN-45 at the membrane if recruitment by Rho is sufficient.

Another possibility is that ERK is regulated by PKC. Multiple reports suggest that PKC can regulate the ERK pathway downstream of Gαq both in mammals and *C. elegans* (van Biesen *et al.* 1996; Ueda *et al.* 1996; You *et al.* 2006; Hyde *et al.* 2011). While the precise mechanisms are unclear, activation of PKC typically occurs through Gαq-PLCβ signaling. In line with our results, *in vitro* studies show that Gαq- or PKC-dependent MAPK activation is unaffected by expression of dominant negative Ras (van Biesen *et al.* 1996; Ueda *et al.* 1996). In contrast to MAPK alleles, loss of *pkc-1* does not strongly suppress Gαq-dependent “loopy” locomotion. Comparing the locomotion phenotypes of *pkc-1* and MAPK alleles suggests it is unlikely that PKC-1 acts linearly with MAPK signaling downstream of Gαq. Despite having different effects on locomotion, PKC-1 and ERK signaling both affect synaptic acetylcholine release. Loss of PKC-1 function reduces sensitivity to aldicarb while activated PKC-1 mutants are hypersensitive to aldicarb (Sieburth *et al.* 2007). To test more directly if *pkc-1* interacts with ERK signaling in the locomotor circuit, we could create transgenic lines expressing activated PKC-1 specifically in cholinergic neurons and assay for locomotion defects and increased sensitivity to aldicarb

(Sieburth *et al.* 2007). If PKC-1 acts in some of the same cells as ERK MAPK signaling, we would expect activated PKC-1 locomotion and aldicarb sensitivity to be suppressed by loss of ERK activity. Conversely, we would expect loss of Ras to not affect PKC-1 phenotypes if acting in the same genetic pathway as Gαq and RHO-1.

Where does ERK signaling act to regulate locomotion? Our rescue experiments show that *ksr-1* signaling acts primarily in the cholinergic interneurons of the motor circuit to regulate Gαq-dependent locomotion. Experiments using transgenic lines expressing *ksr-1* and activated Raf in head acetylcholine neurons show that activation of ERK MAPK signaling in these cells is sufficient to affect locomotion and acetylcholine signaling. While this helps to identify relevant neurons and neurotransmitters, many of the cholinergic interneurons that are expressed under this head acetylcholine neuron promoter may not be physiologically relevant to Gαq-ERK-dependent locomotion. Relevant interneurons can be identified using several approaches. First, we can identify the expression pattern of our genes of interest by tagging them at their endogenous genomic loci using CRISPR. We currently believe that ERK signaling occurs at some level in most, if not all, neurons. We can identify the cholinergic neurons relevant to Gαq-ERK signaling by modifying LIN-45 or MPK-1 with GFP via CRISPR/Cas9. By crossing to lines that express RFP in certain subsets of neurons, we can identify neurons where MAPK pathway components are expressed (Topalidou *et al.* 2017). If certain classes of interneurons in the motor circuit can be identified, the cells important for Gαq-dependent locomotion can be further delineated through genetic rescue experiments. *ksr-1* can again be expressed under promoters specific for individual sets of neurons, then tested if the expression rescues the Gαq*; *ksr-1* phenotype. This can be further confirmed by degrading the levels of Gαq, Rho, or MAPK proteins via degron-tags (Zhang *et al.* 2015) in neurons of interest. If we identify cells where *ksr-1* signaling is required for Gαq-dependent locomotion, we would then test if this pathway is sufficient to act in those neurons using transgenic lines expressing activated Raf/LIN-45 under a promoter specific to those cells. We would expect these experiments to to see similar aldicarb hypersensitivity as the lines that express activated Raf/LIN-45 in all cholinergic neurons. If so, identifying signaling Gq-ERK individual or subsets of neurons could allow us to model where this pathway in the locomotor circuit.

Expression of constitutively active Raf/LIN-45 or Rho/RHO-1 in cholinergic neurons each cause similar locomotion defects and hypersensitivity to aldicarb. It is possible that some of these effects are due to non-physiologically relevant signaling in certain neurons, so it will be interesting to see if Raf or Rho signaling in individual neurons is sufficient to affect locomotion. Similar to the rescue experiments above, transgenic overactivation of Raf or Rho under certain promoters may be sufficient to regulate locomotion and sensitivity to aldicarb. If acting linearly, depletion of ERK proteins in the same cell type using AID would be expected to suppress the activated Rho and Raf phenotypes.

Does Gαq-RHO-ERK signaling act cell-autonomously in the locomotor circuit? Our genetic experiments use promoters that express in many cells despite being limited to a certain class of neurotransmitter. Gαq, Rho, and ERK signaling may occur in the same cells, or cell nonautonomously. Direct ERK activation through a Gαq-dependent pathway can occur through PKC signaling in the pharyngeal muscle (You *et al.* 2006) and via the RasGRP RGEF-1 in the AWC sensory neuron (Chen *et al.* 2011; Uozumi *et al.* 2012). To understand the relevant cells for this Gαq signaling in the locomotor circuit, we could express the activated Gαq/EGL-30(tg26) mutation in subsets of neurons as previously described. If we determine the physiologically relevant cells for Gαq to promote locomotion, we can then test if reduction of Ras or ERK activity in the same cells or downstream neurons affects the locomotor phenotypes and neuronal activation.

It is unclear if Gαq and ERK pathway members act in a linear or a parallel pathway that converges downstream. If acting linearly, we expect Gαq or Rho activation to cause increased ERK activity. Reduction of Gαq or Rho activity leads to aldicarb resistance, as do *mpk-1* mutations (Lackner *et al.* 1999; Sieburth *et al.* 2005; McMullan *et al.* 2006). If acting in parallel, we expect double mutants to show increased aldicarb resistance, while no compound effect would be seen if acting linearly.

How does Gαq-Rho activation increase neuronal activity via ERK MAPK? The experiments above allow identification of neurons of interest for Gαq, Rho, or MAPK signaling. Is ERK activity affected by Gαq and Rho signaling? Several methods can be used to monitor ERK activation. First, activation of Ras/Raf triggers localization of Raf and KSR-1 to the membrane (A Clapéron 2007). Cells with

hyperactive ERK pathway signaling will increase localization of a fluorophore-tagged KSR-1 or Raf. Membrane localization can be quantified by comparing overlap of KSR-1::GFP with a transgene expressing RFP in the cell membrane. We expect to see increased membrane-colocalization of LIN-45 in worms that are also expressing activated Gαq or Rho. Second, ERK activity in specific cells can be quantified using fluorescent sensors in the cytosol versus the nucleus. In normal conditions, active phospho-ERK translocates to the nucleus where it phosphorylates targets such as transcription factors. The genetically encoded ERK-nKTR (kinase translocation reporter) allows quantification of ERK activity. The ERK-nKTR fluorescent sensor remains in the nucleus until active ERK binds and phosphorylates ERK-nKTR, triggering its transfer from the nucleus into the cytosol. An RFP tagged histone marker is co-transcribed with ERK-nKTR and remains in the nucleus. The level of ERK activation is quantified using a fluorescent sensor ERK-KTR, which is excluded from the nucleus when phosphorylated by active ERK. ERK activity is measured by the ratio of nuclear RFP to nuclear ERK-KTR GFP (de la Cova *et al.* 2017). This sensor allows us to test if transgenes that activate Gαq or Rho in certain neurons affect the ERK activity in neurons of interest. Likewise, reduction of Gαq or Rho function via AID-mediated protein degradation would also test if Gαq or Rho is necessary for ERK activation. If Gαq or Rho affect ERK activity, the involvement of Ras/LET-60 in this pathway could further be tested to see if degradation of Ras via AID reduces ERK activation in a Gαq*/Rho* background.

While we might identify neurons where Gαq /Rho affect ERK activity levels, it is still unclear if and how ERK activity leads to neuronal activation. *ksr-1* on its own is not hypersensitive to aldicarb and does not suppress the aldicarb hypersensitivity of activated Gαq (Figure 2-8). This suggests that *ksr-1* is not necessary for synaptic transmission. However, over activation of LIN-45 in acetylcholine neurons is sufficient to increase hypersensitivity to aldicarb and thus promote neurotransmitter release at the neuromuscular junction.

Because we have identified KSR-1 primarily acting in head cholinergic neurons for Gαq-dependent locomotion, it seems that KSR-1 signaling in head interneurons increases acetylcholine release in motorneurons downstream that control locomotion. If true, we would expect to see increased activity of cholinergic motorneurons when activated Raf is expressed in premotor interneurons. Neuronal activity can be assayed using a fluorescently encoded calcium sensor GCaMP in neurons of interest

(Tian *et al.* 2009). Under this model, expression of activated Gαq, Rho, or Raf in the upstream interneurons is expected to increase the frequency or intensity of synaptic signaling of downstream cholinergic motoneurons, while AID-dependent depletion of Gαq, Rho, or Raf would decrease neuronal activity.

In conclusion, my project has characterized complex genetic interactions between different signal transduction cascades. I identified a new role for ERK MAPK signaling in regulating cholinergic locomotion in *C. elegans,* expanding on the relatively few known neuronal roles of ERKs. While the neuronal circuitry controlling locomotion is well characterized, my work introduces Gαq-Rho-MAPK signaling as an important regulator in cholinergic interneurons. This pathway provides a platform for future work to describe new roles of Gαq-ERK signaling and the regulation of synaptic signaling.

Appendix 1

C. elegans Strain List

N2	Bristol wild strain
XZ1151	*egl-30(tg26) I*
EG6699	*ttTi5605 II; unc-119(ed3) III*
EG6207	*unc-119(ed3) III*
RB915	*ksr-1(ok786) X*
EG4782	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II*
MT4866	*let-60(n2021) IV*
PS436	*let-60(sy93) IV*
PS427	*lin-45(sy96) IV*
MT8666	*mek-2(n1989) I*
SD378	*mpk-1(ga117) / dpy-17(e164) unc-79(e1068) III*
CB1417	*lin-3(e1417) IV*
PS5131	*let-23(sy12) / mIn1[dpy-10(e128) mIs14] II*
UP604	*sos-1(cs41) V*
VC450	*ras-1(gk243) II*
RB852	*ras-2(ok682) III*
TZ181	*rap-1(pk2082) IV*
RB848	*rgef-1(ok675) V*
MH538	*mek-2(ku114) I; let-60(n1046) IV*
MT8186	*mpk-1(oz140)/unc-79(e1068) dpy-17(e164) III*

The following strains were produced during this study:

EG314	*ksr-1(ox314) X*
XZ10	*ksr-1(yak10) X*
EG4198	*egl-30(tg26) I ; ksr-1(ox314) X*
XZ1340	*egl-30(tg26) I ; ksr-1(yak10) X*
XZ1511	*egl-30(tg26) I; ksr-1(ok786) X*
XZ1465	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; ksr-1(ox314) X*
XZ1547	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; ksr-1(yak10) X*
XZ2042	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; ksr-1(ok786) X*
XZ1615	*egl-30(tg26) I; let-60(n2021) / nT1[qIs51(Pmyo-2::GFP; Ppes-10::GFP; F22B7.9::GFP)] IV*
XZ1626	*egl-30(tg26) I; lin-3(e1417) IV*
XZ1630	*egl-30(tg26) I; let-23(sy12) / mIn1[dpy-10(e128) mIs14] II*
XZ1677	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; let-60(n2021) / nT1[qIs51(Pmyo-2::GFP; Ppes-10::GFP; F22B7.9::GFP)] IV*
XZ1548	*egl-30(tg26) I; lin-45(sy96) IV*
XZ1556	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; lin-45(sy96) IV*
XZ1850	*egl-30(tg26) mek-2(n1989) / hT2[bli-4(e937) let(q782) qIs48] I*
XZ1851	*mek-2(n1989) / hT2[bli-4(e937) let(q782) qIs48] I; nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II*
XZ1700	*mpk-1(ga117) / qC1[dpy-19(e1259) glp-1(q339) qIs26(Plag-2::GFP, rol-6(su1006))] III*
XZ1668	*egl-30(tg26) I; mpk-1(ga117) / qC1[dpy-19(e1259) glp-1(q339) qIs26(Plag-2::GFP, rol-6(su1006))] III*
XZ2043	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; mpk-1(ga117) / qC1[dpy-19(e1259) glp-1(q339) qIs26(Plag-2::GFP, rol-6(su1006))] III*
XZ2046	*egl-30(tg26) I; sos-1(cs41) V*
XZ1855	*egl-30(tg26) I; ras-1(gk243) II*
XZ1856	*egl-30(tg26) I; ras-2(ok682) III*

XZ2045	*egl-30(tg26) I; rap-1(pk2082) IV*
XZ2101	*egl-30(tg26) I; rgef-1(ok675) V*
XZ1921	*egl-30(tg26) sur-6(sv30) / hT2[bli-4(e937) let(q782) qIs48] I*
XZ1857	*egl-30(tg26) I ; sur-7(ku119) X*
XZ1854	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; ras-2(ok682) III*
XZ1852	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; rap-1(pk2082) IV*
XZ1880	*yakSi26[Punc-17::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II*
XZ1881	*yakSi27[Punc-17H::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II*
XZ1882	*yakSi28[Punc-17β::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II*
XZ1883	*yakSi29[Punc-47::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II*
XZ1884	*egl-30(tg26) I; yakSi26[Punc-17::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II; ksr-1(ok786) X*
XZ1885	*egl-30(tg26) I; yakSi27[Punc-17H::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II; ksr-1(ok786) X*
XZ1946	*egl-30(tg26) I; yakSi28[Punc-17β::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II; ksr-1(ok786) X*
XZ1947	*egl-30(tg26) I; yakSi29[Punc-47::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II; ksr-1(ok786) X*
XZ2015	*unc-119(ed3) III; yakEx122[Punc-17::lin-45* cDNA::tbb-2 3'UTR::OPERON::GFP, Pmyo-3::mCherry]*
XZ2077	*egl-30(tg26) I; let-60(sy93) IV*
XZ2106	*egl-30(tg26) I; let-60(sy93) / nT1[qIs51(Pmyo-2::GFP; Ppes-10::GFP; F22B7.9::GFP)] IV*
XZ2050	*unc-119(ed3) III; yakIs34[Punc-17::lin-45* cDNA::tbb-2 3'UTR::OPERON::GFP, Pmyo-3::mCherry] X*
XZ2130	*egl-30(tg26) I; mpk-1(oz140) / oxTi619[Peft-3::TdTomato::H2B cb-unc-119(+)] III*
XZ2131	*egl-30(tg26) mek-2(ku114) I / hT2[bli-4(e937) let(q782) qIs48] I;III*
XZ2119	*ttTi5605 II; unc-119(ed3) III; yakEx154[Punc-17H::lin-45*::tbb-2 3'UTR::OPERON::GFP, Pmyo-2::mCherry, Pmyo-3::mCherry]*

Appendix 2

List of Plasmids

Gateway destination vectors

pCFJ150	Gateway destination vector for insertion at chr II Mos site ttTi5605

Gateway entry clones

pEGB05	*Prab-3* [4-1]
pGH1	*Punc-17* [4-1]
pADA180	*Punc-17H* [4-1] (head acetylcholine neurons)
pMA23	*Punc-17β* [4-1] (body acetylcholine neurons)
pMH522	*Punc-47* [4-1]
pBC11	*ksr-1* cDNA [1-2]
pBC26	*lin-45* cDNA [1-2]
pBC35	*lin-45** (T626E/T629D) cDNA [1-2]
pCFJ326	*tbb-2* 3'UTR::OPERON::*GFP* [2-3]

Gateway expression constructs

pBC13	*Prab-3::ksr-1* cDNA::*tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC31	*Punc-17::ksr-1* cDNA*::tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC32	*Punc-17H*::*ksr-1* cDNA::*tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC33	*Punc-17β*::*ksr-1* cDNA::*tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC34	*Punc-47*::*ksr-1* cDNA::*tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC37	*Punc-17::lin-45** cDNA*::tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC44	*Punc-17H::lin-45** cDNA*::tbb-2* 3'UTR::OPERON::GFP_pCFJ150

List of primers

oBC011	Forward *ksr-1* cDNA for Gateway [1-2] ENTR vector	GGGGACAAGTTTGTACAAAAAAGCAG GCTCAatgatgcaaacccaagttgc
oBC012	Reverse *ksr-1* cDNA for Gateway [1-2] ENTR vector	GGGGACCACTTTGTACAAGAAAGCTG GGTGaaatgtcgactcgtaacttttcatc
oBC085	Forward *lin-45* cDNA for Gateway	GGGGACAAGTTTGTACAAAAAAGCAG

	[1-2] ENTR vector	GCTcaATGAGTCGGATTAATTTCAAAAAG
oBC086	Reverse *lin-45* cDNA for Gateway [1-2] ENTR vector	GGGGACCACTTTGTACAAGAAAGCTGGGTgCTAAATGAGACCATAGACATTGTAGTATG
oBC094	Reverse *lin-45* T626E mutagenesis	gttcactgtccatttcgttttgacctctgccaagccgaaatctccaatttt
oBC095	Forward *lin-45* T626E mutagenesis	aaaattggagatttcggcttggcagaggtcaaaacgaaatggacagtgaac
oBC096	Reverse *lin-45* T629D mutagenesis	cctccgttcactgtccatttatctttgacctctgccaagccga
oBC097	Forward *lin-45* T629D mutagenesis	tcggcttggcagaggtcaaagataaatggacagtgaacggagg

Materials and Methods

C. elegans strains

All strains were cultured using standard methods and were maintained at 20°C. Table S1 contains all the strains used in this study.

Isolation and mapping of the ksr-1(ox314) and ksr-1(yak10) mutations

The *ox314* and *yak10* mutants were isolated from an ENU mutagenesis suppressor screen of the activated Gq mutant *egl-30(tg26)* (Ailion *et al.* 2014). We mapped the *ox314* mutation by its activated Gq suppression phenotype using single nucleotide polymorphisms (SNPs) in the Hawaiian strain CB4856 as described (Davis *et al.* 2005).The *ox314* mutation was mapped to an approximately 709 kb region in the middle of the X chromosome between SNPs on cosmids F45E1 and F53A9 (SNPs F45E1[1] and pkP6158). This region included 159 predicted protein-coding genes. A complementation test of *ox314* and *yak10* in the *egl-30(tg26)* background showed these to be alleles of the same gene. Whole genome sequencing (see below) identified these as mutations in *ksr-1*, and we confirmed this by performing a complementation test with the deletion allele *ksr-1(ok786).*

Whole genome sequencing

Strains EG4198 *egl-30(tg26); ox314* and XZ1340 *egl-30(tg26); yak10* were sequenced to identify candidate mutations. DNA was purified according to the Hobert Lab protocol (http://hobertlab.org/whole-genome-sequencing/). Ion Torrent sequencing was performed at the University of Utah DNA Sequencing Core Facility. Each data set contained roughly 18,400,000 reads of a mean read length of 160 bases, resulting in about 30X average coverage of the *C. elegans* genome. The sequencing data were processed on the Galaxy server at usegalaxy.org (Afgan *et al.* 2016). SNPs and indels were identified and annotated using the Unified Genotyper and SnpEff tools (DePristo *et al.* 2011; Cingolani *et al.* 2012). After filtering for mutations in open reading frames, we found each strain to have unique stop mutations in *ksr-1*, in the middle of the interval where we mapped *ox314*. *ox314* is a G to A transition that causes a stop codon at amino acid K463, and *yak10* is an A to T transversion that causes a stop codon at W254.

Locomotion assays

Track waveform and radial locomotion assays were performed on 10 cm nematode growth medium (NGM) plates seeded with 400 µl of OP50 *E. coli* culture and spread with sterile glass beads. Bacterial lawns were grown at 37°C for 16 hrs and the plates were stored at 4°C until needed. For track waveform measurements, five first day adult animals were placed on a plate and allowed to roam for 2-5 min. We then recorded each animal's tracks following forward locomotion. Track pictures were taken at 40X on a Nikon SMZ18 microscope with the DS-L3 camera control system. Pictures of worm tracks were processed using ImageJ. Period and 2X amplitude were measured freehand using the line tool. For each worm, we calculated the average period/amplitude ratio of five individual track bends (Figure 1C). For assays with the temperature sensitive allele *sos-1(cs41)*, all strains were grown at 20°C and shifted to the non-permissive temperature of 25°C for 24 hours before being assayed. For radial locomotion assays, ten to fifteen first day adult animals were picked to the center of a plate and were then allowed to move freely for 40 minutes. The positions of worms were marked and the distances of the worms from the starting point were measured. For all waveform and radial locomotion assays, the experimenter was blind to the genotypes of the strains assayed.

Microscopy

Photographs of moving worms were taken at 60X on a Nikon SMZ18 microscope with the DS-L3 camera control system. The worms were age-matched as first day adults grown at 20°C.

Constructs and transgenes

Plasmids were constructed using the three-slot multisite Gateway cloning system (Invitrogen). Plasmids and primers used are found in Tables S2 and S3. The *ksr-1* and *lin-45* cDNAs were amplified by PCR from worm cDNA library and cloned into [1-2] Gateway entry vectors. Activating Raf mutations T626E/T629D were introduced into the *lin-45* cDNA vector by two sequential site-directed mutagenesis reactions (Q5 NEB) with primers oBC094/095 and oBC096/097, respectively, and then confirmed by sequencing. The *ksr-1* and activated *lin-45* cDNAs were cloned into expression constructs under different

neuronal promoters using the multisite Gateway system. Proper expression of *ksr-1* and activated *lin-45* was confirmed by including an operon GFP::H2B in the [2-3] slot of the expression constructs. The operon GFP template *tbb-2 3'utr::gpd-2 operon::GFP::H2B:cye-1 3'utr* (Frøkjær-Jensen *et al.* 2012) results in untagged proteins whose expression can be monitored by GFP expression.

Injections and chromosomal integrations

Worms carrying the activated *lin-45* transgenes *Punc-17::lin-45** and *Punc-17H::lin-45** as extrachromosomal arrays were generated by injecting pBC37 or pBC44 at 20 ng/μL or 10 ng/ μL respectively along with co-injection markers pCFJ104 (*Pmyo-3::mCherry*) at 5 ng/μL, pCFJ90 (*Pmyo-2::mCherry*) at 2.5 ng/μL, and the carrier DNA Litmus 38i to a final concentration of 100 ng/μL DNA (Mello *et al.* 1991; Frøkjær-Jensen *et al.* 2012). MosSCI lines were generated as described (Frøkjær-Jensen *et al.* 2012) using an injection mix containing 10-15 ng/μL targeting vector, 50 ng/μL pCFJ601 (*Peft-3::Mos1* transposase), negative selection markers pGH8 (*Prab-3::mCherry*) at 10 ng/μL, pCFJ104 (*Pmyo-3::mCherry*) at 5 ng/μL, pCFJ90 (*Pmyo-2::mCherry*) at 2.5 ng/μL, pMA122 (*Phsp16.41::peel-1*) at 10 ng/μL, and carrier DNA Litmus 38i to a final concentration of 100 ng/μL DNA.

Extrachromosomal arrays were integrated into the genome by exposure to 4000 rads of gamma irradiation. Irradiated young adult hermaphrodites were transferred to 10 cm OP50 plates (5 worms/plate) and grown to starvation. The plates were chunked and grown to starvation twice more to enrich for stably expressing lines. When nearly starved, 8 animals per plate were picked to individual plates. The progeny were then screened for 100% stable transmission, indicating integration into the genome. Integration was confirmed by mapping the transgene to a chromosome.

Aldicarb assays

35 mm aldicarb assay plates were poured with NGM supplemented with 1 mM aldicarb. The plates were seeded with 5 uL OP50 and dried at room temperature overnight. Animals were picked onto the OP50 lawn to begin the assay (time 0) and then kept at room temperature. Every 15 minutes, animals were scored for paralysis by lightly touching the nose of the animal with an eyebrow hair. Animals were

scored as paralyzed if the worm displayed no locomotor response to three nose touches and had no pharyngeal pumping. Animals that left the OP50 lawn were picked back onto the food.

Statistical analysis

P values were determined using GraphPad Prism 5. Normally distributed data sets were analyzed with a one-way ANOVA and Bonferroni's *post hoc* test when group size was unequal, or with Tukey's *post hoc* test when group size was equal. Data sets with non-normal distribution (using the Shapiro-Wilk normality test) were analyzed with a Kruskal-Wallis test and Dunn's *post hoc* test. Data sets with multiple independent variables were analyzed by two-way ANOVA and Bonferroni's *post hoc* test.

References

A Clapéron M. T., 2007 Claperon A, Therrien M.. KSR and CNK: two scaffolds regulating RAS-mediated RAF activation. Oncogene 26: 3143-3158. Oncogene 26: 3143–58.

Abreu-Blanco M. T., Verboon J. M., Parkhurst S. M., 2014 Coordination of Rho family GTPase activities to orchestrate cytoskeleton responses during cell wound repair. Curr. Biol. CB 24: 144–155.

Afgan E., Baker D., Beek M. van den, Blankenberg D., Bouvier D., *et al.*, 2016 The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res. 44: W3–W10.

Ailion M., Hannemann M., Dalton S., Pappas A., Watanabe S., *et al.*, 2014 Two Rab2 interactors regulate dense-core vesicle maturation. Neuron 82: 167–180.

Bastiani C. A., Gharib S., Simon M. I., Sternberg P. W., 2003 Caenorhabditis elegans Gαq Regulates Egg-Laying Behavior via a PLCβ-Independent and Serotonin-Dependent Signaling Pathway and Likely Functions Both in the Nervous System and in Muscle. Genetics 165: 1805–1822.

Beitel G. J., Clark S. G., Horvitz H. R., 1990 Caenorhabditis elegans ras gene let-60 acts as a switch in the pathway of vulval induction. Nature 348: 503–509.

Biesen T. van, Hawes B. E., Raymond J. R., Luttrell L. M., Koch W. J., *et al.*, 1996 G(o)-protein alpha-subunits activate mitogen-activated protein kinase via a novel protein kinase C-dependent mechanism. J. Biol. Chem. 271: 1266–1269.

Bos J. L., 1989 ras oncogenes in human cancer: a review. Cancer Res. 49: 4682–4689.

Bos J. L., Rehmann H., Wittinghofer A., 2007 GEFs and GAPs: Critical Elements in the Control of Small G Proteins. Cell 129: 865–877.

Brenner S., 1974 The genetics of Caenorhabditis elegans. Genetics 77: 71–94.

Brundage L., Avery L., Katz A., Kim U. J., Mendel J. E., *et al.*, 1996 Mutations in a C. elegans Gqalpha gene disrupt movement, egg laying, and viability. Neuron 16: 999–1009.

Chalasani S. H., Chronis N., Tsunozaki M., Gray J. M., Ramot D., *et al.*, 2007 Dissecting a circuit for olfactory behaviour in *Caenorhabditis elegans*. Nature 450: 63–70.

Chalfie M., Sulston J. E., White J. G., Southgate E., Thomson J. N., *et al.*, 1985 The neural circuit for touch sensitivity in Caenorhabditis elegans. J. Neurosci. 5: 956–964.

Chan J. P., Hu Z., Sieburth D., 2012 Recruitment of sphingosine kinase to presynaptic terminals by a conserved muscarinic signaling pathway promotes neurotransmitter release. Genes Dev. 26: 1070–1085.

Chen L., Fu Y., Ren M., Xiao B., Rubin C. S., 2011 A RasGRP, C. elegans RGEF-1b, Couples External Stimuli to Behavior by Activating LET-60 (Ras) in Sensory Neurons. Neuron 70: 51–65.

Chong H., Lee J., Guan K. L., 2001 Positive and negative regulation of Raf kinase activity and function by phosphorylation. EMBO J. 20: 3716–3727.

Cingolani P., Platts A., Wang L. L., Coon M., Nguyen T., *et al.*, 2012 A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 6: 80–92.

Coleman B., Topalidou I., Ailion M., 2017 The ERK MAPK pathway modulates Gq-dependent locomotion in Caenorhabditis elegans. bioRxiv: 231746.

Collins K. M., Bode A., Fernandez R. W., Tanis J. E., Brewer J. C., *et al.*, 2016 Activity of the C. elegans egg-laying behavior circuit is controlled by competing activation and feedback inhibition. eLife 5.

Comi A. M., 2015 Sturge-Weber syndrome. Handb. Clin. Neurol. 132: 157–168.

Consortium* T. C. elegans S., 1998 Genome Sequence of the Nematode C. elegans: A Platform for Investigating Biology. Science 282: 2012–2018.

Cova C. de la, Townley R., Regot S., Greenwald I., 2017 A Real-Time Biosensor for ERK Activity Reveals Signaling Dynamics during C. elegans Cell Fate Specification. Dev. Cell 42: 542–553.e4.

Davis M. W., Hammarlund M., Harrach T., Hullett P., Olsen S., *et al.*, 2005 Rapid single nucleotide polymorphism mapping in C. elegans. BMC Genomics 6: 118.

De Luca A., Maiello M. R., D'Alessio A., Pergameno M., Normanno N., 2012 The RAS/RAF/MEK/ERK and the PI3K/AKT signalling pathways: role in cancer pathogenesis and implications for therapeutic approaches. Expert Opin. Ther. Targets 16 Suppl 2: S17-27.

Dent J. A., Han M., 1998 Post-embryonic expression pattern of C. elegans let-60 ras reporter constructs. Mech. Dev. 72: 179–182.

DePristo M. A., Banks E., Poplin R., Garimella K. V., Maguire J. R., *et al.*, 2011 A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 43: 491–498.

Deschênes-Simard X., Kottakis F., Meloche S., Ferbeyre G., 2014 ERKs in cancer: friends or foes? Cancer Res. 74: 412–419.

Doi M., Iwasaki K., 2002 Regulation of retrograde signaling at neuromuscular junctions by the novel C2 domain protein AEX-1. Neuron 33: 249–259.

Drosten M., Dhawahir A., Sum E. Y. M., Urosevic J., Lechuga C. G., *et al.*, 2010 Genetic analysis of Ras signalling pathways in cell proliferation, migration and survival. EMBO J. 29: 1091–1104.

Drosten M., Sum E. Y. M., Lechuga C. G., Simón-Carrasco L., Jacob H. K. C., *et al.*, 2014 Loss of p53 induces cell proliferation via Ras-independent activation of the Raf/Mek/Erk signaling pathway. Proc. Natl. Acad. Sci. U. S. A. 111: 15155–15160.

Ferguson E. L., Horvitz H. R., 1989 The multivulva phenotype of certain Caenorhabditis elegans mutants results from defects in two functionally redundant pathways. Genetics 123: 109–121.

Frøkjær-Jensen C., Davis M. W., Ailion M., Jorgensen E. M., 2012 Improved Mos1-mediated transgenesis in C. elegans. Nat. Methods 9: 117–118.

Garcia-Marcos M., Ghosh P., Farquhar M. G., 2015 GIV/Girdin transmits signals from multiple receptors by triggering trimeric G protein activation. J. Biol. Chem. 290: 6697–6704.

Gilman A. G., 1987 G Proteins: Transducers of Receptor-Generated Signals. Annu. Rev. Biochem. 56: 615–649.

Gray J. M., Hill J. J., Bargmann C. I., 2005 A circuit for navigation in Caenorhabditis elegans. Proc. Natl. Acad. Sci. 102: 3184–3191.

Hamakawa M., Uozumi T., Ueda N., Iino Y., Hirotsu T., 2015 A role for Ras in inhibiting circular foraging behavior as revealed by a new method for time and cell-specific RNAi. BMC Biol. 13: 6.

Han M., Sternberg P. W., 1990 let-60, a gene that specifies cell fates during C. elegans vulval induction, encodes a ras protein. Cell 63: 921–931.

Han M., Sternberg P. W., 1991 Analysis of dominant-negative mutations of the Caenorhabditis elegans let-60 ras gene. Genes Dev. 5: 2188–2198.

Han M., Golden A., Han Y., Sternberg P. W., 1993 C. elegans lin-45 raf gene participates in let-60 ras-stimulated vulval differentiation. Nature 363: 133–140.

Herzog D., Loetscher P., Hengel J. van, Knüsel S., Brakebusch C., *et al.*, 2011 The small GTPase RhoA is required to maintain spinal cord neuroepithelium organization and the neural stem cell pool. J. Neurosci. Off. J. Soc. Neurosci. 31: 5120–5130.

Hiley E., McMullan R., Nurrish S. J., 2006 The Galpha12-RGS RhoGEF-RhoA signalling pathway regulates neurotransmitter release in C. elegans. EMBO J. 25: 5884–5895.

Hirotsu T., Saeki S., Yamamoto M., Iino Y., 2000 The Ras-MAPK pathway is important for olfaction in Caenorhabditis elegans. Nature 404: 289–293.

Hirotsu T., Iino Y., 2005 Neural circuit-dependent odor adaptation in C. elegans is regulated by the Ras-MAPK pathway. Genes Cells 10: 517–530.

Hu S., Pawson T., Steven R. M., 2011 UNC-73/trio RhoGEF-2 activity modulates Caenorhabditis elegans motility through changes in neurotransmitter signaling upstream of the GSA-1/Galphas pathway. Genetics 189: 137–151.

Hu Z., Vashlishan-Murray A. B., Kaplan J. M., 2015 NLP-12 engages different UNC-13 proteins to potentiate tonic and evoked release. J. Neurosci. Off. J. Soc. Neurosci. 35: 1038–1042.

Huang J. L.-Y., Urtatiz O., Van Raamsdonk C. D., 2015 Oncogenic G Protein GNAQ Induces Uveal Melanoma and Intravasation in Mice. Cancer Res. 75: 3384–3397.

Hunt-Newbury R., Viveiros R., Johnsen R., Mah A., Anastas D., *et al.*, 2007 High-throughput in vivo analysis of gene expression in Caenorhabditis elegans. PLoS Biol. 5: e237.

Hyde R., Corkins M. E., Somers G. A., Hart A. C., 2011 PKC-1 acts with the ERK MAPK signaling pathway to regulate C. elegans mechanosensory response. Genes Brain Behav. 10: 286–298.

Jarrell T. A., Wang Y., Bloniarz A. E., Brittin C. A., Xu M., *et al.*, 2012 The Connectome of a Decision-Making Neural Network. Science 337: 437–444.

Jorgensen E. M., Mango S. E., 2002 The art and design of genetic screens: *Caenorhabditis elegans*. Nat. Rev. Genet. 3: 356.

Kornfeld K., Hom D. B., Horvitz H. R., 1995 The ksr-1 gene encodes a novel protein kinase involved in Ras-mediated signaling in C. elegans. Cell 83: 903–913.

Lackner M. R., Nurrish S. J., Kaplan J. M., 1999a Facilitation of synaptic transmission by EGL-30 Gqalpha and EGL-8 PLCbeta: DAG binding to UNC-13 is required to stimulate acetylcholine release. Neuron 24: 335–346.

Lackner M. R., Nurrish S. J., Kaplan J. M., 1999b Facilitation of Synaptic Transmission by EGL-30 Gqα and EGL-8 PLCβ: DAG Binding to UNC-13 Is Required to Stimulate Acetylcholine Release. Neuron 24: 335–346.

Lutz S., Freichel-Blomquist A., Yang Y., Rümenapp U., Jakobs K. H., *et al.*, 2005 The guanine nucleotide exchange factor p63RhoGEF, a specific link between Gq/11-coupled receptor signaling and RhoA. J. Biol. Chem. 280: 11134–11139.

Lutz S., Shankaranarayanan A., Coco C., Ridilla M., Nance M. R., *et al.*, 2007 Structure of Galphaq-p63RhoGEF-RhoA complex reveals a pathway for the activation of RhoA by GPCRs. Science 318: 1923–1927.

Mahoney T. R., Luo S., Nonet M. L., 2006 Analysis of synaptic transmission in Caenorhabditis elegans using an aldicarb-sensitivity assay. Nat. Protoc. 1: 1772–1777.

Mandal R., Becker S., Strebhardt K., 2016 Stamping out RAF and MEK1/2 to inhibit the ERK1/2 pathway: an emerging threat to anticancer therapy. Oncogene 35: 2547–2561.

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

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

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

Montagut C., Sharma S. V., Shioda T., McDermott U., Ulman M., *et al.*, 2008 Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. Cancer Res. 68: 4853–4861.

Nazarian R., Shi H., Wang Q., Kong X., Koya R. C., *et al.*, 2010 Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature 468: 973–977.

Nguyen A., Burack W. R., Stock J. L., Kortum R., Chaika O. V., *et al.*, 2002 Kinase Suppressor of Ras (KSR) Is a Scaffold Which Facilitates Mitogen-Activated Protein Kinase Activation In Vivo. Mol. Cell. Biol. 22: 3035–3045.

Nishimura K., Fukagawa T., Takisawa H., Kakimoto T., Kanemaki M., 2009 An auxin-based degron system for the rapid depletion of proteins in nonplant cells. Nat. Methods 6: 917–922.

Nobes C. D., Hall A., 1995 Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 81: 53–62.

O'Donovan K. J., Ma K., Guo H., Wang C., Sun F., *et al.*, 2014 B-RAF kinase drives developmental axon growth and promotes axon regeneration in the injured mature CNS. J. Exp. Med. 211: 801–814.

Plaza S. M., Scheffer L. K., Chklovskii D. B., 2014 Toward large-scale connectome reconstructions. Curr. Opin. Neurobiol. 25: 201–210.

Reiner D., Lundquist E. A., 2016 Small GTPases. WormBook Online Rev. C Elegans Biol.

Reynolds N. K., Schade M. A., Miller K. G., 2005 Convergent, RIC-8-Dependent Gα Signaling Pathways in the Caenorhabditis elegans Synaptic Signaling Network. Genetics 169: 651–670.

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

Richmond J. E., Jorgensen E. M., 1999 One GABA and two acetylcholine receptors function at the C. elegans neuromuscular junction. Nat. Neurosci. 2: 791–797.

Roberts W. M., Augustine S. B., Lawton K. J., Lindsay T. H., Thiele T. R., *et al.*, 2016 A stochastic neuronal model predicts random search behaviors at multiple spatial scales in C. elegans. eLife 5: e12572.

Siderovski D. P., Willard F. S., 2005 The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int. J. Biol. Sci. 1: 51–66.

Sieburth D. S., Sundaram M., Howard R. M., Han M., 1999 A PP2A regulatory subunit positively regulates Ras-mediated signaling during Caenorhabditis elegans vulval induction. Genes Dev. 13: 2562–2569.

Sieburth D., Ch'ng Q., Dybbs M., Tavazoie M., Kennedy S., *et al.*, 2005 Systematic analysis of genes required for synapse structure and function. Nature 436: 510–517.

Sieburth D., Madison J. M., Kaplan J. M., 2007 PKC-1 regulates secretion of neuropeptides. Nat. Neurosci. 10: 49–57.

Sisley K., Doherty R., Cross N. A., 2011 What hope for the future? GNAQ and uveal melanoma. Br. J. Ophthalmol. 95: 620–623.

Sprang S. R., 2016 Activation of G proteins by GTP and the mechanism of Gα-catalyzed GTP hydrolysis. Biopolymers 105: 449–462.

Stankiewicz T. R., Linseman D. A., 2014 Rho family GTPases: key players in neuronal development, neuronal survival, and neurodegeneration. Front. Cell. Neurosci. 8.

Sternberg P. W., Han M., 1998 Genetics of RAS signaling in C. elegans. Trends Genet. TIG 14: 466–472.

Stokoe D., Macdonald S. G., Cadwallader K., Symons M., Hancock J. F., 1994 Activation of Raf as a result of recruitment to the plasma membrane. Science 264: 1463–1467.

Sulston J., Dew M., Brenner S., 1975 Dopaminergic neurons in the nematode Caenorhabditis elegans. J. Comp. Neurol. 163: 215–226.

Sun Y., Liu W.-Z., Liu T., Feng X., Yang N., *et al.*, 2015 Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. J. Recept. Signal Transduct. Res. 35: 600–604.

Sundaram M., Han M., 1995 The C. elegans ksr-1 gene encodes a novel Raf-related kinase involved in Ras-mediated signal transduction. Cell 83: 889–901.

Sundaram M. V., 2013 Canonical RTK-Ras-ERK signaling and related alternative pathways. WormBook Online Rev. C Elegans Biol.: 1–38.

Takashima A., Faller D. V., 2013 Targeting the RAS oncogene. Expert Opin. Ther. Targets 17: 507–531.

Tall G. G., Krumins A. M., Gilman A. G., 2003 Mammalian Ric-8A (synembryn) is a heterotrimeric Galpha protein guanine nucleotide exchange factor. J. Biol. Chem. 278: 8356–8362.

Tall G. G., 2013 Ric-8 regulation of heterotrimeric G proteins. J. Recept. Signal Transduct. Res. 33: 139–143.

Tian L., Hires S. A., Mao T., Huber D., Chiappe M. E., *et al.*, 2009 Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat. Methods 6: 875–881.

Tomida T., Oda S., Takekawa M., Iino Y., Saito H., 2012 The Temporal Pattern of Stimulation Determines the Extent and Duration of MAPK Activation in a Caenorhabditis elegans Sensory Neuron. Sci Signal 5: ra76-ra76.

Tomioka M., Adachi T., Suzuki H., Kunitomo H., Schafer W. R., *et al.*, 2006 The insulin/PI 3-kinase pathway regulates salt chemotaxis learning in Caenorhabditis elegans. Neuron 51: 613–625.

Topalidou I., Chen P.-A., Cooper K., Watanabe S., Jorgensen E. M., *et al.*, 2017a The NCA-1 and NCA-2 Ion Channels Function Downstream of Gq and Rho To Regulate Locomotion in Caenorhabditis elegans. Genetics.

Topalidou I., Cooper K., Pereira L., Ailion M., 2017b Dopamine negatively modulates the NCA ion channels in C. elegans. PLoS Genet. 13: e1007032.

Ueda Y., Hirai S. i, Osada S. i, Suzuki A., Mizuno K., *et al.*, 1996 Protein kinase C activates the MEK-ERK pathway in a manner independent of Ras and dependent on Raf. J. Biol. Chem. 271: 23512–23519.

Uozumi T., Hirotsu T., Yoshida K., Yamada R., Suzuki A., *et al.*, 2012 Temporally-regulated quick activation and inactivation of Ras is important for olfactory behaviour. Sci. Rep. 2: 500.

Wedegaertner P. B., Wilson P. T., Bourne H. R., 1995 Lipid modifications of trimeric G proteins. J. Biol. Chem. 270: 503–506.

White J. G., Southgate E., Thomson J. N., Brenner S., 1976 The structure of the ventral nerve cord of Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 275: 327–348.

White J. G., Southgate E., Thomson J. N., Brenner S., 1986 The structure of the nervous system of the nematode Caenorhabditis elegans. Phil Trans R Soc Lond B 314: 1–340.

Wicks S. R., Rankin C. H., 1995 Integration of mechanosensory stimuli in Caenorhabditis elegans. J. Neurosci. Off. J. Soc. Neurosci. 15: 2434–2444.

Wicks S. R., Rankin C. H., 1996 The integration of antagonistic reflexes revealed by laser ablation of identified neurons determines habituation kinetics of the Caenorhabditis elegans tap withdrawal response. J. Comp. Physiol. [A] 179: 675–685.

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

Wittinghofer A., Vetter I. R., 2011 Structure-function relationships of the G domain, a canonical switch motif. Annu. Rev. Biochem. 80: 943–971.

Wittinghofer A. (Ed.), 2014 *Ras Superfamily Small G Proteins: Biology and Mechanisms 1: General Features, Signaling*. Springer-Verlag, Wien.

Yoder J. H., Chong H., Guan K.-L., Han M., 2004 Modulation of KSR activity in Caenorhabditis elegans by Zn ions, PAR-1 kinase and PP2A phosphatase. EMBO J. 23: 111–119.

You Y., Kim J., Cobb M., Avery L., 2006 Starvation activates MAP kinase through the muscarinic acetylcholine pathway in Caenorhabditis elegans pharynx. Cell Metab. 3: 237–245.

Zallen J. A., Peckol E. L., Tobin D. M., Bargmann C. I., 2000 Neuronal cell shape and neurite initiation are regulated by the Ndr kinase SAX-1, a member of the Orb6/COT-1/warts serine/threonine kinase family. Mol. Biol. Cell 11: 3177–3190.

Zhang H., Koo C. Y., Stebbing J., Giamas G., 2013 The dual function of KSR1: a pseudokinase and beyond. Biochem. Soc. Trans. 41: 1078–1082.

Zhang L., Ward J. D., Cheng Z., Dernburg A. F., 2015 The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in C. elegans. Dev. Camb. Engl. 142: 4374–4384.

Zhen M., Samuel A. D., 2015 C. elegans locomotion: small circuits, complex functions. Curr. Opin. Neurobiol. 33: 117–126.

Zhong J., Li X., McNamee C., Chen A. P., Baccarini M., *et al.*, 2007 Raf kinase signaling functions in sensory neuron differentiation and axon growth in vivo. Nat. Neurosci. 10: 598–607.

Zhong J., 2016 RAS and downstream RAF-MEK and PI3K-AKT signaling in neuronal development, function and dysfunction. Biol. Chem. 397: 215.