UNIVERSITY OF CALIFORNIA SAN DIEGO

Investigating roles of the protein translation initiation factor EIF-3.G in regulating neuronal circuit activity in *Caenorhabditis elegans*.

A Thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Biology

by

Yan Zhao

Committee in charge:

Professor Yishi Jin, Chair
Professor Susan Ackerman
Professor Gulcin Pekkurnaz

2020

The Thesis of Yan Zhao is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

_____

_____

_____

Chair

University of California San Diego

2020

DEDICATION

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to acknowledge all members of Jin & Chisholm lab for supporting me throughout my program. I would be utterly lost without their willingness to help.

Data involving the *eif-3.g(ju807)* and *acr-2(gf)* animals were provided with permission by Dr. Stephen Blazie. The fluorescent report of *ins-29* was provided with permission by Dr. Katherine McCulloch. The thesis author was the primary author of this material.

ABSTRACT OF THE THESIS

Investigating roles of the protein translation initiation factor EIF-3.G in regulating neuronal circuit activity in *Caenorhabditis elegans*.

by

Yan Zhao

Master of Science in Biology

University of California San Diego, 2020

Professor Yishi Jin, Chair

Regulation of neural circuit activity is essential for cognitive ability and behavioral activities, as aberrant circuit function is a hallmark of disorders, including Autism Spectrum Disorders and epilepsy. This study uses a unique *C. elegans* model of circuit hyperactivity to dissect molecular mechanisms contributing to neural circuit function. A gain-of-function mutation in a nicotinic cholinergic subunit, *acr-2(gf)*

functions in acetylcholine motor neurons and results in a disrupted neural circuit by hyperactivating the acetylcholine excitatory motor neurons. The *acr-2(gf)* mutant animals show impaired locomotion with stochastic muscle convulsions. From previous genetic screening, a functional linkage has been found between neuronal activity and a missense mutation within the conserved translation initiation factor *eif-3.g*. The aim of my master thesis is to identify genes affecting motor neural circuit function through *eif-3.g* and *acr-2(gf)*. Specifically, I have analyzed mutations isolated from a genetic suppressor screen for genes that restored convulsion rate in *eif-3.g(gf); acr-2(gf)* animals. I identified one *eif-3.g* suppressor as protein translation repressor *lin-66*, which encodes for a novel protein that promotes tissue differentiation. We speculate that the *lin-66* gene is one of the targets of, or participates in, *eif-3.g* function in motor neurons. Our studies provide further insights into the regulation of motor neurons by translational initiation factors.

Introduction

Regulated neural circuit is required for functional phenotypes.

A neural circuit is composed of neurons connected via synapses, serving as an organized functional unit for relaying synaptic information. The human nervous system contains multiple, interconnected circuits that are required for distinct behaviors. For instance, a neural circuit located within the entorhinal cortex and hippocampus region of the brain (the entorhinal-hippocampal circuit), is involved in encoding spatial memory (Tonegawa and McHugh, 2008). Another type of neural circuit involves motor neurons and one example is the knee-jerk response. This response is categorized as muscle stretch reflex, which happens by conducting neural signaling to or from muscle neural cells (Zimmerman and Hubbard, 2018). Functional activities and phenotypes of neural circuits requires balanced excitatory and inhibitory neurotransmission.

Disruption in excitatory and inhibitory signaling results in imbalanced neural circuit, and often, neurological disorders. Autism Spectrum Disorder (ASD), with manifestation on impaired social interaction, learning disability, and repetitive movements, is one possible consequence of circuit imbalance. Some patients with ASD have been diagnosed with increased neocortical excitatory/inhibitory ratio caused by the malfunctions of parvalbumin inhibitory transmission. These results suggest that excitatory/inhibitory imbalance could contribute to the development of ASD. (Wei *et al*., 2012; Lee *et al*., 2017). In addition, patients with epilepsy have shown similar impairment in brain with abnormal excitatory and inhibitory neurons (Fisher *et al*., 2005). Neural circuit imbalances have been implicated in other neurological diseases, such as

Huntington's Disease and Down Syndrome (Kojima and Shirao, 2007). In my project, my major goal was to identify and characterize new players involved in regulating excitatory/inhibitory (E/I) circuits. These studies will aid in understanding the relationship between E/I circuits and pathology of E/I imbalance related disease.

Balanced neurotransmitter release is essential for regulated neural circuit activity.

The process of synaptic neurotransmission in sending and receiving information requires critical messengers known as neurotransmitters, which are released from the presynaptic axon terminal of one neuron that then interact with dendrite receptors of the postsynaptic neuron (Regis *et al*., 1993). Multiple neurotransmitters have been identified which can be either excitatory or inhibitory in action. Excitatory neurotransmitters generate an activating effect to carry out downstream neuron networking and muscle contraction. In contrast, inhibitory neurotransmitters inhibit information sending.

Acetylcholine is a commonly known excitatory neurotransmitter and modulates signaling activities in brain and muscle. In central nervous system, acetylcholine is released from forebrain neurons to hippocampus for regulating cognitive ability and supporting memory and learning. Deficits in acetylcholine transmission caused by decreased acetylcholine receptor expression leads to dysfunctional circuit, which has been associated with Alzheimer's Disease (Levey, 1996). In the peripheral nervous system, acetylcholine is released from the brain to motor neurons for carrying out muscle response and contraction. To activate skeletal muscles, motor neurons connect with muscle fibers forming a synapse called a neuromuscular junction (NMJ) (Drachman *et al*. 1980). Acetylcholine is released at NMJs from motor neurons to muscle fibers,

generating action potential to produce muscle contraction. Abnormal NMJ activity has been associated with myasthenia gravis disease. In the myasthenic patients, acetylcholine receptor expression is downregulated, leading inhibition of acetylcholine transmission. Therefore, myasthenic patients are often characterized with muscle weakness and facial paralysis (Drachman *et al*. 1980). The diseases caused by acetylcholine receptors implicate its role and importance in neural circuit. The regulatory mechanisms of maintaining balanced neuronal circuit remain obscure. My project focused on the translational regulation of neuronal circuit, which would implicate novel genes participating in genetic regulatory pathway and provide fundamental study of investigating regulation between translational factors and motor neurons.

***C. elegans acr-2(gf)* mutation disrupts neural circuit.**

C. elegans was established almost forty years ago as a model for understanding neuronal function (Brenner, 1974). Many of the genes involved in synaptic function and neurotransmission are conserved from *C. elegans* to humans, providing basic model circuit for investigation. In *C. elegans*, cholinergic neurotransmission participates in multiple events, such as locomotion and feeding (Rand, *Wormbook*). The proper function of the motor circuit requires balanced excitatory/inhibitory neurotransmission and appropriate muscle coordination that patterns sinusoidal movement. Acetylcholine transmission could be facilitated by two types of receptors, which are nicotinic and muscarinic receptors. Nicotinic receptors are ion channel-dependent whereas muscarinic receptor involves G-protein-coupled signaling (Albuquerque *et al*., 2009). In the motor circuit, the nicotinic receptor consists of five subunits regulating presynaptic

neurotransmitter release (Albuquerque *et al*., 2009). The composition and assembly of acetylcholine receptor subunits are critical for receptor function (Jospin *et al*., 2009). Disruption in any subunit causes unfunctional acetylcholine receptor leading to uncoordinated muscle movement. One missense gain-of-function mutation in acetylcholine receptor subunit 2 (*acr-2*) causes permanent channel opening, allowing acetylcholine to diffuse through the ion channel consistently and eventually resulting in imbalanced neural circuit, characterized by increased excitatory acetylcholine transmission while showing a spontaneous shrinking of "convulsion" phenotype in *C. elegans* (Jospin *et al*., 2009). This imbalance is characterized by increased excitatory acetylcholine transmission as well as a cell non-autonomous decrease in inhibitory transmission. Previous studies have used convulsion rate to assay circuit activity and identify other genes that modulate E/I imbalance in the motor circuit.

A gain-of-function mutation in *eif-3.g* reduces convulsion of *acr-2(gf).*

Previous genetic suppressor screens of *acr-2(gf)*- induced neural circuit hyperactivation identified a single nucleotide polymorphism (SNP) mutation in *eif-3.g*, assigned with allele number *ju807* (McCulloch *et al*., 2017). Worms with *eif-3.g(ju807); acr-2(gf)* exhibit with significantly lower convulsion rates than *acr-2(gf)* alone. The *eif.3g* is orthologous to the eukaryotic EIF-3.g gene, which is one of 13 subunits of eukaryotic initiation factor complex 3 (EIF-3) (Asano *et al*., 2002; Hinnebusch, 2006). EIF-3 is known to interact with 40S ribosomal subunits to form complexes for recruiting the initiator complex to 5' Untranslated Regions to initiate translation. The newly formed complex binds to mRNA through RNA binding domain and scans for the start codon to

initiate translation (Asano *et al*., 2002; Hinnebusch, 2006). Knockdown of *eif-3.g* using RNAi causes larval arrest during L1 stage in *C. elegans*, suggesting that this subunit is essential for maintaining viability and protein translation machinery (Kamath et al, 2003).

In addition to the phenotypical observation of *eif-3.g(ju807)* suppresses convulsions induced by *acr-2(gf)*, a corresponding transcriptional expression level change was also observed using fluorescent transgene *ins-29* (*juEx7742*). This transgene was driven by the *ins-29* promoter to express GFP as a transcriptional reporter (McCulloch *et al.,* 2019). In the wildtype animals, the *ins-29* expression was weakly observed in head neurons. However, the *ins-29* expression showed clearly and consistently increased expression level in the same head neurons under the neuronal circuit overexcitation of *acr-2(gf)* animals. In the *eif-3.g(ju807); acr-2(gf); Pins-29::GFP* strain, the *ins*-29 expression decreased with the presence of *eif-3.g(ju807)*. The observation of *ins-29* expression change suggests that *eif-3.g(ju807)* suppress *acr-2(gf)* induced expression, indicating *eif-3.g(ju807)* is able to suppress overexcitation of motor neuronal circuit. However, the function of *eif-3*.g and how it could regulate motor circuit activity in the context of *acr-2* remains obscure.

The fluorescent report of *ins-29* was provided with permission by Dr. Katherine McCulloch. The thesis author was the primary author of this material.

Further investigation on novel genes affecting motor neural circuit function through *eif-3.g* and *acr-2(gf)*.

In order to study the interaction between *eif-3.g* and *acr-2(gf)* in motor neural circuit, a forward genetic screen was performed to identify the novels genes that affect motor neural circuit function through *eif-3.g* (S. Blazie, unpublished). The phenotypical suppression represents the disruption in particular genes contributing to the phenotype. The gene *eif-3*.g suppresses convulsions caused by *acr-2(gf)* in motor neurons. Once suppressor mutants of *eif-3.g* are present, *eif-3.g* lose ability of suppressing *acr-2(gf)* because suppressor mutants might disrupt the function of genes located at upstream or downstream of *eif-3.g*'s genetic signaling pathway. In this project, my goal is to use *C. elegans* genetics to identify and study these novel mutants that suppress the *eif-3.g* effect on motor circuit activity. Mapping of suppressors of *eif-3.g* will potentially help me to locate genes affecting motor neuron circuit through *acr-2(gf)* and *eif-3.g* and begin to understand how these genes affect motor neuron function. Once we revealed the interaction within motor neuron circuit, our study could provide fundamental mechanism for future for future study on translational regulation in motor neuron.

Material and Methods.

Construction of Recombinant Strains:

All worm strains were maintained on NGM plates with *E. coli* OP 50 and grew at 20˚C (Brenner, 1974). Recombinant strains containing *eif-3.g(ju807)(*II*); acr-2(n2420gf)(*X*); ju1661* were generated from CZ26711[*eif-3.g(ju807)(*II*); acr-2(n2420gf)(*X*); ju1661*] crossing with wildtype N2 males. In the F1 generation, heterozygous animals had an indistinguishable phenotype compared to wildtype. In the F2 generation, convulsing worms were selected indicating homozygous *acr-2(n2420gf)*(X) allele was present. In the F3 generation, non-convulsing worms were selected to isolate homozygous *eif-3.g(ju807)(*II*)*; *acr-2(n2420gf)(*X*)*. In the F4 generation, convulsing worms were selected indicating the presence of homozygous *ju1661*. Both *ju807* and *ju1661* alleles were verified through sequencing using primers/phenotype listed in the table below.

Convulsion Assay:

To assay convulsion, L4 worms (n=15) were prepared onto fresh plate with OP50. At the next day, convulsion rate was quantified in young adult. Each worm was quantified separately within 90 seconds and then normalized to 60 seconds scale.

Rescue Experiment and Cloning:

Plasmids used to rescue *lin-66* contained pCR8 (isolated with 2555bp) as vector backbone. Inserts contained the wildtype genomic *lin-66* fragments, comprising 2000bp

promoter region upstream from *lin-66* and 4148 bp fragment consisting of coding sequences and 3' untranslated region, generating total of 6148 bp genomic *lin-66* fragments (assigned as *juEx8032* and *juEX8033*). Vector and inserts were amplified using Phusion High-Fidelity DNA Polymerase. Cloning primers are included in Table 1. Constructed recombinant plasmid was ligated using Gibson Assembly.

Plasmids used for rescue *eat-9* contained pCR8 (isolated with 2555bp) as vector backbone. Inserts contained the wildtype genomic *eat-9* fragments, comprising 1971bp promoter region upstream from *eat-9* and 2313 bp fragment consisting of coding sequences and 3' untranslated region, generating total of 6148 bp genomic *eat-9* fragments. Vector and inserts were amplified using Phusion High-Fidelity DNA Polymerase. Cloning primers are included in Table 1. Constructed recombinant plasmid was ligated using Gibson Assembly.

Molecular Cloning:

RNA from CZ26711 strain was extracted using TRIzol reagents. Worm lysate was mixed with 3 volume of TRIzol solution and then centrifuged at room temperature for 5 minutes. Chloroform was added and mixed via gentle inverting. Mixed solution was centrifuged at 4˚C for 15 minutes. The top aqueous layer was transferred out and treated with GlycoBlue and Isopropanol. After an hour incubation at -20˚C and centrifuge, the pellet was kept and washed with 75% ethanol. The RNA pellet was then resuspended with dI RNAse-free water. RNA was set up with 50 μM polydT and 10 mM dNTPs first, then incubated at 65˚C for 5 minutes. Reverse-transcription of RNA was performed with the Invitrogen SuperScript III kit Reactions were performed essentially as described in

the manufacturer protocol, with polydT and 10 mM dNTPs first. For performing the TA cloning, the insert fragment was amplified using DreamTaq polymerase were mixed with salt solution and TOPO vector. After incubation at room temperature for 10 minutes, ligated plasmids containing insert and TOPO vector were transform into DH5alphs cells and grew on the LB plates with Spectinomycin antibiotics.

Statistical Analysis:

All statistical analyses were performed using one-way ANOVA with Bonferroni test for multiple comparisons in GraphPad Prism 7.0. All error bars were generated based on the standard error of the mean.

Results

Identification of two suppressors of *eif-3.g(ju807); acr-2(gf)* animals.

In order to investigate the regulatory role of *C. elegans eif-3.g* on *acr-2 (gf)-induced* in neural circuit imbalance, Dr. Steve Blazie carried out a genetic screen to search for the potential genes interacting with *eif-3.g*. Briefly, the *eif-3.g(ju807);acr-2(gf)* strain was treated with the chemical mutagen EMS for generating random mutations in its genomic DNA through guanine alkylation of nucleotide (Flibotte *et al.,* 2010). Disruption in *eif-3.g* genetic pathway interdicts *eif-3.g* suppression on *acr-2(gf),* therefore the *eif-3.g(ju807); acr-2(gf); mut* presents convulsion phenotype. Based on the phenotype, novel mutant strains presenting "convulsion" were selected. Dr. Steve Blazie also prepared and obtained whole genome sequences from new mutant strains. His analysis generated a list of candidate SNPs, which guided me to design genetic mapping strategies to identify the suppressors.

Below, I describe my work in identifying and analyzing two genetic suppressors. *acr-2(gf)* animals exhibit impaired locomotion and stochastic convulsions whereas the double mutant *eif-3.g(ju807)*; *acr-2(gf)* showed significantly suppressed convulsion rate (Fig.1). The newly generated suppressors prohibit *eif-3.g*'s regulatory pathway, leading to restoration of *acr-2 (gf)* convulsion phenotype. The suppression on *eif-3.g* could either be direct or indirect regulation. A total of seven suppressor mutants were identified that restored convulsion (Fig. 1). In my project, I started with three suppressors *ju1491, ju1661,* and *ju1608*. In order to map the casuative allele of each suppressors, the original suppressor strains was outcrossed with wildtype to generate recombinants. During the process of generating recombinants for *ju1491,* the progeny of all recombinants showed

diluted convulsion phenotype through multiple generation, suggesting that the originally observed suppression effects of *ju1491* might not be due to single genetic locus. Then, the suppressors *ju1608* and *ju1661*were selected for further study and genetic mapping due to their consistent convulsion rate.

The suppressor *ju1608* is linked to the genetic position of *eat-9*.

For mapping the candidate gene for suppressor *ju1608*, I generated multiple recombinants containing *ju1608* by outcrossing parental *acr-2(gf); eif-3.g(ju807);ju1608* strain with wildtype N2 strain. In order to reveal the identity of suppressor *ju1608*, I used *ju1608* recombinants to find a common mutated gene which could be linked to suppressor *ju1608*. Based on whole genome sequencing data, single nucleotide polymorphisms (SNP) in coding regions were first considered as candidates. The *ju1608* recombinants were examined through restriction enzyme mapping and I found that a single base (C to T) nonsense mutation located within *eat-9* (also referred as *F09C3.2*) on Chromosome I (+24.2) could be a possible candidate for suppressor *ju1608* because all recombinants contained this same mutation with zero segregation, which indicated the linkage with suppressor mutant *ju1608*.

The gene *eat-9* has been previously shown to modulate pharyngeal muscle activity. EAT-9 is required for motor neuron excitation located within pharynx to maintain normal pharyngeal pumping behavior and rhythm (Raizen *et al*., 1995). Mutation within *eat-9* coding region is likely to lead to mRNA mediated degradation og the transcript and disrupt the open reading frame, causing rhythmic pumping with irregular pumping rate. In order to verify *ju1608* is the causative allele of *eat-9,* one

nonsense mutation *eat-9*(*e2337*) was used to replace *ju1608* to see if *e2337* could phenocopy *ju1608*. The triple mutant *eat-9(e2337); eif-3.g(ju807); acr-2(gf)* showed similar convulsion rate as *eif-3.g(ju807); acr-2(gf); ju1608,* indicating that disrupting *eat-9* could eventually restore convulsion phenotype. This data confirm that *ju1608* is genetically linked to *eat-9* (Fig. 2). In addition, rescue experiment was also conducted to confirm the linkage between *ju1608* and *eat-9*. The molecular construct contains 2555bp of vector backbone, 1971bp upstream genomic promoter region, and 2313bp of genomic CDS with 3'UTR (Fig. 3). The mCherry was injected with rescue construct into *eif-3.g(ju807); acr-2(gf); ju1608* strain as co-injection marker, proving the presence of rescue transgene. After the injection, the *eif-3.g(ju807); acr-2(gf); ju1608* worms showed comparable convulsion rate as *acr-2(gf)* strain, suggesting that *ju1608* is only link to the genetic position of *eat-9* or the rescue construct was not appropriately prepared (Fig. 4).

The suppressor *ju1661* is an allele of *lin-66*.

For mapping the candidate gene for suppressor *ju1661*, I generated multiple *acr-2(gf); eif-3.g(ju807);ju1661* recombinant strains. Similar to mapping suppressor *ju1608*, I examined the single nucleotide polymorphisms (SNP) in coding regions first because these SNPs are more likely to cause amino acid change, which further lead to change in gene function. The *ju1661* recombinants were examined through restriction enzyme digest and I found that a single base (G to A) mutation located within *lin-66* on Chromosome IV (+10.9) could be a possible candidate because all recombinants contained this same mutation with zero segregation. This indicates linkage between *ju1661* and *lin-66*. The *lin-66* encodes a nematode-specific protein that had previously be

shown to regulate developmental timing through regulation of target mRNA stability in C. elegans (Morita and Han 2006).

In order to verify *ju1661* is the causative allele of *lin-66,* the rescue experiment was conducted containing 2555bp of vector backbone, 2000bp upstream genomic promoter region, and 4148bp of genomic CDS with 3'UTR (Fig. 6). The mCherry was injected with rescue construct into *eif-3.g(ju807); acr-2(gf); ju1661* strain as co-injection marker, proving the presence of rescue transgene. If *ju1661* is mutation in gene *lin-66,* the wildtype transgene would cause a reduction of convulsion rate in *acr-2(gf); eif-3.g(ju807);ju1661* strain. The rescued mutants will decrease convulsion rate because releasing repression on *eif-3.g* enables *eif-3.g* suppression on *acr-2(gf)*. The strain *eif-3.g(ju807);acr-2(gf);ju1661* injected with wildtype copies showed significant reduced convulsion rate, suggesting that *ju1661* is a mutation in gene *lin-66* (Fig. 5).

The *lin-66(ju1661)* mutation disrupts *lin-66* splicing.

I next investigated the nature of the *lin-66 (ju1661)* mutation on *lin-66* expression and function. In order to study the mutation effect on gene expression, knowing the location of mutation is critical because the position determines alteration of gene function. In gene translation, accurate splicing is essential for gene function and is controlled by exon-intron junctions including donor and acceptor sites (Burge *et al*., 1999). Disrupting exon-intron junction potentially leads to abnormal alternative splicing, exon exclusion, and intron retained as coding sequence. Aberrant intron splicing events manipulate gene transcript, causing non-sense mediated decay and extensive alternative splicing (Scotti and Swanson, 2016). Our *lin-66* (*ju1661*) mutation is located at 3' end of

an acceptor splice site and was predicted to disrupt correct splicing leading to a premature stop codon before fifth exon and eventually attenuate functional protein synthesis (Fig. 6).

Through RT-PCR and Sanger sequencing analysis, four alternative splicing events between exon 2 and exon 3 were identified in *acr-2(gf); eif-3.g(ju807); ju1661* strain (Fig. 7). These alternative splicing events consist of wildtype splicing, full intron retention, and partial intron retention, which allows certain mRNA transcript still remained in frame. Since both in frame and frameshift were observed, it lead to my next hypothesis that *lin-66 (ju1661)* could serve as loss of function mutation because the frameshift would cause premature stop codon to appear, leading these short transcripts to be degraded through mRNA mediated degradation.

The *lin-66(ju1661)* is a partial loss of function.

Previous studies on *lin-66* isolated a null mutation (*ku423*) at 5' end donor splice site inducing nonsense mutation within first intron and cause L4 (late larval stage) arrest (Morita and Han 2006). The *lin-66* is a heterochronic gene that negatively regulates gene expression at early larval development (Morita and Han 2006). Therefore, mutations in *lin-66* are predicted to contribute to impairment in developing cell lineages. Since both previous identified *lin-66* mutation (*ku423*) and our *lin-66* (*ju1661*) mutation involved in disrupting intron splicing sites, we expected to observe similar consequence and phenotype.

In order to study the *lin-66* (*ju1661*) mutations, we generated a strain that only contained *lin-66* (*ju1661*) mutation in the absence of *eif-3.g(ju807);acr-2(gf)*. This strain

showed egg laying defective phenotype but the L4 stage arrest was not observed (Fig.8). This contradictory observation between *lin-66 (ku423)* and *lin-66* (*ju1661*) suggests an alternative hypothesis that mutation site could be a partial loss-of-function, retaining some protein function. I used *lin-66 (ku423)* to study the property of *ju1661* by generating trans-heterozygotes. *ku423* was originally maintained with balancer nT1(Unc) to avoid lethality. In my observation, I found that the animals with *ku423* from viable homozygous *ku423* parent showed higher rate of L4 lethality than the *ku423* animals from the heterozygous balancer strain, which could be explained by the rescue of maternal effect. The heterozygous strain *ju1661/ku423* was observed with a combination of both Egl (egg laying defect) and L4 lethality, indicating that *ju1661* is a partial loss of function mutation that tolerates null allele. Combining the transcript identification from cDNA and the phenotypical observation of *ku423*/*ju1661*, *ju1661* could be concluded as partial loss-of-function in consistent with the presence of in frame transcripts producing some functional protein. Our study has revealed *lin-66* as one player involving in regulatory pathway of *eif-3.g* and *acr-2 (gf)*.

Discussion

The suppressor *ju1608* is linked to *eat-9*.

The SNP mapping data among *ju1608* recombinants showed that the suppressor *ju1608* is genetically linked to *eat-9* on Chromosome I (+24.2). By replacing *ju1608* with one nonsense mutation of *eat-9(e2337)*, the *eat-9(e2337); eif-3.g(ju807); acr-2(gf)* strain was able to restore the convulsion rate similar to *ju1608*, providing the evidence for proving *ju1608* is genetically linked to *eat-9* locus. Although positional mapping and genetic analyses suggested that ju1608 was linked to a mutation in eat-9 on Chromosome I, I was unable to rescue suppression by transgenic expression of wild type eat-9. Further work involving testing of eat-9 alleles and other rescue experiments are needed to verify the identity of ju1608. For example, the promoter used for rescue included 2kb upstream of eat-9, which may not be sufficient for expression. Little is known about eat-9 structure or function. However, eat-9 mutants are defective in pharyngeal pumping, a circuit that involves cholinergic signaling

The suppressor *ju1661* is a partial loss-of-function mutation in *lin-66*.

In this study, I have identified the suppressor *ju1661* is the causative allele of *lin-66* on Chromosome IV (+10.9), which was verified through rescue experiment. The *ju1661* mutation at 3' acceptor splicing site could potentially activate multiple cryptic sites within one intron or multiple introns, leading to various of alternative splicing events. In the transcript identification of cDNA, both wildtype and in frame splicing were observed in *ju1661* animals, indicating that some functional products of LIN-66 still

exist. The phenotypical quantification of *ku423/ju1661* revealed that *ju1661* behaves differently from the null allele *ku423* of *lin-66*. The null allele has distinguished phenotype of L4 lethality whereas the *lin-66 (ju1661)* only has egg laying defective phenotype. Taken together, our data shows that *ju1661* is a partial loss-of-function mutation in *lin-66* because it tolerates the L4 lethality in null allele by consistently producing a fraction of functional protein.

Regulatory interaction between *lin-66, eif-3.g,* and *acr-2.*

Since both *lin-66* and *eif-3.g* are involved with translational regulation, further investigation of translational level change in this pathway could help us to understand how does the translational pathway participates in regulating motor neuron. In the future, we can test the LIN-66 protein level change in the *eif-3.g (ju807)* background to determine whether *eif-3.g* regulates *lin-66* translation. On the other hand, we can also test the EIF-3.G protein level change in the *lin-66 (ju1661)* background to determine whether *lin-66* regulates *eif-3.g*. If the expression level change is detected, it would suggest that the *lin-66* and *eif-3.g* directly regulate each other and participate in a linear pathway. It is also possible that either LIN-66 or EIF-3.G expression would change, suggesting that *lin-66* and *eif-3.g* might work individually in a parallel pathway to regulate hyperactivation of motor circuit. Another possibility is that LIN-66 and EIF-3.G interact together to carry out further downstream translational regulation.

LIN-66 has been shown to be a translational suppressor, which negatively regulates the protein LIN-28 through its 3'UTR region (Morita and Han, 2006). LIN-28 regulates stage-specific development by inhibiting later cell fates from occurring too

early in development. . Since *lin-66* and *eif-3.g* showed genetic interaction and participate in translational regulation, one possibility is that they act on a shared target like lin-28. The interaction between *lin-28* and *eif-3.g* would implicate that *lin-28* is likely to be involved with regulating hyperactivation motor neuronal circuit.

Through genetic screening, we have identified multiple factors involved in translation, such as eif-3.g and lin-66, as important for regulating motor circuit function. Further study of these factors will potentially contribute valuable information about how the motor neural circuit is regulated under translational mechanism.

Data involving the *eif-3.g(ju807)* and *acr-2(gf)* animals were provided with permission by Dr. Stephen Blazie. The fluorescent report of *ins-29* was provided with permission by Dr. Katherine McCulloch. The thesis author was the primary author of this material.

Table 1. Primers used for genotyping

	Gene (allele)	Mutation	Analysis method	PCR Size	Primer name	Sequence (5' to 3')
Genotyping *n2420*	EIF-3.g(ju807)	G --> A	Sequencing	1698bp	eif3gout_fwd	GATTTCCAGCCGTGTTTTTGTTGTAG
					eif3gout_rev	GGACAGAAACCCAACTCGATTGG
					eif3gseq3_rev	CTTCACTTGCCAACTCTGATAACC
Genotyping *ju807*	acr-2(n2420)	G --> A	Sequencing	788bp	n2420_fwd	GGAATATGGGACGTGATTGGTAA
					n2420_rev	ATTATTTCTCTATTGACCGTGGTCC
					acr2_seqrev	GGTATCCTTTCTGGTCGTTTCATC
SNP genotyping primers for ju1661	**Gene (allele)**	**Mutation**	**Analysis method**	**PCR Size**	**Primer name**	**Sequence (5' to 3')**
	B0432.7	C --> T	Sequencing	1044bp	B04327_fwd	AATTCGTTCATCTTTCAGCAGTTGC
					B04327_seq	CCATTAGGTTACTCTCTCACCTGC
	F57F4-1	C --> T	Gel: Loss of BccI cut site in mutant (493bp); wildtype (129bp+364bp)	493bp	F57F41_fwd	TGGATGGAACTAAGAAGATTCGCG
					F57F41_rev	GTGTAAGTGACGAATGAATCCAAGTG
	K10D11.2	G --> A	Sequencing (with forward primer)	676bp	K10D11.2_fwd	AAGCAGTGGTTCTCAAAAACTGGC
					K10D11.2_rev	CCTTTGAAAGTAAAGTCAGCTTGTCCAC
	lin-66	G --> A	Sequencing	491bp	lin-66_fwd	CTTTGGGAACCACGCGGTC
					lin-66_rev	GGGACCATAGATTGGTGAGACAATG
					lin-66_seq	GGCTGAACAACAGAGAGCCG
	ptr-14	G --> A	Gel: Loss of MwoI cut site in mutant (781bp); wildtype (536bp+245bp)	781bp	ptr-14_fwd	CCTTGGCCATCACCTCTATTTGC
					ptr-14_rev	GGTAGGCAGATACGAAAGCCCA
	R04D3.2	C --> T	Gel: Loss of EarI cut site in mutant (1039bp); wildtype (297bp+742bp)	1039bp	R04D32_fwd	CATATGACGAACAATTCGCTTCCG
					R04D32_rev	CTGAGCTCGGAGGGACTGTC
	T07D10.1	G --> A	Sequencing	1028bp	T07D101_fwd	AACTGTATTTTCGTGGGTCTCACC
					T07D101_rev	CCTAAACCTACGCCTAATTCTAAGCC
					T07D101_seq	TCGTAGGTAAATGCAGATCCCCG
	Tln-1	G --> A	Sequencing	656bp	tln1_fwd	TGCAGGAGAACACGGTGAGC
					tln1_rev	GACTATCGGGATCGGCTCTCAC
					tln1_seq	GGGCAGGTAATGGCTGGTAAG
	Y105C5B.12	G --> A	Gel: Gain of Hpy188I cut site in mutant (246bp+345bp); wildtype (591bp)	591bp	Y105C5B.12_fwd	GATTTGACCAGTGACTTGGCAACC
					Y105C5B.12_rev	GATGTCGGAGCTGGAGATCAAGG
SNP genotyping primers for ju1608	**Gene (allele)**	**Mutation**	**Analysis method**	**PCR Size**	**Primer name**	**Sequence (5' to 3')**
	C54E4.5	C --> T	Gel: Loss of Fnu4HI cut site in mutant (662bp); wildtype (309bp+353bp)	662bp	C54E45_fwd	GCAAAATGAAGCTGAGGACGC
					C54E45_rev	CGAATTTGAATTGGGCTTGTGG
	C27D9.1	G --> A	Gel: Loss of Hpy188I cut site in mutant (557bp); wildtype (83bp+474bp)	557bp	C27D9.1_fwd	GAAGTAGGCAGGCAACTCAGG
					C27D9.1_rev	CCTTCCGCACAAGCCACAAATC
	daf-19	G --> C	Gel: Gain of Fnu4HI cut site in mutant (298bp+242bp); wildtype (540bp)	540bp	daf-19_fwd	GGTTGCCTAGGTGACAAGTACTTTACG
					daf-19_rev	CACCTGTACGGTCTCCACCTC
	daf-21	C --> T	Gel: Loss of BstEII cut site in mutant(578bp); wildtype (279bp+299bp)	578bp	daf-21_fwd	CCCTTACCATTATGGATACCGGAATCG
					daf-21_rev	CCAGATTGGCTTGGTCTTGTTAAGC
	eat-9	C --> T	Gel: Gain of EarI cut site in mutant(194bp+199bp); wildtype (393bp)	393bp	gk612856_fwd	GGTGCACACGGCAATCTTGAT
					gk612856_rev	CCTAAAATCTTGATGTGGCGTAGGC
	eat-20	C --> T	Gel: Loss of HaeIII cut site in mutant(1270bp); wildtype (490bp+780bp)	1270bp	eat20_snp_fwd	AGATGGAAACGAGTACGGAAGG
					eat20_snp_rev	GAAAAACATTACGCAACCCGTTGTG
	F09C3.2	G --> A	Gel: Loss of AccI cut site in mutant(521bp); wildtype (222bp+299bp)	521bp	F09C3.2_fwd	GTGCACAAATGGCTCAGTGC
					F09C3.2_rev	CACCTAGATTTCTGTTTCAGAGCAGC
	F57F4.1	C --> T	Gel: Loss of BccI cut site in mutant(493bp); wildtype (124bp+369bp)	493bp	F57F41_fwd	TGGATGGAACTAAGAAGATTCGCG
					F57F41_rev	GTGTAAGTGACGAATGAATCCAAGTG
	frpr-2	C --> T	Gel: Loss of HhaI cut site in mutant(844bp); wildtype (337bp+507bp)	844bp	frpr2_snp_fwd	GGACGATCCATTCTTCAAGTGC
					frpr2_snp_rev	CCGACGAAGCATTACAATGATCG
	K08D8.5	C --> T	Sequencing (with forward primer)	682bp	K08D8.5_fwd	CGGTAGCATCAAATAATCAGGTTGTCTG
					K08D8.5_rev	GGTTTAAACCTCAAAGATCAGGCTGC
	lem-4	C --> T	Gel: Loss of EcoRI cut site in mutant(563bp); wildtype (306bp+257bp)	563bp	lem4_fwd	CTCGAAAAAGTCTGGTTTTTTTGGG
					lem4_rev	CGTGGCATATTCCATGGAAGATATGC
	lgc-1	G --> A	Sequencing	473bp	lgc-1_fwd	CCAGGTTGAGGTCGAAGAGAAAGTC
					lgc-1_rev	CGCTCACACCACATTCATGGC
					lgc-1_seq	GGACAGACCAACGCTTGAAATGG
	npa-1	G --> A	Sequencing (with forward primer)	643bp	npa-1_fwd	CAATGGATGACCCCTGAACAACTG
					npa-1_rev	GTCTCCGGATGCAACATGTTCC
	sru-1	C --> T	Gel: Gain of Hpy188I cut site in mutant(146bp+389bp); wildtype (535bp)	535bp	sru1_fwd	TTACCTAACTCCGGGAAGCAGGC
					sru1_rev	CCTAACAATTGGCATGAAAAACGG
	T04C4.1	T --> C	Gel: Loss of HindIII cut site in mutant(1114bp); wildtype (353bp+761bp)	1114bp	T04C4.1_fwd	CAAAGCTCTCTCGGTGAGCC
					T04C4.1_rev	TGCGAGTCCGAAGAATCCGAC
	Y51H4A.25	G --> A	Gel: Loss of HindIII cut site in mutant(416bp); wildtype (203bp+213bp)	416bp	Y51H4A.25_fwd	CGGAGGAGCCAATGATGTGAATATTG
					Y51H4A.25_rev	TGGAACACTCGTCCGTAAAGTGTC
	ZC376.6	C --> T	Gel: Loss of AgeI cut site in mutant(1307bp); wildtype (551bp+756bp)	1307bp	ZC3766_fwd	AACTATATCCATACTTTGCACGCCG
					ZC3766_rev	ACTCCGAAAGCTCTTCGATTCG

Table 2. Primers used for cloning.

Molecular Cloning				
	Region	**PCR Size**	**Primer Name**	**Sequencing (5‘ to 3')**
lin-66 rescue construct primers	pCZGy3009_lin-66	2555bp	pCZGY3009_lin66_fwd	AATTTAACAAGTCATAGCTGTTTCCTGGC
			pCZGY3009_lin66_rev	GCTGAGAATTACTGGCCGTCGTTTTACAAC
	Promoter for lin-66	2014bp	Plin-66_fwd	GACGGCCAGTAATTCTCAGCTACAGTACC
			Plin-66_rev	GAGACTATTCATTTCGTAAGACATCCCG
	CDS & 3'UTR for lin-66	4791bp	lin-66_CDS_fwd	CTTACGAAATGAATAGTCTCTTCTCGTC
			lin-66_CDS_rev	CAGCTATGACTTGTTAAATTGGGATCCC
eat-9 rescue construct primers	pCZGy3009_eat-9	2555bp	pCZGY3009_fwd	TTTTTAGCTGGTCATAGCTGTTTCCTGGC
			pCZGY3009_rev	TCAATTGTAGACTGGCCGTCGTTTTACAAC
	Promoter for eat-9	1991bp	Peat-9_fwd	GACGGCCAGTCTACAATTGAAAAATATGTTACCAATAATG
			Peat-9_rev	GGGTCGTCATTGTTTTACCTGAAGAATTCG
	CDS & 3'UTR for eat-9	2365bp	eat-9_CDS_fwd	AGGTAAAACAATGACGACCCCTAACAAC
			eat-9_CDS_rev	CAGCTATGACCAGCTAAAAACTAAATTCGTACAC

Table 3. List of strains.

Strain number	Genotype	Notes (purpose and what data is used for)
CZ10402	*acr-2(n2420) X jmjd3.1(ju1201) X*	Used for mapping *ju1608* and testing the linkage between *frpr-2* and Chr. X .
CZ11819	*eif-3.g(ju807) II; acr-2(n2420) X*	
CZ21759	*eif-3.g(ju807) II; acr-2(n2420) X*	Used for convulsion rate test.
CZ22197	*eif-3.g(ju807) II*	
CZ24652	*Peif-3.g-GFP-loxp-EIF-3.G(C130Y)-loxp(juSi331) IV; acr-2(n2420) X*	
CZ24729	*Peif-3.g-GFP-loxp-EIF-3.G-loxp(juSi320) IV; acr-2(n2420) X*	
CZ25184	*eif-3.g(ju807) II; acr-2(n2420) X; ju1491*	
CZ25777	*Pins-29::GFP(juEX7741)*	
CZ25778	*Pins-29::GFP(juEX7742)*	
CZ25779	*Pins-29::GFP(juEX7743)*	
CZ25780	*acr-2 (n2420) X; Pins-29::GFP(juEx7741)*	
CZ25904	*eif-3.g(ju807) II; acr-2(n2420) X; ju1491*	2X cross from CZ25184
CZ26189	*eif-3.g(ju807) II; acr-2(n2420) X; ju1606*	
CZ26190	*eif-3.g(ju807) II; acr-2(n2420) X; ju1607*	
CZ26192	*eif-3.g(ju807) II; acr-2(n2420) X; ju1607*	1X cross from CZ26190
CZ26710	*eif-3.g(ju807) II; acr-2(n2420) X; ju1661*	
CZ26711	*eif-3.g(ju807) II; acr-2(n2420) X; ju1661*	1X cross from CZ26710; used for convulsion rate test
CZ26734	*eif-3.g(ju807) II; acr-2(n2420) X; ju1608*	
CZ26920	*acr-2(n2420) X; Pins-29::GFP(juEx7742)*	Used to observed GFP expression change in *acr-2(n2420)* background
CZ26921	*eif-3.g(ju807) II; acr-2(n2420) X; Pins-29::GFP(juEx7742)*	To use as marker for generating recombinants and mapping
CZ26922	*eif-3.g(ju807) II; Pins-29::GFP(juEx7742)*	
CZ26923	*eif-3.g(ju807) II; Pins-29::GFP(juEx7743)*	Used for observing GFP expression change in *eif-3.g(ju807)* background
CZ26924	*acr-2(n2420) X; Pins-29::GFP(juEx7743)*	Used for observing GFP expression change in *acr-2(n2420)* background
CZ26925	*eif-3.g(ju807) II; acr-2(n2420) X; Pins-29::GFP(juEx7743)*	To use as marker for generating recombinants and mapping
CZ27229	*eif-3.g(ju807) II; ju1661*	To test if *ju1661* had effect on *eif-3.g*
CZ27230	*ju1661 IV*	Isolated from CZ26711
CZ27386	*eat-9(gk612856) I*	1X cross from VC40338
CZ27387	*eat-9(e2337) I; eif-3.g(ju807)II; acr-2(n2420) X*	
DA563	*eat-9(e2337) I; him-8(e1489) IV*	Used for complementary cross to replace *ju1608* and for testing if *eat-9* is causative allele for *ju1608*
MH2285	*lin-66(ku423)/nT1(unc) IV*	Used for testing the property of *lin-66(ju1661)* mutation.
MT6241	*acr-2(n2420) X*	
MT6448	*lon-2(e678) X acr-2(n2420) X*	Used in testing ChrX linkage for *ju1608*
NH2246	*Pegl-17-GFP(ayls4) I; dpy-20(e1282ts) IV*	
VC145	*pes-7 (gk123) I*	
VC2554	*lin-66(ok3326)/nT1[qIs51]*	
VC40338	*eat-9(gk612856) I*	

Figures.

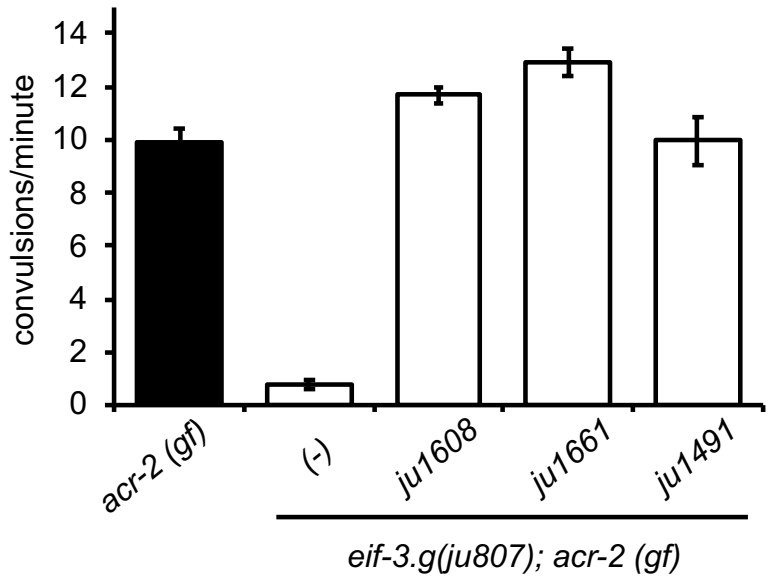

Figure 1. Mutants *ju1608, ju1661, ju1491* restore *eif-3.g(ju807); acr-2(gf)* *convulsion*. Shown are average convulsion rates of suppressed lines compared to acr-2(gf) and un-mutagenized eif-3g(ju807); acr-2(gf) controls. Each *ju1608, ju1661, ju1491* represents and independent suppressor of *eif-3.g (ju807).* Error bars represent standard deviation of mean. For each experimental group, n=15 worms.

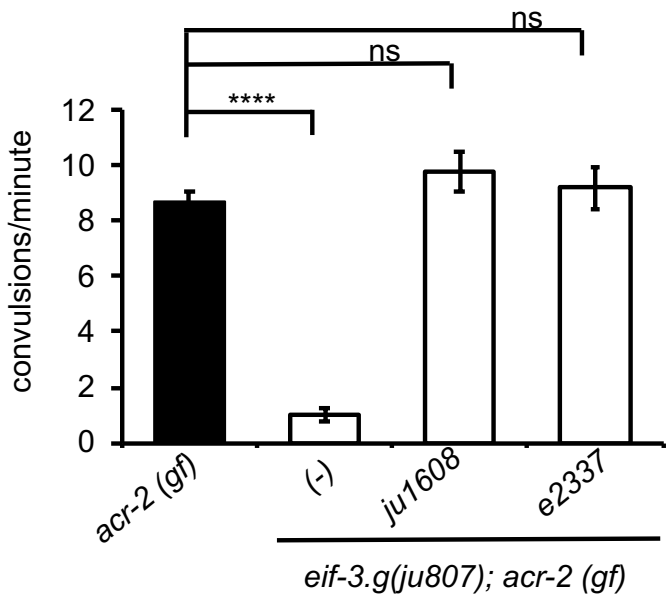

Figure 2. One nonsense mutation *e2337* in *eat-9* phenocopies *ju1608*. Triple mutant *eat-9(e2337); eif-3.g(ju807); acr-2(gf)* showed comparable convulsion rate to *eif-3.g(ju807); acr-2(gf); ju1608*. Convulsion rates were quantified within a minute for each strain by counting. Top and bottom bars represent standard deviation of mean. Statistical test was analyzed using ANOVA, followed by Bonferroni test. Middle bar represents the mean. For each experimental group, n=15 worms. P-value < 0.0001 (****).

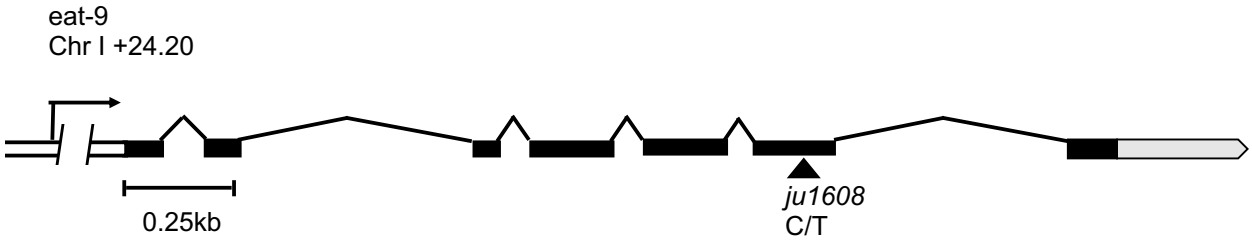

Figure 3. Suppressor *ju1608* was linked to gene *eat-9* located at +24.20 genetic position on Chromosome I of *C. elegans*. Figure above represents gene construct of *eat-9*, including 2kb upstream promoter region indicated by arrow. Nonsense mutation *ju1608* is located within exon 6.

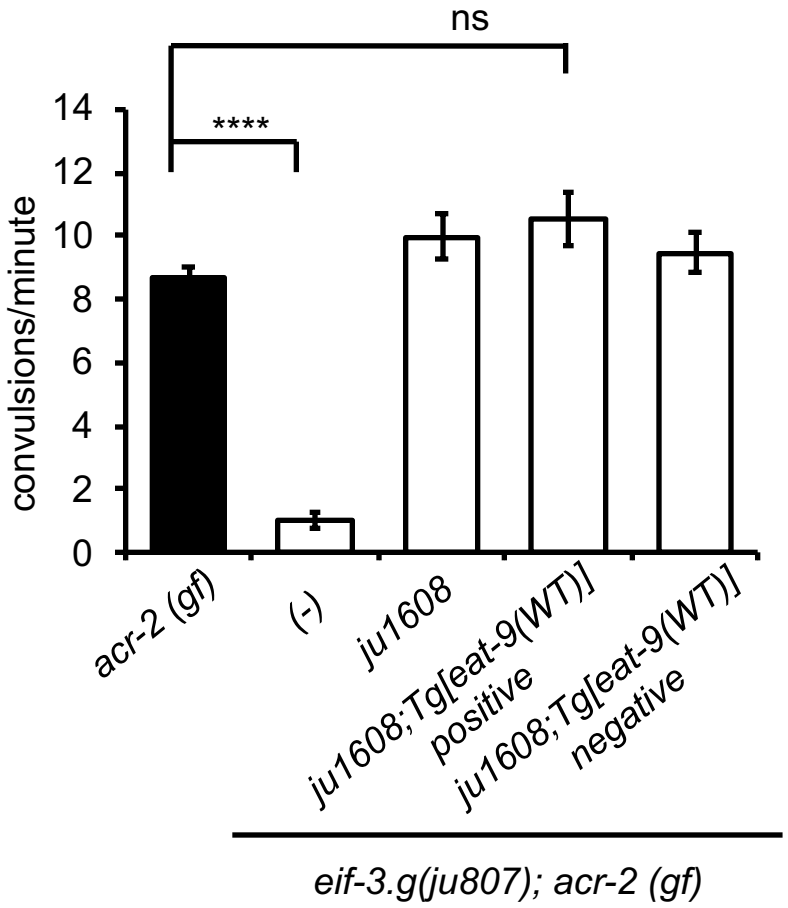

Figure 4. Wildtype *eat-9* construct could not sufficiently rescue *eif-3.g(ju807); acr-2(gf); ju1608*. Plasmids contain transgene (Tg) of wildtype copies of *eat-9* were introduced into strain with *acr-2(gf); eif-3.g(ju807); ju1608*. Convulsion rates were quantified within a minute for each strain by counting. Top and bottom bars represent standard deviation of mean. Statistical test was analyzed using ANOVA, followed by Bonferroni test. Middle bar represents the mean. For each experimental group, n=15 worms. P-value < 0.0001 (****).

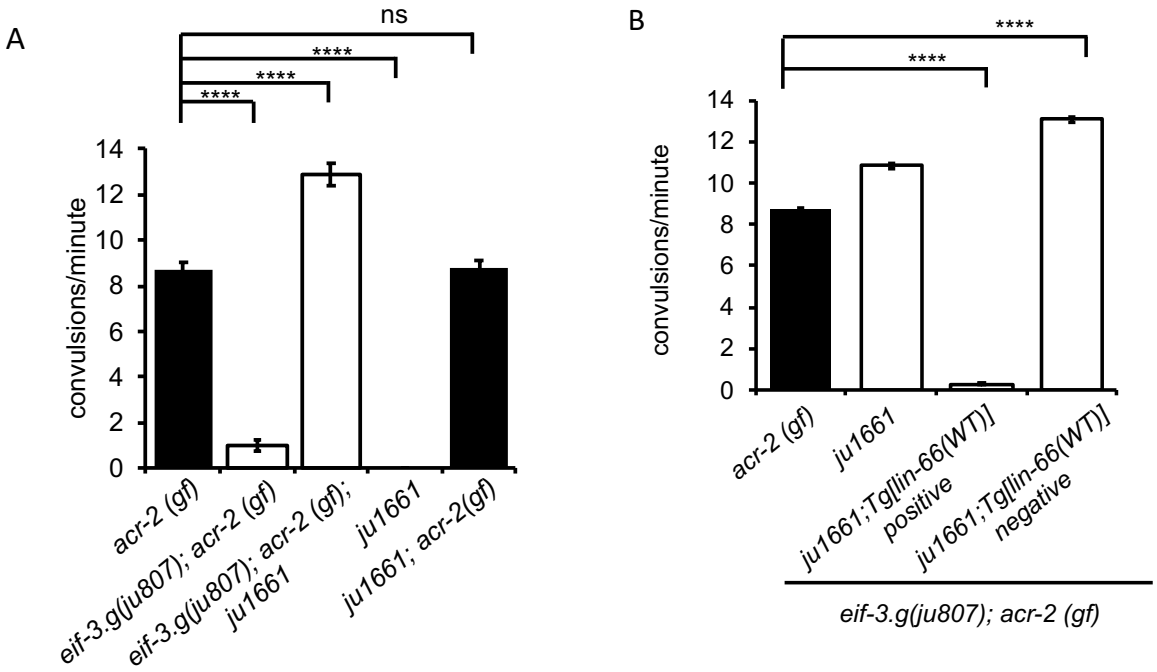

Figure 5. *ju1661* is one causative allele of *lin-66*. Plasmids contain transgene (Tg) of wildtype copies of *lin-66* were introduced into strain with *acr-2(gf); eif-3.g(ju807); ju1661*. Convulsion rates were quantified within a minute for each strain by counting. Top and bottom bars represent standard deviation of mean. Statistical test was analyzed using ANOVA, followed by Bonferroni test. Middle bar represents the mean. For each experimental group, n=15 worms. P-value < 0.0001 (****).

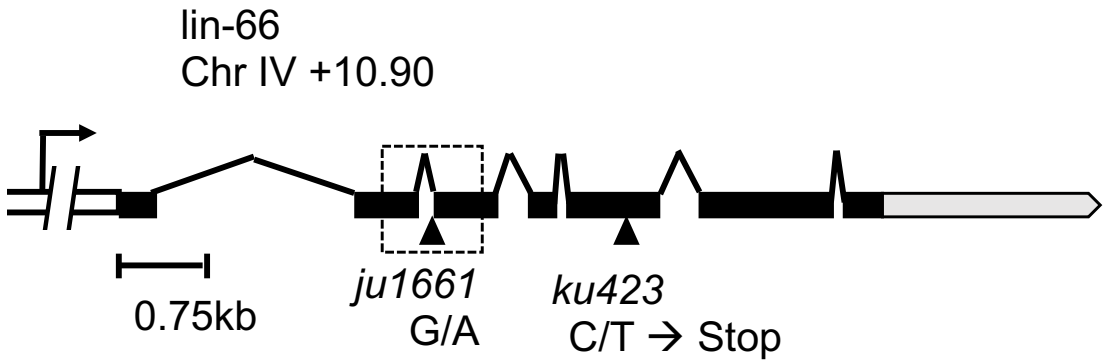

Figure 6. Suppressor *ju1661* was linked to gene *lin-66* located at +10.9 genetic position on Chromosome IV of *C. elegans*. Figure above represents gene construct of *lin-66*, including 2kb upstream promoter region indicated by arrow. Missense mutation *ju1661* is located at the 3'splicing site near exon 3 whereas null allele *ku423* is located within exon 5.

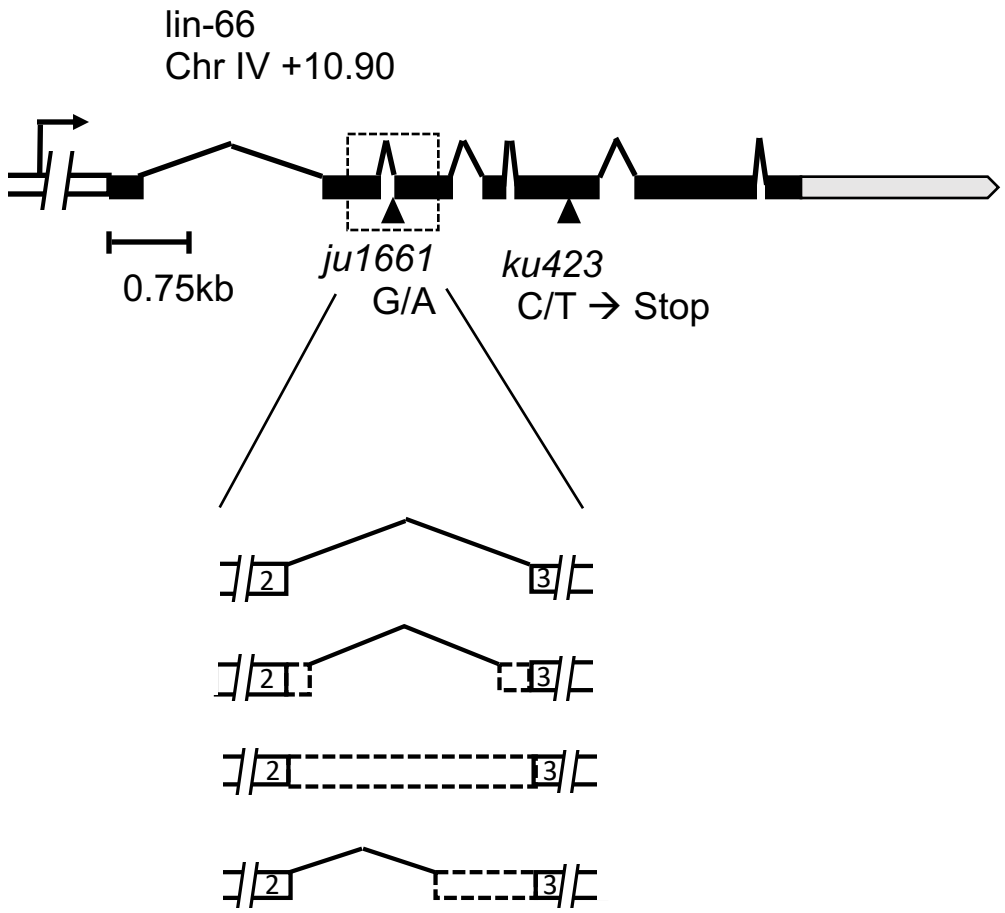

Figure 7. Four alternative splicing events were detected in *acr-2(gf); eif-3.g(ju807);ju1661* between exon 2 and exon 3. Alternative splicing events were detected after RT-PCR and sequencing analysis. Dash lines represent the retained introns. Continuous lines represent introns spliced out.

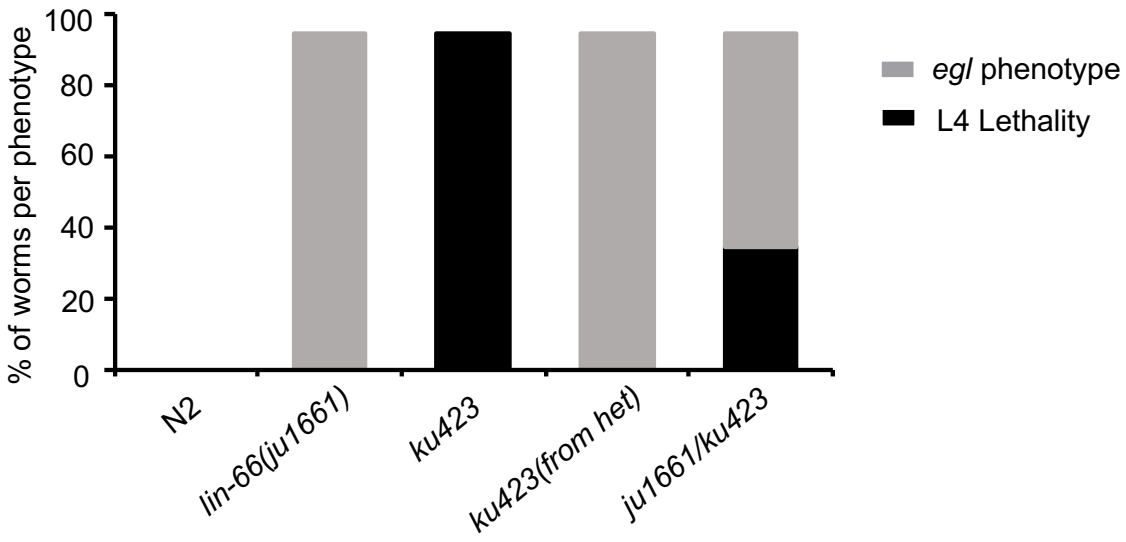

Figure 8. *lin-66(ju1661)* allele is partial loss-of-function. Each genotype group was quantified with the phenotype observation. The wildtype strain, N2, was used as control. The *ku423* strain was originally maintained with balance nT1(*unc*). The *ku423* (from homo) represents the progeny from viable homozygous *ku423* parent whereas the *ku423* (from het) represents the progeny form *ku423/nT1(unc)* balance strain. The heterozygous strain *ju1661/ku423* was observed with a combination of both *egl* (egg laying defect) and L4 lethality. n=30-58 worms.

Reference:

Albuquerque, Edson X., Edna F. R. Pereira, Manickavasagom Alkondon, and Scott W. Rogers. 2009. “Mammalian Nicotinic Acetylcholine Receptors: From Structure to Function.” *Physiological Reviews* 89 (1): 73–120. https://doi.org/10.1152/physrev.00015.2008.

Asano, Katsura, Hans-Peter Vornlocher, Nancy J. Richter-Cook, William C. Merrick, Alan G. Hinnebusch, and John W. B. Hershey. 2002. “Structure of CDNAs Encoding Human Eukaryotic Initiation Factor 3 Subunits.” *Journal of Biological Chemistry* 272 (43): 27042–52. https://doi.org/10.1074/jbc.272.43.27042.

Brenner, Sydney. 1974. "The genetics of Caenorhabditis elegans." *Genetics* 77.1: 71-94.

Drachman, Daniel B., Robert N. Adams, Elis F. Stanley, and Alan Pestronk. 1980. “Mechanisms of Acetylcholine Receptor Loss in Myasthenia Gravis.” *Journal of Neurology, Neurosurgery and Psychiatry* 43 (7): 601–10. https://doi.org/10.1136/jnnp.43.7.601.

Fisher, Robert S., Walter Van Emde Boas, Warren Blume, Christian Elger, Pierre Genton, Phillip Lee, and Jerome Engel Jr. 2005. “Epileptic Seizures and Epilepsy: Definitions Proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE).” *Epilepsia* 46 (10): 1698–99; author reply 1701-2. https://doi.org/10.1111/j.1528-1167.2005.00273_1.x.

Flibotte, Stephane, Mark L. Edgley, Iasha Chaudhry, Jon Taylor, Sarah E. Neil, Aleksandra Rogula, Rick Zapf, Martin Hirst, Yaron Butterfield, Steven J. Jones, Marco A. Marra, Robert J. Barstead and Donal G. Moerman. 2010. “Whole-Genome Profiling of Mutagenesis in Caenorhabditis Elegans.” *Genetics* 185 (2): 431–41. https://doi.org/10.1534/genetics.110.116616.

Hinnebusch, Alan G. 2006. “EIF3: A Versatile Scaffold for Translation Initiation Complexes.” *Trends in Biochemical Sciences* 31 (10): 553–62. https://doi.org/10.1016/j.tibs.2006.08.005.

Jospin, Maelle, Yingchuan B. Qi, Tamara M. Stawicki, Thomas Boulin, Kim R. Schuske, H. Robert Horvitz, Jean Louis Bessereau, Erik M. Jorgensen, and Yishi Jin. 2009. “A Neuronal Acetylcholine Receptor Regulates the Balance of Muscle Excitation and Inhibition in Caenorhabditis Elegans.” *PLoS Biology* 7 (12). https://doi.org/10.1371/journal.pbio.1000265.

Kojima, Nobuhiko, and Tomoaki Shirao. 2007. “Synaptic Dysfunction and Disruption of Postsynaptic Drebrin-Actin Complex: A Study of Neurological Disorders Accompanied by Cognitive Deficits.” *Neuroscience Research* 58 (1): 1–5. https://doi.org/10.1016/j.neures.2007.02.003.

Lee, Eunee, Jiseok Lee, and Eunjoon Kim. 2017. “Excitation/Inhibition Imbalance in Animal Models of Autism Spectrum Disorders.” *Biological Psychiatry* 81 (10): 838–47. https://doi.org/10.1016/j.biopsych.2016.05.011.

Levey, A I. 1996. “Muscarinic Acetylcholine Receptor Expression in Memory Circuits: Implications for Treatment of Alzheimer Disease.” *Proceedings of the National Academy of Sciences of the United States of America* 93 (24): 13541–46. http://www.ncbi.nlm.nih.gov/pubmed/8942969%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC33643.

McCulloch, Katherine A., Yingchuan B. Qi, Seika Takayanagi-Kiya, Yishi Jin, and Salvatore J. Cherra. 2017. “Novel Mutations in Synaptic Transmission Genes Suppress Neuronal Hyperexcitation in Caenorhabditis Elegans.” *G3: Genes, Genomes, Genetics* 7 (7): 2055–63. https://doi.org/10.1534/g3.117.042598.

McCulloch, Katherine A., Kingston Zhou, Yishi Jin. “Neuronal transcriptome analyses reveal novel neuropeptide modulators of excitation and inhibition imbalance in *C. elegans*.” *bioRxiv* (2019): 821769.

Morita, Kiyokazu, and Min Han. 2006. “Multiple Mechanisms Are Involved in Regulating the Expression of the Developmental Timing Regulator Lin-28 in Caenorhabditis Elegans.” *EMBO Journal* 25 (24): 5794–5804. https://doi.org/10.1038/sj.emboj.7601451.

Raizen, David M., R. Y. Lee, and Leon Avery. "Interacting genes required for pharyngeal excitation by motor neuron MC in Caenorhabditis elegans." *Genetics* 141, no. 4 (1995): 1365-1382.

Rand, James. 2007. “Acetycholine.” *Wormbook*.

Tonegawa, Susumu, and Thomas J. McHugh. 2008. “The Ins and Outs of Hippocampal Circuits.” *Neuron* 57 (2): 175–77. https://doi.org/10.1016/j.neuron.2008.01.005.

Wei, Hongen, Kathryn K. Chadman, Daniel P. McCloskey, Ashfaq M. Sheikh, Mazhar Malik, W. Ted Brown, and Xiaohong Li. 2012. “Brain IL-6 Elevation Causes Neuronal Circuitry Imbalances and Mediates Autism-like Behaviors.” *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1822 (6): 831–42. https://doi.org/10.1016/j.bbadis.2012.01.011.

Zimmerman, Barret, and John B. Hubbard. 2018. “Anatomy, Deep Tendon Reflexes (Stretch Reflexes).” *StatPearls* 5: 1–5. http://www.ncbi.nlm.nih.gov/pubmed/30285397.