Spring 2019

The Role of KLP-4 in Regulating Cholinergic Signaling in vivo

Catherine Deuchler
cdeuchler@rollins.edu

The Role of KLP-4 in Regulating Cholinergic Signaling *in vivo*

Catherine Deuchler and
Dr. Jay Pieczynski

Rollins College Department of Biology
Winter Park, FL

Abstract

Cellular trafficking is dominated by the use of motor proteins trafficking cargo along the microtubules that make up the cellular cytoskeleton. Polarity within these cells controls the direction of the trafficking, and the cargo consists of organelles, new proteins, neurotransmitters, etc.. Motor proteins differ in the cargo they carry, the direction that they move, as well as the rate and distance that they can travel. The trafficking of neurotransmitters occurs along neurons, highly polarized cells, and this research focuses on neuronal trafficking as a method of controlling the release and degradation of acetylcholine and thus regulating the signals that they propagate. KLP-4 is currently identified as a motor protein that contributes to the regulation of acetylcholine trafficking. KLP-4 as a part of the kinesin-3 super family of motor proteins is especially important due cells' reliance upon its function as a long distance and highly processive motor protein. This characteristic of the kinesin-3 family is essential, especially when considering the extraordinary lengths that neurons can cover in a given organism. Having identified hypersensitivity to acetylcholine was present in mutant *klp-4 Caenorhabditis elegans*, rescues of the mutant *klp-4* in *C. elegans* were performed in order to further establish the role that the motor protein has in the regulation of this heavily used signaling pathway. *C. elegans* were used as the model organism due to their homology to the human nervous system as well as the extent of literature on their success as *in vivo* models. Rescue crosses as well as transgenic *C. elegans* were created in order to test their behavior and the activity of their cholinergic signaling in comparison to the aforementioned mutant *klp-4* counterparts. The rescued crosses showed recovery of the phenotype, consistent with the known literature that describes the complex and heavily regulated cholinergic signaling pathway. Discovering the an in-frame 200 amino acid deletion and one amino acid insertion

within the cargo binding domain of *klp-4*, leads to the hypothesis that the KLP-4 motor is constitutively active and thus contributing to the hypersensitivity phenotype. The participation of CHA1 and Unc17 as co regulators in cholinergic signaling will be explored as possible opposite mutants (hyposensitive mutants to the effects of acetylcholine) when crossed with the original *klp-4* mutants will show a true rescue of the cholinergic signaling mutant phenotype.

Lay Person Summary

The neuronal circuitry of organisms controls the information and relay controls of the bodily commands. The specialized cells within the system, neurons, are tasked with the intake of information from the external environment in the form of stimuli. The information of the stimuli is relayed from nerve to nerve in a cascade via chemical messengers, neurotransmitters. From sensory neurons through interneurons and finally relayed to motor neurons in order to elicit a physical response. The motor neurons are responsible for triggering muscle contraction by communicating the neuronal signal through the release specific neurotransmitters onto muscle fibers. The activity of neurotransmitters and connectivity between neurons, therefore, is of the utmost importance within neuronal signaling and motor control. One of the mechanisms that perpetuates the signal throughout these neuronal cascades is the work of motor proteins. The study of these proteins and how connections are maintained and operatively sustained through their activity will shed light on the more detailed innerworkings of neurons and neuronal circuitry. The function and purpose of the motor protein KLP-4, which is operates along the forward locomotion responsible AVB interneuron, is the specific topic of study within *C. elegans*. This model organism is especially suited for studying the nervous system as the entirety of the organism's neuronal

connections are mapped out. The gene *klp-4* was originally hypothesized to affect the physical structure of the neuron, but mutations within *klp4* did not affect the neuron's morphology nor its develop. Now it has been experimentally shown that the effect is within subcellular changes of the neuron, and that KLP-4 has a marked effect on cholinergic signaling within the neuron. Thus, the focus of this research is to discover if the production and or transport of acetylcholinesterase is also affected by mutations of *klp-4*.

Introduction

The nervous system is based upon a circuitry that is both electric and chemical in nature. Neuronal signals are passed from one neuron to the other by the use of neurotransmitters as chemical messengers released by the pre-synaptic neuron to be received by the post-synaptic neuron to continue the signal. This signaling mechanism can be manipulated through chemical and physical structure of the synapses, keeping the activity of the nervous system flexible and adaptable, but also meaning that the balance and the regulation of these changes have a wide impact (Van Spronsen and Hoogenraad, 2010).

The propagation of signals from neuron to neuron is reliant upon the release of the appropriate neurotransmitters. This process of getting the appropriate proteins, organelles, and neurotransmitters from one end of the neuron to the other relies upon a two-component intracellular transport system. The cytoskeleton is responsible for both transport within the cell as well as structure and support, as it acts as a "roadway" for trafficking cargo, provides structural support, assists in neuronal development, and act as polar signaling devices (Kapitein and Hoogenraad, 2015). The major components of the cytoskeleton are polymers of proteins that form filaments, termed microtubules. Microtubule filaments are polarized structures, with

the plus end of the microtubules at the synaptic end of the neuron and the minus end at the soma of the neuron. The second component of the intracellular transport system is the movement of motor proteins on microtubules. Motor protein movement is directed by the polarity of microtubule, thus allowing for location directed development. Motor proteins traffic their cargos on the microtubule system which is akin to a railway of freight trains (Hirokawa et al., 2009). Cytoskeletal motor proteins carry diverse cargo such as organelles, newly made proteins, and synaptic vesicle precursors.

Microtubule Structure and Function

Microtubules are constructed from alpha and beta heterodimer subunits that are connected from tip to tail in a repetitive pattern that lends to their characteristic rail-like structure, and thus microtubules are described as polarized, with distinct plus (+) and minus (-) ends. Polarized microtubules have directionally different characteristics which cells can co-op for their development. For example, the early development of neurons is influenced by the polarized nature of the neuronal cytoskeleton and its extension from an actin-rich growth cone which encourages neuronal outgrowth (Kapitein and Hoogenraad, 2015). When the neuronal tip growth cone is developing, cyclic nucleotide concentration is elevated on one side of the growth cone, increasing attraction and growth or repulsion and retraction depending on which cyclic nucleotide has an effective concentration at respective ends of the microtubule (Akiyama et al., 2016). This system of different cyclic nucleotides working at the edge of the microtubule creates the polar gradient and therefore is fundamental in creating the bidirectional trafficking of cargo along the microtubules. The cyclic nucleotide, cAMP, is the stimulator of microtubule growth and development of the (+) end and cGMP as the developer of microtubule retraction and growth of the (-) end (Akiyama et al., 2016). In highly polarized cells, such as

neurons, the plus end of microtubules are arrayed towards the cell's cortex, and in the axon, the plus ends are directed outwards of the neuron (Maday et al., 2014). The system of polarized microtubules creates a highly functional bidirectional trafficking structure within the neuron, which facilitates a diverse range of polarized cellular signaling processes keeping the neurons functional (Hirokawa et al., 2010). Tubulin isoforms differ between axonal and dendritic microtubules which would contribute to preferential binding for different kinesins. The microtubule structure is also post translationally modified, creating more specific binding domains and particular affinities for different families of kinesins. The structural dynamics of microtubules is dictated by the state of the bound GTP and GDP. Anterograde trafficking kinesins and microtubules have been associated with GTP bound microtubules, as anterograde trafficking follows the + end of the growing microtubule where GTP hydrolysis takes place, as well as anti-GTP antibodies prohibit anterograde kinesin trafficking (Siddiqui and Straube, 2017).

Kinesins and Neuronal Development

Directed trafficking within neurons is especially important during development, when the number of synapses is regulated by kinesin activity due to the association of apoptotic and repair factors with kinesins (Tanaka, 2015). Neurogenesis is affected as nuclear migration is dependent upon microtubule orientation. The correct growth of the microtubule for this process is dependent upon certain kinesins, as the activity of kinesins at the tip of the growth cone can regulate the microtubule dynamics (Homma et al., 2003). Kinesins take part in the regulation of the growth of the microtubules within the neuron specifically depolymerizing at the plus end of the microtubules, as well as the association with certain microtubule associated proteins (MAPs) that contribute to the stability of the microtubule (Chen, 2018; Homma et al., 2003).

Early on, the plus end of the developing microtubule is under regulation by certain kinesins that act in this depolymerization manner. This action allows the microtubule and neuron to control its growth and extend to its singular size. The expression of kinesins is controlled, then, during repair to the microtubule in order to prevent any further depolarization (Ghosh-Roy et al., 2012). During the development of the neuron, kinesin binding to the microtubule promotes earlier trafficking which in turn promotes increased neuronal polarization (Lasser et al., 2018). There is a strong correlation between the activity along the microtubule and the ability for the neuron to polarize and differentiate, as higher activity along the microtubule means increased trafficking of necessary materials during development of the cell.

Kinesin-3 Structure and Function

Signal transduction plays an especially crucial role in neurons; the trafficking of synaptic vesicles containing neurotransmitters along the neuron by motor proteins will affect the availability of neurotransmitters and thus control the signaling of the neuron. Two classes of motor proteins move cargo on microtubules: dyneins and kinesins. This thesis will focus on kinesin motors. Evolutionarily conserved throughout all kinesins are three domains: motor, neck, and cargo. Kinesins are defined by their genetically conserved motor domains, whereas the neck and cargo binding domains that carry the cargo are diverse (Wickstead et al., 2010). The motion of kinesins is powered through ATP binding and hydrolysis in a conserved motor domain. Kinesins are specified by the placement of the motor domain within the protein. N-kinesins have their motor domains at the amino terminus, C-kinesins have their motor domain at the carboxyl terminus, M-kinesins have their motor domain in the middle of the protein. Kinesins can act in directional trafficking of cargo mostly in the anterograde direction towards the cell periphery, but some kinesins move in a retrograde direction toward the cells interior,

or act in the depolymerization of the microtubules (Tanaka, 2015). Interestingly, some kinesins operate some kinesins are minus end directed, but most are associated with anterograde (+ end directed) trafficking, although they are still mostly associated with anterograde trafficking. The bidirectional trafficking has been theorized to be the result of cooperation between dyneins and kinesins. Two proposed models suggest that this cooperation takes place through mechanical activation via the opposite motor or steric inhibition as the opposite motor relieves autoinhibition and contributes to the processive motion (Siddiqui and Straube, 2017). In humans, there are at least 45 different kinesins, divided into at least 14 different superfamilies based on sequence homologies.

For this work, we are focusing on the kinesin-3 superfamily, which is one of the most studied groups of motors and contains the prototypical kinesin, KIF1A/UNC-104. The kinesin 3 family is an especially reliable transporter within the cell as it is able to transport cargo for long distances at efficient speeds, demonstrating a high degree of processivity (Soppina and Verhey, 2014). The structures of the neck and motor domain of the kinesin-3 superfamily are thought to contribute to the highly processive capability of this family of kinesins and dictate which cargo will be bound to the motor protein. The kinesin-3 motor domains include a lysine-rich loop (K-loop) that interacts with the negative glycine-rich E-hook of the ß-tubulin of the microtubules (Siddiqui and Straube, 2017). This high affinity between the kinesin and the microtubule allows for the increase in the processivity of the kinesin, so the affinity between the K-loop and the E-hook increases the rate at which the kinesin will bind to the microtubule (Siddiqui and Straube, 2017). The specific lysine residues that are responsible for the K-loop and E-hook interaction differ from family to family, but in the case of the kinesin-3 family, the residues contribute to a 200-fold affinity increase (Siddiqui and Straube, 2017). The cargo

binding domain of the kinesin is affected by the forkhead-associated (FHA) domain as well as the short and interspersed coiled-coil (CC) domains.

The processive ability of kinesin-3 motors likely resides in their ability to dimerize, which the neck coil region is responsible for those interactions that lead to dimerization (Soppina et al., 2014). As a monomer, kinesin-3 motors are not active and in a state of autoinhibition. There are two models for that explain kinesin autoinhibition, and kinesins can follow either this first model, the second model, or operate through a combination of the two models. The first model proposes the switch from monomer to dimer (and therefore from completely inactive to active) is moderated by interactions within the neck and tail domains that hold the kinesin in the inactive, monomeric state (Yue et al., 2013). Once dimerized, the interactions between the neck and tail regions of the previously monomeric kinesins contribute heavily to sustaining the dimerization (Soppina et al., 2014). In the second part of the model, the motor units are constitutively dimerized but inhibited by interactions with the tail domains. Cargo binding, then, occupies the tail domain and therefore releases the motor domain to its active state. There is evidence that specific kinesins within the kinesin-3 family use one or the other model, or a mixture of the two (Figure 1) (Siddiqui and Straube, 2017). Altering the structure of the tail, specifically truncating it, will result in processive dimeric motor units (Soppina et al., 2014). Similarly, the deletion of a proline at the CC 1 and neck coil junction inhibit the flexibility of the protein and therefore will inhibit the interactions that autoinhibit the kinesin. If the motor would dimerize, the proline-rich kink must be released between the neck and the CC domain. During this transition, the FHA becomes unbound from the folded CC domain allowing kinesin-3 monomers to dimerize (Lee et al., 2004; Soppina et al., 2014). Dimerization between separate CC domains and FHA domains impair synaptic vesicle

transport and locomotion (Siddiqui and Straube, 2017). Further specific inhibition mechanisms require the participation of other microtubule affinity regulating kinases.

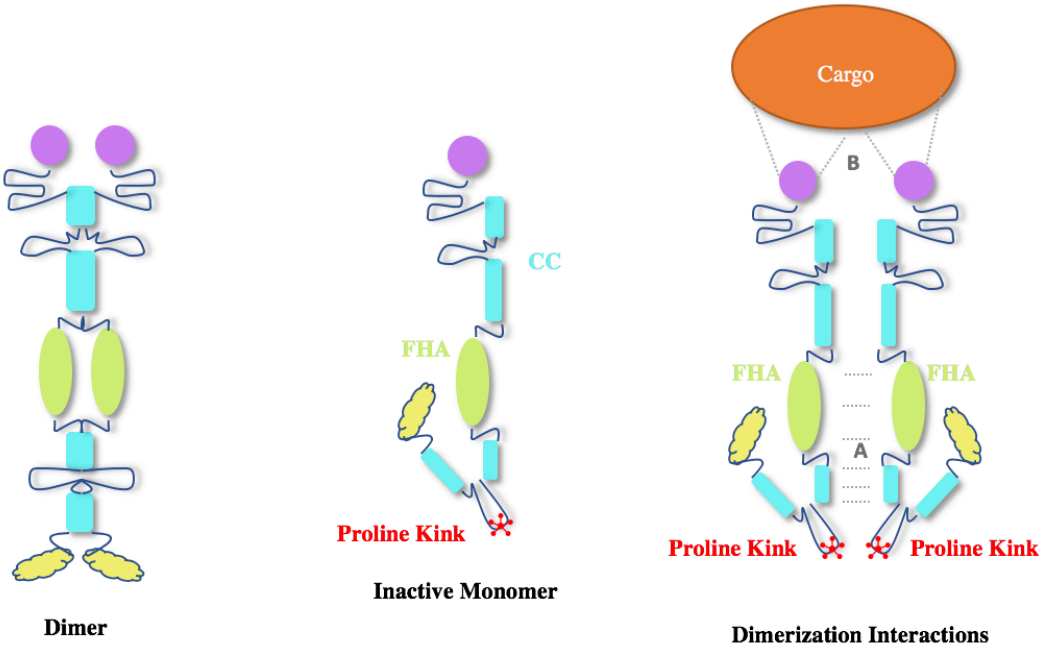


Figure 1. The dimerization models for kinesin-3 families. Either the interactions at A or B, or both, release the kinesin from its autoinhibited state and allow for it to take its dimerized processive shape.

The extent of cargo binding and its control over kinesin activation is as varied as the cargo binding domains for each kinesin. Many of the cargoes carried by kinesins are proteins for cellular signaling and establishing cellular polarity (Vale, 2003). To complicate matters, cargo binding also is assisted by cargo adaptor proteins that recruit cargo and also influence the directionality of their transport. Specific cargo adapter protein and kinesin interactions can lead to multiple different uses of the kinesin. The association with cargo adapter proteins, and their diverse relationships increases the complexity and the inter-relatability of motor proteins (Hirokawa et al., 2009). As another mode of control of kinesins by outside molecular agents, Rab GTPases contribute to the functionality of kinesins, as they assist in vesicle and organelle localization. For example, Rab3 controls the exocytosis of synaptic vesicles. The kinesin-3

motor, KIF13A, contributes to the cycle of Rab GTPases by binding to recycling endosomes that regulate recycling and localization of endosomal cargo that is loaded into the vesicles at the synapse (Siddiqui and Straube, 2017).

Kinesin Trafficking and Synaptic Activity

Intracellular/endomembrane trafficking becomes increasingly important when understood in the context of how neurotransmitters are transported within the cell. If neurotransmitters control responses within an organism via their signal release and reception, and kinesins control the availability of neurotransmitters, then kinesins play a crucial role in what signaling affects. Within the nervous system, there is a cyclic relationship between signal reception and transcription of genes. If there is an increase in signaling, there will usually be an upregulation in transcription of an involved gene. Vice versa, if there is a change in transcription within a gene, this can change the synaptic signaling by increasing or decreasing availability of a necessary gene product. The relationship between synaptic activity and transcription of kinesin genes suggests intricate feedback mechanisms between synaptic vesicle trafficking, signal release, and reception (Tanaka, 2015). Environmental factors regulate microtubule structural and related proteins, such as MAPs that have control over the run length of certain kinesins (Tanaka, 2015). Synaptic activity is also altered in this relationship between kinesin expression and synaptic activity. Mice housed within an environment rich in cognitive stimuli showed morphological changes within the neuronal structure, and this was closely tied to fluctuations of both neurotrophins and kinesin expression presumably because of the increased synaptic load (Kondo et al., 2012). Increasing the presynaptic transport of synaptic vesicle proteins will induce and increase kinesin expression within the enriched environment (Kondo et al., 2018). As morphological changes take place,

kinesin motor proteins are increasingly utilized to traffic the signals that contribute to neuronal plasticity seen in learning or memory or muscular action. Further studies suggest that interrupting kinesin function can affect the expression level of multiple parts within the signaling cascade, including components of the synaptic vesicles (Li et al., 2017). To deal with the stress of a malfunctioning kinesin-3 family gene, proteins regulated by the motor domain are suppressed, while different signaling pathways are upregulated (Li et al., 2017). Overall, there is a connection between the expression of kinesin genes and the stimulus load which the neuron is subjected to, leading to activation of other signaling pathways, allowing the cell to produce more kinesins and their required cargo.

As kinesins control and regulate neurotransmitter activity, they contribute to diseases caused by irregular signaling. Tubulin defects tend to have dominant negative effects as the system of trafficking is dependent upon them. This causes inappropriate localization by kinesins as well as inhibiting axon elongation. Understandably, defects within this signaling system create disorder within the entire nervous system, or even the species of cell that the defect is present in. Diseases that have been linked to kinesin defects are schizophrenia, autism, and mental retardation as well as anxiety and even metabolic disorders. Disorders that present themselves during development are linked to disordered neuronal development which is linked to and affected by transport (Lau and Zukin, 2007; Van Spronsen and Hoogenraad, 2010; Tanaka, 2015).

Kinesin-3 Motors in vivo

Caenorhabditis elegans (*C. elegans*) is a free living, non-parasitic soil nematode that provides a highly predictable model system for studying the function of gene products *in vivo.* There is approximately 60-80% similarity between the genomes of the *C. elegans* and humans.

Additionally, *C. elegans* have a simplified, yet fully mapped out nervous system of 302 cells (Ma et al., 2018). The relative ease by which *C. elegans* take up foreign DNA has allowed for the production of transgenic lines that can be used as models for representative expression patterns and is useful in studying neurological similarities between worms and humans (Ma et al., 2018).

C. elegans contain homologous genes for many kinesin-3 superfamily members, including *klp-4*, which is the worm homolog of human kinesins KIF13A and KIF13B. KLP-4, and all other worm kinesin-3 motors, are anterograde traffickers with the long distance and high processive ability like the rest of the kinesin-3 family. KLP-4 is highly expressed in *C. elegans* neurons, but especially along the AVB interneuron which is responsible for most of the *C. elegans'* locomotion (Rakowski et al., 2013). The AVB interneuron is only one of four major interneurons forming the animal's ventral nerve cord (VNC). The axons AVB and other VNC neurons extend almost the entire length of the worm, allowing for visualization of neuronal processes. Combining genetic, qualitive analysis and quantitative locomotive behavior patterns have allowed us to study the role of the KLP-4 motor protein *in vivo*. Specifically, the mutant strain that we have focused our studies on, *ok3537*, exhibits abnormal locomotive movements, and irregular neuronal morphology.

Aim and Goals

This study of KLP-4 has been focused around its role in cholinergic signaling, which can be seen by the mutant locomotive phenotypes in *klp-4* mutants. Previously, it has been established that these locomotive phenotypes were linked to cholinergic signaling. In order to further ensure that this locomotive defect and hypersensitivity to the acetylcholinesterase inhibitor was truly attributable to the mutant *klp-4,* the next step consisted of rescuing the

phenotype through various methods. Non-complementation crosses as well as creation of transgenics through injection were attempted in order to rescue the mutant *klp-4* worms, and the original aldicarb assay was performed on all these worms in order to compare them to the wild type cholinergic signaling. After the successful transgenic rescues

Materials and Methods

C. elegans strains and transformations

Caenorhabditis elegans strains were obtained from the *Caenorhabditis* Genetics Center (CGC, University of Minnesota-Twin Cities), funded by the NIH Office of Research Infrastructure Programs (P40 OD010440). *C. elegans* mutant strain RB2546 *(klp-4 (ok3537)* was provided by the *C. elegans* Gene Knockout Consortium (Oklahoma Medical Research Foundation, Oklahoma City, OK) to the CGC. Strains VC41006, VC40502, and VC20255 were deposited in the CGC via the Million Mutation Project (MMP, Vancouver, BC Canada). All other strains used were deposited in the CGC from lab of origin.

Strain Designation	**Genotype**
N2	Wild Type
RB2546	*ok3537* mutant
TH215	GFP tagged *klp-4*
PVX60	Transgenic rescue of *ok3537* mutant, clone line 1
PVX62	Transgenic rescue of *ok3537* mutant, clone line 2
CB113	*unc17* null
LX919	GFP tagged *unc-17*

Table 1. Relevant *C. elegan* strains. *C. elegan* strains that were used in the course of this experiment and were part of the meaningful results of this research project.

Any background mutations in *klp-4* mutants were eliminated via outcrossing to wild type N2 worms. Outcrossed *klp-4* mutant strains were then crossed with *rab-3::eGFP* worms (strain NM2415) to generate *klp-4* mutant strains PVX38 and PVX39 with green fluorescent

protein synaptic markers. After crossing, strains were genotyped to verify the presence of *klp-4* mutation.

F_1 Non-Complementation

Non-complementation crosses were performed on crossed mutant *klp-4* strains with N2 strains. The F_1 male progeny were collected and tested in Aldicarb sensitivity assays.

Transgenesis

A fosmid (UBC-f80C1340Q, Sourcebioscience) containing the genomic sequence of *klp-4* was inoculated in an overnight LB medium supplemented with 50 ug/mL chloramphenicol. The culture was and the DNA was isolated through a mini prep protocol (Ahn et al., 2000). The purified fosmid was injected into young adult hermaphrodites at 50 ng/µL. The coinjection marker, pJNP9 *(Punc-122::unc-122::eGFP*), was used at final concentration of 30 ng/µL. Injections done using a Tritech MINJ-1000 complete injection system. The worms were allowed to recover and then imaged under a fluorescent microscope to look for the eGFP marker. GFP positive worms were then cloned and the F_1 offspring were monitored in order to watch for the germline passage of the fosmid, indicated by the successful passage of the fosmid into the F_2 offspring,

Microscopy

Neuronal morphology of the ventral nerve chord (VNC) neurons were visualized in mutant *klp-4* strains PVX38 and PVX39 and the control strain NM2415 using a Zeiss ISM 700 laser scanning confocal microscope. VNC pictures were taken immediately posterior to the vulva. The collected images were processed using Adobe Photoshop.

Behavior assays

Aldicarb stock (100 mM in 70% ethanol) was added to agar plates to a final concentration of 0.5 mM during the plate pouring process. At least twenty worms were transferred to a 0.1M Aldicarb plate and the time course of worm paralysis was monitored in 30-minute intervals over three hours. Paralysis was indicated by a failure of the animal to move when prodded with a platinum wire. Data obtained were plotted using Microsoft Excel.

Spontaneous Reversal

In order to further test the locomotive behavior and response of the mutant *ok3537*, a locomotive behavior assay was conducted to confirm that neurological defects were present in the strain of *C. elegans*. *C. elegans* naturally exhibit behaviors of spontaneous reversal either as a method of avoidance as well as part of their natural sinusoidal movement. A reduction in the spontaneous reversals as compared to the wild type are indicative of a defect in the neurologic signaling pathway.

CRISPR Cas-9 Truncations

In vitro transcription was used to create the truncated RNA for injection. The arms of homology were created with PCR using the primer sets for the 603 amino acid, 474 amino acid, the 390 amino acid truncations, as well as the reporter. Then these arms of homology were PCR purified using the GeneJet purification system. The plasmids (pJNP10-13) were linearized using DraI. These linearized plasmids were then purified using the GeneJet Purification system rather than the traditional phenol-chloroform extraction. The purified linearized plasmids were used for in vitro transcription of the desired RNAs using the HiScribe T7 *in vitro* transcription kit (NEB). The sgRNAs were purified with RNA Clean and Concentrator-5 (Zymo Technologies) with the modifications that the volumes of reagents were doubled before being transferred to the spin column.

Outcrossing

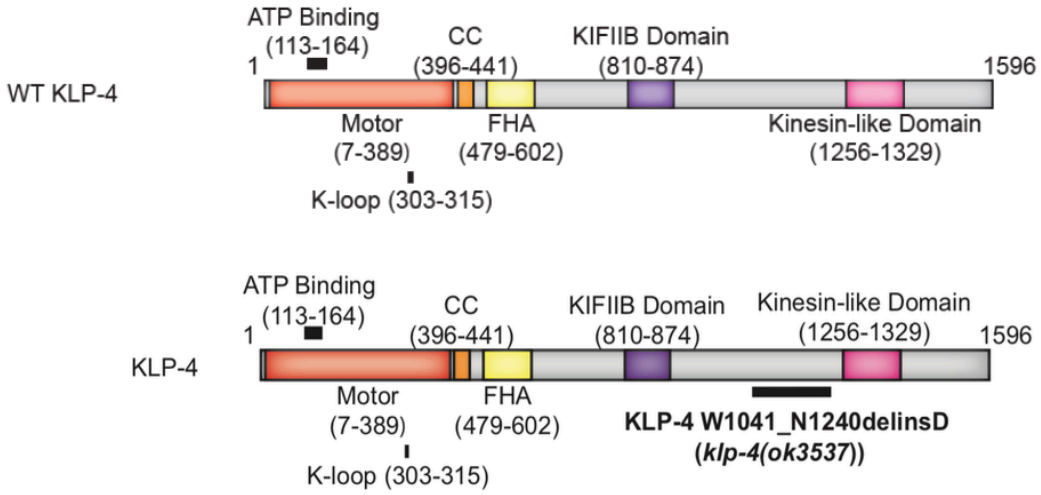
To ensure the integrity of the experiments, the *ok3537 C. elegans* were crossed with male wild type. By outcrossing the mutant strain with the wild type, it ensures that, besides the *klp-4* mutation of interest, the *ok3537* strain is genetically the same to the wild type, which strengthens any results found in this project regarding the mutation *ok3537*.

Results and Discussion

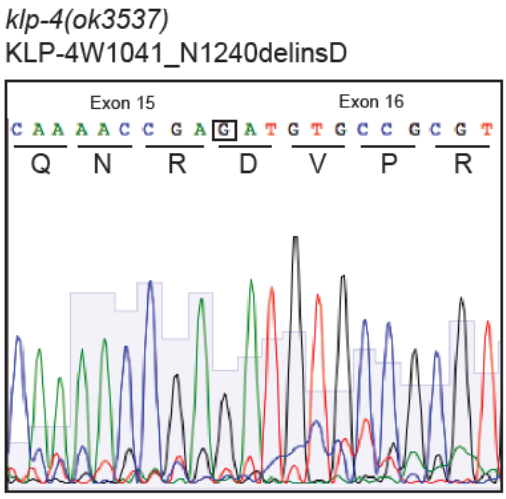
Previous mutants of KLP-4 have been considered null mutants so the research into *ok3537* focused on how it could be functional as well as what role it played. The opportunity of having a functional mutant KLP-4 allowed an investigation into what type of pathway the motor protein was involved with and its place within the signaling cascade. A previous research student obtained *C elegans* strains containing *klp-4* mutations and eliminated background mutations via outcrossing to wild type N2 worms. The *klp-4* mutants were then assayed for locomotive defects using a nose touch and spontaneous reversal assay (data not shown). From these previous experiments, we determined that *klp-4* mutants display general locomotive behavioral defects consistent with defects in neuronal signaling.

Since the allele *klp-4(ok3537)* had previously not been analyzed, we wanted to determine the molecular nature. Since the *klp-4(ok3537)* allele is a large deletion, it was previously assumed to be a null allele. However, genotyping of the *klp-4(ok3537)* mutants showed a mutation which results in a 200 amino acid deletion and an insertion (200/1 del/in) of one amino acid, resulting in an in-frame deletion inside the cargo binding domain (Figure 2 A and B).

A)


WT KLP-4
ATP Binding
(113-164)
CC
(396-441)
KIFIIB Domain
(810-874)
1
1596
Motor
(7-389)
FHA
(479-602)
Kinesin-like Domain
(1256-1329)
K-loop (303-315)
KLP-4
ATP Binding
(113-164)
CC
(396-441)
KIFIIB Domain
(810-874)
Kinesin-like Domain
(1256-1329)
1
1596
Motor
(7-389)
FHA
(479-602)
KLP-4 W1041_N1240delinsD
(klp-4(ok3537))
K-loop (303-315)

B)


klp-4(ok3537)
KLP-4W1041_N1240delinsD
Exon 15
Exon 16
C A A A A C C G A G A T G T G C C G C G T
Q N R D V P R

C)

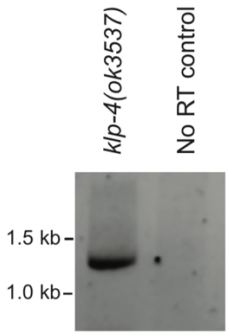

klp-4(ok3537)
No RT control
1.5 kb
1.0 kb

Figure 2. The *klp-4* mutant strain RB2546 sequencing showing an in-frame 200 amino acid deletion and single amino acid insertion. A) Schematic of *klp-4*, comparing wildtype to *ok3537* with labeled domains of interest. B) The sequencing of *klp-4(ok3537)* mutant. C) One step RT-PCR using primers 100bp upstream and within the 3' UTR.

How does this large in-frame deletion contribute to the previous observation that *klp-4* mutants have neuronal signaling defects (M. Magaletta, unpublished). To determine the effect of the *klp-4 (ok3537)* on neuronal structure, mutant *klp-4* strains were then crossed with *rab-3::eGFP* worms (strain NM2415) to generate *klp-4* mutant strains PVX38 and PVX39 with green fluorescent protein synaptic markers.

Images of the VNC taken posterior to the vulva in mutant strains (PVX38 and PVX39) and control strain (NM2415) indicate that *klp-4* mutants experience irregularly spaced, aggregated puncta (Figure 3). This differential spacing and aggregation of *rab-3::GFP* synaptic marker indicates that *klp-4* mutants experience misorganization of VNC synapses.

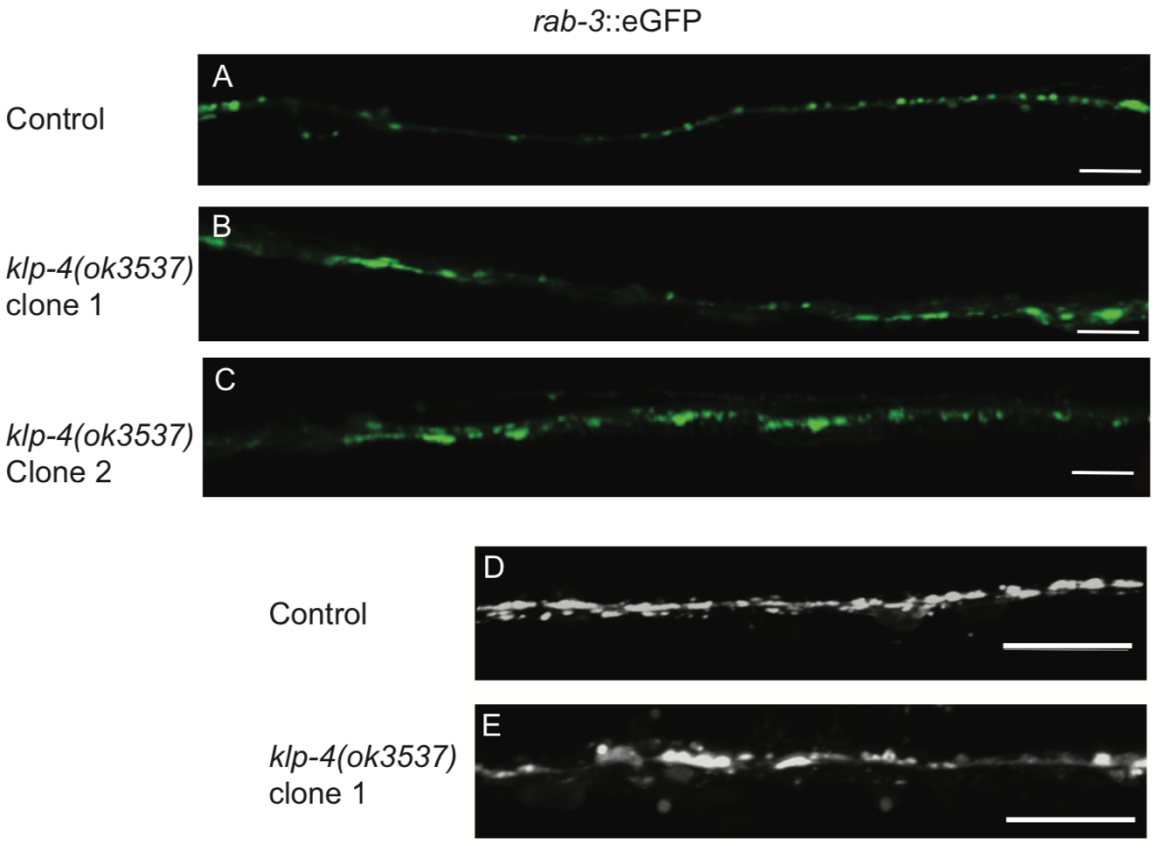


Figure 3. *klp-4* mutants experience ventral nerve chord (VNC) synaptic misorganization. The effect of *klp-4* mutation on neuronal morphology was visualized in the ventral nerve chord (VNC) posterior to the vulva. VNC visualization of *rab-3::GFP* synaptic marker showed synaptic misorganization in *klp-4 ok3537* mutants of two independent lines (A and B), indicated by irregular puncta aggregates and spacing in mutants. The 3D images (D and E), max intensity projections, show the full view of the irregular puncta and spacing throughout the entire breadth of the worms in comparison to the wildtype.

To determine if *klp-4* mutants experience a global synaptic misorganization phenotype, *rab-3::GFP* was also visualized in the PVD sensory neuron of mutants (PVX38 and PVX39) and control strain (NM2415) (Figure 4). PVD sensory neuron images taken anterior to the vulva revealed irregular *rab-3::GFP* puncta spacing and aggregation in *klp-4* mutants, as well as an additional arboring phenotype (Figure 4). These irregular puncta spacing, and

aggregation observed in both the VNC and PVD sensory neuron of mutants (PVX38 and PVX39) suggests that *klp-4* mutants experience global neuronal morphology defects.

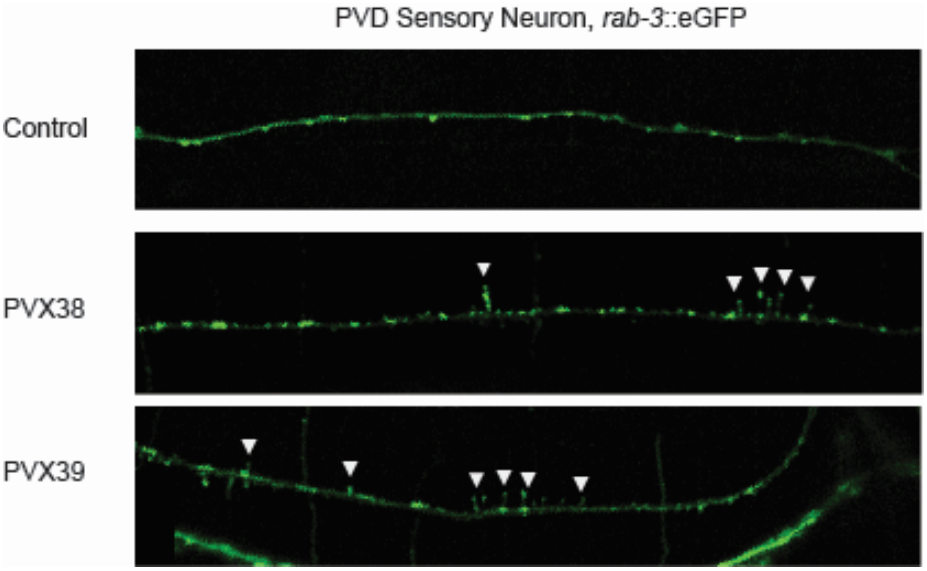


Figure 4. *klp-4* mutants have sensory neuron morphological defects. The effect of *klp-4* mutation on neuronal morphology was visualized in PVD sensory neuron anterior to the vulva. PVD sensory neuron visualization of *rab-3::GFP* synaptic marker showed synaptic misorganization in *klp-4* null mutants PVX38 and PVX39, indicated by irregular puncta aggregates and spacing in mutants. An additional arboring phenotype was also visualized, indicated by white triangles above.

Irregular puncta spacing and aggregation observed in both the VNC and PVD sensory neuron of mutants (PVX38 and PVX39) suggests that *klp-4* mutants experience global neuronal subcellular morphology defects. However, how these morphology defects might contribute to aberrant neuronal signaling is still unknown. Considering that the neurons we are assaying are known members of the *C. elegans* locomotive circuit, we wanted to determine if locomotive signaling is defective in *klp-4(ok3537)* mutants. Locomotive behavior is mainly controlled by cholinergic signaling and the neurotransmitter acetylcholine. To determine if

cholinergic signaling is affected in the *ok3537* strain, we used an aldicarb assay (Figure 5). At the neuromuscular junction, the excitatory motor neuron releases the neurotransmitter acetylcholine which binds with the acetylcholine receptor on the corresponding muscle cell. This causes an excitatory signal, which is terminated upon acetylcholine degradation by acetylcholinesterase. Aldicarb is an inhibitor of acetylcholinesterase, and in wild type N2 *C. elegans* causes a prolonged excitatory signal and eventual paralysis.

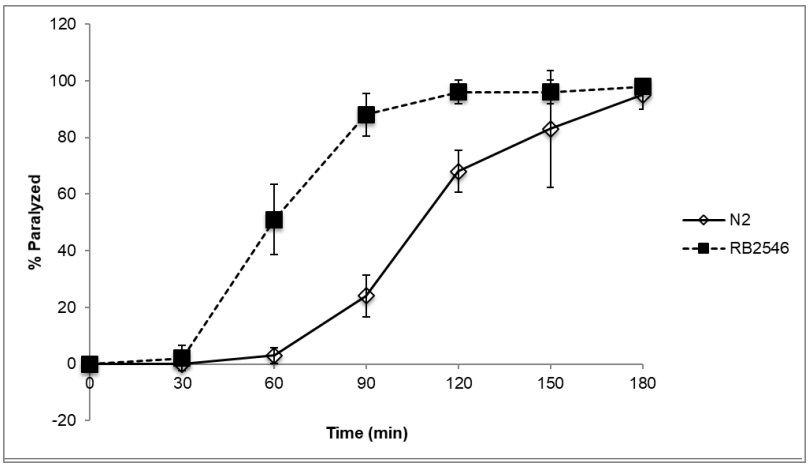
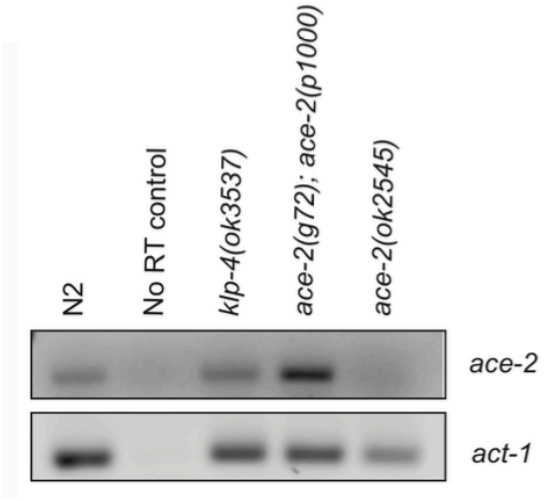


Figure 5. *klp-4* mutant worms are hypersensitive to aldicarb. Aldicarb assays were performed on *klp*-4 null mutants (RB2546) and wild type N2 worms using 0.5 mM aldicarb plates. The course of paralysis of twenty worms on each aldicarb plate was measured over the course of three hours in 30-minute increments, where paralysis was indicated by a failure of the *C. elegan* to move when prodded with a platinum wire. RB2546 worms exhibited an overall increase in percent paralysis in comparison to wild type N2 worms in both determinations, indicating that worms lacking functional KLP-4 experience aldicarb hypersensitivity.

Aldicarb assays were performed on *klp*-4 mutant (RB2546) and wild type N2 worms by monitoring the time course paralysis of worms every thirty minutes for three hours on 0.5 mM aldicarb plates (Figure 5). Overall, *klp-4* mutant (RB2546) worms were hypersensitive to aldicarb when compared to the wild type N2 worms paralyzed over time. Hypersensitivity of

klp-4 mutant worms suggests KLP-4 affects the trafficking of molecules related to acetylcholine signaling: acetylcholine receptor, acetylcholinesterase, or acetylcholine synaptic organization. The exact mechanism by which KLP-4 influences synaptic organization, however, is unknown. As confirmation that *ok3537* was producing acetylcholinesterase, RT-PCR was performed to ensure there was not an *ace-2* null in *ok3537* that was responsible for the hypersensitivity to aldicarb (Figure 6).

A)



B)

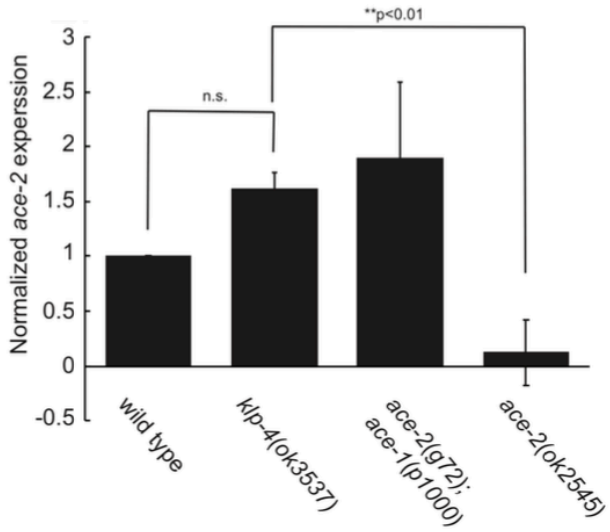


Figure 6. The RT-PCR of *ace-2* within *ok3537,* normalized with actin expression, shows no significant difference from the wildtype expression. A) The RT-PCR of *ace-2* within the wild type, *ok3537*, an *ace-2* functional null, and an *ace-2* genetic null strains, compared to the transcription of RNA of actin within the respective strains. B) The quantification of the imaging data using the LICOR densitor program, with *act-1* as the normalizing value, from data collected from triplicate triplicates.

With no significant difference found between the mutant and the wildtype in regard to *ace-2* expression, the reason for the aldicarb hypersensitivity could not be either a functional or a genetic null of acetylcholinesterase (Figure 6). On the other hand, within the context of the uploaded cholinergic system, the higher expression of acetylcholinesterase would be expected due to the increased load.

Since *klp-4* is located on the X-chromosome, we took advantage of the XO sex determination system of *C. elegans,* where males contain only one copy of the X-chromosome. To determine if the resultant aldicarb hypersensitivity phenotype of *klp-4* mutants could be rescued by swapping X-chromosomes, we performed F_1 non-complementation crosses between male *klp-4* mutants (RB2546) and hermaphrodites of either wild type (N2) or GFP

tagged *klp-4* (TH215). By crossing *klp-4* mutant males and then screening the resultant F_1 male progeny, we can check that the X-chromosomes in the F_1 male progeny came solely from the hermaphrodite parent. When the F_1 progeny were isolated and allowed to age to the L4 stage, they were placed in the aldicarb sensitivity assay against the positive controls of L4 hermaphrodite N2 and TH215 worms and the negative control of L4 RB2546 hermaphrodite worms. The control N2 (Figure 7) and TH215 (Figure 8) are both independent wild type strains, but TH215 has GFP-tagged KLP-4. The rescued crosses showed a partial rescue of the phenotype as they resisted paralysis until the 60-minute mark, like the positive controls, but then the rate of paralysis became similar to the negative controls at the 90-minute mark (Figures 7 and 8). These confounding results lead to the interesting possibility of a complex genetic interaction between *klp-4* and the cholinergic signaling processes. This complexity has already been previously demonstrated given that the foundational portions of signaling mechanisms are regulated by a common factor. For example, the transcription factor UNC-3 acts as a general regulator for cholinergic signaling along the ventral nerve cord of *C elegans*, with multiple target genes as parts of the mechanism that it regulates (Kratsios et al., 2012). This type of regulation could also be responsible for differentiation between nerve type in a way to further coordinate the nervous system (Kratsios et al., 2017). Signaling along cholinergic nerves, as one of the most important signaling pathways within the nervous system should be intensely regulated and under multiple levels of control, and there are two models proposed for how UNC-3 participates in modulating the signaling within which KLP-4 operates. These two models propose either a modular or a coregulatory control of signaling along the neuron which would influence the way KLP-4 traffics its cargo, and thus the signaling conditions (Kratsios et al., 2015).

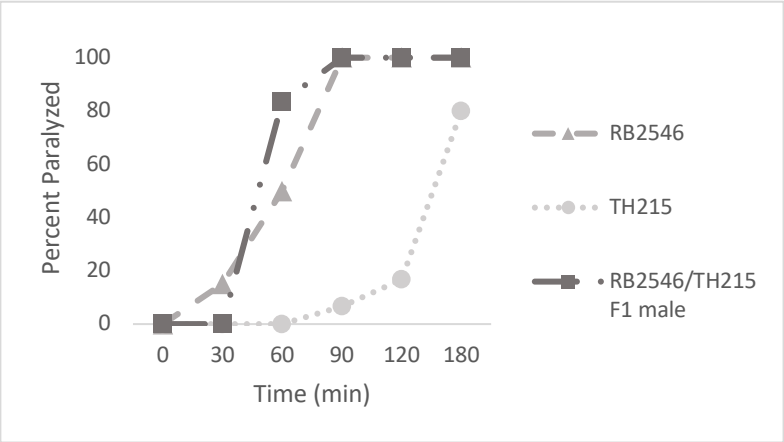


Figure 7. F_1 non-complementation cross of RB2546xTH215 show a partial rescue of the aldicarb sensitivity phenotype. Aldicarb assays were performed on *klp*-4 mutants (RB2546), *klp-4* wild type (N2) and GFP tagged *klp-4* (TH215) worms as well as the F_1 male progeny of RB2546xTH215 using 0.5 mM aldicarb plates. Each aldicarb plate contained 20 worms, with 3 plates of each representative strain. The rate of paralysis was measured over the course of three hours in 30-minute increments. Paralysis was indicated by a failure of the *C. elegans* to move when prodded with a platinum wire. RB2546 worms exhibited an overall increased rate of paralysis in comparison to wild type N2 and TH215 worms. This indicates that worms lacking functional KLP-4 experience aldicarb hypersensitivity.

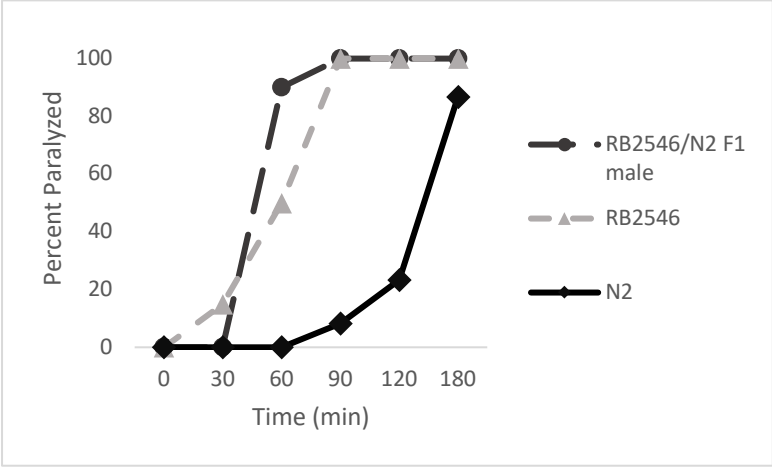
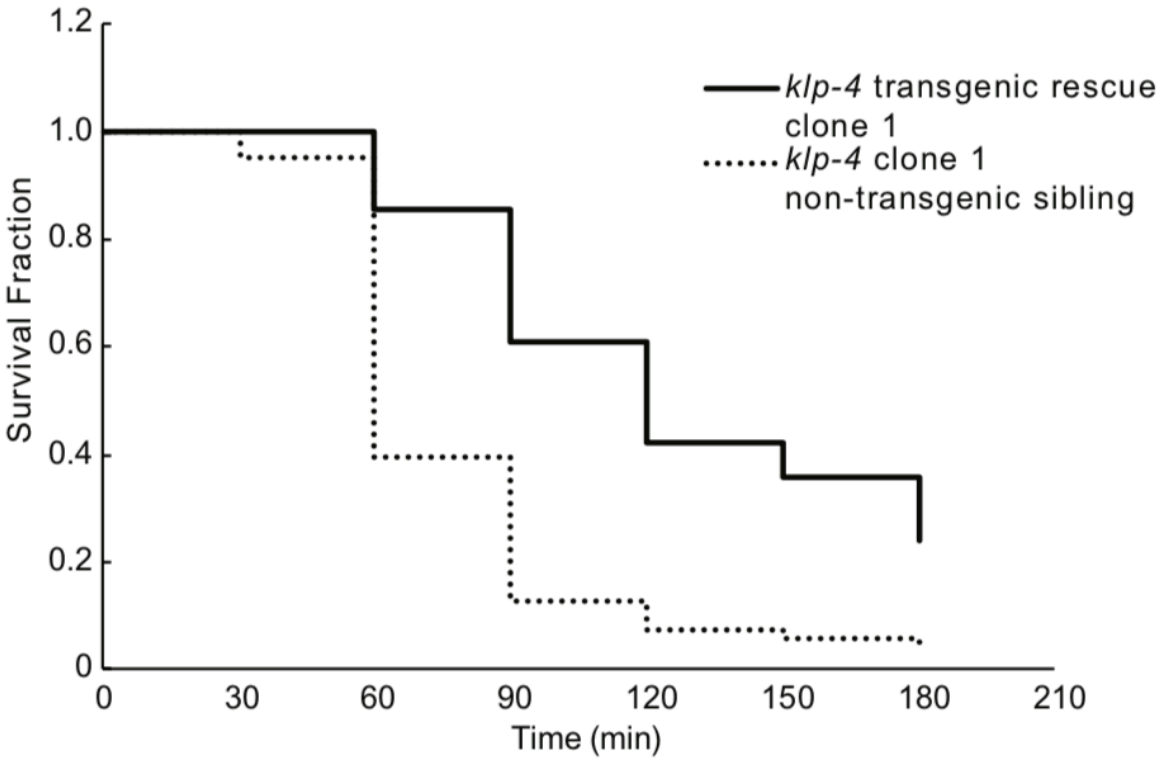


Figure 8. F_1 non-complementation cross of RB2546xN2 show a partial rescue of the aldicarb sensitivity phenotype. Aldicarb assays were performed on *klp*-4 mutants (RB2546) and *klp-4* wild type (N2) worms, and the F_1 male progeny of RB2546xN2 using 0.5 mM aldicarb plates. Each aldicarb plate contained 20 worms, with

3 plates of each representative strain. The rate of paralysis was measured over the course of three hours in 30-minute increments. Paralysis was indicated by a failure of the *C. elegans* to move when prodded with a platinum wire. RB2546 worms exhibited an overall increase in rate of percent paralysis in comparison to wild type N2 and TH215 worms, indicating that worms lacking functional KLP-4 experience aldicarb hypersensitivity.

Since our complementation tests suggest a highly complex genetic interaction between *klp-4* and other members of the cholinergic signaling pathway, we wanted to directly rescue worms with only wild-type version of the *klp-4* gene. To determine if the aldicarb hypersensitivity phenotype observed in *klp-4* mutants was directly due to a mutation in the *klp-4* gene, we produced transgenic strains using a fosmid that contains the wild-type version of the *klp-4* gene (Figure 9). The fosmid was successfully injected, and 15 independent clonal transgenic lines were produced. Animals expressing the *klp-4* transgene were assayed for aldicarb sensitivity compared to non-transgenic sibling controls. Expression of the *klp-4* transgene was able to rescue the aldicarb hypersensitivity phenotype, suggesting *klp-4* directly modulates cholinergic signaling *in vivo* (Figure 9). Furthermore, this result suggests the hypothesis that the *klp-4 (ok3537)* mutation might result in a constitutively active motor, and that overexpression of a wild-type copy of the motor acts as a "braking" mechanism to regulate the delivery of synaptic cargoes, including components of the cholinergic signaling pathway, in neurons.

A)


1.2
1.0
0.8
0.6
0.4
0.2
0
Survival Fraction
0
30
60
90
120
150
180
210
Time (min)
klp-4 transgenic rescue clone 1
klp-4 clone 1 non-transgenic sibling

B)

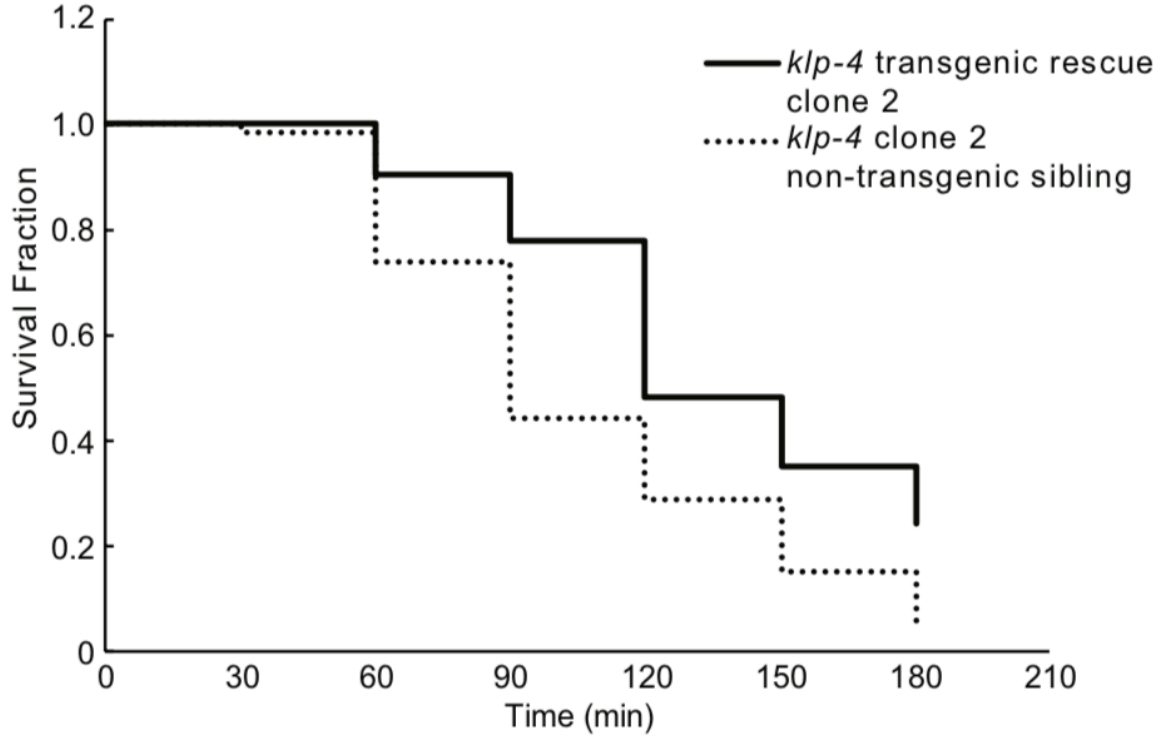

1.2
1.0
0.8
0.6
0.4
0.2
0
Survival Fraction
0
30
60
90
120
150
180
210
Time (min)
klp-4 transgenic rescue clone 2
klp-4 clone 2 non-transgenic sibling

Figure 9. A *klp-4* transgene can rescue aldicarb hypersensitivity *in vivo.* A) Aldicarb sensitivity assay of Clone 1 (PVX60) compared to non-transgenic siblings. B) Aldicarb sensitivity assay of Clone 2 (PVX62) compared to non-transgenic siblings. Error bars represent standard deviations of means from three different trials.

Thus far, our result supported the hypothesis that the *klp-4(ok3537)* in-frame deletion mutation is a gain of function mutation that results in a constitutively active motor, possibly due to lack of autoinhibition. The deletion that is present within the mutant strain (Figure 2) is consistent with previously published data suggesting that this mutant motor cannot fold back in neck to tail autoinhibition, resulting in constitutive dimerization, and therefore activity, of the motor. According to the current theories of kinesin autoinhibition, the modes of autoinhibition are dependent upon the availability of the cargo binding domain to bind and thus release from the inactive conformation (Siddiqui and Straube, 2017). The binding of cargo allows for dimerization and the high processive motion that is characteristic of KLP-4 (Horiguchi et al., 2006; Soppina et al., 2014). The sequencing results of the indel and the results of the functional assay suggest that the hypersensitivity to acetylcholine could be disruption autoinhibition. With the inability to retract from cargo binding, the kinesin is left in a constitutively active state.

To ensure that the age of the worms is not called into question, I performed age discrimination assays upon the N2 (wild type) and RB2546 strains of worms in order to show that the assays done at the L4 stage are the most representative of the worm. There were no differences between the L3 stage worms in the aldicarb assay from the L4 which was previously used to establish the hypersensitivity to the *ok3537* mutants. Worms younger than L3 were exempt from the test due to their size and the ability to accurately determine whether or not they have been paralyzed.

After completing replicate behavioral assays for statistical analysis, I performed further outcrossings of the *ok3537* with the wildtype. These were incomplete due to trouble with the primers that were used during the genotyping, but this type of outcrossing will provide further evidence of the integrity of the research (Figure 12). This type of quality control work will become important during the next publication of this project.

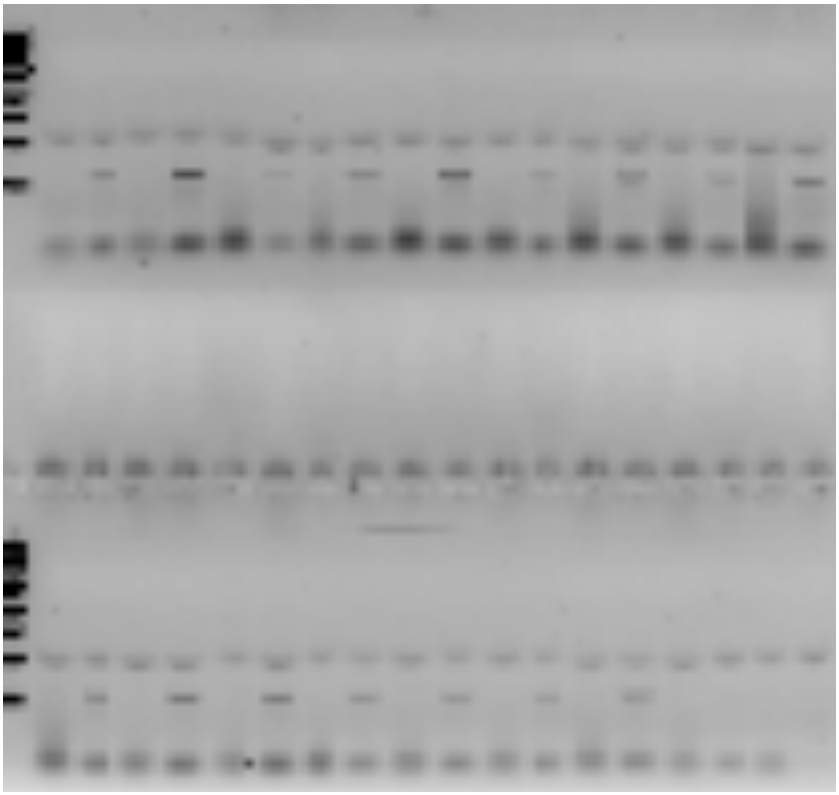


Figure 12. Genotyping of N2 male x RB2546 hermaphrodite. The outcrossing of N2 males and RB2456 hermaphrodites showed successful outcrossing of the wild type with the mutant strain, *ok3537*, of sixteen successful independent lines.

The next step of my research focused on looking at the interactions between KLP-4 and its cargo. In order to accomplish this, I created serially truncated sgRNAs of *klp-4*. I prepared the arms of homology by PCR and created the sgRNA through in vitro transcription (Figure 10 and 11).

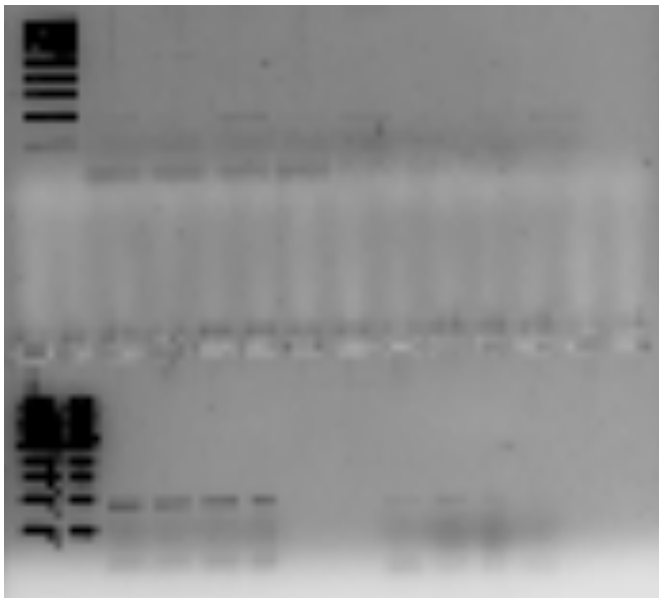


Figure 10. HDR templates for CRISPR Cas 9 *klp-4* truncations. The PCR products of the templates for the CRISPR Cas-9 target.

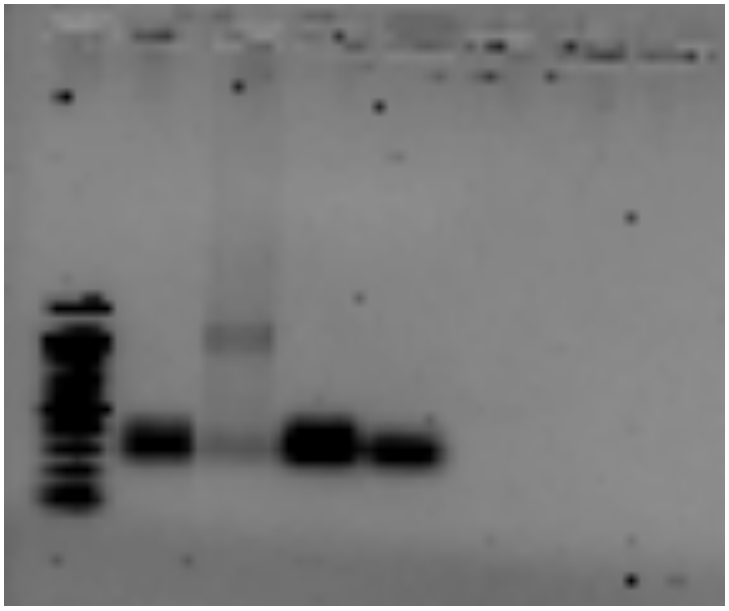


Figure 11. sgRNA for CRISPR Cas 9 *klp-4* truncations. The sgRNAs transcribed through invitro transcription.

By creating these serially truncated sgRNA (at 603, 474, 390 amino acids, as well as the reporter) and injecting the worms with this, will allow research into how the different domains contribute to the overall role of the protein. By taking away domain by domain of

KLP-4 and comparing the behavioral assays of the established worm strains, this will provide further information into how each portion of the protein functions within the entire motor protein. Comparing the sensitivity of acetylcholine of the truncated strains would also shed light onto how cholinergic signaling is regulated. Understanding which truncation keeps function or loses function, and therefore which domain is responsible, can explain which model of activation is relevant for KLP-4 and thus what kind of activity the mutant has as a result.

Future Directions

The continued confirmation of the hypothesis that acetylcholinesterase is regulated by KLP-4 *in vivo* leads to the investigation of the 200/1 del/in mutation. The mutation within the cargo binding domain has not been experimentally defined and verified as to how it affects the tertiary and quaternary structure of the kinesin and therefore its ability to operate and regulate as normal. Also, the cargo binding domain could be altered enough to change the actual cargo that is bound and carried by KLP-4. The cholinergic signaling pathway is also controlled by other factors such as channel regulators and vesicles. The vesicles carrying neurotransmitters are controlled by channels that load and release the vesicle's contents. This is another layer of control within the cholinergic signaling pathway that could be affected by a mutation within vesicle trafficking like *klp-4(ok3537)*. Understanding the how *klp-4(ok3537)* regulates cholinergic signaling would be accomplished through CAD-cell assays trafficking assays (see below).

Unc17 and CHA1

The focus of our study, cholinergic signaling regulation by KLP-4, rests on the

neurotransmitter acetylcholine (ACh). This is under heavy regulation by multiple different factors but specifically looking at the factors that synthesize and load the neurotransmitter, choline acetyltransferase (ChAT) and vesicular ACh transporter (VAChT) respectively. These factors influence KLP-4 by influencing the availability of acetylcholine as well as facilitating the synaptic release of the neurotransmitter after trafficking, all of these factors playing a role in any observed locomotive phenotype. ChAT is encoded by *cha1* and VAChT is encoded by *unc17*, and the expression of these genes are closely linked due to the fact that they are transcribed from the cholinergic gene locus (CGL), which is a unique organization in comparison to other neurotransmitters (Mathews et al., 2015). The presence of both *unc17* and *cha1* determine the characteristic of cholinergic neurons and are present at the earliest point of development.

Unc17

Unc17 codes for VACHTA, an acetylcholinesterase vesicular loader and transporter. A null *unc17* then is hyposensitive to acetylcholine, as neither loading nor transporting of the neurotransmitter will occur. This resulting a cessation in the signal forces the signal to be transmitted by to alternative acetylcholine pathways. In order to understand the interactions between proteins and regulations within cholinergic signaling, I will perform crosses between wild type strains, N2, and *unc17* null strains, CB113, hyposensitive to acetylcholine. I will establish the crossed gene-line as well as perform F_1 Non-Complementation tests on the crossed F_1 males. The hope in this cross is to see whether or not the hypo and hyper sensitive strains can end up regulating each other and establishing a normal phenotypic response to Aldicarb, the acetylcholinesterase inhibitor. I will also perform crosses with wild type strains, N2, as well as with *unc17* GFP-tagged strains, LX919. With this cross I will establish

independent lines as well as perform imaging on created strains. The hope of looking at the localization of VACht is to understand where it localizes within the cells (Mathews et al., 2012).

Thrashing Assay

As a different quantification assay for the locomotive defect among our worms, I will perform thrashing assays, or body bend assays, as a different way to quantify the effect that acetylcholine has on a given strain (Lee et al., 2008). After transferring worms onto a clean plate in order to clean off any possible bacteria, I will transfer the worms to a plate with M9 with Aldicarb (Nawa et al., 2012). Under a camera, I will record the swimming or thrashing behavior of the worms. By scoring the amount of body bends, this will provide a different way to quantify any type of sensitivity to acetylcholine.

CAD cell assay

I will use CAD cells to screen for the serial truncation's processive ability. Using differentiated neuronal CAD cells, the minus ends of the microtubule at the cell body and the plus ends of the microtubule at the synapse, I will transfect the cells with plasmids of the CRISPRed serial truncations of KLP-4 (Soppina et al., 2014). The goal of this assay is to determine the how the kinesins process along the axon, and if and where there are any accumulations of the truncated kinesins.

References and Acknowledgements

Ahn, S.C., Baek, B.S., Oh, T., Song, C.S., and Chatterjee, B. (2000). Rapid mini-scale plasmid isolation for DNA sequencing and restriction mapping. Biotechniques *29*, 466–468.
Akiyama, H., Fukuda, T., Tojima, T., Nikolaev, V.O., and Kamiguchi, H. (2016). Cyclic Nucleotide Control of Microtubule Dynamics for Axon Guidance. J. Neurosci. *36*, 5636–5649.

Chen, L. (2018). Microtubules and axon regeneration in C. elegans. Mol. Cell. Neurosci. *91*, 160–166.

Ghosh-Roy, A., Goncharov, A., Jin, Y., and Chisholm, A.D. (2012). Article Kinesin-13 and Tubulin Posttranslational Modifications Regulate Microtubule Growth in Axon Regeneration. Dev. Cell *23*, 716–728.

Hirokawa, N., Noda, Y., Tanaka, Y., and Niwa, S. (2009). Kinesin superfamily motor proteins and intracellular transport. Nat. Rev. Mol. Cell Biol. *10*, 682–696.

Hirokawa, N., Niwa, S., and Tanaka, Y. (2010). Molecular Motors in Neurons: Transport Mechanisms and Roles in Brain Function, Development, and Disease. Neuron *68*, 610–638.

Homma, N., Takei, Y., Tanaka, Y., Nakata, T., Terada, S., Kikkawa, M., Noda, Y., and Hirokawa, N. (2003). Kinesin Superfamily Protein 2A (KIF2A) Functions in Suppression of Collateral Branch Extension. Cell *114*, 229–239.

Horiguchi, K., Hanada, T., Fukui, Y., and Chishti, A.H. (2006). Transport of PIP $_3$ by GAKIN, a kinesin-3 family protein, regulates neuronal cell polarity. J. Cell Biol. *174*, 425–436.

Huo, L., Yue, Y., Ren, J., Yu, J., Liu, J., Yu, Y., Ye, F., Xu, T., Zhang, M., and Feng, W. (2012). The CC1-FHA tandem as a central hub for controlling the dimerization and activation of kinesin-3 KIF1A. Structure *20*, 1550–1561.

Kapitein, L.C., and Hoogenraad, C.C. (2015). Building the Neuronal Microtubule Cytoskeleton. Neuron *87*, 492–506.

Kondo, M., Takei, Y., and Hirokawa, N. (2012). Motor Protein KIF1A Is Essential for Hippocampal Synaptogenesis and Learning Enhancement in an Enriched Environment. Neuron *73*, 743–757.

Kondo, M., Tanabe, K., Amo-Shiinoki, K., Hatanaka, M., Morii, T., Takahashi, H., Seino, S., Yamada, Y., and Tanizawa, Y. (2018). Activation of GLP-1 receptor signalling alleviates cellular stresses and improves beta cell function in a mouse model of Wolfram syndrome. Diabetologia *61*, 2189–2201.

Kratsios, P., Stolfi, A., Levine, M., and Hobert, O. (2012). Coordinated regulation of

cholinergic motor neuron traits through a conserved terminal selector gene. Nat. Neurosci. *15*, 205–214.

Kratsios, P., Pinan-Lucarré, B., Kerk, S.Y., Weinreb, A., Bessereau, J.-L., and Hobert, O. (2015). Transcriptional Coordination of Synaptogenesis and Neurotransmitter Signaling. Curr. Biol. *25*, 1282–1295.

Kratsios, P., Yen Kerk, S., Catela, C., Liang, J., Vidal, B., Bayer, E.A., Feng, W., Daniel De La Cruz, E., Croci, L., Giacomo Consalez, G., et al. (2017). An intersectional gene regulatory strategy defines subclass diversity of C. elegans motor neurons.

Lasser, M., Tiber, J., and Lowery, L.A. (2018). The Role of the Microtubule Cytoskeleton in Neurodevelopmental Disorders. Front. Cell. Neurosci. *12*, 165.

Lau, C.G., and Zukin, R.S. (2007). NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat. Rev. Neurosci. *8*, 413–426.

Lee, H., Cho, J.S., Lambacher, N., Lee, J., Lee, S.-J., Lee, T.H., Gartner, A., and Koo, H.-S. (2008). The Caenorhabditis elegans AMP-activated protein kinase AAK-2 is phosphorylated by LKB1 and is required for resistance to oxidative stress and for normal motility and foraging behavior. J. Biol. Chem. *283*, 14988–14993.

Lee, J.-R., Shin, H., Choi, J., Ko, J., Kim, S., Lee, H.W., Kim, K., Rho, S.-H., Lee, J.H., Song, H.-E., et al. (2004). An intramolecular interaction between the FHA domain and a coiled coil negatively regulates the kinesin motor KIF1A. EMBO J. *23*, 1506–1515.

Li, J., Zhang, Y. V, Asghari Adib, E., Stanchev, D.T., Xiong, X., Klinedinst, S., Soppina, P., Jahn, T.R., Hume, R.I., Rasse, T.M., et al. (2017). Restraint of presynaptic protein levels by Wnd/DLK signaling mediates synaptic defects associated with the kinesin-3 motor Unc-104. Elife *6*, e24271.

Liu, H., Li, L., Wang, W., Gong, J., Yang, X., and Hu, Z. (2018). Spontaneous Vesicle Fusion Is Differentially Regulated at Cholinergic and GABAergic Synapses. Cell Rep. *22*, 2334–2345.

Ma, L., Zhao, Y., Chen, Y., Cheng, B., Peng, A., and Huang, K. (2018). Caenorhabditis elegans as a model system for target identification and drug screening against neurodegenerative diseases. Eur. J. Pharmacol. *819*, 169–180.

Maday, S., Twelvetrees, A.E., Moughamian, A.J., and Holzbaur, E.L.F. (2014). Axonal Transport: Cargo-Specific Mechanisms of Motility and Regulation. Neuron *84*, 292–309.

Mathews, E.A., Mullen, G.P., Hodgkin, J., Duerr, J.S., and Rand, J.B. (2012). Genetic interactions between UNC-17/VAChT and a novel transmembrane protein in Caenorhabditis elegans. Genetics *192*, 1315–1325.

Mathews, E.A., Mullen, G.P., Manjarrez, J.R., and Rand, J.B. (2015). Unusual regulation of splicing of the cholinergic locus in Caenorhabditis elegans. Genetics *199*, 729–737.

Nawa, M., Kage-Nakadai, E., Aiso, S., Okamoto, K., Mitani, S., and Matsuoka, M. (2012). Reduced expression of BTBD10, an Akt activator, leads to motor neuron death. Cell Death Differ. *19*, 1398–1407.

Rakowski, F., Srinivasan, J., Sternberg, P.W., Karbowski, J., Troyer, T., and Balaguer-Ballester, E. (2013). Synaptic polarity of the interneuron circuit controlling C. elegans locomotion.

Siddiqui, N., and Straube, A. (2017). Intracellular cargo transport by kinesin-3 motors. Biochem. *82*, 803–815.

Solinger, J.A., Paolinelli, R., Klöß, H., Scorza, F.B., Marchesi, S., Sauder, U., Mitsushima, D., Capuani, F., Stürzenbaum, S.R., and Cassata, G. (2010). The Caenorhabditis elegans Elongator Complex Regulates Neuronal α-tubulin Acetylation. PLoS Genet. *6*, e1000820.

Soppina, V., and Verhey, K.J. (2014). The family-specific K-loop influences the microtubule on-rate but not the superprocessivity of kinesin-3 motors. *25*.

Soppina, V., Norris, S.R., Dizaji, A.S., Kortus, M., Veatch, S., Peckham, M., Verhey, K.J., and Mcintosh, J.R. (2014). Dimerization of mammalian kinesin-3 motors results in superprocessive motion. Proc. Natl. Acad. Sci. U. S. A. *111*, 5562–5567.

Van Spronsen, M., and Hoogenraad, C.C. (2010). Synapse Pathology in Psychiatric and Neurologic Disease.

Tanaka, Y. (2015). Kinesin superfamily proteins (KIFs): Various functions and their relevance for important phenomena in life and diseases. Exp. Cell Res. *334*, 16–25.

Vale, R.D. (2003). The Molecular Motor Toolbox for Intracellular Transport. Cell *112*, 467–480.

Wickstead, B., Gull, K., and Richards, T.A. (2010). Patterns of kinesin evolution reveal a complex ancestral eukaryote with a multifunctional cytoskeleton. BMC Evol. Biol. *10*, 110.

Yue, Y., Sheng, Y., Zhang, H.-N., Yu, Y., Huo, L., Feng, W., and Xu, T. (2013). The CC1-FHA dimer is essential for KIF1A-mediated axonal transport of synaptic vesicles in C. elegans. Biochem. Biophys. Res. Commun. *435*, 441–446.

Thanks to:
Margaret Magaletta and Kendall Perkins