**Statement of Research Interests**

I use a combination of molecular genetics, cell biology, and electrophysiology in the nematode *Caenorhabditis elegans* to answer important and difficult questions in neuroscience. Although I have a long-standing interest in how receptors and ion channels function in sensory neurons, my recent work has emphasized that intracellular transport is exceptionally important for delivery of receptors and other cargos along the long dendrites and axons of neurons. Therefore, I have a strong interest in how neurons regulate microtubule-based intracellular transport, neurite outgrowth, and especially regeneration after injury. *C. elegans* is an excellent system for both forward and reverse genetics, and most assaysare well suited to student researchers and require only basic equipment, such as stereo-dissecting microscopes and compound epifluorescence-equipped microscopes. Using the unique attributes of *C. elegans,* I can investigate the function of molecules in an animal nervous system at a level of detail not possible in any other system.

**Background**

I previously found that post-translational glutamylation of microtubules (MTs) can regulate both cellular traffic and cytoskeletal stability in cilia, antenna-like sensory organelles. Localization and trafficking of ciliary receptors in worm neurons is defective in *ccpp-1* mutants [[1](#_ENREF_1)]. The *ccpp-1* gene encodes CCPP-1, a deglutamylase that reduces glutamylation on MTs. CCPP-1 regulates the function of particular kinesins and stability of ciliary MTs [[1](#_ENREF_1)].

In mammals, loss of the homolog of CCPP-1 causes progressive neurodegeneration [[2](#_ENREF_2)]. The same deglutamylase gene is “turned on” in injured spinal cord neurons mice [[3](#_ENREF_3), [4](#_ENREF_4)], suggesting MT glutamylation is a determinant of neuronal survival and regeneration. My research to unravel the functions of MT glutamylation should be of medical importance in treatment of neurodegenerative diseases and spinal cord injury.

**Future Research Goals**

**1) Determine the molecular pathways by which post-translational glutamylation controls MT stability and transport in cilia, dendrites, and axons.**

Loss of the deglutamylase CCPP-1 leads to hyperglutamylation of MTs, which causes the progressive deterioration of amphid cilia. Mutations in the glutamate ligase-encoding genes *ttll-4*, *ttll-5,* or *ttll-11* suppress the degeneration of amphid cilia, suggesting that a balance of glutamylation and deglutamylation of MTs regulates the stability of cilia.

**Fig. 1 Dye-filling of amphid neurons with intact cilia**

Loss of amphid neuron cilia is complete in *ccpp-1* mutant

adults, as shown by dye-filling assay. Suppressor mutants

displayed a wild-type phenotype.

To determine what other factors are involved in regulation of MT stability by post-translational glutamylation, I led a team of undergraduates (Winnie Zhang, Maggie Morash, and Sebastian Bellotti) to perform a mutagenesis screen for suppressors of the ciliary degeneration in *ccpp-1* mutants.

This analysis relies on a “Dye-filling Assay,” in which animals take up dye into the amphid neurons only if their sensory cilia are intact (See Fig. 1; [[5](#_ENREF_5)]). We screened for mutants that suppressed *ccpp-1,* which appeared “bright” among all the “dark” animals and identified fifteen promising mutants that will form the basis of my future independent work.

**Fig. 2**

**Hypothetical Suppressors of *ccpp-1***

I hope to identify molecules that

interpret the Tubulin Code to modulate

transport and MT stability

I plan to identify these *ccpp-1* suppressors, which may include glutamylation cofactors, essential residues in tubulins, MT-associated proteins, MT-severing enzymes, and molecular motors (Fig. 2). Molecules we identify in our screen may be of medical importance as therapeutic targets in treating neurodegeneration and promoting regeneration after neuronal injury.

**2) Determine the function of polycystins and other ion channels in**

***C. elegans* neurons**

In mammalian kidney epithelia and in *C. elegans* sensory neurons, the polycystin complex localizes to cilia [[6](#_ENREF_6), [7](#_ENREF_7)]. In humans, mutations that affect the polycystins PKD1 or PKD2 cause autosomal dominant polycystic kidney disease (ADPKD), in which kidneys become filled with cysts and progressively cease functioning [[8](#_ENREF_8)]. In *C. elegans*, both polycystins LOV-1 and PKD-2 are required in male-specific ciliated sensory neurons for normal mating behavior [[6](#_ENREF_6), [7](#_ENREF_7)]. Because the function of the polycystins in both *C. elegans* male neurons and in the human kidney has not been clearly established, I plan to use electrophysiology in vivo in *C. elegans* neurons to elucidate the function of the LOV-1/PKD-2 complex.

The polycystin complex was previously proposed to transduce mechanical stimuli associated with urine flowing through the kidneys, as kidney cells lacking PKD1 lacked Ca2+ responses to fluid flow in vitro [[9](#_ENREF_9)]. Polycystins have also been proposed to transduce chemical stimuli, possibly as sour taste receptors: a polycystin complex formed by PKD1L3 (PKD1-Like-3) and PKD2L1 (PKD2-Like-1) can open in response to application of acid when co-expressed in heterologous cells [[10](#_ENREF_10), [11](#_ENREF_11)]. However, it remains unclear whether a polycystin channel directly transduces either mechanical or chemical cues in vivo in animals.

Since polycystin-expressing neurons in *C. elegans* are required in males for mating behavior, the polycystins are thought to sense cues to detect hermaphrodite mates [[12](#_ENREF_12)]. I plan to determine if *C. elegans* polycystin neurons respond to pheromones, acid, or other chemical stimuli and then use electrophysiology to determine if sensation requires the LOV-1/PKD-2 channel complex (Fig.3).

The polycystins may also be involved in a role other than sensory transduction: for example, non-selective polycystin cation channels have been shown to control the activity BK potassium channels, both by mediating Ca2+ influx and by putative protein interactions [[13](#_ENREF_13)]. In this case, activation of a polycystin channel could actually promote hyperpolarization and silencing of neurons by activating BK channels. Might polycystins regulate the excitability of worm neurons by activating SLO-1, the BK K+ channel subunit encoded in the *C. elegans* genome [[14](#_ENREF_14)]? Electrophysiology in *C. elegans* neurons, when combined with powerful genetics, and convenient behavioral assays, has the resolution to answer such questions in fine detail and unravel how molecules function in neurons, leading, in turn, to a behavioral output.

Determination of the function of the PKD-2 TRP channel in *C. elegans* should be informative for understanding the role of the polycystins in kidney function, in both health and disease. The functions of many other types of ion channels are amenable to similar investigation in *C. elegans.* Although electrophysiology in *C. elegans* neurons in vivo is definitely the more challenging project I plan to tackle, talented researchers may have an aptitude for it.

**Fig. 3 Electrophysiology of sensory neurons in the *C. elegans*****male tail**

After dissection to gain access to the cell body,whole-cell voltage clamp

recording is used to measure responses to voltage stimuli, as shown at right.

In the future, I plan to activate sensory neurons with chemical stimuli.

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