Stress-Dependent Cilia Remodeling in *C. elegans*

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Abstract

Primary cilia are sensory organelles that act as signaling hubs found on the surface of most cells in vertebrates. The goal of this project was to use *C. elegans* as a model to study the mechanisms by which cilia respond to physiological stress. *C. elegans* were subjected to heat and cold-stress assays, and their ciliated neurons were imaged to examine stress response. Primary cilia exhibited shortening in response to both heat and cold shock. Understanding the mechanisms by which cilia respond to stress may allow researchers to reverse adverse effects of environmental stress on ciliary signaling and associated disorders.

Acknowledgements

I would like to thank first and foremost both of my advisors, Professors Inna Nechipurenko and Jim Doyle, for their hard work guidance throughout this year long culmination of my undergraduate experience, as well as their support and patience through what proved to be a difficult and complicated year. I am also thankful to the Nechipurenko lab in helping me learn the techniques and practices needed to accomplish this project, especially Eric Peet and graduate student Christina Campagna, who helped me troubleshoot potential technical difficulties that arose over the course of the project and instructed me in how to image cilia on the Leica Thunder microscope and how to create graphs and statistical analyses using the Prism software. I would also like to extend my gratitude to the Psychological Sciences department and faculty who provided additional guidance and support week after week, as well as my fellow Psych majors who never failed to be positive, supportive, and encouraging as we tackled our projects side-by-side.

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Introduction

Primary cilia are found on the surface of most vertebrate cell types, and they are essential for proper communication between cells and their environment (Venkatesh, 2017). Primary cilia contain molecular machinery of most major signal transduction pathways and therefore play critical roles during development and in adult tissues (Venkatesh, 2017). The critical importance of cilia for human health is highlighted by the fact that defects in cilia structure or function lead to genetic disorders known as ciliopathies (Waters & Beales, 2011). Ciliopathies can affect most organ systems, and commonly manifest in retinal degeneration, renal disease, and neurodevelopmental defects (Water & Beales, 2011). In many cell types, primary cilia are dynamic and can shorten or elongate during normal tissue development and in response to environmental stress. For example, heat shock of mammalian cells *in vitro* and zebrafish *in vivo* induces resorption of primary cilia (Prodromou et al., 2012). Additionally, increased production of reactive oxygen species (ROS) by the mitochondria impairs cilia assembly in cultured mouse epithelial cells (Moruzzi et al., 2022). Further studies highlight the role of ROS on primary cilia length and show that ischemic kidney injury, which is associated with ROS overproduction, causes cilia shortening in mouse kidney cells, while treatment of ischemic kidneys with the antioxidant MnTMPyP facilitates recovery of cilia length (Kim et al., 2013). Importantly, changes in cilia length are associated with altered cellular signaling during development (Toro-Tapia & Das, 2020) and in the adult tissues (Blitzer et al., 2011; Tu et al., 2023). Despite these findings, the mechanisms by which certain stressors facilitate resorption or remodeling of primary cilia remain largely unknown. The goal of this project is to establish *C. elegans* as a model system for studying stress-dependent cilia remodeling and to begin uncovering the mechanisms that mediate cilia remodeling in response to environmental stress. This would

ultimately allow for the better understanding of ciliopathies and may eventually be the foundation for researchers to develop therapeutics to combat cilia dysfunction and cilia-related disorders that cause severe impairment to vital organs and tissues.

Primary cilia are signaling hubs present on the surface of most vertebrate cells.

Primary cilia are non-motile and range in length from 2-12 µm (Macarelli et al., 2023). At the core of the primary cilium is the microtubule-based structure called the axoneme. The axoneme is made up of nine doublet microtubules extending from a basal body, a modified centriole that nucleates the cilium (Figure 1) on the cell surface.

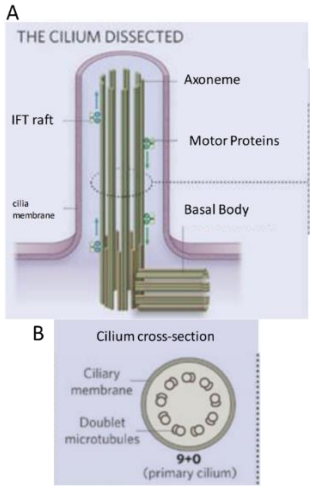

Figure 1. Primary cilia structure (A) IFT rafts and motor proteins mediate cilium assembly and move ciliary protein cargoes along the axoneme. The basal body anchors the cilium to the cell surface (Adams, 2010). (B) Cross-section view of a primary cilium. The axoneme is comprised of 9 doublet microtubules and surrounded by specialized ciliary membrane.

Intraflagellar transport (IFT) is the major intra-ciliary transport system composed of multisubunit protein particles, IFT-A and IFT-B. These particles move bidirectionally along an axoneme and carry structural components needed for axonemal assembly as well as signaling molecules required for normal cilia function (Anvarian et al., 2019). IFT-B complexes polymerize on their own at the base of an axoneme; this event then acts as a catalysis for the polymerization of IFT-A complex and the recruitment of kinesin 2 motor proteins, which help move the assembled IFT complex inside the cilia (Lacey et al., 2023). Structural and signaling molecules, acting as cargo, bind to the “train” of the IFT complex and are carried to the tip of the cilia, at which point the

complex remodels and takes up new “cargo” and is carried back to the cilia base by dynein-2 motor proteins (Lacey et al., 2023, Figure 2).

Primary cilia contain components of many signaling pathways including G protein-coupled receptors (GPCR), Sonic hedgehog (SHH), Wingless-related integration site (Wnt) and transforming growth factor-beta (TGF-β)/bone morphogenetic protein (BMP) that play important roles in neurodevelopment, cell proliferation, and cell differentiation (Macarelli et al., 2023; Anvarian et al., 2019). Primary cilia are also responsible for sensing external/environmental signals, such as light, odorants, hormones, mechanical signals, morphogens, growth factors, and fluid movement, and for releasing extracellular vesicles to communicate with other cells or organisms within the species (Anvarian et al., 2019; Wang et al., 2021). Primary cilia can also have a protective effect against some types of environmental stress (Bae et al., 2023; Ishii et al, 2020). For example, in mouse embryos neuronal primary cilia activated insulin-like growth factor 1 (IGF-1) receptor and downstream AKT signaling upon fetal exposure to alcohol and ketamine to protect neurons from the harmful effects of these substances (Bae et al., 2023). In human neuroblastoma cell line, SH-SY5Y, the loss of heat shock protein HSPA9, led to impairment of the mitochondrial function and overproduction of ROS, which was concomitant with increased ciliogenesis (Ishii et al., 2020). The increased ciliogenesis in this context led to activation of the AKT pathway to counter the ROS-mediated neurotoxicity and promote cell survival (Ishii et al., 2020).

Mechanisms that modulate cilia length

Actin

F actin and actin polymerization play a critical role in regulating cilia length (Smith et al., 2020). A stable actin and microtubule network helps promote both ciliogenesis and proper

positioning of the centrosome (Brücker et al., 2020). However, polymerization of actin and F-actin branching inhibit primary cilia formation, while actin depolymerization typically enhances ciliogenesis. Actin polymerization, mediated by ARP2/3 and WASp, acts as a physical barrier to cilia-targeted vesicle transport and to the membrane remodeling that is essential for ciliogenesis (Smith et al., 2020). F actin also actively helps to create stress fibers which further restrict cilia growth (Smith et al., 2020). Recently a study on protein LUPZ1 has explored its complex relationship with cilia length (Bozal-Basterra et al., 2020). LUPZ1 localizes to centrosomes and the actin skeleton and helps to regulate primary cilia dynamics, and while in the absence of LUPZI there are longer cilia, increased ciliogenesis and increased SHH signaling, researchers also hypothesize that LUPZ1 may form complexes with actin and filamin that may help to strengthen the axoneme in times of elongation or stress (Bozal-Basterra et al., 2020).

Actin polymerization has also been implicated in cilia decapitation or fragmentation, which is when fragments of primary cilia, or in some cases the entire cilia, break off from the cilia base, severely shortening the cilia and therefore limiting and disrupting its normal functionality (Phua et al., 2017).

IFT

IFT is required for ciliogenesis, specifically loss of many IFT-B complex components results in shortened cilia (Wang et al., 2021; Lechtreck, 2015). Once the basal body has docked at the surface of a cell, IFT and transition zone proteins are recruited to the basal body, along with Rab8a which promote cilia assembly (Wang et al., 2021). IFT transports tubulin – the building block of the axoneme – to the cilia tip, thus facilitating cilia elongation (Wang et al., 2021). IFT-A most likely functions mainly to carry IFT-B back to the basal body from the cilia tip, where IFT-B is recycled (Figure 2). However, IFT-A mutants also exhibit depletion of

certain membrane proteins, which implies that IFT-A contributes to trafficking of ciliary membrane proteins (Lechtreck, 2015, Figure 2). IFT is also responsible for the maintenance of protein homeostasis in cilia and the transport of signaling proteins to and from the cilia to regulate different cilia signaling pathways (Lechtreck, 2015).

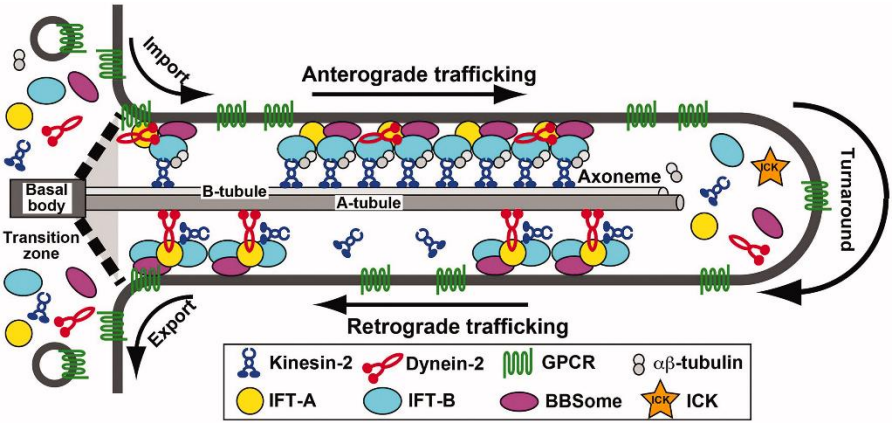

Figure 2. Schematic diagram of IFT and associated proteins within the cilia (Nakayama & Katoh, 2020). IFT-A and IFT-B complex together at cilia base where they are transported up the axoneme by motor protein kinesin-2 while carrying proteins essential for cilia maintenance and assembly, such as αβ-tubulin. Once the IFT complex reaches the cilia tip, the cargo is released, and the complex is transported back to the basal body via motor protein dynein-2.

PI(4,5)P2 signaling

Polyphosphoinositides (PPIs) are signaling lipids that populate distinct membrane domains in cells. For example, the ciliary membrane contains high levels of PI(4)P, while PI(4,5)P2 is enriched in plasma membrane at the cilia base. INPP5E is an enzyme that dephosphorylates PI(4,5)P2 and converts it into PI(4)P in the cilia membrane (Chen et al., 2022; Nechipurenko, 2020). Under certain conditions, such as serum stimulation, PI(4,5)P can accumulate in distal cilia inducing actin polymerization and subsequent cilium ‘decapitation’ (Phua et al., 2017; Nechipurenko, 2020). It is thought that the actin polymerization caused by

actin regulators binding to PI(4,5)P2 molecules is regulated by the AurA/HDAC6 pathway (Chen et al., 2022). The increased levels of PI(4,5)P2 in cilia results in shorter cilia length and is associated with a ciliopathy (Stilling et al., 2022). In contrast, the absence of PI(4,5)P2 from the ciliary membrane is associated with cilia elongation. Notably, ciliopathies caused by excess intraciliary PI(4,5)P2 may be ameliorated by small molecule inhibitors of AurA and HDAC6 (Stilling et al., 2022).

C. elegans is a promising model organism for studying stress response in cilia.

C. elegans constitute a promising model organism for studying cilia response to stress. First, the only ciliated cells in *C. elegans* are sensory neurons (Inglis et al., 2018); as a result, cilia are not required for viability in this organism unlike in mammals. Second, molecular mechanisms of cilia assembly tend to be highly conserved across evolution making findings in *C. elegans* generally translatable to higher-order organisms. Third, the extensive genetic toolkit available in C. elegans allows one to easily manipulate the C. elegans genome to determine the roles of different genes in cilia biology.

For this project I focused on cilia found on a subset of sensory neurons in the worm head (ASH (Figure 3)) and tail (PHA or PHB (Figure 3)) (Inglis et al., 2018). ASH neurons are a pair of nociceptive neurons in the amphid – the main sensory organ in *C. elegans* (Bae & Barr, 2008). ASH neurons have simple rod-like cilia that are exposed to the environment and are ~7µm in length (Bae & Barr, 2008). PHA/PHB neurons share similar properties and cilia structure with the ASH neurons (Bae & Barr, 2008).

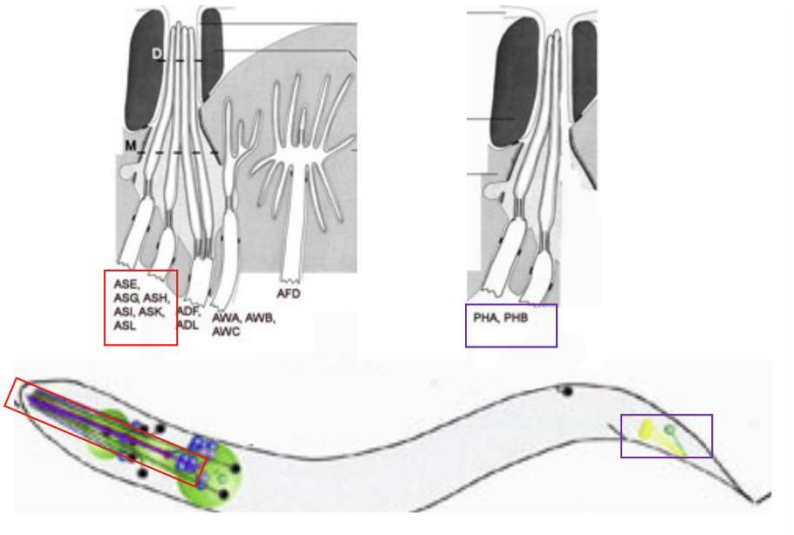

Figure 3. Location and morphology of ciliated neurons in *C. elegans* (Inglis et al., 2018). In this project, cilia were examined in the ASH neurons (morphology and location denoted in red) and PHA/PHB neurons (denoted in purple).

Ric-8 regulates cilia morphology in C. elegans neurons.

C. elegans RIC-8 is homologous to the human RIC8A and RIC8B proteins. RIC8 proteins function as chaperones and guanine nucleotide exchange factors (GEF), to facilitate folding and catalyzes the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) in heterotrimeric G alpha proteins, respectively (Seven et al., 2020). Additionally, in *Cryptococcus deneoformans* the RIC8 protein was implicated in increased thermal tolerance and resistance to oxidative stress, hinting at RIC8's role as a possible cellular stress protectant (Roth et al., 2021).

RIC-8 plays a critical role in the development and morphology of specialized olfactory cilia (AWA, AWB, AWC) in *C. elegans* (Campagna et al., 2023). In *ric-8* null worms, the size of the specialized olfactory ('wing') cilia was dramatically reduced. On the other hand, the length of rod ASH cilia was only mildly affected, and the overall morphology of ASH cilia in *ric-8* null neurons showed no obvious differences from wild type (Campagna et al., 2023). Additionally,

RIC-8 localizes within the primary cilia of sensory neurons, specifically in the proximal ~4 μm of the rod cilia with its highest concentration at ~1 μm from the cilia base (Campagna et al., 2023). RIC-8's chaperone and Gα-binding motifs are necessary for its proper functioning in cilia morphogenesis (Campagna et al., 2023).

Project Goals

The goals of this project were as follows:

1. To establish *C. elegans* as an experimental model to study cilia remodeling in response to stress.
2. To define the role of RIC-8 in regulating cilia stress response
3. Test the role of actin regulatory proteins in stress-dependent cilia remodeling

Methods

Maintenance of *C. elegans*

C. *elegans* strains were grown and maintained at 20C on standard nematode growth media plates seeded with OP50 strain of *E. coli*. *C. elegans* strain N2 (variety Bristol) with the extrachromosomal array *Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp::dsRed]* was used as wildtype. Transgenic mutant strains were created using the standard germline transformation technique. New strains were confirmed using PCR and gel electrophoresis. The following strains were created for this project: *ric-8(md1909); Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp::dsRed], ags-3(ok1169); Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp:: dsRed], glsn-1(ok2979); Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp:: dsRed]*.

Heat-shock and cold-shock population assays

C. *elegans* were subjected to multiple conditions of heat or cold shock prior to imaging. For heat-shock assays, 20-30 L4 hermaphrodite *C. elegans* were picked onto a new plate and left overnight at 20C to age to one-day old adults. The next day the petri dishes containing one-day old adult *C. elegans* were parafilmed and placed carefully cap side up into a water bath at 35C for either 30 min or 3 hrs to induce heat shock. After heat shock, the plate was removed from the water bath, and heat shocked worms were allowed to recover in the 20 C incubator for 20 minutes prior to imaging. For cold-shock assays, 20-30 L4 *C. elegans* were picked onto a new plate and immediately placed in the 4C cold room for 24 hours to induce cold shock. After the 24-hr cold shock, the animals were allowed to recover in a 20C incubator for 30 minutes prior to imaging.

Microscopy

On the day of imaging, 10% agarose pads (10mL M9, 1g agarose) were placed on top of the microscope slides. For imaging, *C. elegans* were anesthetized using a freshly prepared 10mM tetramisole solution that was applied directly on to a coverslip. *C. elegans* were then collected in a 3uL drop of the tetramisole solution and the microscope slide was very gently placed onto the coverslip with the agarose pad against the worms. The animals were imaged on upright THUNDER imager 3D tissue (Leica). Complete z stacks of ciliated neurons (ASH, PHA/PHB) were acquired at 0.22µm intervals with K5 sCMOS camera (Leica) in Leica Application Suite X software using an HC Plan Apochromat 63X NA 1.4-0.60 oil immersion objective.

Results

Primary cilia in *C. elegans* neurons shorten in response to heat and cold stress.

To determine whether *C. elegans* could be used as a model to study cilia remodeling in response to environmental stress, I subjected wild-type *C. elegans* (N2) expressing a fluorescent marker that labels ASH neurons (*sra-6*p*::myr-gfp*) (Figure 4A) to a 3-hour heat shock or a 24-hour cold shock (see Methods for experimental details). ASH cilia showed significant shortening in response to both the heat and cold shock in wild-type animals (Figure 4B and 4C). Specifically, in the control animals that were not subjected to stress, ASH cilia were a mean of 5.9 μm long and show a continuous rod-like morphology (Figure 4C). However, after both the 3-hour heat shock and the 24-hour cold shock, ASH cilia are significantly shorter with 3.85 μm mean length in 3hr HS condition ($p = < 0.0001$) and 4.08 μm mean length in 24hr CS condition ($p = < 0.0001$) (Figure 4B) and exhibit distorted morphology characterized by bulging, cilia branches, and fragmentation (Figure 4C, Table 1, Table 2). Taken together, these results demonstrate that cilia in *C. elegans* neurons shorten in response to environmental stress as was previously established in vertebrate models (Prodromou et al., 2012) and suggest that *C. elegans* can be used as an effective model to investigate cilia remodeling in response to stress.

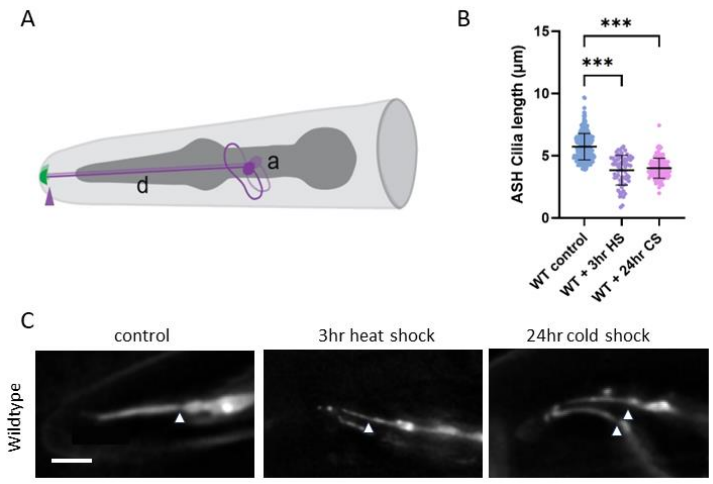

Figure 4. Cilia in ASH neurons shorten in response to heat and cold shock. (A) Diagram of head neuron in *C. elegans*; cilia are labeled in green (Campagna et al., 2023) **(B)** Quantification of ASH cilia length in *Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp::dsRed]* (WT, wild type) animals after the 3-hour heat shock and 24-hour cold shock. *** indicates different between marked conditions at p<0.001 (Kruskal-Wallis test) **(C)** Representative images of ASH neurons in *C. elegans* after indicated stress paradigms. White arrow marks cilia base; scale bar: 3 µm

ASH cilia in *ric-8(md1909)* mutant animals exhibit greater shortening and fragmentation compared to wildtype.

In the fungal pathogen *C. deneoformans*, RIC8 was demonstrated to regulate thermal tolerance and oxidative stress resistance (Roth et al., 2021). So next I wanted to test whether *C. elegans* RIC-8, which localizes to neuronal cilia, contributes to cilia remodeling in response to stress. To this end, *ric-8(md1909)* mutants were subjected to 3-hour heat shock, and their cilia length and overall morphology were compared to those of wildtype animals. Both wildtype and *ric-8(md1909*) mutants showed significant shortening of ASH cilia in response to 3-hour heat shock compared to their respective non-heat-shocked controls (Figure 5). Furthermore, heatshocked *ric-8* mutants exhibited shorter cilia compared to heat-shocked wildtype after a heat shock event

(*ric-8* mutant: 3.22 µm long cilia, wildtype: 3.85 µm) (Figure 5), suggesting that *ric-8* may contribute to cilia remodeling after heat stress.

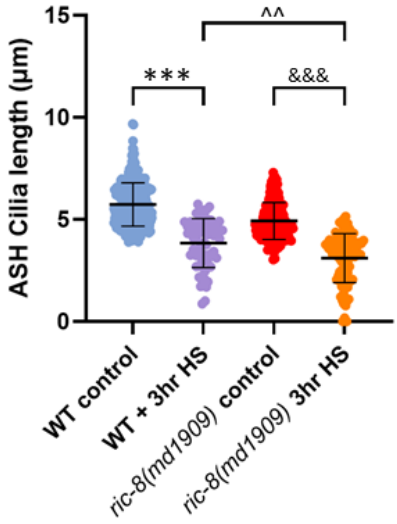

Figure 5, ASH cilia in *ric-8(md1909)* mutants shorten to a greater extent than in wildtype after heat stress. Quantification of ASH cilia length in *Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp::dsRed]* (WT) and *ric-8(md1909); Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp::dsRed]* after the 3-hour heat shock. *** indicates difference between marked conditions at p<0.001 (Kruskal-Wallis). *** and &&& represent p = < 0.0001, ^^ represents p = 0.0098.

In addition to changes in cilia length, I noted that 3-hour heat shock increased cilia fragmentation in wildtype, and *ric-8(md1909)* mutant animals (Table 1). For example, only 4.77% of wild-type ASH cilia exhibited fragmentation under control conditions, while after heat shock 52.9% of ASH cilia were fragmented. Similarly, in *ric-8(md1909)* mutants, 2.47% of ASH cilia were fragmented under control conditions, and 61.9% of cilia were fragmented after heat shock (Table 1), suggesting that ASH cilia may shorten in response to stress by shedding their axonemes.

Table 1. Cilia fragmentation increases after a heat stress event. Cilia fragmentation was defined here as when any piece of cilia was disconnected from the neuron. WT: *Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp::dsRed]; ric-8(md1909): ric-8(md1909); Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp::dsRed].*

Condition	Total visible cilia	Cilia Fragmentation	% fragmented cilia
WT control	461	22	4.77
WT 3hr HS	121	64	52.89
***ric-8(md1909)* control**	243	6	2.47
***ric-8(md1909)* 3hr HS**	126	78	61.9

Levels of RIC-8 inside cilia relative to dendrites increase after a heat stress event.

Given the increased shortening of ASH cilia in *ric-8(md1909)* mutants compared to wild type after heat stress, I next wanted to determine if RIC-8 levels or sub-cellular localization change in response to heat stress. To address this question, I subjected wild type animals to either a 30-minute or 3-hour heat shock and examined localization of RIC-8::TagRFP in *C. elegans* tail neurons (PHA/B). The TagRFP fluorescence intensity was measured in the proximal 3.5-µm cilia segment and 2-µm distal dendrite segment and expressed as a ratio of cilia to dendrite fluorescence intensity (Figure 6). The relative RIC-8::TagRFP levels inside cilia compared to dendrites significantly increased after 3-hour heat shock ($p = < 0.0001$), although there was no significant difference after 30 minutes heat shock ($p = 0.667$) Figure 6A and 6B). This result suggests that RIC-8 ciliary localization is regulated by stress.

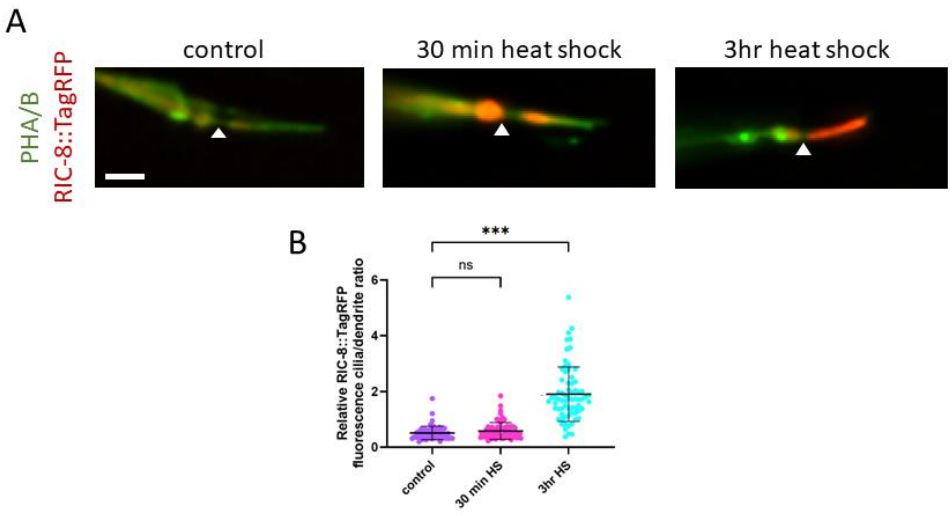

Figure 6. Relative levels of RIC-8 inside cilia increase after a heat shock event. (A) Images of PHA/B neurons in *Ex[bbs-8*p*::myr-gfp, bbs-8*p*::ric-8::tagrfp, unc-122Dp::gfp]* under the indicated conditions; PHA/B neurons are labeled in green (GFP), RIC-8::TagRFP protein is shown in red (RFP). White arrow marks cilia base; Scale bar: 2 µm. **(B)** Quantification of RIC-8::TagRFP fluorescence intensity in cilia vs dendrite. *** indicates different between marked conditions at p<0.001 (Kruskal-Wallis test)

gsnl-1 is not required for heat-shock-dependent cilia shortening.

Actin plays an important role in cilia length regulation and fragmentation (Smith et al., 2020; Phua et al., 2017). Additionally, transcript levels of several actin regulators with established roles in ciliogenesis have been reported to be upregulated following a 3-hour heat shock in *C. elegans* (Jovic et al., 2017). So next I wanted to test whether *C. elegans* gelsolin-like protein 1 (*gsnl-1*), whose transcript levels were increased after heat stress (Jovic et al., 2017) contributes to stress-dependent cilia remodeling. g*snl-1(ok2979)* mutants were subjected to 3-hour heat shock and their ASH cilia were compared to those of wildtype. Of note, under control conditions, *gsnl-1* mutant ASH cilia appear slightly longer compared to wildtype. I found that *gsnl-1(ok2979)* mutants showed no significant difference in ASH cilia length after a heat stress event compared to wildtype (gsnl-1 3hr heat shock mean length: 3.18 µm, p = 0.2405) (Figure 7). Additionally,

ASH cilia in *gsnl-1* mutants exhibited cilia fragmentation after 3-hour heat shock with the penetrance similar to that observed in wild type and *ric-8* mutants (Table 2). Together, these results suggest that GSNL-1 does not play a major role in cilia shortening in response to heat stress.

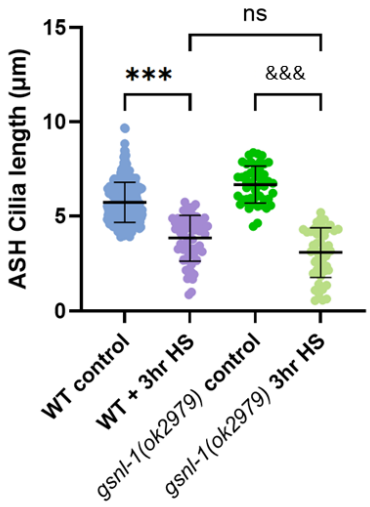

Figure 7. *gsnl-1* is not required for ASH cilia shortening in response to heat shock. Quantification of ASH cilia length in *Ex[sra-6p::myr-gfp, sra-6p::mksr-2::tagrfp, unc-122Dp::dsRed]* (WT) and *gsnl-1(ok2979); Ex[sra-6p::myr-gfp, sra-6p::mksr-2::tagrfp, unc-122Dp::dsRed]* animals following 3-hour heat shock. ***/&&& indicates different between marked conditions at p<0.001 (Kruskal-Wallis test). *** and &&& represent $p = < 0.0001$.

In addition to changes in cilia morphology after heat shock, I noted dendritic defects that included branching, bulging, fragmentation, other distortions, and expansions in ASH neurons of all examined genotypes (Table 2), suggesting that dendrite integrity could be similarly compromised after heat shock.

Table 2. Summary of defects in cilia and dendrite morphology in the indicated genotypes are after a 3-hour heat stress event. WT: *Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122Dp::dsRed]; ric-8(md1909): ric-8(md1909); Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122D p::dsRed]; gsnl-1(ok2979):gsnl-1(ok2979); Ex[sra-6*p*::myr-gfp, sra-6*p*::mksr-2::tagrfp, unc-122D p::dsRed]*

Condition	Number of quantified cilia	Non-uniform thickness/ bulging(%)	Cilia branching (%)	Dendritic Defects (%)
WT control	301	1.66	0.33	2.66
WT 3hr HS	67	35.82	2.99	11.94
***ric-8(md1909)* control**	194	1.03	0	3.09
***ric-8(md1909)* 3hr HS**	90	26.67	2.22	8.89
***gsnl-1(ok2979)* control**	49	2.04	0	0
***gsnl-1(ok2979)* 3hr HS**	46	43.48	4.35	17.39

Discussion

My MQP project demonstrated that cilia in *C. elegans* neurons shorten in response to heat and cold stress similarly to what has been shown in the vertebrate models. Thus, *C. elegans* can be used as a valuable genetic model to study cilia remodeling in response to stress. In the future, candidate-gene approach and forward genetic screens can be conducted to identify molecular mechanisms that underlie cilia remodeling in response to heat and/or cold stress. These mechanisms may also lend insight into the other environmental and cellular stresses, such as oxidative stress, that may impact cilia regulation and cause ciliopathies.

Additionally, I showed that after 3-hour heat shock, ASH cilia in *ric-8(md1909)* mutants were significantly shorter than in wild type and that RIC-8 ciliary levels in phasmid (tail) cilia were increased relative to the dendrite. The shorter cilia found in *ric-8(md1909)* mutants may indicate a potential protectant effect RIC-8 has on the cilia after a stress event occurs, similar to the way RIC-8 functions to protect against oxidative stress in *C. deneoformans* (Roth et al., 2021). The most interesting result from this project is perhaps the increased localization of RIC-8 to the cilia after heat stress. Primary cilia concentrate G protein coupled receptors (GPCRs) and their downstream transducers such as G alpha proteins (Schou et al., 2015). RIC-8 is a chaperone that facilitates G alpha protein folding and therefore G protein signaling. Perhaps RIC-8 is recruited to cilia after stress to help re-fold G alpha proteins and ensure continued cilia signaling. The next step would be to monitor ciliary G protein folding and signaling after heat shock in the presence and absence of RIC-8.

Actin regulatory proteins play important roles in cilia length control, and transcripts of several actin regulators are increased after 3-hour heat shock in *C. elegans* (Jovic et al., 2017)

suggesting that actin dynamics may contribute to cilia remodeling in response to stress. I examined mutants in one actin regulator that is transcriptionally upregulated after heat stress in *C. elegans gsnl-1* and found no significant difference in cilia shortening in response to heat shock in this background compared to wildtype. In the future, it would be interesting to test additional actin regulatory proteins in stress-dependent cilia remodeling.

Preliminary data from our lab demonstrate that cilia length recovers to some degree within 24 hours following a 3-hour heat shock event. My data also establishes the impact of heat shock on cilia fragmentation and preliminary data from our lab suggested increased cilia fragmentation occurs as a result of cold shock as well. Some researchers have suggested that this fragmentation may cause a loss of necessary cilia assembly proteins such as IFT-B (Phua et al., 2017), which could impact how cilia recover from a stress event. So future work could focus on studying the mechanisms that are responsible for the recovery of cilia length after stress, and also to determine the impact of cilia fragmentation on the recovery process. Finally, even if we know what is happening to the *C. elegans* cilia at a cellular level, we have yet to understand how a stress event that damages neuronal cilia affects cilia-mediated neuronal functions including behavior. It may be interesting to follow up the heat shock experiments with functional assays.

Ciliopathies can be very debilitating, especially those that impact the brain, heart, kidneys, liver, and other vital organs and tissues. By conducting research as completed in this project, we not only learn more about cilia, cells, neurodevelopment, and the body, but we also begin to build a framework of these disorders and understand how and why they occur. Moving forward, research into cilia and their stress response, as well as the potential for their recovery, will be crucial for creating therapeutics that can help restore the function of these impacted cilia, and therefore increase the quality of life for those afflicted.

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