Defining the Role of a Conserved G Protein Activator RIC-8 in Neuronal Heat Shock Response

A Major Qualifying Project submitted to the faculty of
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Abstract

Stress response, which is a complex interconnection of various pathways and proteins, has the capacity to protect an organism from cellular stress. Previous research in the Nechipurenko lab has shown that a non-canonical Gα protein guanine nucleotide exchange factor RIC-8 localizes to the primary cilium, and when heat shocked, the cilia of *ric-8* mutant *C. elegans* are shorter than wild-type cilia. These preliminary findings suggest that RIC-8 contributes to protecting cilia against heat stress. The aims of this project were to determine whether RIC-8 is required for stress-dependent cilia remodeling and to identify additional genes involved in this process.

Acknowledgements

I am thankful to the members of the Nechipurenko lab for their scientific input and technical assistance as well as discovering the preliminary data that my project was based on. I would like to thank my advisor Inna Nechipurenko for allowing me to work in her lab and providing guidance and insight into my project alongside my advisor Elizabeth Ryder, who I would like to thank for aiding me with the bioinformatics and statistical components of my project. As for the members of our lab, I would like to thank Hayley McMahon and Eric Peet for imaging many of the *C. elegans* strains that I quantified, and Christina Campagna for helping me learn to use the Prism software that I conducted the graphing and statistical analysis with.

Introduction

Despite the important and far-reaching benefits of stress response, the components and pathways of this process remain poorly understood. Many neurological disorders such as Alzheimer's and Parkinson's diseases, which involve proteins that are altered by oxidative stress building up in the brain of the affected individual, are influenced by cellular stress (Jomova et al, 2010). The prevalence of cellular stress-linked neurological disorders underscores the importance of studying stress response pathways that may protect against the stressor. Immotile cilia, also called primary cilia, are cellular organelles that are responsible for sensory perception and signaling, and as such play a significant role in the nervous system (Liu et al. 2021). Previous research in the Nechipurenko lab has shown that the cilia in a subset of *C. elegans* sensory neurons undergo shortening following heat stress. Furthermore, cilia of *ric-8* mutants undergo more pronounced shortening compared to those of wild-type animals following heat stress, suggesting a role for RIC-8 in regulating cilia integrity in response to cellular stress. Similarly, cilia in mammalian cells are known to shorten in response to cellular stress (Liang et al, 2016). Thus, the findings from this research project may identify a new role for RIC-8 in regulating stress-mediated cilia remodeling and inform future work in the mammalian models.

Stress Response Pathways and the Effects of Stress on Neurons

The effect of stress on neurons can be profound for both the cell and organism itself. Neurons attempt protective and reconstructive stress responses when exposed to mild stressors, but more extreme stress often results in cell death. Additionally, as cellular signaling centers, primary cilia have the capacity to initiate autophagy, or the breakdown of cellular components and organelles, in response to cell stress (Zemirli et al, 2019), which demonstrates the importance of primary cilia in mediating a stress response. The signal transduction pathways associated with neuronal stress response are often complex and are not fully understood, but there are several pathways which are more well defined. The Sonic Hedgehog (Shh) pathway involves binding of the Patched1 (PTCH1) receptor by Shh glycoproteins; this interaction prevents PTCH1-mediated inhibition of Smoothened, allowing for GLI transcription factors to localize to the nucleus and activate transcription of genes associated with cellular functions such as proliferation and apoptosis (Rimkus et al, 2016; Park et al 2019). The Shh pathway has been shown to be activated in cortical neurons under oxidative stress, and this activation was shown to increase cell survival under this oxidative stress condition, demonstrating the likely role of Shh

in stress response (Dai, 2010). The Wnt pathway involves the binding of Wnt proteins to Frizzled/LRP receptor complex which signals the Dishevelled (Dvl) and Axin proteins, thus causing the accumulation of β-Catenin, which interacts with transcription factors to effect intercellular signaling and maintenance (Logan and Nusse, 2004). The Wnt pathway has also been shown to be activated in response to oxidative stress in the retina of mice affected by diabetic neuropathy (Zhou, 2011).

Heat shock is one of the best studied forms of cell stress, and involves transcriptional regulators known as heat shock factors. Cells initiate a heat shock response when cell temperature rises above accepted thresholds, but this stress response can also be triggered by oxidative stress. In dealing with heat shock, cells deploy chaperone proteins to reduce protein damage. General protein synthesis is impeded by the heat shock response, but the heat shock factors, which aid in the stress response, increase transcription of the heat shock protein genes (Gorman et al, 2009). The heat shock transcription factor HSF-1 controls the heat shock response in *C. elegans*. Although HSF-1 is the sole heat-shock transcription factor in *C. elegans*, there are four heat-shock transcription factor homologues that control the heat shock response in mammals. HSF-1 has the capacity to upregulate certain genes in response to heat shock, with the most upregulated genes corresponding to heat shock proteins (HSP). HSPs function as molecular chaperones to refold other proteins unfolded by cellular stress. Many stress response pathways such as Wnt and the insulin/insulin-like growth factor signaling pathway, which boosts cell survival in response to thermal stress are highly conserved (Kyriakou et al, 2022).

RIC-8, GPCRs, and their Role in Stress Response

G protein signaling plays an important role in stress response. Activated GPCRs promote exchange of GDP on the Gα subunit for GTP leading to disengagement of Gα protein subunits from G β/γ and pathway activation (Syrovatkina et al, 2016). GPCR pathways are critical in cellular signaling, with a large variety of diseases and disorders as well as most systems in the human body being directly affected by GPCRs via their capacity to detect environmental stimuli and evoke a cellular response (Kumari et al, 2021). Additionally, over forty percent of approved drugs target GPCRs (Filmore 2004). GPCRs have the capacity to both inhibit and aid in stress response. For example, the *C. elegans* GPCR *gtr-1* has been shown to induce heat shock protein (HSP) genes and is responsible for increased animal survival and resistance to heat stress (Maman et al, 2013). Additionally, GprC and GprD have been shown to aid in the thermal and

oxidative stress responses in the fungus *A. fumigatus*, where deletion mutants were more sensitive to temperature and reactive oxygen intermediates (Gerhke et al, 2010), while oncogenic GPCRs such as LPAR inhibit stress-induced apoptosis in mesenchymal cells (Kumari et al, 2021).

RIC-8 is a guanine nucleotide exchange factor (GEF) which can activate heterotrimeric G proteins independently of GPCRs (Fig 1). RIC-8 also acts as a chaperone to promote the folding of Gα proteins and thus positively regulate G protein signaling (Tall, 2013) and has been implicated in cell division and synaptic function in *C. elegans* (Hinrichs et al, 2012). Specifically, RIC-8 has been shown to aid in the release of neurotransmitter at the neuromuscular junction via potentiating activity of a Gα protein EGL-30. In mice, the RIC-8 homolog Ric8a is important for proper embryotic development, as heterozygous (+/-) *Ric8a* mutants have mood and memory defects, and homozygous (-/-) mutants are inviable (Hinrichs et al, 2012). Although RIC-8 is known to regulate G protein signaling (Roth et al, 2021), the role for RIC-8 in cellular stress response remains to be expanded upon. RIC-8 has been shown to play a role in increasing thermal tolerance and resistance to oxidative stress in *Cryptococcus deneoformans*; with *ric8*Δ mutants having reduced growth rates in response to heat shock (Roth et al, 2021).

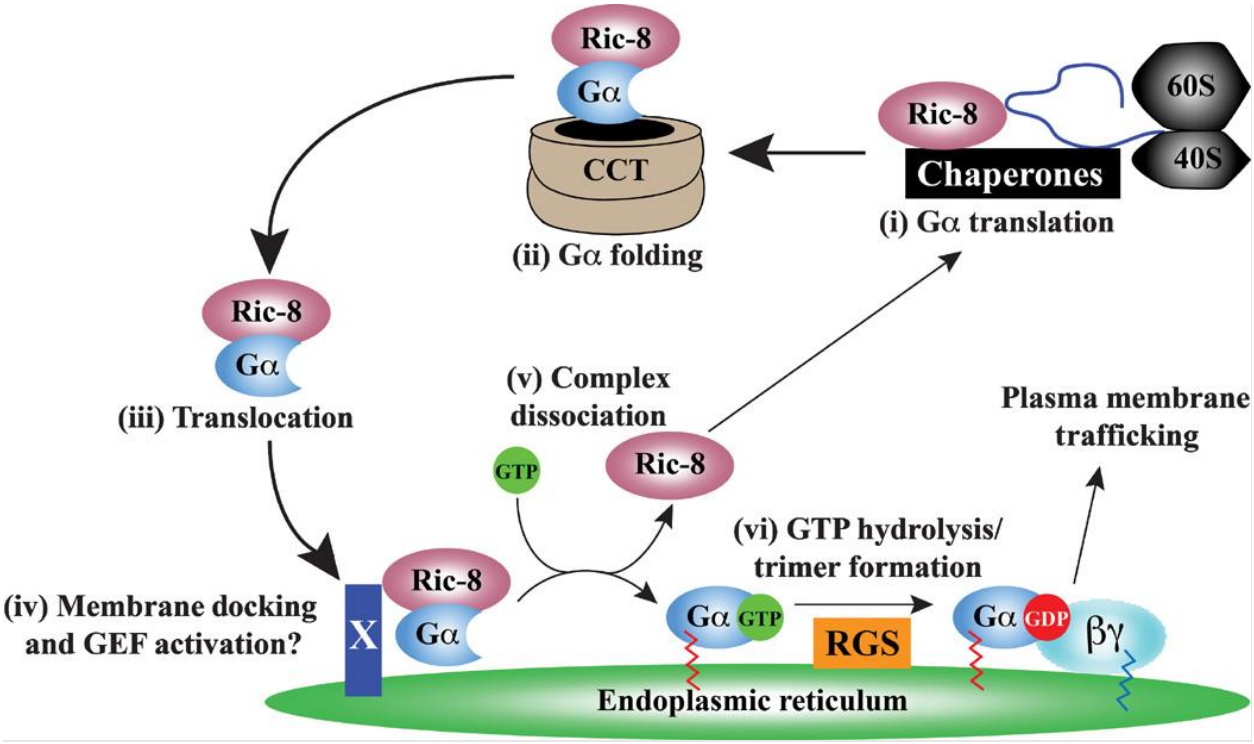

Figure 1: A diagram of how RIC-8 functions as a GEF and chaperone to regulate G protein signaling and folding, respectively.

RIC-8 binds to the nascent Gα protein and facilitates its folding. The protein complex then likely moves to an unknown docking factor x at the endoplasmic reticulum, which activates RIC-8 nucleotide exchange and the subsequent production of Gα-GTP, which is hydrolyzed with the aid of a regulator of G-protein signaling. Gα-GDP binds the Gβγ subunit, and the heterotrimer then relocates to the plasma membrane (Gabay et al, 2011).

Structure and Function of Cilia

Cilia are ubiquitous eukaryotic organelles that mediate signaling by all major pathways in mammals (Berbari et al, 2009) (Fig. 2). As cilia are important for the transduction of signals for cellular development, defective cilia are responsible for a range of birth defects as well as degenerative disorders (Eguether et al, 2014). Dysfunction in cilia contributes to genetic disorders called ciliopathies which are often associated with polydactyly, situs inversus, and retinitis pigmentosa (Baker and Beales, 2019).

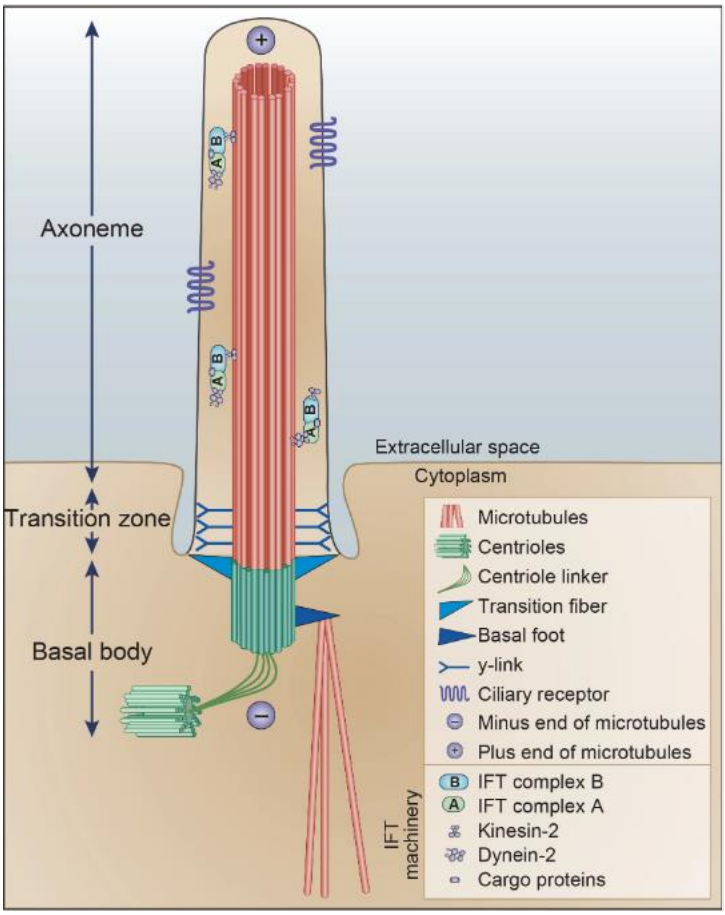

Figure 2: A diagram of a primary cilium with main components labeled. The cilium is divided into three major compartments; the axoneme, which is a microtubule-based core of the cilium, the transition zone, which regulates entry and exit of molecules into and out of the cilium, respectively, and the basal body, which templates the cilium assembly and anchors it to the cell membrane. The intraflagellar transport (IFT) is composed of multiprotein complexes that are responsible for trafficking of many ciliary components between the tip of the cilia and the base and required for cilia assembly and maintenance **(**Girardet et al, 2019).

The Role of Cilia in Stress Response

As the primary cilium is a sensory organelle, it is responsible for detecting stressors and subsequently activating stress response pathways to act against the stressor. For example, the protein channel TRPV4 which localizes to the cilia in cholangiocytes has been shown to detect osmotic change and subsequently cause a calcium influx to begin the osmotic response to restore cell homeostasis (Siroky et al, 2017). Additionally, the ciliary membrane contains components of

the Shh and Wnt signaling pathways among others, which can be activated in response to external stressors (Pampleiga and Cuervo, 2016).

The mode of autophagy activation that primary cilia utilize is not fully understood, but it has been shown that autophagy is initiated by cilia via the Shh signaling pathway (Cao and Zhong, 2015). Autophagy can protect the cell from initiating apoptosis, or cell death, but the precise means by which this occurs is unknown. Autophagy and apoptosis also have many of the same inducers, such as p53, and repressors, such as the PI3 kinase/Akt pathway (Thorburn 2008).

Autophagy also has the capacity to positively and negatively regulate ciliogenesis. Autophagy can break down ciliogenesis-associated proteins, and thus decrease ciliary length, but it can also break down ciliogenesis blockers, so the type of change that autophagy has on ciliogenesis can change based on various circumstances from cell type to cellular conditions. Interestingly, p53 has also been shown to play a part in regulating the shortening of cilia during acute kidney injury (S. Han et al 2017) in addition to its role in inducing autophagy and apoptosis, which furthers the connection between autophagy, stress, and cilia shortening. Although cilia may play a part in the autophagic response and thus reduce the damage caused by stress (Cao and Zhong, 2015), stress conditions also have the capacity to reduce the length of primary cilia (Zemirli et al, 2019), so stress may have the capacity to reduce a cell's ability to respond to stress by damaging cilia. Oxidative stress has been shown to decrease cilia length and lead to the fragmentation of cilia in mice lung cells (Y. Han et al, 2021). Cilia have the capacity to recognize stress and control stress response pathways but are also impacted by stress themselves.

C. elegans as a Model to Study Cilia and Stress

The one-mm long roundworm *C. elegans* is a great model for studying sensory cilia as *C. elegans* only have non-motile (sensory) cilia, located on the sixty ciliated neurons of hermaphrodites (108 in males), of the 302 total neurons (Fig. 3). *C. elegans* are also translucent, so the process of imaging structures such as cilia *in vivo* is made much easier (Mondal et al, 2011). A great advantage of *C. elegans* in studying stress response is their small size, which allows for any experimentally imposed stressors to affect the entire organism, and the effects can be measured *in vivo.* Finally, since stress response pathways are highly conserved (Prahlad and Morimoto, 2009), and mammalian cilia also experience stress-dependent shortening (Mirvis et

al, 2019), much of what is gained from studying stress and cilia in *C. elegans* can be potentially extended to humans. Taken together, these aspects of *C. elegans* make it an excellent model for research into cilia and stress response.

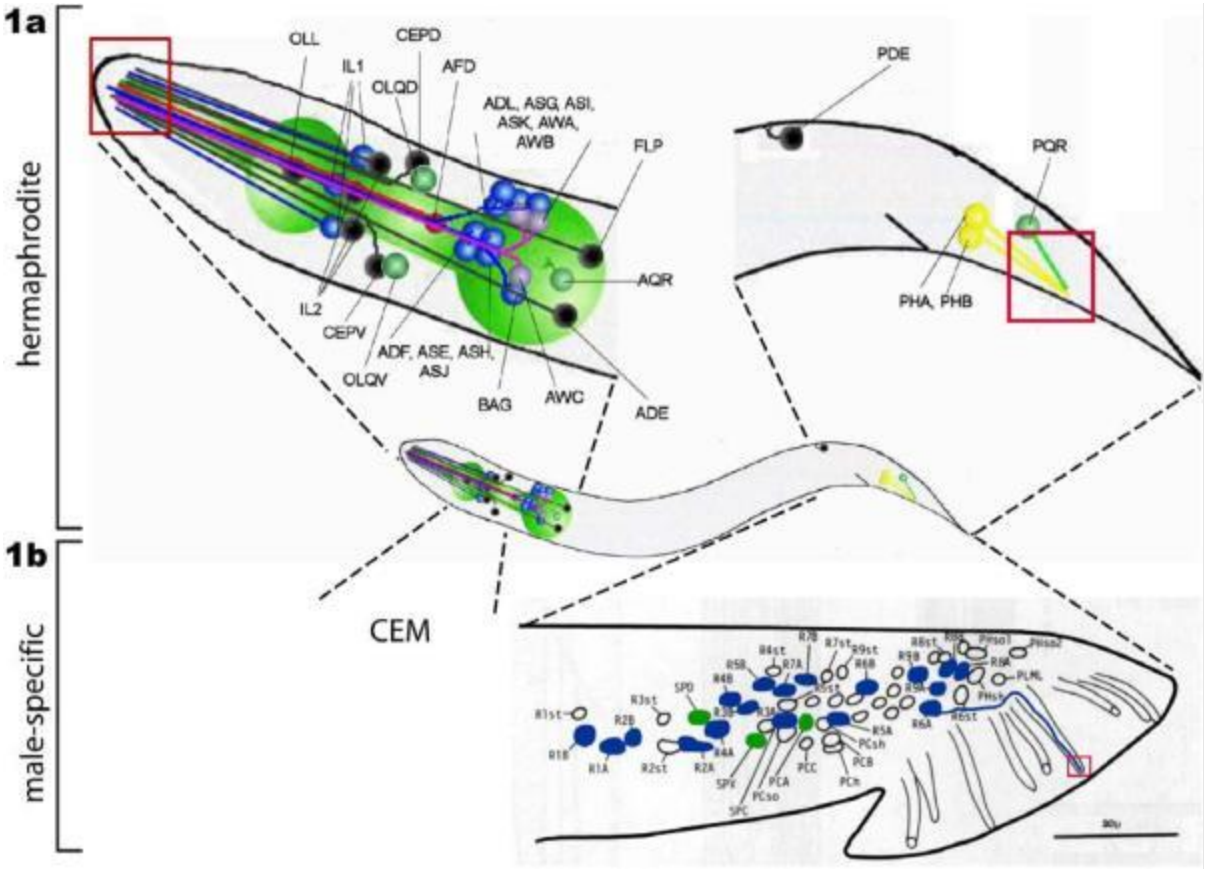

Figure 3: A diagram of ciliated neurons in *C. elegans*. (1a) The hermaphrodite *C. elegans* has 60 ciliated sensory neurons. Pictured here are the neurons labeled in the head. (1b) Male *C. elegans* possess 48 additional ciliated neurons for a total of 108 ciliated neurons. Most of these additional neurons are present in the tail, as pictured here. The red boxes mark the ciliary endings (Bae and Barr, 2008).

Project Goals

This project uses live imaging in the nematode *C. elegans* to examine cilia remodeling in sensory neurons in response to heat stress and to define the role for the *ric-8* gene in this cellular process. Additionally, this project uses bioinformatics approaches to identify cilia gene candidates that may be involved in stress-dependent cilia remodeling.

Hypothesis

My hypothesis is that the *ric-8* gene functions to promote cilia integrity in a subset of *C. elegans* sensory neurons in response to heat stress. The findings from my project will contribute to furthering knowledge of *ric-8* function and the effects of heat shock on ciliary length as well as identify a list of candidate cilia genes that may mediate stress-dependent cilia remodeling.

Methods

C. elegans Genetics and Maintenance

One-day-old hermaphrodites of the following strains were used for experiments in this project:

Strain ID	Source	Genotype
NWM169	Nechipurenko lab	*ric-8(md303); nuIs11[osm-10p::gfp, lin-15(+)]*
NWM227	Nechipurenko lab	*mks-3(ok2142); nuIs11[osm-10p::gfp, lin-15(+)]*
NWM340	Nechipurenko lab	*odr-3(n1605); nuIs11[osm-10p::gfp, lin-15(+)]*
HA3	CGC	*nuIs11[osm-10p::gfp, lin-15(+)]*

The worms were maintained at 20C on Nematode Growth Medium (NGM) plates seeded with a lawn of *E. coli* OP50 as food source.

Heat Stress Assays

Approximately twenty hermaphrodite L4 animals per genotype were transferred onto fresh NGM plates seeded with OP50. The plates were sealed with parafilm and floated in the water bath set at 35C for 3 hours. Following a three-hour shock, the animals were allowed to recover at 20C for at least 20 minutes prior to imaging.

Microscopy

For imaging, 10-20 one-day-old hermaphrodite animals were mounted on 10% agarose pads set on microscope slides and anesthetized in a 5-microliter drop of 10 mM tetramisole. The slides were imaged using Leica's Thunder Imager Tissue with a HC Plan Apochromat 63X NA 1.40-0.60 oil immersion objective. For each worm, a z-stack was taken of the cilia in the worm head.

Image Analysis

The cilia length of ASI and ASH neurons was measured using the software ImageJ. Optical z-sections containing the cilium were projected at maximum intensity in ImageJ. Segmented lines were drawn in the resultant projected images from cilia base to tip to measure cilia length.

Statistics

GraphPad's Prism 9 was used for statistical analysis and to create scatter plots. Data were plotted with both treatments grouped per genotype, and bars for the mean and standard deviation for each set included. A two-way ANOVA was conducted to determine whether cilia length varied depending on the genotype and the treatment (heat shocked or control temperature). Because the interaction between genotype and treatment factors was significant, a one-way ANOVA with Tukey's multiple comparisons test was used to determine the significance of the difference in cilia length of the heat-shocked vs control worms for each genotype and comparing mutant genotypes to wild type for heat-shocked worms.

Bioinformatics

We took a list of 6966 Human cilia genes from the Chlamyfp database at http://chlamyfp.org/ (Pazour et al, 2005) and ran it through Alliance of Genome Resources SimpleMine at https://www.alliancegenome.org (Adzhubei et al, 2013) to get a list of all *C. elegans* orthologues of genes from the initial list. The input was set to be case insensitive, duplicate entries were set to be merged, retrieved information was set to gene name, and the result was displayed in HTML format. As the resulting list included both the common name and Wormbase ID of the orthologues, the table was copied into Microsoft Excel and run through a query to remove the Wormbase ID from each cell, leaving the common name of the gene. The list of *C. elegans* orthologues was then checked against a small subset of *C. elegans* genes that were differentially expressed during heat shock from a study done by Jovic et al. (Jovic et al, 2017) using Excel. Eighty-five genes were found to be in common between the two lists. We then created a list of the human orthologues of genes in the two GO term categories with the highest fold enrichment (signal transducer activity and actin binding) from the Jovic data by searching the Alliance of Genome Resources database for the best human orthologues for each gene. Genes from the protein binding GO term were also matched with human orthologues in this manner as they were also all upregulated in response to heat shock, but this list was not included in the final list of five genes as it had a lower fold enrichment than signal transducer

activity or actin binding. When these human orthologues were searched in the Chlamyfp database, the number of reference papers from the database was recorded for each gene, and it was noted whether the gene was in the data from one of the primary cilia papers (Ishikawa et al, 2012; Mick et al, 2015; May et al, 2021; Breslow et al, 2018; Pusapati et al, 2018). The genes in the actin binding and signal transducer activity GO term lists that were listed as being included in one of the primary cilia papers were added to the final list of genes which could be examined in future heat shock experiments. The human orthologues of protein binding genes were also narrowed down according to their presence in one or more of the primary cilia papers.

Results

ric-8 Mutants Were Unique Among Tested Genotypes for Having Shorter Cilia than Wild Type Following Heat Shock.

We tested the effect of heat shock on cilia length in worms of several genotypes (Figs 4, 5). Consistent with the preliminary findings from the Nechipurenko lab which informed this research, *ric-8* mutant worms displayed shorter cilia than wild-type worms when heat shocked (Figs 4, 5). We wanted to test whether *ric-8* was unique among cilia genes in its effect on preventing cilia shortening after heat shock. MKS-3 is a structural component of the cilium and was tested to see if *ric-8* had a unique effect on heat shock-dependent cilia shortening. *odr-3* encodes a Gα protein, which localizes to cilia and is activated by RIC-8 (Campagna et al., in preparation). Following heat shock, the cilia of *mks-3* mutants were not significantly shorter than heat-shocked wild-type cilia, suggesting that the shorter cilia observed in heat-shocked *ric-8* mutants are not a common consequence of mutating cilia genes (Fig. 4 and 5). Surprisingly, the cilia of *odr-3* mutants were not shorter than cilia of heat-shocked wild type animals, suggesting that *odr-3* does not function in the same pathway with *ric-8* in this cellular context.

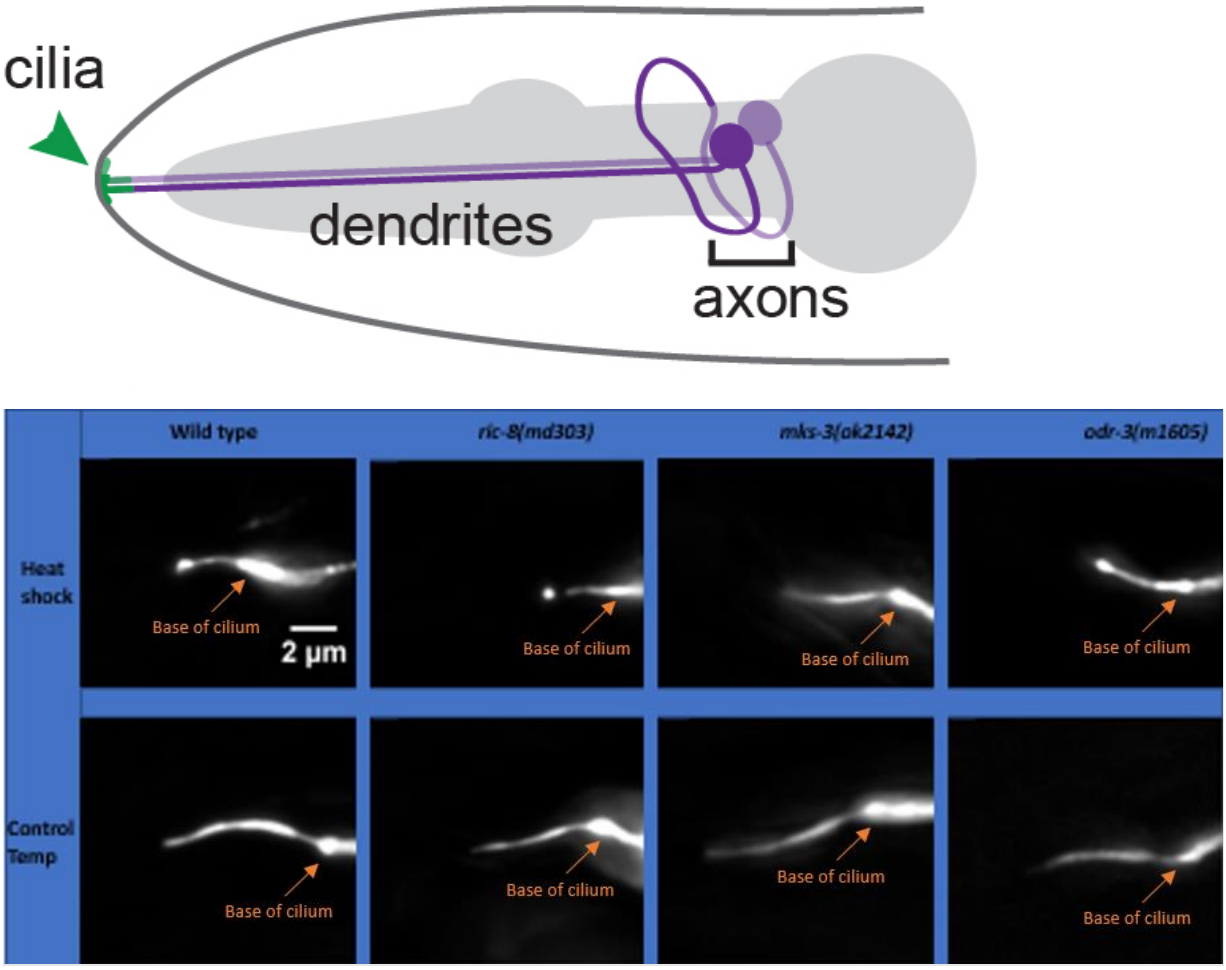

Figure 4. **(Top)** A simplified model of cilia, dendrites, and axons of *C. elegans* head sensory neurons (Campagna et al, in preparation). **(Bottom)** Representative images of the indicated genotypes and treatment conditions. Heat-shocked worms of all genotypes (top row) display shorter cilia compared to the respective non-heat-shocked controls (bottom row). Cilia were measured from the base (marked with an orange arrow) to the tip of the nose. The anterior is to the left.

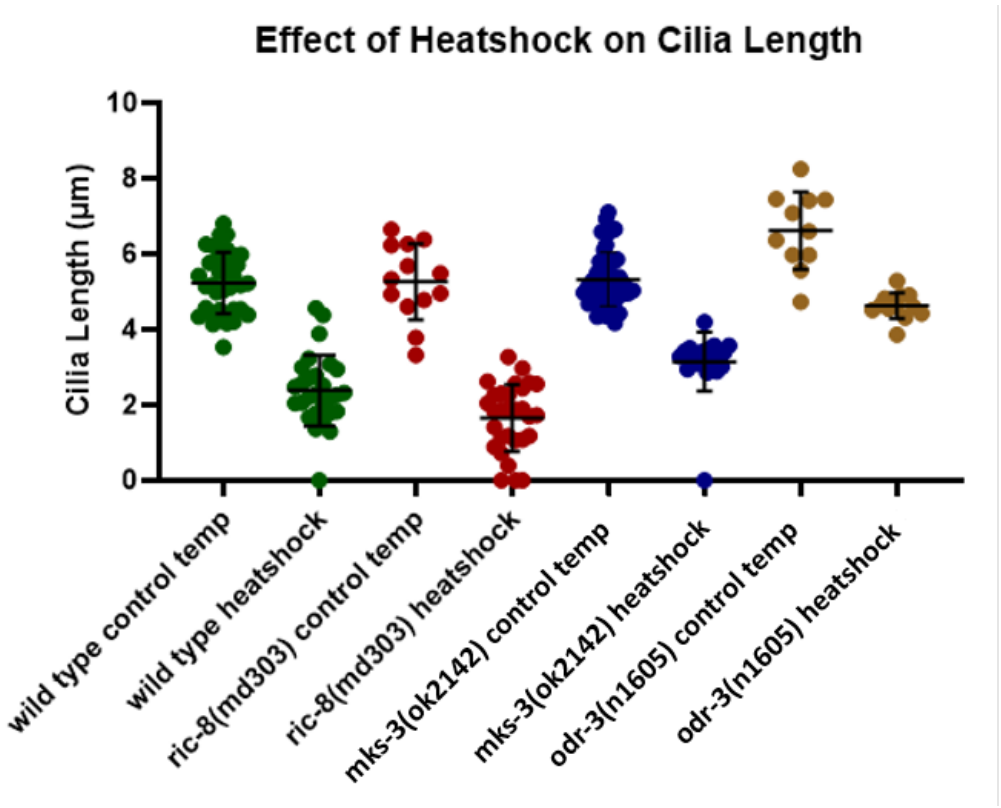

Figure 5. Quantification of cilia length in response to heat shock across genotypes. Mean length and standard deviation are shown for each set. The control temperature treatment is on the left for every genotype pair and is consistently higher on average for all genotypes than the heat-shocked treatment. Two-way ANOVA showed a significant interaction between genotype and treatment ($p = 0.001$). Heat-shocked wild-type and *ric-8* mutant worms also had significantly different cilia lengths (Tukey's multiple comparison test, $p = 0.0063$). Many of the *C. elegans* strains were imaged by other members of the lab, including *odr-3, mks-3,* and some of the *ric-8* and wild-type worms as described in the acknowledgements section. All quantifications and statistical tests were done by the author.

Several Primary Cilia Genes are Good Candidates for Future Experiments on Heat-shock Dependent Cilia Remodeling.

I conducted a database search to create a list of eighty-five known primary cilia genes which were differentially regulated in response to heat stress based on their occurrence in several key datasets (Chlamyfp and Jovic et al., 2017; see Methods for details). This list should provide promising candidate genes to conduct experiments similar to how we tested *ric-8* for its role in

the heat shock response. Figure 6 includes the final list of genes from the primary cilia papers in the Chlamyfp database and that were found to have the most highly enriched GO terms (actin binding and signal transducer activity) from the Jovic analysis. It is worth noting that *ric-8* was among the list of eighty-five genes present in both the Chlamyfp and Jovic datasets. Additionally, while the final list of five genes is of most interest, the total list of eighty-five genes from all the GO term categories may also present good candidates for heat shock experiments, especially in the protein binding GO term category which was also enriched in the heat shock study.

Next, we used the list of genetic and molecular interactions from Alliance of Genome Resources for each of the genes on the final list to create a graphic of shared interactions between them (Fig 7). All shown interactions including one of the five genes from the final list were physical except for the interaction of ARPC3 with KRAS, which is genetic. Genes were included if they interacted with at least two genes from the final list of five genes or the protein binding genes from the list of 85, which were also all upregulated. EGFR, which is involved in actin binding in addition to other processes, was an interactor for many of the actin binding genes but was not included in the diagram as it had far more interactions in general than any of the other interactors, both with other genes in the diagram and with unrelated genes. The inclusion of EGFR in the diagram would render the diagram difficult to read due to its number of interactions, and thus it is reported here as being an interactor for many of the genes to not overcomplicate the diagram and take away from the other interactions.

Go term and up or down regulation	C. elegans	Best Human Ortholog	number of refrences	Primary cilia paper
actin binding (up)	COR-1	CORO1B	4	Mick
actin binding (up)	LEV-11	TPM3	3	Mick
actin binding (up)	ARX-5	ARPC3	5	Ishikawa
actin binding (up)	GSNL-1	CAPG	4	Mick
signal transducer activity (down)	STA-2	STAT3	3	Mick

Figure 6. The final list of genes from the database search. Included from left to right are the GO terms (Jovic et al, 2017), the *C elegans* gene names, the best human ortholog, the number of reference papers for the human ortholog listed in the Chlamyfp database, and the first author of the paper (from the Clamyfp database) reporting the discovery of that cilia gene.

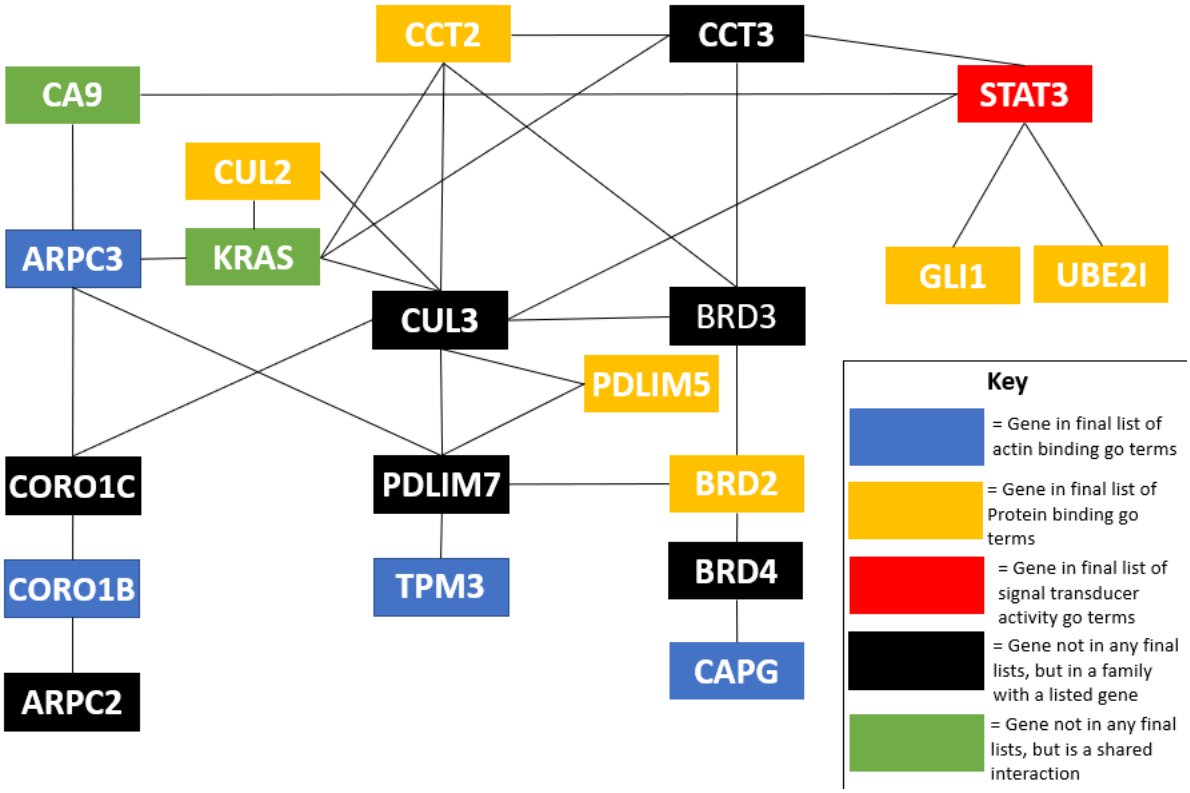

Figure 7. Diagram of predicted interactions for the final list of candidate genes. Both physical and genetic interactions are included and are from the interactions listed in Alliance of Genome Resources. Every line represents an interaction between the two genes that it connects. Genes from the protein binding GO term present in the primary cilia papers on the Chlamyfp database are marked in yellow, as they were also all upregulated due to heat shock but were not included in the final list of five genes. Protein binding genes were included only if they shared interactors with other genes present in the list and were present in a primary cilia paper listed on Chlamyfp (Fig 9).

Discussion

ric-8 Mutant Worms Displayed Shorter Cilia Than Wild Type After Heat Shock.

My results confirm that *ric-8* is necessary for maintaining the integrity of the cilia in response to heat shock, and that the reduction of *ric-8* function leads to decreased cilia length following heat stress compared to wild type. Assessing the effects of other known *ric-8* interactors, particularly G protein subunits such as *gpa-16* or *goa-1,* for effects on cilia shortening in response to heat shock would be a good extension of this research to map the *ric-8*-dependent pathway of heat-induced cilia shortening. Another extension of my work would be to assess the shortening of *ric-8* mutants when exposed to stressors besides heat such as cold or oxidative stress.

ric-8 Mutants Were Unique Among Tested Genotypes for Displaying Shorter Than Wild-type Cilia Following Heat Shock.

As the *mks-3* mutant did not have shorter cilia than wild type after heat shock, we can conclude that the cilia shortening displayed by the *ric-8* mutant is a feature that cannot be generalized to all cilia genes and is thus specific to the function of *ric-8*. The fact that the heat-shocked *odr-3* mutants did not demonstrate cilia shortening comparable to *ric-8* mutants implies that *odr-3* is not involved in regulating cilia integrity during heat stress in the same pathway as *ric-8.*

Several Primary Cilia Genes Are Good Candidates for Future Experiments on Mechanisms of Heat-shock-dependent Cilia Remodeling.

The final list of five genes from the database search included four genes that encode actin binding proteins (CORO1B, TPM3, ARPC3 and CAPG) and one transcription factor (STAT3). Actin binding is undoubtably relevant to experiments on modulation of ciliary length due to the role of actin, particularly the F-actin network, in regulating ciliogenesis and ciliary length (Smith et al, 2020). As actin is relevant to ciliogenesis and regulation of cilia length, the four actin binding genes in the final list are likely the best candidates for future experiments on the effects of heat shock on the primary cilium such as we conducted with *ric-8*. CAPG in particular presents as an attractive option for further experiments as it codes for a gelsolin family protein, and gelsolin has been shown to be a main regulator of ciliogenesis and ciliary length (Smith et al, 2020). Actin stability is known to regulate ciliogenesis positively and negatively, and both gelsolin and tropomyosin, the latter of which represents the family of proteins that TPM3 codes

for, have been shown to influence actin stability, as well as being shown to exist proximal to the ciliary membrane (Smith et al, 2020). ARPC3 is a component of the ARP2/3 complex, and CORO1B enables binding of this complex. The ARP2/3 complex is known to be involved in F-actin branching, which is important for cilia formation, and actin polymerization, which is known to regulate ciliogenesis both positively and negatively. (Smith et al, 2020). As these genes are all connected to actin-dependent ciliogenesis and cilia length regulation, they constitute excellent candidates for regulating the length of the primary cilium in response to heat shock.

Appendix

GO term														
signal transducer activity (downregulated)	npr-26	gpa-3	egl-8	sta-2	gpa-12	gpa-14	plc-3	dao-5	mgl-1	nmur-1				
dendrite (downregulated)	vps-35	lin-10	pct-1	sad-1	tax-4	tax-6	che-12							
GEF activity (downregulated)	ric-8	exc-5	eif-2Bbeta	eif-2Balpha	ced-5	aex-3	F46H5.4							
protien binding (upregulated)	C40A11.7	deb-1	pfkb-1.1	elc-1	ntl-2	par-6	atf-2	mksr-2	T01G9.2	ife-1	lec-1	unc-64	sta-1	lec-3
	taf-4	age-1	pat-4	cyk-4	rpl-6	alp-1	syp-1	tra-1	afd-1	bet-1	F57C9.4	nrf-5	stn-1	baf-1
	usp-46	atg-13	fbf-1	snfc-5	pxl-1	gpch-1	fem-2	D2063.1	snx-6	wdr-83	cul-2	psr-1	apg-1	
	mdf-2	dsbn-1	unc-108	ubc-9	shn-1	ced-9	vps-33.1	tsg-101	dyrb-1	phm-2	R07G3.8	cct-2	unc-45	
actin binding (upregulated)	cor-1	unc-78	lev-11	arx-5	arx-2	gsnl-1	unc-60							

Figure 8. List of *C. elegans* genes in both the Chlamyfp and Jovic et. al. datasets.

Go term and up or down regulation	C. elegans gene	Best Human Ortholog(s)	Primary cilia paper(s)
protien binding(up)	ntl-2	CNOT2	BRESLOW
protien binding(up)	mksr-2	B9D2	MAY,BRESLOW,PUSAPATI
protien binding(up)	alp-1	PDLIM5	MAY
protien binding(up)	tra-1	GLI1,GLI2,GLI3	BRESLOW,BRESLOW+PUSAPATI,BRESLOW
protien binding(up)	bet-1	BRD2	BRESLOW+PUSAPATI
protien binding(up)	stn-1	SNTB2	ISHIKAWA
protien binding(up)	atg-13	ATG13	PUSAPATI
protien binding(up)	cul-2	CUL2	PUSAPATI,ISHIKAWA
protien binding(up)	psr-1	JMJD6	PUSAPATI
protien binding(up)	ubc-9	UBE2I	PUSAPATI
protien binding(up)	vps-33.1	VPS33A	PUSAPATI
protien binding(up)	tsg-101	TSG101	MICK,MAY
protien binding(up)	R07G3.8	CYRIB	ISHIKAWA
protien binding(up)	cct-2	CCT2	PUSAPATI

Figure 9. Final list of protein binding genes from the database search. Included from left to right is the protein binding GO term (Jovic et al, 2017), the *C elegans* gene names, the best human ortholog, and the first author of the primary cilia papers that listed the human ortholog. *tra-1* has three best orthologs, and thus the commas in the primary cilia paper(s) section for that row separate the papers for each ortholog, with GLI2 being cited in both Breslow and Pusapati, as denoted by the plus sign.

Bibliography

Adzhubei I., Jordan D. M., Sunyaev S. R. (2013). Predicting functional effect of human missense mutations using PolyPhen-2. Curr. Protoc. Hum. Genet. Chapter 7, Unit7.20.

Bae YK, Barr MM. Sensory roles of neuronal cilia: cilia development, morphogenesis, and function in C. elegans. Front Biosci. 2008 May 1;13:5959-74. doi: 10.2741/3129. PMID: 18508635; PMCID: PMC3124812.

Baker K, Beales PL. 2009. Making sense of cilia in disease: The human ciliopathies. Am J Med Genet Part C Semin Med Genet 151C:281–295.

Berbari N.F. et al. The Primary Cilium as a Complex Signaling Center, Current Biology, Volume 19, Issue 13 2009, doi: 10.1016/j.cub.2009.05.025

Breslow, D., Hoogendoorn, S., Kopp, A., Morgens, D., Vu, B., & Kennedy, M. et al. (2018). A CRISPR-based screen for Hedgehog signaling provides insights into ciliary function and ciliopathies. *Nature Genetics*, *50*(3), 460-471. doi: 10.1038/s41588-018-0054-7

Campagna, C., McMahon, H., May, I., Nechipurenko, I. (2023). RIC-8 modulates cilia morphology in *C. elegans* sensory neurons

Cao, M., Zhong, Q. Cilia in autophagy and cancer. *Cilia* 5, 4 (2015). **https://doi.org/10.1186/s13630-016-0027-3**

Dai, R., Zhu, S., Xia, Y., Mao, L., Mei, Y., & Yao, Y. et al. (2010). Sonic Hedgehog Protects Cortical Neurons Against Oxidative Stress. *Neurochemical Research*, *36*(1), 67-75. doi: 10.1007/s11064-010-0264-6

Eguether, T., San Agustin, J., Keady, B., Jonassen, J., Liang, Y., & Francis, R. et al. (2014). IFT27 Links the BBSome to IFT for Maintenance of the Ciliary Signaling Compartment. *Developmental Cell*, *31*(3), 279-290. doi: 10.1016/j.devcel.2014.09.011

Filmore, D. (2004) It's a GPCR world. Modern Drug Discovery, 11, 24-28. Retrieved from http://pubsapp.acs.org/subscribe/archive/mdd/v07/i11/html/1104feature_filmore.html?

Gabay M, Pinter ME, Wright FA, Chan P, Murphy AJ, Valenzuela DM, Yancopoulos GD, Tall GG. Ric-8 proteins are molecular chaperones that direct nascent G protein α subunit membrane association. Sci Signal. 2011 Nov 22;4(200):ra79. doi: 10.1126/scisignal.2002223. PMID: 22114146; PMCID: PMC3870195.

Gehrke, A, Eheinekamp, T, Jacobsen, I, Brakhage, A. (2010). Heptahelical receptors GprC and GprD of *Aspergillus fumigatus* are essential regulators of colony growth, hyphal morphogenesis, and virulence. *Appl. Environ. Microbiol.*

Girardet L, Augière C, Asselin M-P, Belleannée C (2019). Primary cilia: biosensors of the male reproductive tract. Andrology. https://doi.org/10.1111/andr.12650

Gregory G. Tall (2013). Ric-8 regulation of heterotrimeric G proteins, Journal of Receptors and Signal Transduction, 33:3, 139-143, DOI: 10.3109/10799893.2013.763828

Han, S., Jang, H., Seu, S., Cho, H., Hwang, Y., Kim, J., & Park, K. (2017). Hepatic ischemia/reperfusion injury disrupts the homeostasis of kidney primary cilia via oxidative stress. *Biochimica Et Biophysica Acta (BBA) - Molecular Basis Of Disease*, *1863*(7), 1817-1828. doi: 10.1016/j.bbadis.2017.05.004

Han, Y., Kim, J., Jang, G., Park, K., Cisplatin Induces Lung Cell Cilia Disruption and Lung Damage via Oxidative Stress, Free Radical Biology and Medicine,Volume 177, 2021, Pages 270-277, ISSN 0891-5849, https://doi.org/10.1016/j.freeradbiomed.2021.10.032. Retrieved from https://www.sciencedirect.com/science/article/pii/S0891584921007826

Hinrichs, M., Torrejón, M., Montecino, M., J. Olate, J. (2012). Ric-8: Different cellular roles for a heterotrimeric G-protein GEF. *Journal of Cellular Biochemistry.*Retrieved from Ric-8: Different cellular roles for a heterotrimeric G-protein GEF - Hinrichs - 2012 - Journal of Cellular Biochemistry - Wiley Online Library

Inglis, P., Ou, G., Leroux, M., & Scholey, J. (2018). The sensory cilia of Caenorhabditis elegans. *Wormbook*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK19729/

Ishikawa, H., Thompson, J., Yates, J., & Marshall, W. (2012). Proteomic Analysis of Mammalian Primary Cilia. *Current Biology*, *22*(5), 414-419. doi: 10.1016/j.cub.2012.01.031

Jomova, K., Vondrakova, D., Lawson, M., & Valko, M. (2010). Metals, oxidative stress and neurodegenerative disorders. *Molecular And Cellular Biochemistry*, *345*(1-2), 91-104. doi: 10.1007/s11010-010-0563-x

Jovic, K., Sterken, M., Grilli, J., Bevers, R., Rodriguez, M., & Riksen, J. et al. (2017). Temporal dynamics of gene expression in heat-stressed Caenorhabditis elegans. *PLOS ONE*, *12*(12), e0189445. doi: 10.1371/journal.pone.0189445

Kyriakou, E., Taouktsi, E., & Syntichaki, P. (2022). The Thermal Stress Coping Network of the Nematode Caenorhabditis elegans. *International Journal Of Molecular Sciences*, *23*(23), 14907. doi: 10.3390/ijms232314907

Logan, C., Nusse, R. (2004). THE WNT SIGNALING PATHWAY IN DEVELOPMENT AND DISEASE. Annu. Rev. Cell Dev. Biol. 2004. 20:781–810 doi: 10.1146/annurev.cellbio.20.010403.113126

Liang, Y., Meng, D., Zhu, B., & Pan, J. (2016). Mechanism of ciliary disassembly. *Cellular And Molecular Life Sciences*, *73*(9), 1787-1802. doi: 10.1007/s00018-016-2148-7

Liu, Siling et al. *The Essential Role of Primary Cilia in Cerebral Cortical Development and Disorders*. Vol. 142. New York :: Academic Press 2021, Web.

Machiela, E., Jeloka, R., Caron, N., Mehta, S., Schmidt, M., & Baddeley, H. et al. (2020). The Interaction of Aging and Cellular Stress Contributes to Pathogenesis in Mouse and Human Huntington Disease Neurons. *Frontiers In Aging Neuroscience*, *12*. doi: 10.3389/fnagi.2020.524369

Maman, M., Carvalhal Marques, F., Volovik, Y., Dubnikov, T., Bejerano-Sagie, M., & Cohen, E. (2013). A Neuronal GPCR is Critical for the Induction of the Heat Shock Response in the NematodeC. elegans. *The Journal Of Neuroscience*, *33*(14), 6102-6111. doi: 10.1523/jneurosci.4023-12.2013

May, E., Kalocsay, M., D'Auriac, I., Schuster, P., Gygi, S., Nachury, M., & Mick, D. (2021). Time-resolved proteomics profiling of the ciliary Hedgehog response. *Journal Of Cell Biology*, *220*(5). doi: 10.1083/jcb.202007207

Mick, D., Rodrigues, R., Leib, R., Adams, C., Chien, A., Gygi, S., & Nachury, M. (2015). Proteomics of Primary Cilia by Proximity Labeling. *Developmental Cell*, *35*(4), 497-512. doi: 10.1016/j.devcel.2015.10.015

Mondal, S., Ahlawat, S., Rau, K., Venkataraman, V., Koushika, S.P. (2011). Imaging in vivo neuronal transport in genetic model organisms using microfluidic devices. Traffic.

Niti Kumari, Somrudee Reabroi, Brian J. North, "Unraveling the Molecular Nexus between GPCRs, ERS, and EMT", *Mediators of Inflammation*, vol. 2021, Article ID 6655417, 23 pages, 2021. https://doi.org/10.1155/2021/6655417

Park, S., Jo, J., Kim, R., Jeong, A., Na, J., Kim, L., Jeong, S., Yun, K., Kim, Y., Kim, G., Kang, H., Oh, C., Lee, H. (2019). Sonic hedgehog pathway activation is associated with cetuximab resistance and EPHB3 receptor induction in colorectal cancer. Theranostics. doi: 10.7150/thno.30678. PMID: 31149041; PMCID: PMC6531304.

Pazour G.J., Agrin N., Leszyk J., Witman G.B. Proteomic analysis of a eukaryotic cilium. *J. Cell Biol*. 2005;170:103–113. doi: 10.1083/jcb.200504008.

Prahlad, V., Morimoto, R. (2009). Integrating the stress response: lessons for neurodegenerative diseases from C. elegans. Trends in Cell Biology, Volume 19, Issue 2, https://doi.org/10.1016/j.tcb.2008.11.002.

Pusapati, G., Kong, J., Patel, B., Krishnan, A., Sagner, A., & Kinnebrew, M. et al. (2018). CRISPR Screens Uncover Genes that Regulate Target Cell Sensitivity to the Morphogen Sonic Hedgehog. *Developmental Cell*, *44*(1), 113-129.e8. doi: 10.1016/j.devcel.2017.12.003

Roth, C., Murray, D., Scott, A., Fu, C., Averette, A., & Sun, S. et al. (2021). Pleiotropy and epistasis within and between signaling pathways defines the genetic architecture of fungal virulence. *PLOS Genetics*, *17*(1), e1009313. doi: 10.1371/journal.pgen.1009313

Rimkus, T., Carpenter, R., Qasem, S., Chan, M., & Lo, H. (2016). Targeting the Sonic Hedgehog Signaling Pathway: Review of Smoothened and GLI Inhibitors. *Cancers*, *8*(2), 22. doi: 10.3390/cancers8020022

Siroky, J., Kleene, K., Kleene, J., Varnell, D., Comer, G., Liu, J., Lu, L., Pachciarz, N., Bissler, J., Dixon, B. (2017). Primary Cilia Regulate the Osmotic Stress Response of Renal Epithelial Cells Through TRPM3. *J. Physiology Renal Physiol.* doi: **10.1152/ajprenal.00465.2015**

Smith, C., Lake, A., & Johnson, C. (2020). Primary Cilia, Ciliogenesis and the Actin Cytoskeleton: A Little Less Resorption, A Little More Actin Please. *Frontiers In Cell And Developmental Biology*, *8*. doi: 10.3389/fcell.2020.622822

Syrovatkina, V., Alegre, K., Dey, R., & Huang, X. (2016). Regulation, Signaling, and Physiological Functions of G-Proteins. *Journal Of Molecular Biology*, *428*(19), 3850-3868. doi: 10.1016/j.jmb.2016.08.002

Thorburn, A. (2008). Apoptosis and autophagy: regulatory connections between two supposedly different processes. *Apoptosis*

Zemirli N, Boukhalfa A, Dupont N, Botti J, Codogno P, Morel E. The primary cilium protein folliculin is part of the autophagy signaling pathway to regulate epithelial cell size in response to fluid flow. Cell Stress. 2019 Feb 25;3(3):100-109. doi: 10.15698/cst2019.03.180. PMID: 31225504; PMCID: PMC6551741.

Zhou, T., Zhou, K., Lee, K., Gao, G., Lyons, T., Kowluru, R., & Ma, J. (2010). The role of lipid peroxidation products and oxidative stress in activation of the canonical wingless-type MMTV integration site (WNT) pathway in a rat model of diabetic retinopathy. *Diabetologia*, *54*(2), 459-468. doi: 10.1007/s00125-010-1943-1