Studying the Effects of Extra Sets of Chromosomes on *C. elegans* Neurons

Using Tetraploid *C. elegans* to Investigate Genetic Abnormalities

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Introduction and Background

Polyploidy is a condition in which organisms possess multiple sets of chromosomes as a result of nondisjunction events during meiosis. In most organisms, polyploidy results in sterility or lethality, making it difficult to study [7].

Caenorhabditis elegans are:

- free-living nematodes with a short lifecycle [2].
- typically diploid (two sets of chromosomes) [2].
- Able to reproduce stably as tetraploids (four sets of chromosomes) [5].

These characteristics make *C. elegans* a good model to investigate the effects of changes in ploidy on living multicellular organisms.

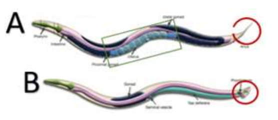


Figure 1: Anatomical Comparison of Adult Hermaphrodite (A) and Male *C. elegans* (B) [2].

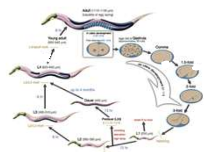


Figure 2: Lifecycle of *C. elegans* [2].

Polyploidy, Primary Cilia, and Polycystic Kidney Disease

Primary cilia are organelles that extend from the surface of some sensory neurons in *C. elegans*. In animals, cilia are thought to function as regulators of cell growth, division and proliferation, and defects in cilia function have been linked to several human diseases and health concerns.

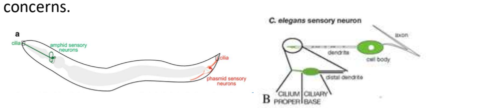


Figure 3: *C. elegans* Ciliated Sensory Neurons. [a] The Amphid head and Phasmid tail of *C. elegans* contain clusters of ciliated sensory neurons [10]. [b] Cilia are Located on the dendrites of *C. elegans* neurons. Sensory neurons are the only ciliated structures possessed by C. elegans. These cilia are non-motile, meaning that they do not move, rather they perceive environmental changes and cues [4].

Polyploidy has been associated with cyst growth in patients with Polycystic Kidney Disorder (PKD) [1].

We hypothesized that analysis of primary cilia structure and function in *C. elegans* might yield insights into connections between ploidy and pathology.

The C. elegans PT443 Strain is Transgenic for PKD2::GFP

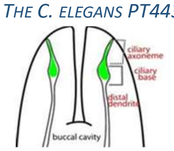


Figure 4: PKD-2 Accumulates at the Cilia Structures of *C. elegans* [3].

Mutations in PKD-2, which encodes the polycystin-2 (PC2) protein found within cilia of human kidneys, are known to cause polycystic kidney disease [1]. The male-specific primary cilia of *PT443 C. elegans* fluoresce green, providing an easy way to assess PC2 localization in a living organism [3], [8].

By assessing PKD-2::GFP localization in polyploid *C. elegans,* we can examine the possible link between polyploidy and cilia structure.

Methods

Reducing rec-8 Gene Expression through RNA-Interference to Produce Tetraploid Mutants

rec-8 is a gene involved in meiotic chromosome segregation in *C. elegans.*

Reduction in *rec-8* expression has been found to induce polyploidy in *C. elegans* [5].

We use RNA interference to reduce expression of *rec-8*, induce nondisjunction, and produce stable lines of tetraploid worms (Fig.5) [5]. The *C. elegans* ingest dsRNA that degrades the *rec-8* mRNA transcript, thus reducing gene expression [5].

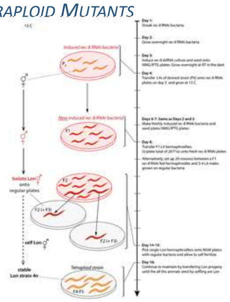


Figure 5 (right): Method for Producing Tetraploid Strains Through RNA-Interference [5].

Identifying Putative Tetraploids (Lon Phenotype)

Before ploidy is confirmed, we screen for putative tetraploid *C. elegans* by identifying worms with the Lon phenotype

Characteristics of Tetraploid (Lon) *C. elegans:*

About 30% larger and longer than diploid worms (Fig.6a) [9].
Have an extra body curve when they move forward (Fig.6b) [5].

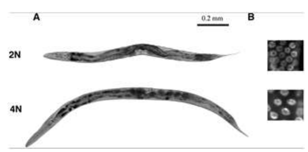
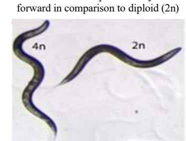


Figure 6a: Comparison of Diploid (2N) and Tetraploid (4N) worms [9]. [A] Tetraploid worms are larger and longer than diploid worms, due to enlarged cell sizes. [B] Comparison of diploid and tetraploid cells shows that tetraploid cells are larger in size.

Figure 6b: Comparison of Diploid (2N) and Tetraploid (4N) worms [5]. Putative polyploid *C. elegans* (4n) have an extra hump when they move forward in comparison to diploid (2n)



Counting Chromosomes with DAPI Staining

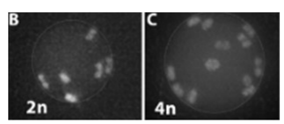


Figure 7: DAPI stained fluorescent chromosomes of diploid (2n) and tetraploid (4n) *C. elegans* [5].

Ploidy of Lon worms is confirmed by counting chromosomes.

Chromosomes are stained with fluorescent DAPI (4′,6-Diamidine-2′-phenylindole dihydrochloride) dye.

Dye-Filling and Fluorescent Analysis

We have hypothesized that the enlarged cell size of polyploid *C. elegans* influences the position of primary cilia.

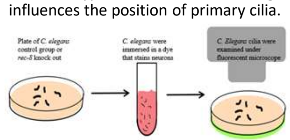


Figure 8: Dye-Filling Method [6], [11].

C. elegans neurons are stained with fluorescent dye that allows us to directly view the position of ciliated neurons under fluorescent microscopes [11].

Current Progress & Future Directions

We have established putative lines of polyploid *C. elegans* and are beginning the process of DAPI staining. Our next focus is to determine ploidy and confirm whether these *C. elegans* are tetraploid, and to characterize the changes in ciliated neurons of *rec-8* knockout mutants.

Past results show that fluorescence was decreased in the tails of *rec-8* knockout *C. elegans* (Fig. 9) [6].

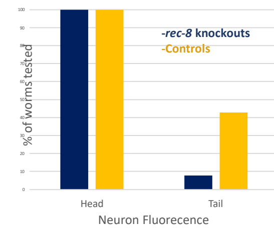


Figure 9: Comparing Cilia Fluorescence of *rec-8* Knock-out and Control *C. elegans* [6]. 13 *rec-8* knock out (blue) and 7 control *C. elegans* (yellow) were examined. *rec-8* data was retrieved from two lines of polyploid *C. elegans.* All cilia within *C. elegans* heads fluoresced, with little difference between knockout and control *C. elegans*. Only 7.7% of the *rec-8* knock out tails fluoresced, compared to 42.9% of the control *C. elegans* tails that fluoresced [6].

We hope to confirm the association of PKD with ploidy by determining that polyploidy has a significant effect on *C. elegans* ciliated neurons.

Understanding the effects of ploidy on primary cilia in *C. elegans* could facilitate a better understanding of PKD in humans [1]. Past results have shown that reduced gene expression due to RNA-interference leads to mislocalization of PKD2::GFP in *C. elegans* (Fig.9) [8].

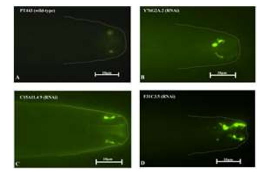


Figure 10: Delocalization of PKD2::GFP to *C. elegans* Primary Cilia. Wild-type (A) *C. elegans* display normal PKD2::GFP localization, while RNAi knockout mutants (B, C, D) show abnormal localization of PKD2::GFP [8].

We have noticed an unusual inability to revive Lon worms that had been left unattended for a few months. While this could be an anomaly, we hypothesize that this could possibly be a result of polyploidy somehow preventing the mutant *C. elegans* from entering the static Dauer phase, and this may be one of the questions that we further investigate in the future.

Acknowledgments

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