Identification of Factors Affecting Proper Localization of *C. elegans* PKD-2

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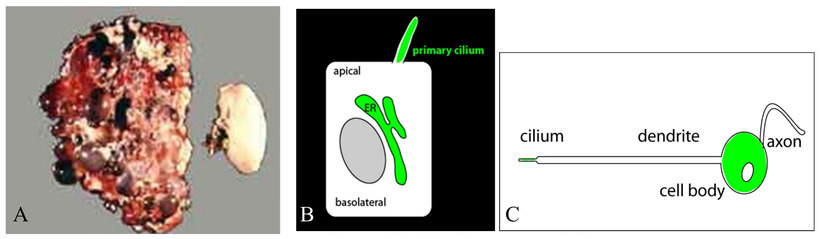
Introduction


C. elegans

Primary cilia are cellular antennae that mediate responses to the external environment. Response requires perception of a cue and transmission of that signal to the cell.

Primary Cilia Share Conserved Structure and Functions in Humans and *C. elegans*



Human Kidney (diseased on left) | Kidney epithelial cell | *C. elegans* sensory neuron

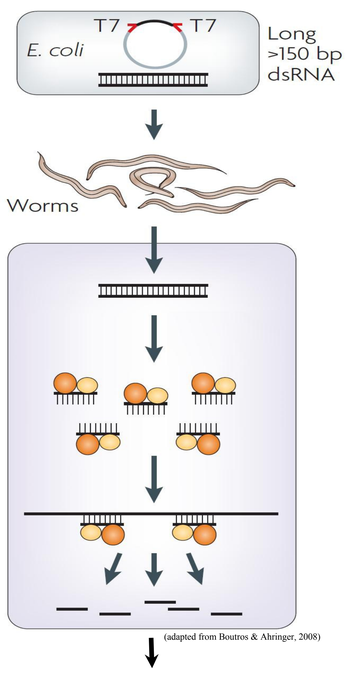
Mutations in polycystin genes cause Polycystic Kidney Diseases (PKD) in humans (A) and male mating behavior defects in *C. elegans*. PKD-2 localizes to primary cilia of human kidney cells (B) and *C. elegans* neurons (C). PKD-2 function is also conserved between these species.

Our Research Questions

1. **Which genes regulate PKD-2 localization?**
   Identify genes using RNAinterference (RNAi).
2. **What are the ciliary functions of those genes?**
   Use dye filling, protein localization, and chemotaxis assays to assess ciliary functions.

RNAinterference reduces function of a specific gene

In order to assess the role of specific genes in ciliary localization of PKD-2, we used a PKD-2::GFP construct (to visualize PKD-2) and RNAinterference (RNAi) to reduce the expression level of the gene in question.



(adapted from Boutros & Ahringer, 2008)

A portion of the gene of interest is cloned into *E. coli.* Both strands of DNA are transcribed producing double-stranded RNA.

The *C. elegans* eat the bacteria and the double stranded RNA is absorbed through the lining of the digestive system.

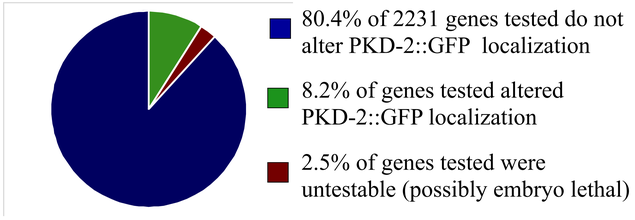
In the nematode cells, the double stranded RNA is rendered single stranded and can bind to the *C. elegans* mRNA with the complementary sequence.

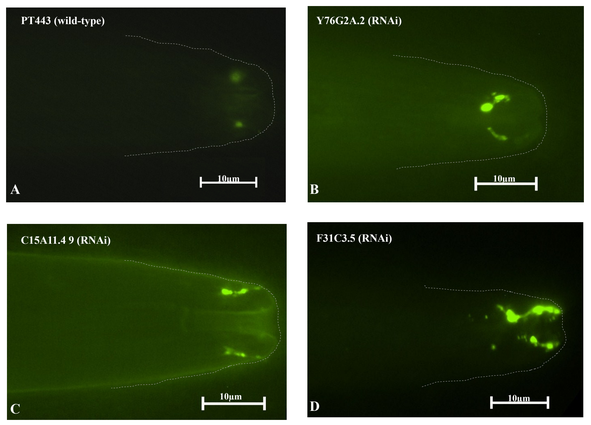
The cells recognize the double stranded RNA and degrade it. Thus, no protein can be translated from this RNA.

Screen worms and progeny for PKD-2::GFP mislocalization.

RNAinterference of chromosome I genes identifies multiple candidate factors

Reduction of Expression of 201 genes on Chromosome I Altered PKD-2::GFP Localization





Wild-type *C. elegans* localize PKD-2::GFP (green) to cilia normally (A) while reduction of specific gene expression by RNAinterference leads to mislocalization of PKD-2::GFP (B,C, and D). Anterior of head to right.

Of the genes that alter PKD-2::GFP localization, 14.9% have been reported to be expressed in neurons or to regulate neuron function while 38.3% are expressed in other cell types and have other functions and 46.8% are uncharacterized.

Further analysis of candidate factors reveals additional evidence of potential roles in ciliated neurons

Serial analysis of gene expression (SAGE) compares gene expression levels

We hypothesized that genes involved in regulation of PKD-2 localization might be expressed more highly in ciliated neurons than in other cell types. 12 of 46 identified candidate factors had increased expression levels in the ciliated neuronal transcriptome compared to other transcriptomes (Blacque et. al, 2005).

Gene	Ciliated Neurons/ Pan-neuronal Cells	Ciliated Neurons/ Muscle Cells	Ciliated Neurons/ Gut Cells
F25H2.5	5.5	2.1	2.5
F55F8.3	6.0	3.0	ns
B0041.2	5.0	5.0	ns
C26C6.3	ns	4.0	ns
B0511.12	ns	5.0	ns
F55F8.2	ns	4.0	ns
ZK973.6	ns	10.7	8.0
F36F2.3	ns	3.2	2.3
F28B3.7	ns	8.0	8.0
R11A5.1	ns	6.0	6.0
C09H6.2	ns	ns	4.0
ZK858.4	ns	ns	6.0

ns= nonsignificant difference

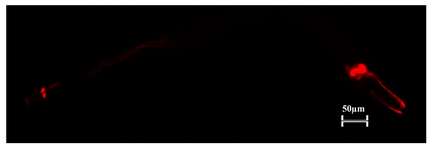
Some candidate PKD-2 localization factors are regulated by DAF-19

In *C. elegans*, *daf-19* encodes a transcription factor that interacts with conserved DNA sequences known as X-boxes. *daf-19* is one of the major regulators of ciliogenesis: *C. elegans* mutant for *daf-19* lack cilia. Thus, the presence of an X-box consensus sequence upstream of a promoter would be a strong indicator that a gene has a role in cilia structure and/or function.

Of the candidate PKD-2 localization factors identified in our screen, 10 have X-boxes within 1500 basepairs upstream of their promoters (Blacque et al. 2005).

In addition, we would expect that the expression level of genes regulated by DAF-19 would change in the absence of DAF-19. We identified an additional 7 candidate factors whose expression is significantly regulated by DAF-19 (Chen et al. 2006). Although X-boxes have not been regulated for these 7 factors, they may still be regulated by DAF-19, albeit indirectly.

We selected these 7 candidate factors for further analysis and analyzed cilia structure using a dye-filling assay. Reduction of expression of 5 candidate factors did not disrupt cilia structure. Reduction of expression of 2 candidate factors (F56W6.2 and C15A11.4) disrupted dye-filling of tail neurons indicating defects in cilia structure in this subset of ciliated neurons.



Ciliated neurons in the head and tail of PT443 (wild-type) *C. elegans* fill with fluorescent dye. Anterior to the right.

Gene	Average fold down-regulation or up-regulation in *daf-19*$^{-/-}$ mutants	Dye-Filling Assay
F56H6.2	5.6	Defects in tail neurons
C15A11.4	2.2	Defects in tail neurons
Y76G2A.2	3.4	Wild-type
K12C11.4	2.9	Wild-type
F31C3.5	1.8	Wild-type
H26D21.1	2.3	Wild-type
C27C7.3	2.8	Wild-type

Summary

Of the 201 candidate PKD-2 localization factors identified:

- 10 contain an X-box element
- 12 have higher expression in ciliated neurons compared to other cell types
- 7 show a significant change in expression level in *daf19*$^{-/-}$ mutants
- 2 have structural defects in ciliated tail neurons (assessed by dye-filling)

Thus, we think that our screen has identified a number of viable candidate PKD-2 localization factors.

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