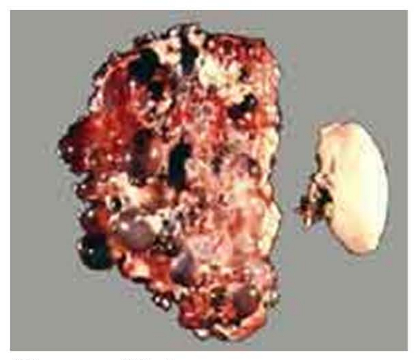
RNAinterference Identifies Genes Important for Cilia Structure and Function

Kara Braunreiter and Casey Gabrhel
Jamie Lyman Gingerich, Ph.D., Department of Biology, University of Wisconsin– Eau Claire

Introduction

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.

Autosomal Dominant Polycystic Kidney Disease model in *C. elegans*



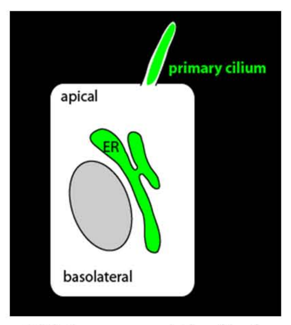
Human Kidney (diseased on left)

C. elegans

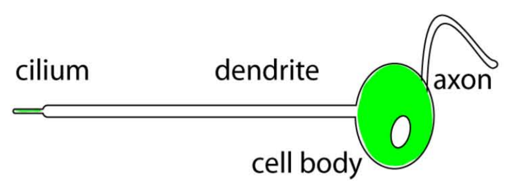
Human PKD-2 = *C. elegans pkd-2*

Mutations in polycystin genes cause ADPKD in humans and male mating behavior defects in *C. elegans.*

PKD-2 ciliary localization is conserved

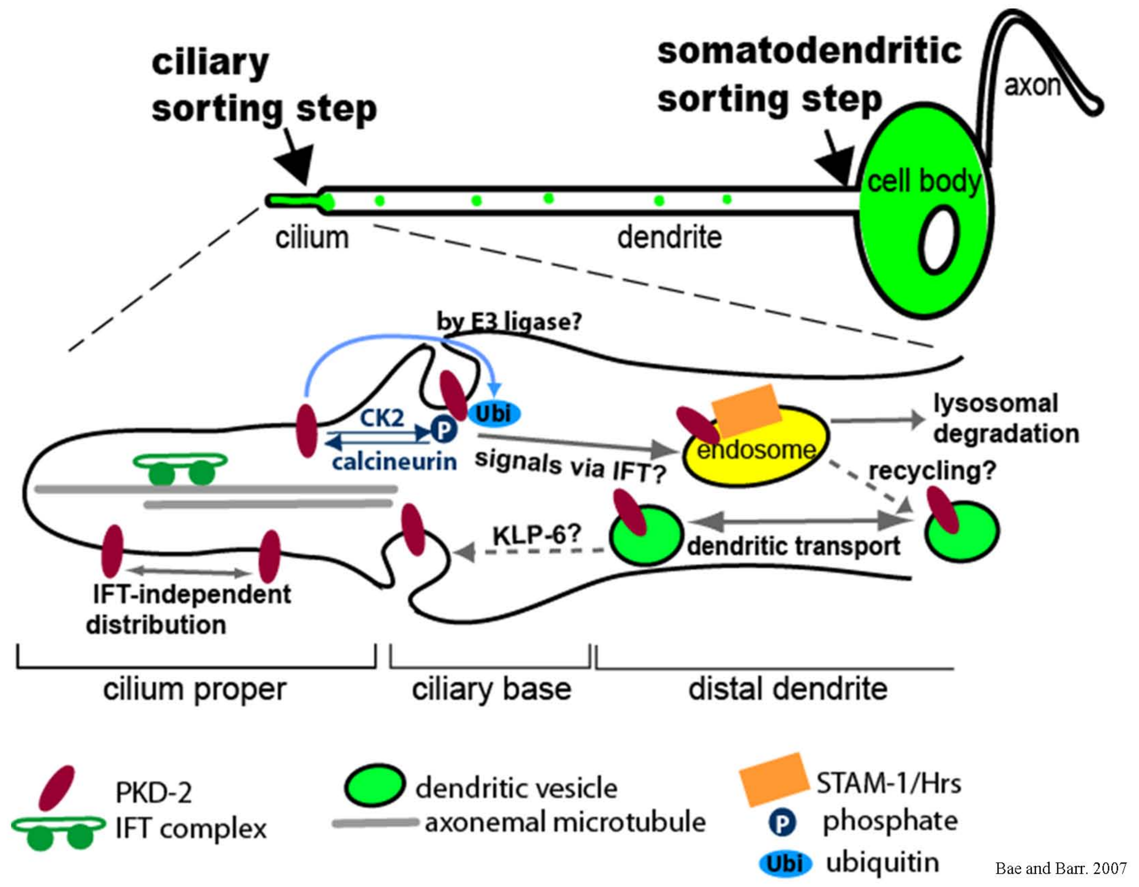


Kidney epithelial cell



C. elegans sensory neuron

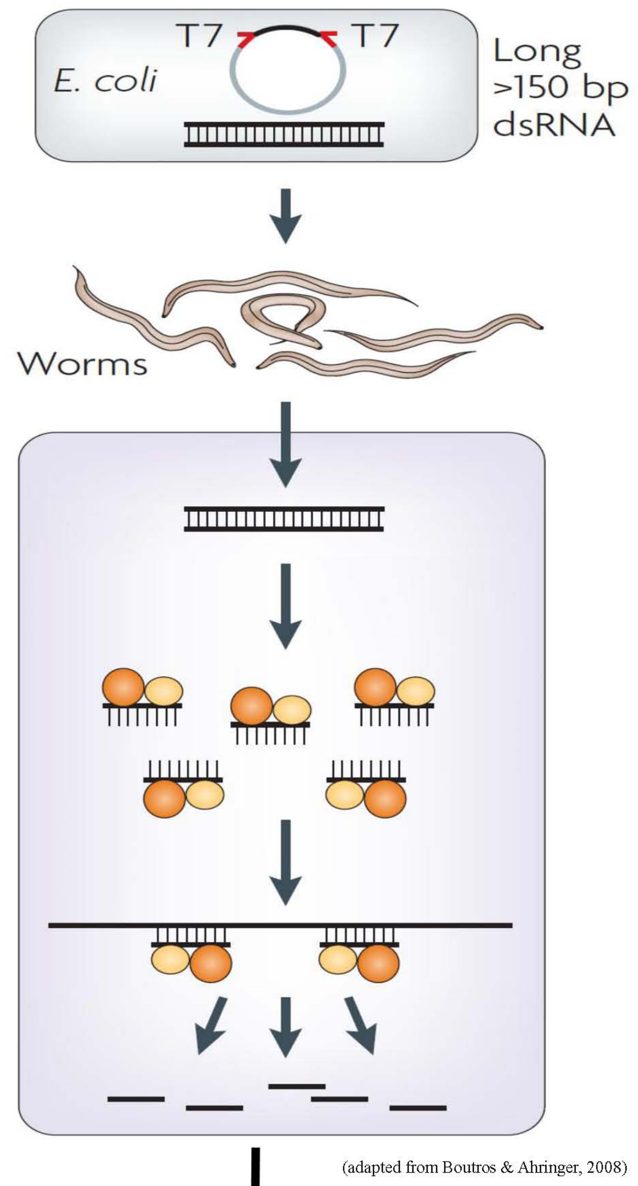
Current model for PKD-2 ciliary localization in *C. elegans*



Our Research Questions

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

RNAinterference systematically reduces function of a specific gene



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.

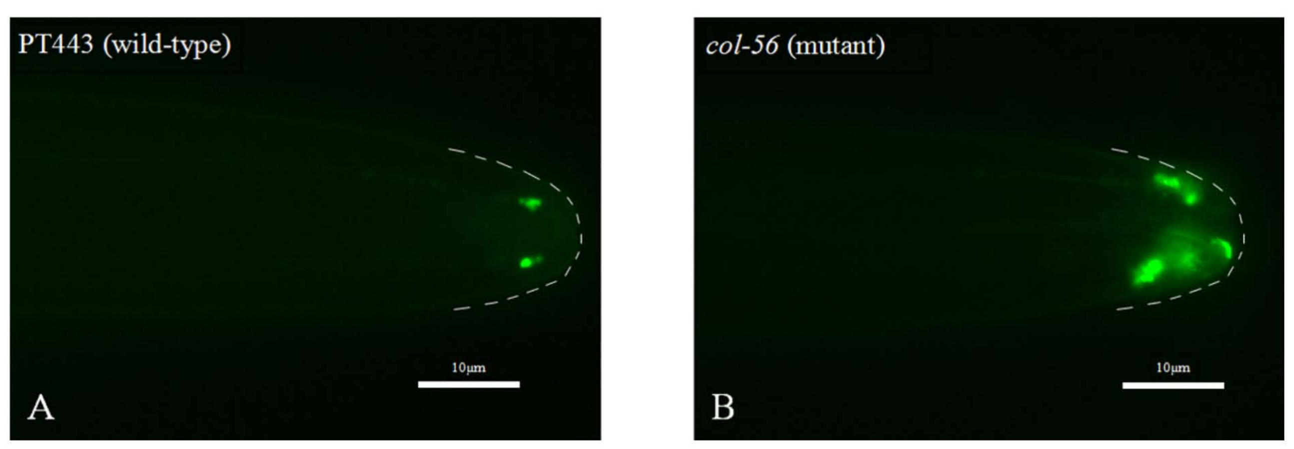
In the nematode cells, the double stranded RNA is rendered single stranded and can bind to the *C. elegans* mRNA with the complementary sequence.

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

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

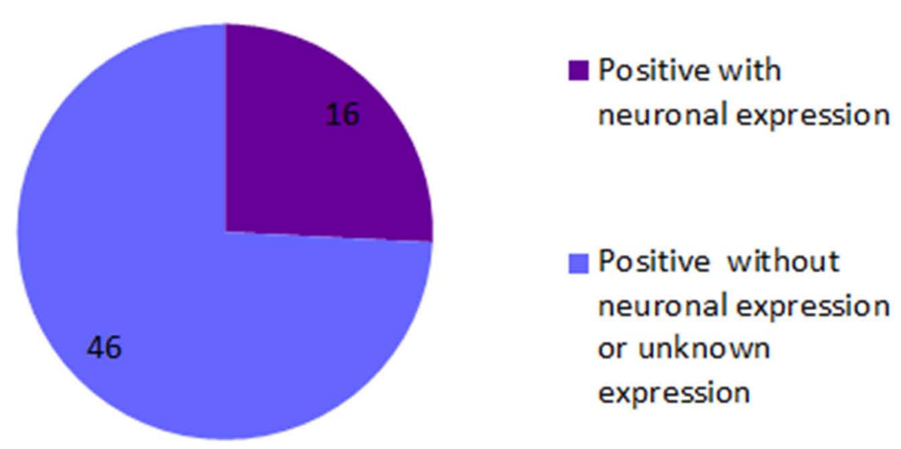
Preliminary Results

We have screened 37% (1066 genes) of linkage group I: 5.8% (62 genes) altered PKD-2::GFP expression.



Wild-type *C. elegans* localize PKD-2::GFP (green) to cilia normally (A) while reduction of *col-56* gene expression by RNAinterference leads to mislocalization of PKD-2::GFP (B). Anterior of head to right.

Of the genes that alter the PKD-2::GFP expression, a subset are expressed in neurons.



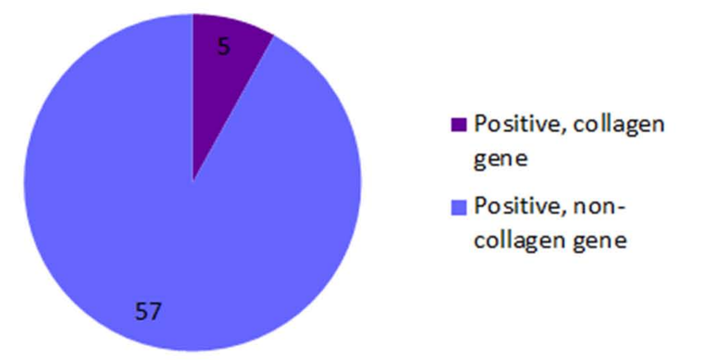
Neuronal expression indicates that our screen is identifying appropriate genes.

Collagen Genes in Relation to Ciliary Function

Analysis of the genes affecting PKD-2::GFP localization revealed a subset of collagen genes.

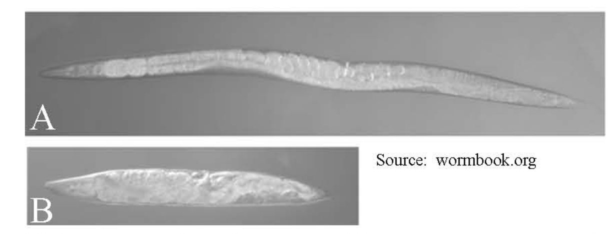
5 of the 62 genes that affect PKD-2::GFP expression are collagen gene family members.

Of the 20 collagen genes analyzed, 13 (65%) do not affect PKD-2::GFP expression.



Collagens have important structural and signaling roles in *C. elegans* and humans.

- Large protein family (over 200 collagen genes in *C. elegans)*
- Classified into different types based on gene structure and function (I-XXVIII)
- Function in tissue scaffolding, structure of bones and joints, and cell adhesion and migration.



Wild-type *C. elegans* (A) compared to a *C. elegans* mutant for a collagen gene (*dpy-5*) (B).

Characteristics of collagen genes affecting PKD-2::GFP localization

C. elegans gene	Collagen Group	Expression / Function in *C. elegans*	Human Homolog	Expression / Result of Mutation in Humans
col-45	I	Cuticle collagen	COL1A1	• Connective tissue • Bone/tissue fragility • Loose joints • Skin hyperextensibility
col-51 *col-56*	III	Cuticle collagen	COL3A1	• Extensible connective tissues • Aneurysms
col-53	VI	Unknown	COL6A2	• Connective tissue • Muscular atrophy
col-54	XIII	Unknown	COL13A1	• Connective tissue • Exact function is still unknown

Mislocalization of PKD-2::GFP in *C. elegans* is not limited to a particular collagen group, confirming that genes affecting localization must be identified experimentally.

Summary and Future Directions

We have identified genes that affect PKD-2 localization:

1. Genes known to be expressed in neurons
2. Genes with previously unidentified neuronal associations

Future Directions:

1. Focus on the role of the collagen genes in ciliary receptor localization:
   - Screen the remaining collagen genes on LGI (26 genes).
   - Characterize ciliary functions and expression patterns of the collagens.
2. Screen the remaining genes on linkage group I (about 2900 genes):
   - Analyze genes for similarities in expression, structure, and function.
   - Characterize ciliary functions and expression patterns.

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