ABSTRACT

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disorder affecting 1/400 to 1/800 individuals in the population. Mutations in the human genes *PKD1* and *PKD2*, which encode Polycystin-1 (PC-1) and Polycystin-2 (PC-2), respectively, account for 95% of ADPKD cases. Defects in sensory receptor localization in cilia, or cilia formation may contribute to many human diseases including ADPKD. The nematode *Caenorhabditis elegans* uses sensory cilia localized to the end of sensory neurons to perceive environmental stimuli. Sensory information is integrated and used to determine appropriate behavioral responses. The *C. elegans* ADPKD gene homologs *lov-1*/PC-1 and *pkd-2*/PC-2 localize and function in male specific sensory cilia, and are required for proper male mating behavior. A pilot mutagenesis screen isolated 21 separate mutations affecting PKD-2 ciliary localization, which were grouped into three categories. This research was done on *my18*, a mutation which effects PKD-2 expression at the transcription level. In a subset of *my18* males, tail fan formation is defective, which maybe responsible for defects in male mating behavior. Furthermore, in *my18* males with normal tail fan formation mating behavior and efficiency was compromised compared to wild type. Linkage analysis mapped *my18* to chromosome IV, and further mapping experiments are in progress.

Thesis Title

Characterization of *my18*, a Mutant Exhibiting Abnormal Expression of the *C. elegans* Polycystic Kidney Disease Gene

(Abstract content here not to exceed 150 words)

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COVER SHEET

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Characterization of *my18*, a Mutant Exhibiting Abnormal Expression of the *C. elegans* Polycystic Kidney Disease Gene

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Abstract
Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disorder affecting 1/400 to 1/800 individuals in the population. Mutations in the human genes *PKD1* and *PKD2*, which encode Polycystin-1 (PC-1) and Polycystin-2 (PC-2), respectively, account for 95% of ADPKD cases. Defects in sensory receptor localization in cilia, or cilia formation may contribute to many human diseases including ADPKD. The nematode *Caenorhabditis elegans* uses sensory cilia localized to the end of sensory neurons to perceive environmental stimuli. Sensory information is integrated and used to determine appropriate behavioral responses. The *C. elegans* ADPKD gene homologs *lov-1*/PC-1 and *pkd-2*/PC-2 localize and function in male specific sensory cilia, and are required for proper male mating behavior. A pilot mutagenesis screen isolated 21 separate mutations affecting PKD-2 ciliary localization, which were grouped into three categories. This research was done on *my18*, a mutation which effects PKD-2 expression at the transcription level. In a subset of *my18* males, tail fan formation is defective, which maybe responsible for defects in male mating behavior. Furthermore, in *my18* males with normal tail fan formation mating behavior and efficiency was compromised compared to wild type. Linkage analysis mapped *my18* to chromosome IV, and further mapping experiments are in progress.

Keywords:
Autosomal Dominant Polycystic Kidney Disease
C. elegans
pkd-2
Expression

Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a lethal ailment which affects more than 1/1000 people in the population today (Solmo et al. 2001). ADPKD causes cysts to form in the nephrons of the kidneys; these cysts fill with fluid and become so abundant that they overcrowd normal kidney tissue (Figure 1). Structural deformities in the kidneys combined with hypertension cause ADPKD to be the fourth leading cause of renal failure. Costs for treatment annually exceed $20,000,000,000; there is no preventative cure so patients must resort to dialysis or kidney transplantation (www.pkdcure.org). In humans, 95% of ADPKD cases are caused by mutations in either *PKD1* or *PKD2*, which encode the transmembrane proteins Polycystin-1 (PC-1) and Polycystin-2 (PC-2), respectively. PC-1 and PC-2 are proposed to form a receptor/channel complex (Igarashi and Solmo 2002). This complex, in mammals, localizes to sensory cilia, and is responsible for sensing urine flow (Nauli et al. 2003).

The model organism, *Caenorhabditis elegans* (*C. elegans*) provides an excellent model to study ADPKD because it is a cheap and efficient way to gain insight into the molecular mechanisms, which when disrupted, cause human diseases. Traits of *C. elegans* which contribute to its ease of genetic manipulation and study are its hermaphroditism, its short generation time (2-3 days from egg to adult), small size, and sequenced genome (Barr 2003). The nervous system of *C. elegans* contains 302 neurons in the hermaphrodite, and 381 neurons in the male. The *C. elegans* homologs for *PKD1* and *PKD2* are *lov-1* and *pkd-2*, respectively, which encode the polycystins LOV-1 and PKD-2, respectively (Barr et al. 2001). *lov-1* and *pkd-2* are expressed in 21 of the male specific neurons involved in mating, and localized to the ciliated regions of the dendrites

(Figure 2). The *pkd-2* expressing cells include the Ray B neurons, R1B-R5B and R7B-R9B, involved in male response to contact with the hermaphrodite, the hook neuron (HOB) associated with location of vulva, and the cephalic male specific neurons (CEMs) in the head, associated with chemotaxis towards hermaphrodites (Chasnov et al 2007). The *C. elegans* polycystins are required for male sensory behavior, which involves a stereotyped mating pattern (Liu and Sternberg 1995). During mating behavior, male response to contact with the hermaphrodite is affected by mutations in *pkd-2*, and LOV (location of vulva) is affected by mutations in *lov-1* (Barr et al. 2001).

To understand the molecular mechanisms of *pkd*-2 expression and ciliary localization, *wild-type* PKD-2::GFP (Green Fluorescent Protein) was integrated into the genome via gamma irradiation (Bae et al. 2006). This transgene rescues *pkd-2* mutant phenotypes and localizes to cilia, consistent with anti-PKD-2 antibody staining (Barr and Sternberg 1999). To further understand the mechanisms whereby *pkd-2* functions, a mutagenesis screen was performed and three classes of mutants were isolated (Figure 3). Class I mutants showed increased ciliary localization of PKD-2::GFP to the cilium proper and ciliary base. In Class II mutants, PKD-2::GFP is abnormally distributed to the entire neuron. Class III mutants are wild type for PKD-2 protein localization, but are defective in *pkd-2* expression. In this study, we focus on one of the Class III mutants, *my18*, which exhibits reduced *pkd-2* expression (Figure 4). In *my18* background, *pkd-2*::GFP is only expressed in Ray B neurons R4B, R8B, and R9B, CEMs and HOB neurons and is absent from Ray B neurons R1B, R2B, R3B, and R7B (Barr lab unpublished data).

This work is a preliminary characterization of *my18*, and includes analysis on the behavioral, anatomical, cellular, and genetic levels. *my18* affects pkd-2 expression in a

subset of ray neurons. Linkage group analysis was completed, and mapping experiments have begun. *my18* males show compromised mating behavior and efficiency, and a subset of *my18* males show defects in tail fan development.

Results

Expression Analysis

To test at which stage *my18* affects *pkd-2* expression, either transcription, translation, or post-translation, *my18* animals was crossed with animals containing a *pkd-2* promoter fusion GFP (Figure 5 and 6). Wild Type *pkd-2* promoter fusion GFP is expressed in all *pkd-2* neurons, and is distributed to the entire cell. The subcellular expression pattern of the resulting homozygosed progeny showed the same expression pattern as *my18* in the PKD-2::GFP background (Figure 6). *pkd-2* promoter fusion GFP can be seen in the entire cell (only in R4B, R8B, and R9B, CEMs, and HOB) because the transcriptional promoter has a GFP tag, whereas PKD-2::GFP is only expressed in the cell body, and localized to cilia.

To further test the range of *my18*'s affects, expression of *osm-6* was examined in a *my18* background. o*sm-6* is a gene required for ciliogenesis and is expressed in most of the ciliated neurons both in males and hermaphrodites, including the CEMs and RnBs where *pkd-2* is expressed. In *my18* background, *osm-6* displayed *wild-type* expression indicating *osm-6* expression is unaltered by *my18*.

Anatomical Analysis

my18 young adult males sometimes show a Mab phenotype (Male abnormal). Males were isolated at the fourth larval stage (L4) and staged at 20° C for 24 hours. 1/3-

1/4 of all young adult males have a deformed tail fan (Figure 7). In some cases there appears to be a cuticle dragged by the deformed tail.

Behavioral Analysis

Male mating behavior is characterized by a stereotyped, step wise behavioral program. Steps include response to contact with the hermaphrodite, backing along its body, turning around the head or tail, location of the vulva, insertion of spicules into the vulva, and sperm transfer (Liu and Sternberg 1995). *my18* males show decreased response compared to wild type; their response to contact with the hermaphrodite was similar to *pkd-2* null mutants. *my18* also showed LOV (location of vulva) defects compared to wild type and *pkd-2* null mutants. Wild type males respond to contact 85% of the time, and locate the vulva 78% of the time. *pkd-2* null mutants respond to contact 13% of the time, and locate the vulva 33% of the time. *my18* males respond to contact 20% of the time, and never locate the vulva.

In addition to defective mating behavior, *my18* males show decreased mating efficiency. In a mating efficiency assay wild type males were 86% efficient, and *my18* males were 35% efficient (Figure 8).

Genetic Analysis

C. elegans has six chromosomes; five autosomes (I-V) and one sex chromosome (X). To determine which chromosome the *my18* mutation maps to, *my18* was crossed with a strain containing three recessive mutations on three different chromosomes (*blistering* on IV, *dumpy* on V, and *long* on X). F_1 cross progeny were isolated and self fertilized. The *my18* phenotype was scored among F_2 males displaying blistering, dumpy and long phenotypes. Among blistering males *my18* was never expressed; among dumpy

males *my18* was expressed 25% of the time; and among long males *my18* was expressed 17% of the time. It can therefore be concluded that the *my18* mutation is located in a gene on chromosome IV (Figure 9). Similar experiments were done to rule out chromosomes I, II, and III as locations for *my18* (not shown). The integrated PKD-2::GFP transgene is also located on chromosome IV. Therefore before any further mapping experiments can be completed a strain containing another reporter of *my18* on a different chromosome must be constructed to avoid losing *my18* or its reporter during a recombination mapping event.

Other Observed Phenotypes

We noticed other gross phenotypes of *my18* mutants. *my18* has a decreased brood size compared to wild type worms. Also the *my18* generation time is slightly longer than wild type. These observations were made on the plate level, they have not been measured.

Discussion

The polycystins and their localization to cilia are conserved from *C. elegnas* to humans. In mammalian kidney epithelial cells *PKD2* is expressed in cilia which project off the apical surface. In *C. elegans, pkd-2* is expressed in the ciliated regions of the male specific neurons such as the CEMs, HOB neuron, and ray neurons. *my18* does not affect PKD-2 expression and localization in the CEMs, and HOB neuron. However *my18* alters *pkd-2* expression in the ray neurons such that it is only expressed in ray neurons R4B, R8B, and R9B; in *my18* background *pkd-2* expression is missing in ray neuron pairs 1, 2, 3, and 5. This suggests that transcription of *pkd-2* is differentially regulated in a subset of ray neurons.

The ray cell-type specific effects of *my18* on *pkd-2* expression were not changed in *pkd-2* promoter fusion GFP background. GFP was expressed in the CEMs, HOB, and ray neurons RB4, RB8, and RB9. As expected for a promoter GFP fusion, it was distributed to the entire cell. This suggests that *my18* affects *pkd-2* expression at the transcription level. Since *osm-6* expression was normal in *my18* background, it can be concluded that *my18* may not affect ciliogenic genes; its effects are probably more specific to the ADPKD genes *lov-1* and *pkd-2,* or ray specific genes.

The Mab phenotype expressed in 1/4-1/3 of *my18* young adult males has two interpretations. The Mab phenotype may be another phenotype of *my18* that is incompletely penetrant. Alternatively, the Mab phenotype could be due to a mutation in a different gene (Figure 3). This distinction can be made by additional outcrossing of *my18*, and from further positional mapping strains on chromosome IV.

The two separate behavioral analysis experiments, *my18* mating assay and *my18* mating efficiency, provided results leading to the same conclusion. *my18* may affect PKD-2 function, and might also affect LOV-1 function. *my18* affects response to contact with the hermaphrodite similar to *pkd-2* null mutants. Furthermore, *my18* affects location of vulva ability more severely than *pkd-2* null mutants. These mating experiments were performed with males not expressing the Mab phenotype, so these two affects of *my18* appear to be unrelated.

Using different reporters is a way to determine the scope of *my18's* affects outside of *pkd-2*. Also since the integrated PKD-2::GFP transgene is located on chromosome IV, before any genetic mapping can continue a strain containing another reporter of *my18* located on a different chromosome must be developed to avoid losing either *my18* or its

reporter in a recombination event. Possible other candidates for a *my18* reporter include promoter fusion *pkd*-2::GFP (Figure 5 and 6), or LOV-1::GFP. *cwp-2* and *cwp-4* (co-expressed with polycystin) might also show altered expression in *my18* background. Finally KLP-6 (Kinesin Like Protein) could be used because it is co expressed with *pkd-2*. The observation that *my18* has a decreased brood size and slower generation time suggests that *my18* may have affects on embryogenesis or development, which have not been carefully studied.

By using the simple model organism *C. elegans* it is possible to study the genetic pathways which affect transcription of human disease gene homologs. Identification of the *my18* locus will provide insight into the molecular mechanisms by which the transcription of the *C. elegans* polycystins is regulated in different neurons. It will be interesting to find out if mammalian homologs of the gene mutated in *my18* have similar effects on polycystin expression.

Materials and Methods

C. elegans

Nematodes were raised, and maintained using standard conditions (Brenner, 1974).

Microscopy

Whole worm mounts were prepared, and images were captured as described previously (Bae et al 2006).

Behavioral Analysis

Male mating assays were performed as described previously (Barr and Sternberg 1999, Bae et al. 2006). Mating efficiency was determined by placing 5 males on a plate containing 20uL feeding *E. coli* with 2 dumpy hermaphrodites. After 24-48 hours the

males and hermaphrodites were removed from the plate. Mating efficiency was quantified by the following equation.

$$MatingEfficiency = [\frac{WildType}{(WT + dumpy)}] \times 100$$

Tables and Figures

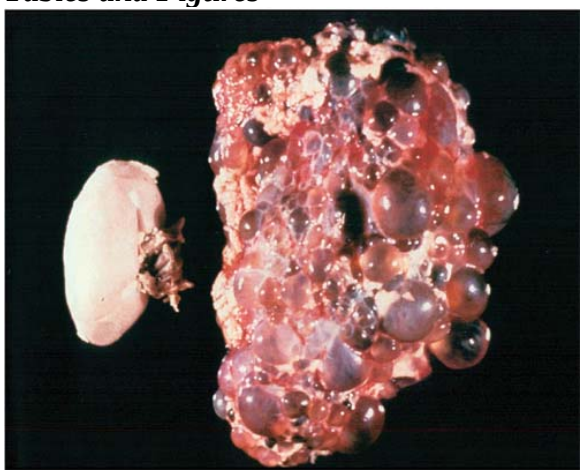


Figure 1. A cystic human kidney (right) next to a normal human kidney (left). (Calvet, 2003).

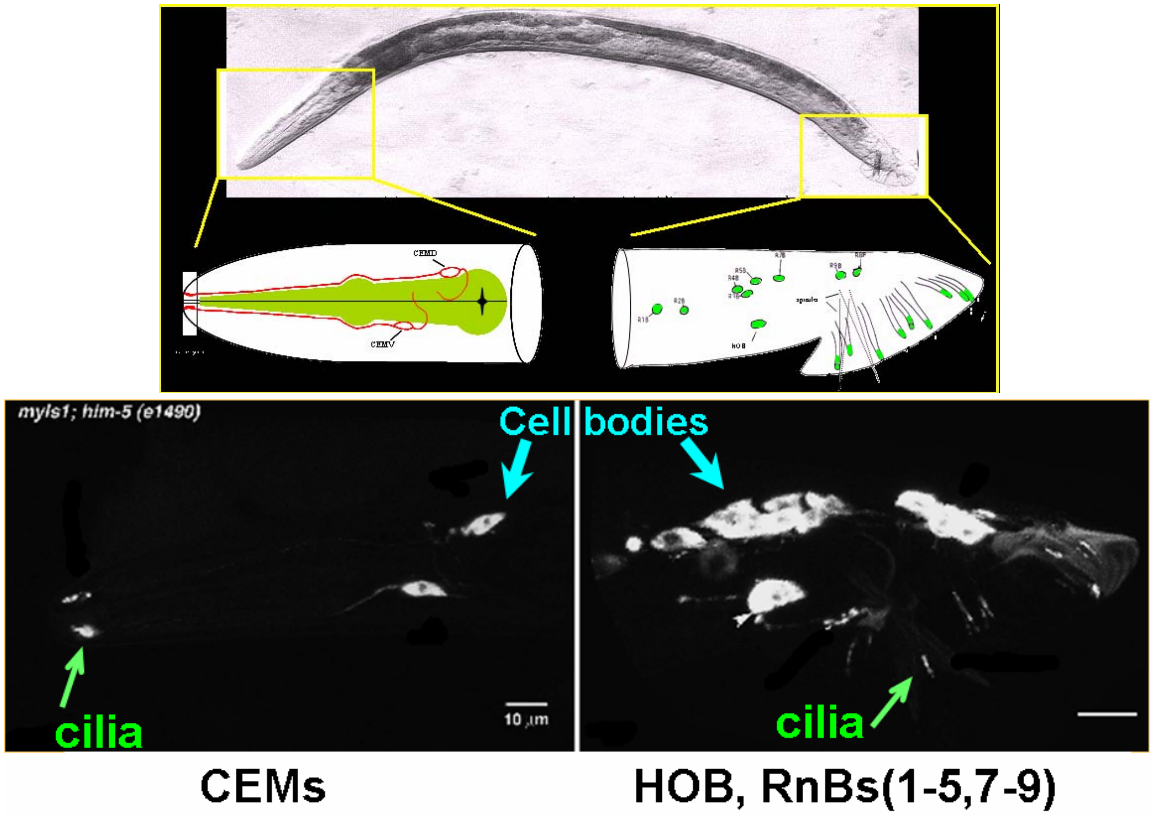
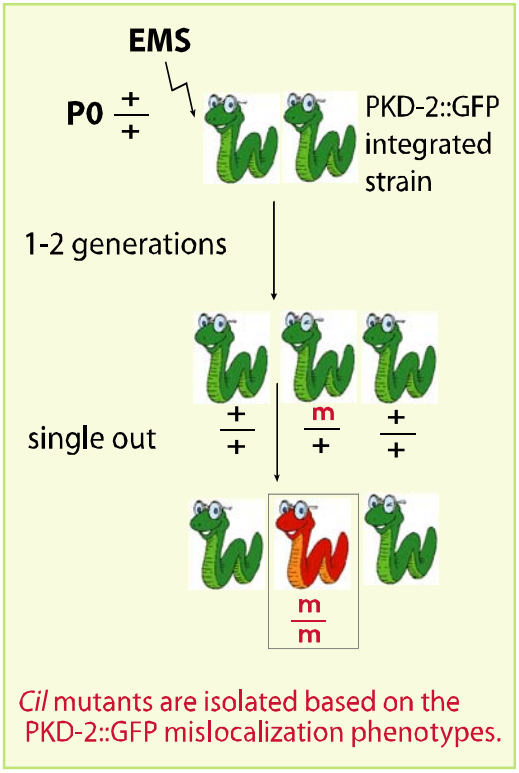


Figure 2. Wild type *pkd-2* expression pattern. CEMs in the head (left), RnBs 1-5, 7-9, and HOB (right). PKD-2::GFP is expressed in the cell bodies, and localized to the ciliated regions of the neurons. (Top: Hu and Barr 2005. Bottom: Bae, 2006)



Class	PKD-2::GFP localization	allele
WT	Cilia & ciliary bases; ER in cell body	
I	Increased levels in cilia & ciliary bases	*my13* *my14* *my16* *my17*
II	Even distribution throughout neuron	*my2* *my15*
III	Defects in *pkd-2* expression	*my18*

Figure 3. Left: Mutagenesis screen performed to isolate three classes of PKD-2::GFP mutants. Right: Classes of PKD-2::GFP mutants, names of alleles, and PKD-2::GFP localization pattern. (Figures courtesy of Young-Kyung Bae)

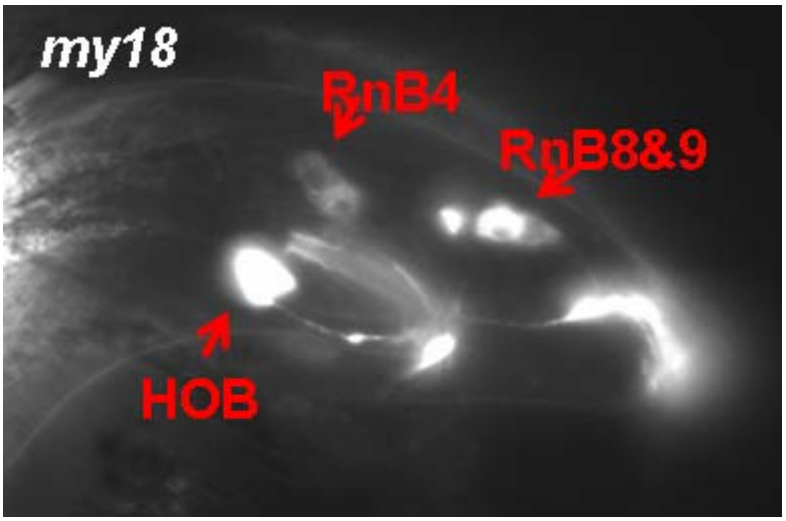


Figure 4. *my18* affects expression of PKD-2::GFP. PKD-2::GFP expression pattern in the male tail in *my18* mutants. PKD-2::GFP is expressed in RnBs 4, 8 and 9, HOB, and CEMs (not shown). *my18* PKD-2::GFP shows normal localization patterns where expressed. (Barr Laboratory unpublished data)

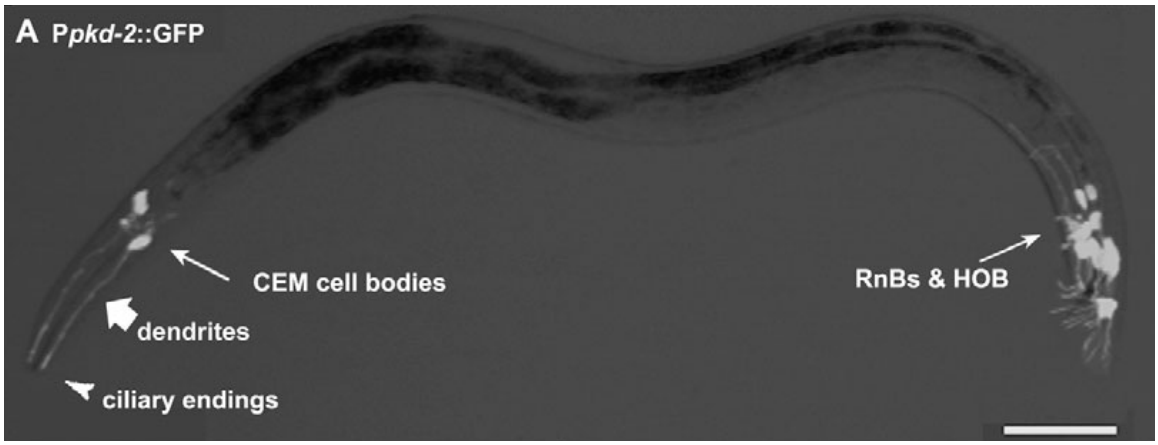


Figure 5. *wild-type pkd-2* promoter fusion GFP. GFP is seen in the entire cell in the CEMs, HOB, RnBs, and not localized to the ciliated regions. (Bae et al. 2006).

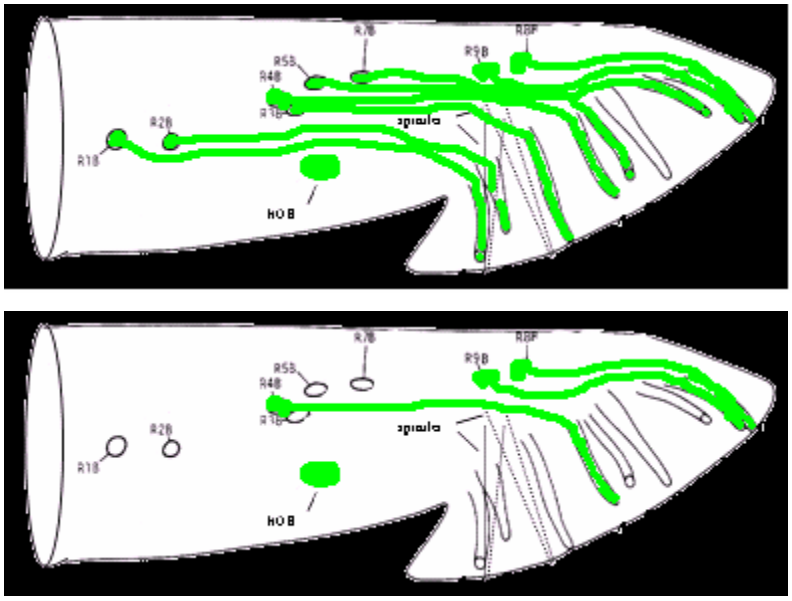


Figure 6. *wild-type pkd*-2 promoter fusion GFP (top) and my18 expression in *pkd-2* promoter fusion GFP background (bottom). *pkd-2* promoter fusion GFP is distributed throughout the cell. In *wild-type pkd-2* promoter fusion is expressed in ray neurons R1B-R5B and R7B-R9B. In *my18* background *pkd-2* promoter fusion is expressed in R4B, R8B, and R9B.

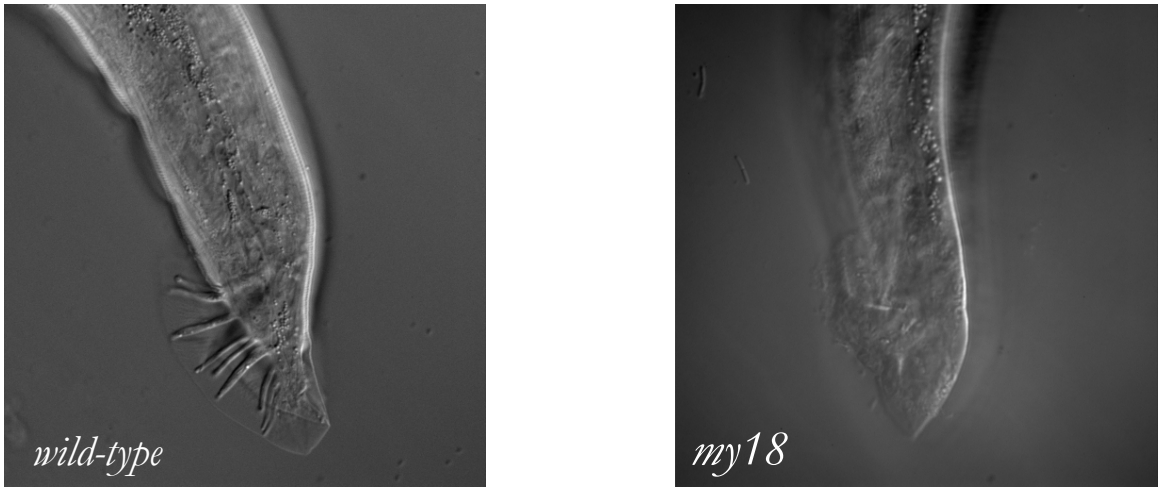


Figure 7. A wild type male tale fan (left) and a typical deformed tail fan seen in 1/4-1/3 of *my18* young adult males (right).

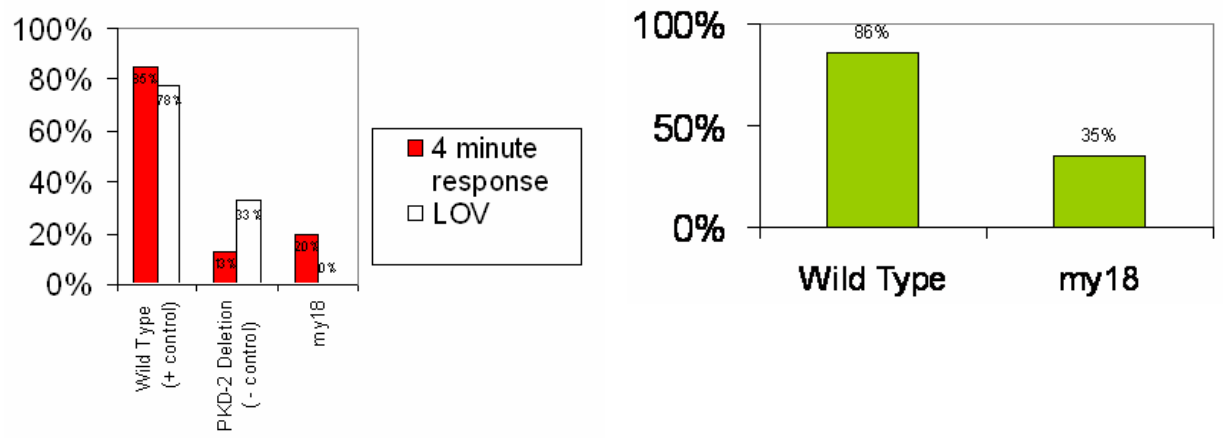


Figure 8. Left: *my18* mating assay. n=21 for wild type males, n=23 for *pkd-2* deletion males, and n=20 for *my18* males. Males that responded to contact and further tested for LOV n=18 for *my18* males, n=3 for *pkd-2* deletion males, and n=4 for *my18* males. Right: *my18* mating efficiency. n=5 for wild type and *my18* males tested.

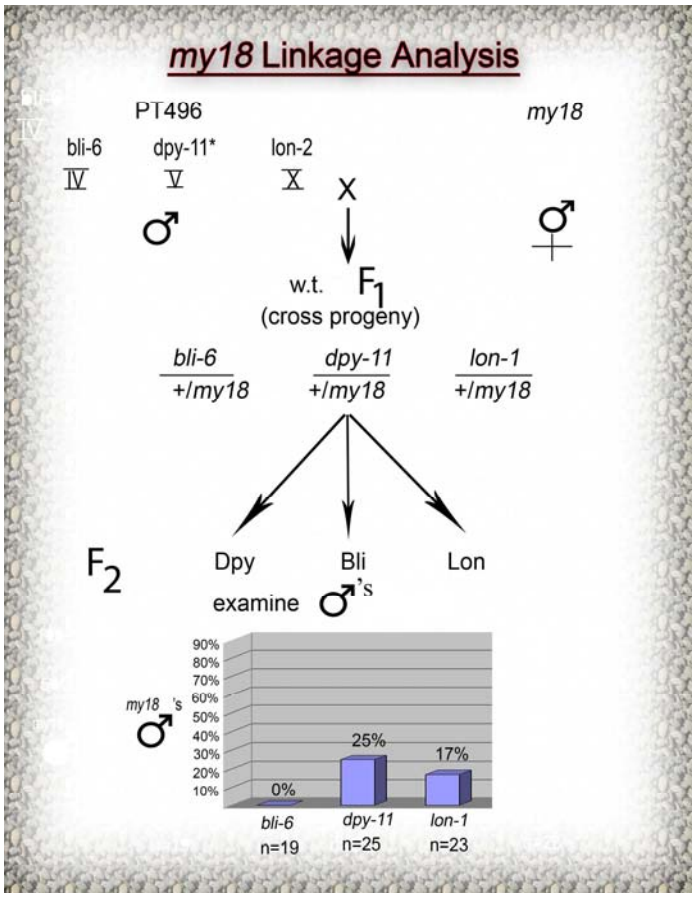


Figure 9. Linkage analysis of *my18* revealed it is a mutation in a gene located on chromosome IV. *my18* hermaphrodites were crossed with PT496 males which contains the recessive mutations blistering, dumpy, and long on chromosomes IV, V, and X, respectively. F_1 cross progeny (not showing expression of any of the four recessive mutations) were isolated and allowed to self fertilize. F_2 males expressing blistering, dumpy, or long phenotypes were isolated and screened for the *my18* phenotype (PKD-2::GFP expression defect).

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