Caenorhabditis elegans as a Model to Study Renal Development and Disease: Sexy Cilia

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The nematode *Caenorhabditis elegans* has no kidney *per se*, yet "the worm" has proved to be an excellent model to study renal-related issues, including tubulogenesis of the excretory canal, membrane transport and ion channel function, and human genetic diseases including autosomal dominant polycystic kidney disease (ADPKD). The goal of this review is to explain how *C. elegans* has provided insight into cilia development, cilia function, and human cystic kidney diseases.

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At first glance, worms and kidneys have as little to do with each other as do *Caenorhabditis elegans* geneticists and practicing medical nephrologists. Sydney Brenner's choice of *C. elegans* as a model organism has provided a means for studying the genetics, molecular biology, and biochemistry of many human diseases. The Nobel laureate Brenner selected *C. elegans* because of its small size (approximately 1 mm), rapid free-living life cycle (2 to 3 d from egg to adult), hermaphroditism, large brood size (self-fertilizing hermaphrodites produce approximately 300 offspring and approximately 1000 progeny when mated with males), genetic amenability, transparency, and simple cellular complexity (<1000 somatic cells).

The constantly expanding *C. elegans* molecular toolkit includes transgenesis, a completely sequenced genome, green fluorescence protein (GFP) to look at gene expression and protein localization *in vivo*, primary cell culture, and RNA interference (RNAi) to knockdown gene function. The *C. elegans* genome project served as a prototype for other sequencing projects (1). Likewise, *C. elegans* systematic genome-wide approaches culminating in effective, public data archiving at WormBase for *C. elegans* genome and biology (http://www.wormbase.org) provide an example for high-throughput functional genomics in more complex biologic systems. Forward or classical genetics is aimed at understanding a biologic process and moves from mutant phenotype to gene/protein identification. Reverse genetics starts with the knowledge of gene/protein sequence and moves to function/phenotype. *C. elegans* is amenable to both approaches, making the worm an attractive model system in which to study your favorite gene (2).

The Ciliary Model of Cystic Kidney Diseases

Renal cysts are observed in a number of human genetic disorders, suggesting that disrupting any one of a number of different cellular processes may result in cyst formation. An alternative hypothesis is that for those disorders that share a subset of renal and extrarenal manifestations, cysts may arise from disruption of a conserved cellular function. Autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive PKD (ARPKD), nephronophthisis (NPHP), and Bardet-Biedl syndrome (BBS) share two common features: Cystic kidneys and ciliary localized gene products (Table 1) (3,4). "Ciliary cystoproteins" (3) include polycystin-1 (PC-1) and PC-2, which are defective in ADPKD (5–7); fibrocystin or polyductin, which is defective in ARPKD (8–10); nephrocystin-1 and nephrocystin-2/inversin, which are defective in nephronophthisis (11,12); and BBS3 and BBS5 through BBS8, which are defective in BBS (13–16).

Cystic kidney diseases, primary cilia dyskinesia, retinitis pigmentosa, and *situs inversus* (reversal of left-right organ asymmetry) are human diseases that result from ciliary defects (17). Establishing and maintaining ciliary function is clearly essential for the well-being of an organism. Cilia are specialized organelles that function in motility (9 + 2 motile cilia or 9 + 0 nodal cilia) or sensation (9 + 0 primary or sensory cilia). Primary kidney cilia project from the apical surfaces of epithelial cells of the kidney nephron and act as flow mechanosensors (18–20). Nodal cilia are located on the embryonic node, exhibit a unique rotation, and are necessary to establish left-right (L-R) asymmetry in vertebrates (21). The greatest challenges to both molecular biologists and nephrologists are determining the mechanisms underlying renal cystic diseases, understanding their interconnections, and designing therapies to prevent, delay, or halt cyst progression.

All Cilia Are Created Equal: Lessons from Algae and Worms

Intraflagellar transport (IFT) is an evolutionarily conserved, microtubule-based motility that is responsible for the assembly and maintenance of all cilia and flagella (22). IFT was first observed by Rosenbaum and colleagues (23) as microscopic particles moving up and down the length of the flagella of the green alga *Chlamydomonas*. The cellular machinery driving IFT was determined using primarily cellular and biochemical approaches (see references within 22). The IFT machinery com-

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Table 1. Mammalian kidney disease genes and *C. elegans* homologs

Gene	*C. elegans* homolog	*C. elegans* expression pattern	Mutant phenotype (Genetic mutation, Δ, and/or RNAi)
Autosomal dominant polycystic kidney disease (ADPKD)			
PKD1	*lov-1* (ZK945.9)	Ciliated sensory neurons (male) (58, 60)	Δ: Male sensory defects, cilia development normal (52,54)
PKD2	*pkd-2* (Y73F8A.1)	Ciliated sensory neurons (male) (58, 60)	Δ: Male sensory defects, cilia development normal (52,54)
Autosomal recessive polycystic kidney disease (ARPKD)			
Tg737orpk	*osm-5* (Y41G9A.3)	Ciliated sensory neurons (all) (39)	Δ: Abnormal cilia development (32)
heterotrimeric	*klp-11* (F20C5.2)	Ciliated sensory neurons (31)	Δ (non-null): cilia development normal (25)
kinesin-II;	*klp-20* (Y50D7A.6)	N.D.	N.D.
KIF3A	*kap-1* (F08F8.3)	Ciliated sensory neurons (31)	Δ: cilia development normal, IFT rates abnormal (25)
Homodimeric kinesin-II KIF17	*osm-3* (M02B7.3)	Ciliated sensory neurons (subset) (31)	Δ: Abnormal cilia development (subset) (32)
PKHD1	None	–	–
cpk/Cystin	None	–	–
Bardet-Biedl Syndrome (BBS)			
BBS1	Y105E8A.5	Ciliated sensory neurons (all) (17)	N.D.
BBS2	F20D12.3	Ciliated sensory neurons (all) (17)	RNAi: WT (65)
BBS3	*arl-6* (C38D4.8)	Ciliated sensory neurons (62)	RNAi: WT (65,66)
BBS4	None	–	–
BBS5	R01H10.6	Ciliated sensory neurons (all) (19)	RNAi: WT (65,66)
BBS6	None	–	–
BBS7	*osm-12* (Y75B8A.12)	Ciliated sensory neurons (all) (17)	Δ: chemotaxis defects and abnormal cilium structure (16)
BBS8	T25F10.5	Ciliated sensory neurons (all) (17)	Δ: chemotaxis defects and abnormal cilium structure (16)
Nephronophthisis (NPH)			
NPHP1	M28.7	Ciliated sensory neurons (subset) (unpublished)	RNAi: WT (65) Δ (non-null): cilia development normal (unpublished)
NPHP2 (inversin)	ZC15.7	N.D.	N.D.
	T28D6.4	N.D.	RNAi: WT (65,66); genome instability (67)
NPHP3	*klc-1* (M7.2)	N.D.	RNAi: WT (65); Embryonic lethal (68)
	klc-2 (C18C4.10)	N.D.	N.D.
NPHP4	R13H4.1	Ciliated sensory neurons (subset) (unpublished)	Δ (non-null): cilia development normal (unpublished)

Mammalian kidney disease genes and *C. elegans* homologs. Column 1 indicates the mammalian disease gene. All are human disease genes with the exception of *Tg737*, *KIF3A*, and cystin, which are mouse ARPKD genes. No renal function or phenotype has been described for KIF17, the mammalian homologue of OSM-3. Column 2 indicates *C. elegans* homolog with gene name and/or cosmid number. There are no *PKHD1*, cystin, *BBS4*, or *BBS6* homologs identified in the *C. elegans* genome. *NPHP2* homology is limited to the amino terminal ankyrin repeats. *NPHP3* homology is restricted to the C-termainal TPR domains. *klc-1* and *klc-2* encode predicted kinesin light chain (KLC) proteins. Column 3 indicates GFP expression patterns. Column 4 indicates gene function as determined by genetic mutation (Δ) or RNA interference (RNAi). WT, wild type; N.D., not determined or no data published.

prises heterotrimeric kinesin-II (consisting of two motor subunits and one nonmotor accessory subunit) and retrograde cytoplasmic dynein motors that move IFT particles and cargo to and from the distal tip of cilia. The IFT particle is composed of two complexes (A or B) that contain 16 to 18 polypeptides. Kinesin-II and complex B polypeptides seem to regulate anterograde transport; dynein and complex A polypeptides may regulate retrograde transport.

Scholey and colleagues (24–29) have performed exquisite experiments to examine the IFT machinery in *C. elegans* using time-lapse microscopy of GFP-tagged IFT motors and polypeptides in conjunction with ciliary mutants. *C. elegans* chemosensory cilia contain two anterograde IFT motors: The canonical heterotrimeric kinesin-II and homodimeric OSM-3 kinesin-II. Surprising, these anterograde motors act cooperatively and redundantly, with kinesin-II and OSM-3 building the cilium from the transition zone/basal body to the middle region of the cilium (25). At the mid-zone, kinesin-II turns back and OSM-3 continues, acting alone to build the distal end of the cilium. Several interesting questions are raised by this study. What regulates the transition from cooperation to autonomy? Is this mechanism specific for worm cilia, or is this an evolutionarily conserved phenomenon?

In *C. elegans*, IFT builds and maintains cilia on dendritic endings of sensory neurons. Ciliated sensory neurons located in the head and tail (Figure 1) sense an extensive variety of environmental signals and mediate a wide spectrum of behaviors. For example, animals must locate food and males must find hermaphrodite mates. Of the 302 neurons in the hermaphrodite, 60 have dendritic endings that terminate in cilia (30). The male possesses an additional 52 ciliated neurons (Figure 1) (31). In *C. elegans*, cilia are sensory and nonmotile, possessing a 9 + 0 doublet microtubule array. Twenty-six of 60 ciliated neurons shared between the sexes have endings that are exposed to the environment and located in the head amphid, inner labial sensilla, and tail phasmid sensilla. It is interesting that many of the genes required for the formation, maintenance, and function of *C. elegans* cilia have human counterparts, which, when mutated, cause diseases with renal pathologies, including ADPKD, ARPKD, BBS, and NPHP (Table 1).

For identifying genes that are required for cilia development in *C. elegans*, classical genetic screens were performed on the

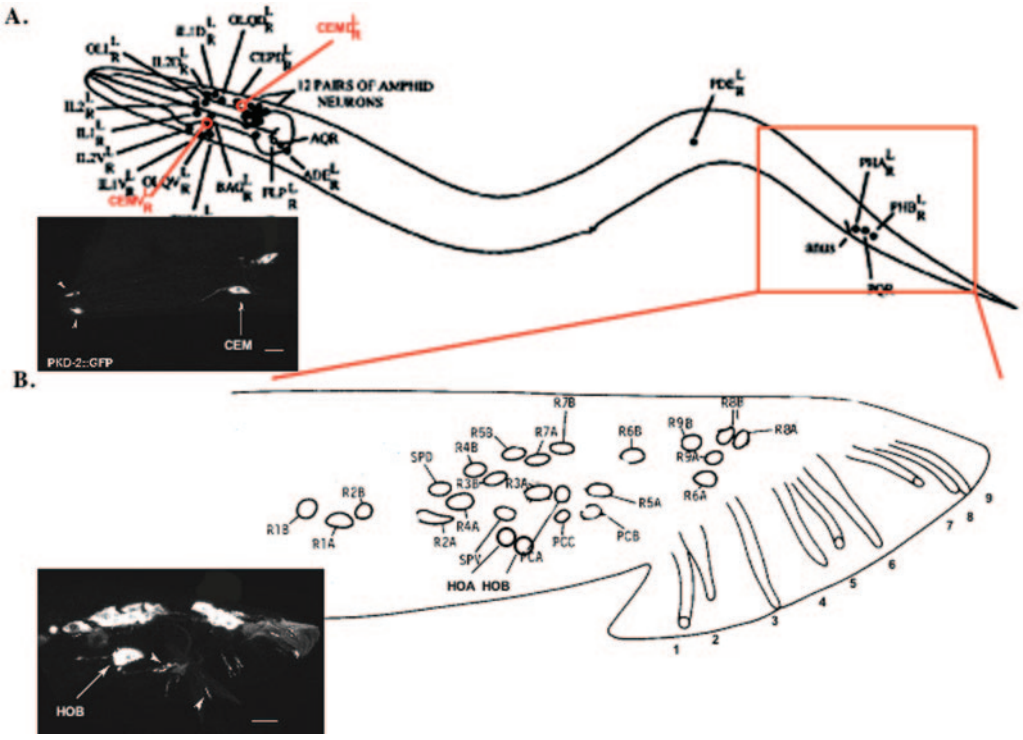

Figure 1. The ciliated nervous system of *Caenorhabditis elegans*. (A) Positions of nuclei of all ciliated neurons in the *C. elegans* hermaphrodite (30), reprinted with permission from reference 53. All but two of the 60 neurons are members of bilateral pairs and are represented by a single cell with L (for left side of animal) and R (for right side of animal). The asymmetric ciliated neurons are AQR and PQR. In the head, the four male-specific ciliated CEM neurons are indicated in red. In the hermaphrodite, the CEM undergo programmed cell death (64). The inset shows PKD-2::green fluorescence protein (GFP) localization in the CEM cilia (arrowheads) and cell bodies (arrow) in an adult *C. elegans* male. Scale bar = 10 μm. (B) Positions of nuclei of all ciliated neurons in the *C. elegans* adult male tail, adapted from reference 31. The male possesses an additional 52 ciliated neurons for a total of 112. All but two of the 52 male-specific ciliated neurons are arranged as left-right bilateral pairs. The asymmetric ciliated neurons are HOA and HOB, which mediate Lov behavior (51). The male tail has nine bilaterally arranged rays (numbered 1 to 9, anterior to posterior) required for response and turning behaviors (51). The inset shows PKD-2::GFP localization in the *C. elegans* adult male tail; indicated are the cell body of HOB (arrow) and cilia of HOB and ray 3 (arrowheads). Scale bar = 10 μm.

basis of the ability of chemosensory amphid and phasmid neurons to fill with fluorescence dyes in living animals. Mutations in 27 genes prevent dye uptake (the "Dyf," or dye filling defective phenotype) (32,33). Mutations in 10 of these genes affect the structure of *all* neuronal cilia (32). These cilium structure genes encode IFT homologs. For example, *osm-5* (for osmotic avoidance defective) is homologous to the *Chlamydomonas* Complex B IFT88 polypeptide and mouse ARPKD *Tg737* gene (Polaris protein product) (34–36). Mutations in *ift88*, *Tg737*, and *osm-5* result in defective ciliogenesis in *Chlamydomonas*, mouse, and worm, respectively (40,41). The *osm-3* homomeric kinesin-II is required *only* for amphid and phasmid cilia development (32) and is expressed in *only* chemosensory neurons (27,37), indicating that ciliogenesis may require at least two levels of regulation: General and specific. First, IFT builds and is common to all cilia. Once the ciliary foundation is laid, cell type–specific machineries perhaps refine the cilium for certain functions (in the case of *osm-3*, chemosensation). In mammals, the KIF3A subunit of heterotrimeric kinesin-II is essential for viability, with conventional knockout mice dying as early embryos and failing to synthesize cilia in the embryonic node (38,39). Kidney-specific inactivation of the KIF3A subunit of heterotrimeric kinesin-II results in PKD in mice (42). In contrast, the human homologue of OSM-3 is KIF17, a neuron-specific molecular motor that acts in neuronal dendrites (43). KIF17 involvement in ciliogenesis or kidney development has not been examined. The cloning and characterization of the remaining *C. elegans dyf* genes will be informative as will genetic screens designed to identify specialization genes.

The RFX transcription factor DAF-19 is required for the formation of all *C. elegans* cilia and regulates the activity of target genes by binding to an X-box promoter motif (15,36,45). The *C. elegans* genome has >200 candidate X-box genes. The fruit fly *Drosophila melanogaster* uses a DAF-19 transcription factor to regulate sensory cilia formation via the X-box motif (46,47). Moreover, the mammalian transcription factor RFX3 is required for the development of nodal cilia and L-R asymmetry (but is not expressed in the kidney) (48). Combined, these data indicate that both IFT machinery and the ciliary transcriptional apparatus may be conserved from worm to human.

Comparative genomics was recently used by two groups to predict the ciliary proteome (15,47). In brief, the genomes of nonciliated organisms were subtracted from ciliates to identify between 200 and 700 cilia-specific genes in the "Flagellar and Basal Body" genome (reviewed in 49 and 50). This approach identified the known players, including IFT components, polyductin/fibrocystin, BBS proteins, nephrocystins, ion channels, and intracellular transport proteins. Model organisms will be essential in determining the function of the ciliary proteome.

C. elegans Male Mating: A Model for ADPKD

Male mating behavior of *C. elegans* offers an intriguing model to study the genetics of sensory behavior, cilia function, and ADPKD. The *C. elegans* polycystins LOV-1 and PKD-2 are required for male sensory behaviors. The *C. elegans* male executes a complex series of stereotyped sub-behaviors to mate with the hermaphrodite. Mating behavior is shown in Supplemental Movie 1. The male nervous system possesses 381 neurons to the hermaphrodite's 302. Sexual dimorphism is reflected in behavior: Many of the 87 male-specific neurons mediate male sensory behaviors (51). Males with severe defects in all sensory neuron cilia, such as the mutant *osm-5*, exhibit pleiotropic male mating defects in response, vulva location, and ejaculation (35,52). The only ciliated cells in *C. elegans* are chemosensory and mechanosensory neurons (30). The male has 48 predicted ciliated sensory neurons in his tail and four in his head (31). *osm-5::gfp* is expressed exclusively in ciliated neurons, including male-specific expression in four CEM head neurons and neurons of the hook and rays (Figure 1) (35).

lov-1 and *pkd-2* mutants are specifically response- and Lov-defective (Table 1, Supplemental Movie 2). *lov-1* encodes the *C. elegans* homolog of the human polycystic kidney disease gene *PKD1* (52). Mutations in *PKD1* or *PKD2* account for 95% of ADPKD, a human genetic disorder that affects 1 in 1000 individuals. PKD-2 is the *C. elegans* homologue of the human PC-2 channel (encoded by the *PKD2* gene) (54). PC-1 (encoded by *PKD1*) and PC-2 are proposed to form a receptor/channel complex (55). PC-2 is a member of the transient receptor potential (TRP) ion channel family that has been implicated in a variety of sensory modalities. *lov-1* and *pkd-2* act in the same genetic pathway (54).

Consistent with a role in male sensation, *lov-1* and *pkd-2* are

Table 2. Supplemental movie available online at http://www.jasn.org

Supplemental Movie 1. Mating behavior of wild-type *C. elegans* male (courtesy of Luis Rene Garcia, Texas A&M University). The male responds to hermaphrodite contact by putting his tail flush on her body; he begins backing until he encounters her head or tail, where he turns via a sharp ventral coil. He continues backing until he reaches the vulva, where he stops, inserts his spicules, and ejaculates into the hermaphrodite uterus. Completion of all sub-behaviors is not mandatory for successful copulation. For example, if the initial contact is on the ventral side of the hermaphrodite, then the male may immediately locate the vulva, insert his spicules, and ejaculate without evoking turning behavior.
Supplemental Movie 2. Mating behavior of *pkd-2* mutant male. When the male tail contacts the hermaphrodite body, *pkd-2* mutants fail to respond and continue moving forward. Occasionally, *pkd-2* mutants will respond. *pkd-2* mutants back and turn normally. However, the *pkd-2* mutant fails to sense and stop at the hermaphrodite's vulva and is categorized as location-of-vulva (Lov)–defective.

expressed in the 21 male-specific exposed ciliated sensory neurons that mediate response (rays), vulva location (hook), and possibly chemotaxis to hermaphrodites (the head CEM; Figure 1). Using a combination of translational GFP fusions and antibodies, we showed that LOV-1 and PKD-2 proteins are enriched in sensory cilia (Figure 1) and that ciliary localization is a requisite for polycystin function (52,54). Stunningly, sensory function and ciliary localization of the *C. elegans* polycystins seem to be evolutionarily conserved. PC-2 localizes to renal cilia (5,6) and forms a mechanosensitive channel with PC-1 in primary cilium of cultured kidney cells (7). The powerful molecular genetic tools of *C. elegans* will enable simultaneous dissection of the molecular basis of male sensory behaviors, ciliary protein localization, and PKD.

LOV-1 and all PC-1 family members share a similar architecture: A large extracellular domain (although there is no primary sequence homology between the extracellular regions of LOV-1 and PC-1), a G protein–coupled receptor proteolysis site (GPS), 11 transmembrane (TM) domains, and an intracellular polycystin-lipoxygenase alpha toxin (PLAT) domain located between TM1 and TM2 (55). The function of the evolutionarily conserved PLAT domain found in all PC-1 family members remains an enigma. On the basis of PLAT sequence homology, a nonredundant genetic PKD pathway, and PC ciliary subcellular localization in both *C. elegans* and mammals, we hypothesize that the PLAT domain may perform an evolutionarily conserved role in mediating PC-1 intracellular signaling pathways. Overexpression of the LOV-1 PLAT domain in transgenic *C. elegans* dominantly interferes with response and vulva location, suggesting that the PLAT domain dominantly interferes with the function of LOV-1 effectors (56).

For identifying targets of the PLAT domain, 1×10^6 cDNA were screened for yeast two-hybrid interaction with the LOV-1 PLAT domain. ATP-2, the β subunit of the ATP synthase, physically associates with the LOV-1 PLAT domain (56). Moreover, *C. elegans* ATP-2 and the human PC-1 PLAT domain physically interact, indicating that this interaction may be evolutionarily conserved. In addition to the expected mitochondria localization, ATP-2 and other ATP synthase components co-localize with LOV-1 and PKD-2 in cilia. Whereas *lov-1* and *pkd-2* mutants are response- and Lov-defective, RNAi treatment of ATP synthase components or overexpression of *atp-2* causes only response but not Lov mating behavior defects. LOV-1 may have tissue-specific effectors. In other words, ATP-2 may be required for polycystin-mediated signaling in ray neurons (with defects resulting in abnormal male response behaviors) but not the HOB hook neuron (as evidenced by wild-type vulva location behavior). Given the diverse clinical manifestation of ADPKD and broad distribution of the polycystins, it is likely that the polycystins will have tissue-specific regulators and targets. We propose that the ciliary localized ATP synthase may play a previously unsuspected role in polycystin signaling. Whether the ATP synthase and PC-1 co-localize in the primary cilium of renal epithelial cells is unknown. Our studies using *C. elegans* as a model for ADPKD promise new avenues to understanding polycystin PLAT function and ciliary sensory signaling.

BBS is a multigenic, pleiotropic disorder characterized by kidney cysts/abnormalities, retinal degeneration, polydactyly, *situs inversus*, obesity, mental retardation, and hypogenitalism (OMIM #209900 at http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id = 209900). Several of these phenotypes bear resemblance to other human ciliary diseases. Six *C. elegans* homologs of the human *BBS* genes (*bbs-1*, *bbs-2*, *bbs-3*, *bbs-5*, *bbs-7*, and *bbs-8*) are expressed in ciliated sensory neurons and contain DAF-19–regulated X-boxes in their promoter regions (Table 1) (6,13,15,57). *bbs-7* and *bbs-8* mutants have abnormal cilia, compromised IFT rates, and defects in chemosensation (16). It is interesting that *bbs* and *osm-3* mutants are similar in that only the distal end of the cilium is lacking (16,32), whereas complex B mutants lack both proximal and distal ciliary regions. Combined, these data suggest that the BBS proteins interact with the IFT machinery, but the physiologic relevance has yet to be determined.

NPHP is an autosomal recessive cystic kidney disease and most frequent genetic cause for end-stage renal failure in infants, children, and young adults (58). NPHP can also be associated with the extrarenal manifestations indicative of ciliary dysfunction, including retinitis pigmentosa and *situs inversus*. Mutation in one of four *NPHP* genes (*NPHP1* through *4*) results in NPHP. The NPHP1 protein product interacts with NPHP2, NPHP3, and NPHP4. NPHP1 and NPHP2 (also known as inversin) localize to cilia (12,59). The molecular functions underlying NPHP biochemical interactions and ciliary localization are not well understood at this time. *C. elegans* has obvious *NPHP1* and *NPHP4* homologs (60,61), whereas NPHP2 and NPHP3 counterparts are less well conserved (Table 1; Jauregui AR, Barr M, unpublished observations). *nphp-1* and *nphp-4* are expressed in a subset of *C. elegans* sensory neurons, and their protein products localize to cilia (Jauregui AR, Barr M, unpublished observations). *nphp-1* and *nphp-4* single and double hypomorphic mutants have wild-type cilia, as judged by dye filling. However, the *nphp-1; nphp-4* double mutants have male mating defects, suggesting genetic redundancy (Jauregui AR, Barr M, unpublished observations).

Worms Don't Have It All: No Cystin (Cys), No Fibrocystin (PKHD1)

The *C. elegans* genome does not contain a cystin or fibrocystin homolog. Cystin, a novel cilia-associated protein, is disrupted in the congenital polycystic kidney (*cpk*) mouse model of ARPKD (6,62). Human ARPKD is caused by mutation in the *PKHD1* gene, which encodes fibrocystin, a novel receptor-like protein expressed on primary cilia (8,9,63). Originally thought to be chordate-specific genes playing specialized roles in higher vertebrates, a fibrocystin homolog is located in the unicellular *Chlamydomonas* genome (Hongmin Qin and Joel Rosenbaum, personal communication). Unlike *Chlamydomonas*, *C. elegans* lacks motile cilia and has a more simple basal body structure. The Flagellar and Basal Body genome contains 326 proteins that are found in humans and *Chlamydomonas* but not *C. elegans*, suggesting that this protein set (including fibrocystin) may be required for motile cilia and complex basal body formation (15).

Conclusion

The genomes of human, rat, and mouse all are known, which allows the cloning of human disease genes by means of comparative genomic analysis (*e.g.*, the identification of *PKHD1* as the gene mutated in ARPKD [8]). A great challenge is ascertaining gene function. *C. elegans* is clearly a powerful model system to study development and diseases of cilia. Systematic RNAi and classic genetic screens have the potential to identify, in an unbiased manner, new genes that are required for cilia development, maintenance, function, and signaling. The worm is your oyster.

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References

1. The *C. elegans* Sequencing Consortium. Genome sequence of the nematode *C. elegans*: A platform for investigating biology. *Science* 282: 2012–2018, 1998
2. Barr MM: Super models. *Physiol Genom* 13: 15–24, 2003
3. Watnick T, Germino G: From cilia to cyst. *Nat Genet* 34: 355–356, 2003
4. Pazour GJ: Intraflagellar transport and cilia-dependent renal disease: The ciliary hypothesis of polycystic kidney disease. *J Am Soc Nephrol* 15: 2528–2536, 2004
5. Pazour GJ, San Agustin JT, Follit JA, Rosenbaum JL, Witman GB: Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Curr Biol* 12: R378–R380, 2002
6. Yoder BK, Hou X, Guay-Woodford LM: The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J Am Soc Nephrol* 13: 2508–2516, 2002
7. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J: Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 33: 129–137, 2003
8. Ward CJ, Hogan MC, Rossetti S, Walker D, Sneddon T, Wang X, Kubly V, Cunningham JM, Bacallao R, Ishibashi M, Milliner DS, Torres VE, Harris PC: The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet* 30: 259–269, 2002
9. Wang S, Luo Y, Wilson PD, Witman GB, Zhou J: The autosomal recessive polycystic kidney disease protein is localized to primary cilia, with concentration in the basal body area. *J Am Soc Nephrol* 15: 592–602, 2004
10. Zhang MZ, Mai W, Li C, Cho SY, Hao C, Moeckel G, Zhao R, Kim I, Wang J, Xiong H, Wang H, Sato Y, Wu Y, Nakanuma Y, Lilova M, Pei Y, Harris RC, Li S, Coffey RJ, Sun L, Wu D, Chen XZ, Breyer MD, Zhao ZJ, McKanna JA, Wu G: PKHD1 protein encoded by the gene for autosomal recessive polycystic kidney disease associates with basal bodies and primary cilia in renal epithelial cells. *Proc Natl Acad Sci U S A* 101: 2311–2316, 2004
11. Ostrowski LE, Blackburn K, Radde KM, Moyer MB, Schlatzer DM, Moseley A, Boucher RC: A proteomic analysis of human cilia: Identification of novel components. *Mol Cell Proteomics* 1: 451–465, 2002
12. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beekmann F, Landau D, Foreman JW, Goodship JA, Strachan T, Kispert A, Wolf MT, Gagnadoux MF, Nivet H, Antignac C, Walz G, Drummond IA, Benzing T, Hildebrandt F: Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. *Nat Genet* 34: 413–420, 2003
13. Ansley SJ, Badano JL, Blacque OE, Hill J, Hoskins BE, Leitch CC, Kim JC, Ross AJ, Eichers ER, Teslovich TM, Mah AK, Johnsen RC, Cavender JC, Lewis RA, Leroux MR, Beales PL, Katsanis N: Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature* 425: 628–633, 2003
14. Kim JC, Badano JL, Sibold S, Esmail MA, Hill J, Hoskins BE, Leitch CC, Venner K, Ansley SJ, Ross AJ, Leroux MR, Katsanis N, Beales PL: The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat Genet* 36: 462–470, 2004
15. Li JB, Gerdes JM, Haycraft CJ, Fan Y, Teslovich TM, May-Simera H, Li H, Blacque OE, Li L, Leitch CC, Lewis RA, Green JS, Parfrey PS, Leroux MR, Davidson WS, Beales PL, Guay-Woodford LM, Yoder BK, Stormo GD, Katsanis N, Dutcher SK: Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell* 117: 541–552, 2004
16. Blacque OE, Reardon MJ, Li C, McCarthy J, Mahjoub MR, Ansley SJ, Badano JL, Mah AK, Beales PL, Davidson WS, Johnsen RC, Audeh M, Plasterk RH, Baillie DL, Katsanis N, Quarmby LM, Wicks SR, Leroux MR: Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev* 18: 1630–1642, 2004
17. Pazour GJ, Rosenbaum JL: Intraflagellar transport and cilia-dependent diseases. *Trends Cell Biol* 12: 551–555, 2002
18. Praetorius HA, Spring KR: Bending the MDCK cell primary cilium increases intracellular calcium. *J Membr Biol* 184: 71–79, 2001
19. Praetorius HA, Spring KR: Removal of the MDCK cell primary cilium abolishes flow sensing. *J Membr Biol* 191: 69–76, 2003
20. Praetorius HA, Spring KR: The renal cell primary cilium functions as a flow sensor. *Curr Opin Nephrol Hypertens* 12: 517–520, 2003
21. McGrath J, Brueckner M: Cilia are at the heart of vertebrate left-right asymmetry. *Curr Opin Genet Dev* 13: 385–392, 2003
22. Rosenbaum JL, Witman GB: Intraflagellar transport. *Nat Rev Mol Cell Biol* 3: 813–825, 2002
23. Kozminski KG, Forscher P, Rosenbaum JL: Three flagellar motilities in *Chlamydomonas* unrelated to flagellar beating. Video supplement. *Cell Motil Cytoskeleton* 39: 347–348 1998
24. Scholey JM, Ou G, Snow J, Gunnarson A: Intraflagellar transport motors in *Caenorhabditis elegans* neurons. *Biochem Soc Trans* 32[Suppl]: 682–684, 2004

25. Snow JJ, Ou G, Gunnarson AL, Walker MR, Zhou HM, Brust-Mascher I, Scholey JM: Two anterograde intraflagellar transport motors cooperate to build sensory cilia on *C. elegans* neurons. *Nat Cell Biol* 6: 1109–1113, 2004
26. Signor D, Rose LS, Scholey JM: Analysis of the roles of kinesin and dynein motors in microtubule-based transport in the *Caenorhabditis elegans* nervous system. *Methods* 22: 317–325, 2000
27. Signor D, Wedaman KP, Rose LS, Scholey JM: Two heteromeric kinesin complexes in chemosensory neurons and sensory cilia of *Caenorhabditis elegans. Mol Biol Cell* 10: 345–360, 1999
28. Signor D, Wedaman KP, Orozco JT, Dwyer ND, Bargmann CI, Rose LS, Scholey JM: Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans. J Cell Biol* 147 519–530, 1999
29. Orozco JT, Wedaman KP, Signor D, Brown H, Rose L, Scholey JM: Movement of motor and cargo along cilia. *Nature* 398: 674, 1999
30. White JG, Southgate E, Thomson JN, Brenner S: The structure of the nervous system of the nematode *Caenorhabditis elegans*: the mind of a worm. *Phil Trans R Soc Lond* 314: 1–340, 1986
31. Sulston JE, Albertson DG, Thomson JN: The *Caenorhabditis elegans* male: Postembryonic development of nongonadal structures. *Dev Biol* 78: 542–576, 1980
32. Perkins LA, Hedgecock EM, Thomson JN, Culotti JG: Mutant sensory cilia in the nematode *Caenorhabditis elegans. Dev Biol* 117: 456–487, 1986
33. Starich TA, Herman RK, Kari CK, Yeh WH, Schackwitz WS, Schuyler MW, Collet J, Thomas JH, Riddle DL: Mutations affecting the chemosensory neurons of *Caenorhabditis elegans. Genetics* 139: 171–188, 1995
34. Pazour GJ, Dickert BL, Vucica Y, Seeley ES, Rosenbaum JL, Witman GB, Cole DG: *Chlamydomonas* IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J Cell Biol* 151: 709–718, 2000
35. Qin H, Rosenbaum JL, Barr MM: An autosomal recessive polycystic kidney disease gene homolog is involved in intraflagellar transport in *C. elegans* ciliated sensory neurons. *Curr Biol* 11: 457–461, 2001
36. Haycraft CJ, Swoboda P, Taulman PD, Thomas JH, Yoder BK: The *C. elegans* homolog of the murine cystic kidney disease gene Tg737 functions in a ciliogenic pathway and is disrupted in *osm-5* mutant worms. *Development* 128: 1493–1505, 2001
37. Tabish M, Siddiqui ZK, Nishikawa K, Siddiqui SS: Exclusive expression of *C. elegans osm-3* kinesin gene in chemosensory neurons open to the external environment. *J Mol Biol* 247: 377–389, 1995
38. Marszalek JR, Ruiz-Lozano P, Roberts E, Chien KR, Goldstein LS: Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc Natl Acad Sci U S A* 96: 5043–5048, 1999
39. Takeda S, Yonekawa Y, Tanaka Y, Okada Y, Nonaka S, Hirokawa N: Left-right asymmetry and kinesin superfamily protein KIF3A: New insights in determination of laterality and mesoderm induction by kif3A−/− mice analysis. *J Cell Biol* 145: 825–836, 1999
40. Murcia NS, Richards WG, Yoder BK, Mucenski ML, Dunlap JR, Woychik RP: The Oak Ridge Polycystic Kidney (orpk) disease gene is required for left-right axis determination. *Development* 127: 2347–2355, 2000
41. Taulman PD, Haycraft CJ, Balkovetz DF, Yoder BK: Polaris, a protein involved in left-right axis patterning, localizes to basal bodies and cilia. *Mol Biol Cell* 12: 589–599, 2001
42. Lin F, Hiesberger T, Cordes K, Sinclair AM, Goldstein LS, Somlo S, Igarashi P: Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc Natl Acad Sci U S A* 100: 5286–5291, 2003
43. Setou M, Nakagawa T, Seog DH, Hirokawa N: Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* 288: 1796–1802, 2000
44. Haycraft CJ, Schafer JC, Zhang Q, Taulman PD, Yoder BK: Identification of CHE-13, a novel intraflagellar transport protein required for cilia formation. *Exp Cell Res* 284: 251–263, 2003
45. Swoboda P, Adler HT, Thomas JH: The RFX-type transcription factor DAF-19 regulates sensory neuron cilium formation in *C. elegans. Mol Cell* 5: 411–421, 2000
46. Dubruille R, Laurencon A, Vandaele C, Shishido E, Coulon-Bublex M, Swoboda P, Couble P, Kernan M, Durand B: *Drosophila* regulatory factor X is necessary for ciliated sensory neuron differentiation. *Development* 129: 5487–5498, 2002
47. Avidor-Reiss T, Maer AM, Koundakjian E, Polyanovsky A, Keil T, Subramaniam S, Zuker CS: Decoding cilia function: Defining specialized genes required for compartmentalized cilia biogenesis. *Cell* 117 527–539, 2004
48. Bonnafe E, M. Touka M, AitLounis A, Baas D, Barras E, Ucla C, Moreau A, Flamant F, Dubruille R, Couble P, Collignon J, Durand B, Reith W: The transcription factor RFX3 directs nodal cilium development and left-right asymmetry specification. *Mol Cell Biol* 24: 4417–4427, 2004
49. Pazour GJ: Comparative genomics: Prediction of the ciliary and basal body proteome. *Curr Biol* 14: R575–R577, 2004
50. Snell WJ, Pan J, Wang Q: Cilia and flagella revealed: From flagellar assembly in *Chlamydomonas* to human obesity disorders. *Cell* 117: 693–697, 2004
51. Liu KS, Sternberg PW: Sensory regulation of male mating behavior in *Caenorhabditis elegans*. *Neuron* 14: 79–89, 1995
52. Barr MM, Sternberg PW: A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans. Nature* 401: 386–389, 1999
53. Collet J, Spike CA, Lundquist EA, Shaw JE, Herman RK: Analysis of *osm-6*, a gene that affects sensory cilium structure and sensory neuron function in *Caenorhabditis elegans. Genetics* 148: 187–200, 1998
54. Barr MM, DeModena J, Braun D, Nguyen CQ, Hall DH, Sternberg PW: The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs *lov-1* and *pkd-2* act in the same pathway. *Curr Biol* 11: 1341–1346, 2001
55. Igarashi P, Somlo S: Genetics and pathogenesis of polycystic kidney disease. *J Am Soc Nephrol* 13: 2384–2398, 2002
56. Hu J, Barr MM: ATP-2 interacts with the PLAT domain of LOV-1 and is involved in *C. elegans* polycystin signaling. *Mol Biol Cell* Nov 24, 2004 [Epub ahead of print]
57. Fan Y, Esmail MA, Ansley SJ, Blacque OE, Boroevich K, Ross AJ, Moore SJ, Badano JL, May-Simera H, Compton

DS, Green JS, Lewis RA, van Haelst MM, Parfrey PS, Baillie DL, Beales PL, Katsanis N, Davidson WS, Leroux MR: Mutations in a member of the Ras superfamily of small GTP-binding proteins causes Bardet-Biedl syndrome. *Nat Genet* 36: 989–993, 2004

58. Hildebrandt F, Omram H: New insights: Nephronophthisis-medullary cystic kidney disease. *Pediatr Nephrol* 16: 168–176, 2001
59. Watanabe D, Saijoh Y, Nonaka S, Sasaki G, Ikawa Y, Yokoyama T, Hamada H: The left-right determinant Inversin is a component of node monocilia and other 9+0 cilia. *Development* 130: 1725–1734, 2003
60. Otto E, Hoefele J, Ruf R, Mueller AM, Hiller KS, Wolf MT, Schuermann MJ, Becker A, Birkenhager R, Sudbrak R, Hennies HC, Nurnberg P, Hildebrandt F: A gene mutated in nephronophthisis and retinitis pigmentosa encodes a novel protein, nephroretinin, conserved in evolution. *Am J Hum Genet* 71: 1161–1167, 2002
61. Hildebrandt F, Otto E: Molecular genetics of nephronophthisis and medullary cystic kidney disease. *J Am Soc Nephrol* 11: 1753–1761, 2000
62. Hou X, Mrug M, Yoder BK, Lefkowitz EJ, Kremmidiotis G, D'Eustachio P, Beier DR, Guay-Woodford LM: Cystin, a novel cilia-associated protein, is disrupted in the cpk mouse model of polycystic kidney disease. *J Clin Invest* 109: 533–540, 2002
63. Ward CJ, Yuan D, Masyuk TV, Wang X, Punyashthiti R, Whelan S, Bacallao R, Torra R, LaRusso NF, Torres VE, Harris PC: Cellular and subcellular localization of the ARPKD protein; fibrocystin is expressed on primary cilia. *Hum Mol Genet* 12: 2703–2710, 2003
64. Sulston JE, Horvitz HR: Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans. Dev Biol* 56: 110–156, 1977
65. Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, LeBot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J: Systemic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* 421: 231–237, 2003
66. Gonczy P, Echeverri C, Oegema K, Coulson A, Jones SJ, Copley RR, Duperon J, Oegema J, Brehm M, Cassin E, Hannak E, Kirkham M, Pichler S, Flohrs K, Goessen A, Leidel S, Alleaume AM, Martin C, Ozlu N, Bork P, Hyman AA: Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. *Nature* 408: 331–336, 2000
67. Pothof J, Van Haaften G, Thijssen K, Kamath RS, Fraser AG, Ahringer J, Plasterk RH, Tijsterman M: Identification of genes that protect the *C. elegans* genome against mutations by genome-wide RNAi. *Genes Dev* 17: 443–448, 2003
68. Piano F, Schetter AJ, Morton DG, Gunsalus KC, Reinke V, Kim SK, Kemphues KJ: Gene clustering based on RNAi phenotypes of ovary-enriched genes in *C. elegans. Curr Biol* 12: 1959–1964, 2002