Nephronophthisis (NPHP), an autosomal-recessive cystic kidney disease, is the most frequent genetic cause of end-stage renal failure in children. NPHP types 1 and 4 are caused by mutations in NPHP1 and NPHP4, encoding the proteins nephrocystin-1 and nephrocystin-4, respectively. Nephrocystin-1 and nephrocystin-4 are expressed in primary cilia of renal epithelial cells. NPHP1 and NPHP4 are highly conserved in *Caenorhabditis elegans*. However, this species does not have a kidney but an excretory system that consists of an excretory cell, an excretory gland cell, a duct cell, and a pore cell. Therefore, cell type–specific expression pattern and function of the nephrocystin homologs in *C. elegans* were of interest. Expression of green fluorescence protein fusion constructs that contain the *C. elegans* promoter regions for *nph-1* and *nph-4* was not found in the excretory system but in ciliated sensory neurons of the head (amphid neurons) and the tail in hermaphrodites (phasmid neurons) and males (sensory ray neurons). As the knockout phenotype for the PKD homologs *lov-1* and *pkd-2* shows impaired male mating behavior, RNAi knockdown animals were analyzed for this phenotype. A similar phenotype was found in the *nph-1* and *nph-4* RNAi knockdown animals compared with the *lov-1* and *pkd-2* knockout phenotype. Thus, it is suggested that renal cyst–causing genes may be part of a shared functional module, highly conserved in evolution. The NPHP homologs may be necessary for initial assembly of the cilium, whereas the polycystic kidney disease homologs may function as sensory transducers.

Expression and Phenotype Analysis of the Nephrocystin-1 and Nephrocystin-4 Homologs in *Caenorhabditis elegans*

Matthias T.F. Wolf,* Jeeyong Lee,† Franziska Panther,* Edgar A. Otto,* Kun-Liang Guan,† and Friedhelm Hildebrandt‡§

Departments of *Pediatrics and Communicable Diseases, †Biological Chemistry, and ‡Human Genetics, University of Michigan, Ann Arbor, Michigan

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Address correspondence to: Dr. Friedhelm Hildebrandt, Department of Pediatrics and Communicable Diseases, University of Michigan, 8220C MSRB III, 1150 West Medical Center Drive, Ann Arbor, MI 48109-0646. Phone: 734-615-7285; Fax: 734-615-1386; E-mail: fhilde@umich.edu

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Nephronophthisis (NPHP), an autosomal-recessive cystic kidney disease, is the most frequent genetic cause of end-stage renal failure in children. NPHP types 1 and 4 are caused by mutations in *NPHP1* and *NPHP4*, encoding the proteins nephrocystin-1 and nephrocystin-4, respectively. Nephrocystin-1 and nephrocystin-4 are expressed in primary cilia of renal epithelial cells. *NPHP1* and *NPHP4* are highly conserved in *Caenorhabditis elegans*. However, this species does not have a kidney but an excretory system that consists of an excretory cell, an excretory gland cell, a duct cell, and a pore cell. Therefore, cell type–specific expression pattern and function of the nephrocystin homologs in *C. elegans* were of interest. Expression of green fluorescence protein fusion constructs that contain the *C. elegans* promoter regions for *nph-1* and *nph-4* was not found in the excretory system but in ciliated sensory neurons of the head (amphid neurons) and the tail in hermaphrodites (phasmid neurons) and males (sensory ray neurons). As the knockout phenotype for the PKD homologs *lov-1* and *pkd-2* shows impaired male mating behavior, RNAi knockdown animals were analyzed for this phenotype. A similar phenotype was found in the *nph-1* and *nph-4* RNAi knockdown animals compared with the *lov-1* and *pkd-2* knockout phenotype. Thus, it is suggested that renal cyst–causing genes may be part of a shared functional module, highly conserved in evolution. The NPHP homologs may be necessary for initial assembly of the cilium, whereas the polycystic kidney disease homologs may function as sensory transducers.
Because of the high degree of conservation of the \textit{NPHP1} and \textit{NPHP4} homologs in \textit{C. elegans}, an animal that has no kidney but an excretory system (consisting of an excretory cell, an excretory gland cell, a pore cell, and a duct cell), we were interested in the cell type–specific expression pattern and the function of the nephrocystin homologs in \textit{C. elegans}. Green fluorescence protein (GFP) expression of \textit{nph-1} and \textit{nph-4} was not found in the excretory system. However, the expression patterns of \textit{nph-1} and \textit{nph-4} were reminiscent of the expression of the \textit{PKD} homologs in \textit{C. elegans}. Because both groups of genes are expressed in mammalian primary cilia, we also compared the knockdown phenotype of \textit{nph-1} and \textit{nph-4} with the published impaired male mating behavior described in the knockouts of the \textit{C. elegans} \textit{PKD} homologs. The \textit{nph-1} and \textit{nph-4} gene products may have additional functions because of their additional expression in hermaphrodites, amphids, and phasmids in contrast to the \textit{PKD} homologs. However, because of a similar expression pattern and knockdown phenotype, we suggest that different groups of renal cyst–causing genes may work within a shared functional module, highly conserved in evolution.

\textbf{Materials and Methods}

\textit{Cloning of the \textit{C. elegans} nph::GFP Expression Constructs}

Homologs of the human \textit{NPHP1} and \textit{NPHP4} genes were identified by means of BlastP search (http://www.ncbi.nlm.nih.gov/BLAST/). The upstream UTR of \textit{C. elegans} genomic sequences \textit{M28.7} (\textit{nph-1}) and \textit{R13H4.1} (\textit{nph-4}) were amplified from N2 genomic DNA (Expand Long Template PCR System; Roche, Mannheim, Germany). Specifically, the \textit{nph-1} construct contains 5064 bp of upstream sequence and the first 69 bp of the coding sequence of exon 1; \textit{nph-4} includes 3551 bp of upstream sequence and the first 54 bp of the coding sequence of exon 1. Both fragments were cloned upstream of a vector that contains GFP. \textit{Nph-1} was cloned into the XbaI/SmaI site of the pPD95.79 vector (provided by A. Fire). \textit{Nph-4} was inserted into the XbaI/SmaI site of the pPD95.70 vector (provided by A. Fire), which also contains a nuclear localization signal sequence 5′/H11032/ to the GFP construct. Clones were verified by restriction enzyme digest and sequencing.

\textit{Injection of \textit{C. elegans} and Maintenance}

Transgenic N2 lines that carry extrachromosomal arrays of the \textit{nph::GFP} expression constructs were generated by the method of Mello \textit{et al.} (22) by co-injection with plasmid pRF4, which contains the semidominant mutation \textit{rol-6} (su1006), which results in the “roller” phenotype. Expression in males was performed by crossing males from the \textit{him-5} (e1490) strain with hermaphrodites that carry the extrachromosomal array \textit{Ex[nph::GFP; pRF4]}. Male and hermaphrodite roller (Rol) worms were analyzed for GFP expression in M9 buffer on thin agarose pad slides, using sodium azide as an anesthetic. For documentation, an inverted confocal microscope (DMIRB; Leica, Bannockburn, IL) was used with ×40 and ×63 lenses. All nematodes were cultured as described previously (23).

\textbf{Dye-Filling Assay}

DiI stock solution was made by dissolving 2 mg of DiI (Molecular Probes, Eugene, OR) in 1 ml of dimethyl formamide and storing at
-20°C. Worms on a growth plate were washed off with M9 buffer into a test tube and then suspended in 500 μl, to which 2.5 μl of DiI solution was added. The tube was shielded from the light with aluminum foil and incubated for 2 to 3 h at room temperature. After incubation, the animals were washed with M9 buffer three times and put onto a growth plate for 1 to 2 h. The animals were analyzed in M9 buffer on thin agarose pad slides, using sodium azide as an anesthetic using a fluorescence microscope (DMIRB). Assignment of the remaining cells was based on the location of the GFP expression in comparison with published anatomy (http://www.wormatlas.org/).

Figure 2. Expression of the nph-1::GFP and nph-4::GFP constructs in ciliated amphid and phasmid neurons. (a) Overlay of the nph-1::GFP expression in the hermaphrodite head (green fluorescence) and the DiI staining of the ciliated amphid neurons (red fluorescence). The cells that are stained by DiI and expressing green fluorescence protein (GFP) are merging to yellow (arrow). (b) Overlay of the nph-1::GFP expression in the hermaphrodite tail (green fluorescence) and the DiI staining of the ciliated phasmid neurons PHA (arrow) and PHB (dashed arrow; red fluorescence). The cells that are stained by DiI and expressing GFP are merging to yellow (arrow). The second pair of phasmid neurons is out of focus. (c) Overlay of the nph-4::GFP expression in the hermaphrodite head (green fluorescence) and the DiI staining of the ciliated amphid neurons (red fluorescence). The cells that are stained by DiI and expressing GFP are merging to yellow (arrow). (d) Overlay of the nph-4::GFP expression in the hermaphrodite tail (green fluorescence) and the DiI staining of the ciliated phasmid neurons PHA (arrow) and PHB (dashed arrow; red fluorescence). The cells that are stained by DiI and expressing GFP are merging to yellow (arrow). The second pair of phasmid neurons is out of focus. Scale bars: a and c = 25 μm; b and d = 5 μm.
Figure 3. In vivo nph-1::GFP expression in a hermaphrodite C. elegans. All panels are dorsoventral views. (a) Brightfield view of an L2 hermaphrodite worm. The dashed arrow indicates the head; the arrow labels the tail. (b) Fluorescence image of (a) indicates nph-1::GFP expression in amphid neurons (arrowhead), the outer labial neurons (dashed arrow), and the two phasmid neurons (arrow). (c) Differential interference contrast (DIC) view of the head of an L4 worm; AB and PB mark at 2 o’clock the rounded structures of pharynx anterior bulb and pharynx posterior bulb, respectively. (d) Fluorescence image of (c). Arrowhead points to amphid neurons; the arrow marks the left outer labial neuron. The ciliated sensory endings are positioned at the tip of the dendrites (dashed arrow). (e) DIC view of the tail of an L4 worm. (f) The PHA (arrow) and the PHB (dashed arrow) phasmid neurons express nph-1::GFP signal. Note expression at the endings at the tip of the dendrite (arrowhead). (g) DIC image and fluorescence image of nph-1::GFP expression (h) during embryogenesis at the 1.5-fold stage. Scale bars: a = 100 μm; c and e = 20 μm; g = 10 μm.
Figure 4. In vivo nph-4::GFP expression in a hermaphrodite C. elegans. All panels are dorsoventral views. (a) Brightfield view of adult hermaphrodite C. elegans. The dashed arrow indicates the head; the arrow labels the tail. (b) Fluorescence image of (a) indicates nph-4::GFP expression in amphid neurons (arrowhead), the outer labial neurons (dashed arrow), and the two phasmid neurons (arrow). (c) DIC view of a head of a worm is shown; AB and PB mark at 2 o'clock the rounded structures of pharynx anterior bulb and pharynx posterior bulb, respectively. (d) Fluorescence image of (c). Arrowhead points to amphid neurons; the arrow marks the left outer labial neuron. (e) DIC view of a hermaphrodite C. elegans tail. (f) The PHA (arrow) and the PHB (dashed arrow) neurons express nph-4::GFP signal. Note expression at the tip of the dendrite ending (arrowhead). (g) DIC image and fluorescence image (h) of nph-4::GFP expression during embryogenesis at the 1.5-fold stage. Scale bars: a = 100 μm; c and e = 20 μm; g = 10 μm.
Knockdown Experiments

For nph-1 exon 4, containing bp 309 to 840 of the cDNA, and for nph-4 exon 7, containing bp 843 to 1715 of the cDNA, were amplified from a C. elegans cDNA library (Invitrogen, Carlsbad, CA). Amplicons were cloned into the XbaI/XhoI sites of vector pLT61 (provided by A. Fire) between two T7 promoter sites. Vector constructs were verified by restriction enzyme digest and sequencing. The constructs were transformed into HT155 Escherichia coli. dsRNA production in bacteria was induced by isopropylthiogalactoside (24,25). Bacteria were grown for an additional 6 h and then seeded on nematode growth medium.

Figure 5. In vivo expression of nph-1::GFP in an adult male C. elegans (him-5). Panels a through d are dorsoventral views; e through f are lateral views. (a) Brightfield image of adult male C. elegans with head (dashed arrow) and tail rays (arrow) indicated. (b) Nph-1::GFP expression is seen in the sensory neurons of head (dashed arrow) and tail (arrow). (c) DIC view of a male head; AB and PB mark at 2 o’clock the rounded structures of pharynx anterior bulb, and PB denotes pharynx posterior bulb, respectively. (d) Arrowhead indicates the position of the amphid neurons, cells that could be compatible with male-specific CEM neurons are labeled by an open dashed arrow. Note expression in the dendrites extending to the ciliary ending of the amphid neurons (dashed arrow). (e) DIC view of male tail. Arrowheads denote the ninth ray on either side of the tail. Arrow marks the hook of male tail. Open dashed arrow labels spicule of male tail. (f) Fluorescence image of (e): In the male tail, nph-1::GFP expression is visible in the cloacal (arrow) and lumbar ganglia (dashed arrow). In the lumbar ganglia, the sensory ray neurons (the ninth sensory ray neurons are labeled by dashed arrows) showed GFP expression. Note the track of fluorescence to the tip of the ninth ray on either side of the tail (both arrowheads). By changing focus, other ray cilia were visible in different planes. Scale bars: a = 100 μm; c = 20 μm; e = 10 μm.
C. elegans Mating Behavior

Knockout experiments for *lov-1* and *pdk-2* showed that they are essential for stereotyped mating behavior ("response" and "vulva location") of male worms mediated by a subgroup of ciliated sensory neurons (26,27). Mating is the most complex behavioral pattern shown by *C. elegans* (28). Males are presumed to find hermaphrodites via chemical cues and sense contact with sensory rays in their tail. Upon contact, the male responds by apposing the ventral part of his tail to her body, followed by swimming backwards along the length of her body to find the vulva (both phases are called "response to contact"). As the male approaches the hermaphrodite’s head or tail, he turns around the head or tail with a sharp arch to the other side of the hermaphrodite (called "turning"). The male continues to swim backwards until the vulva of the hermaphrodite is located ("vulva location"). When the vulva has been located, the male inserts his spicules and transfers sperm to be stored in the hermaphrodite’s spermatheca ("spicule insertion" and "sperm transfer") (28).

Standard assays were performed using hermaphrodite strains N2 and inc-31 with *him-5* (e1490) males (26,28,29). Male offspring of the knockdown *him-5* animals were isolated for several hours. Afterwards, they were analyzed by incubating individual males with two hermaphrodites for 30 min. They were observed until the male responded successfully to one of the hermaphrodites. Then the number of successful locations of the vulva were counted. Observation lasted for a maximum of 10 min or for a maximum of 10 encounters, whichever occurred first. The vulva location ability of a male animal was measured as the number of successful vulva locations (within the 10 min/10 encounters frame) versus the total number of hermaphrodite encounters. Pairwise comparisons were made using Fisher exact test.

Results

Structural Analysis of *C. elegans* Nephronophthisis Gene Homologs *nph-1* and *nph-4* M28.7

was identified as the *NPH1* homolog in *C. elegans* in a previous publication (21). BlastP analysis of the *C. elegans* genome and proteome with *NPH1* was repeated and detected significant similarity to a single predicted transcript M28.7, as described previously (21). The Expect (E) value for the *C. elegans* homologous *nphlp-1* was $5 \times 10^{-21}$ (amino acid identity 23%). We performed BlastP analysis for human nephrocystin-4. The Expect (E) value for *nph-4* was $9 \times 10^{-38}$ (amino acid identity 24%). *NPHP4* is a novel gene (18). No conserved domains could be detected, only a few short motifs in the N-terminal half (a putative nuclear localization site, an E-rich motif, and a proline-rich motif; the last two domains are also present in *NPHP1*) that are too short to test for conservation. To ascertain where these genes are expressed in *C. elegans*, we generated transcriptional GFP expression constructs (*nph-1*-::GFP and *nph-4*-::GFP) and analyzed transgenic lines of *C. elegans*.

Cell Type–Specific Expression of *nph-1* and *nph-4* in *C. elegans*

Expression of GFP under the *nph-1* and *nph-4* promoters was detected in hermaphrodites and males, respectively. In the hermaphrodite and male *C. elegans* head, ciliated amphid neurons (Figure 2) and the outer labial neurons generally exhibited a bright fluorescence indicative of *nph-1* and *nph-4* promoter activity (Figures 3 through 6). In the head of the male animals, an additional group of neurons can be detected anterior to the amphid neurons, which cannot be assigned with certainty but which may be compatible with the male CEM neurons (Figures 5d and 6d).

In *C. elegans* hermaphrodites, we demonstrated *nph-1*-::GFP expression in the ciliated amphid neurons (Figures 2a and 3, c and d), the outer labial neurons (Figure 3, c and d), and the two phasmid neurons in the tail (Figure 2b and 3, a, b, e, and f). In addition, early expression of *nph-1*-::GFP in transgenic embryos was shown (Figure 3, g and h). The earliest point of observation during development was the 1.5-fold embryonic stage. Very similar to *nph-1* expression, *nph-4* expression in hermaphrodites was found in ciliated amphid neurons (Figures 2c and 4, c and d), the outer labial neurons (Figure 4, c and d), and the phasmid neurons in the tail (Figures 2d and 4, e and f). Developmental regulation of expression for *nph-4*-::GFP was found to be similar to *nph-1*, appearing at the 1.5-fold stage and lasting through adulthood (Figure 4, g and h). In *C. elegans* males, *nph-1*-::GFP expression (Figure 5) in the head was detected in amphid neurons and in a second group of neurons that could not be assigned with certainty but that may be compatible with the male-specific CEM neurons (Figure 5, c and d).

In the male tail, *nph-1*-::GFP expression was found in the cloacal and lumbar ganglia. The cloacal ganglia contain the neurons and the structural cells of the postcloacal sensillia and the neurons associated with the spicules. The lumbar ganglia contain the neurons and supporting cells of the rays (30). GFP expression in the lumbar ganglia was interpreted to be in the ninth sensory ray neuron (Figure 5, e and f). Expression in the cloacal ganglia could not be assigned with certainty. In *C. elegans* males, *nph-4*-::GFP expression (Figure 6) in the head was seen in the amphid neurons, the outer labial neurons, and a cell group that could be compatible with the male-specific CEM neurons (Figure 6, c and d). In the male tail, *nph-4*-::GFP expression was seen in the lumbar and cloacal ganglia (Figure 6, e and f).

In conclusion, in *C. elegans* the nephrocystin-1 and nephrocystin-4 homologs *nph-1* and *nph-4* are expressed in hermaphrodites (Figures 2 through 4) and males (Figures 5 and 6) in neurons of the anterior, lateral, and ventral ganglia of the head. In the tail of hermaphrodites, the phasmid sensory neurons express these genes (Figures 2, b and d, 3, e and f, and 4, e and f). In the male tail, labeling was detected for the cloacal ganglia and the lumbar ganglia showing expression in the ray sensory neurons and a group of cells that cannot be annotated with certainty (Figures 5, e and f, and 6, e and f). Expression is localized in the neuronal cell bodies and their ciliated endings (Figures 3d, 5d, and 6d).

Expression analysis of the *nph-1*-::GFP and *nph-4*-::GFP constructs revealed very similar temporal and spatial expression patterns in hermaphrodite and male *C. elegans*. These data, showing an expression pattern of *nph-1* and *nph-4* in the head and the tail of *C. elegans*, demonstrate some overlap with the
Figure 6. *In vivo* expression analysis of *nph-4::GFP* in adult males (*him-5*). All panels are dorsoventral views. (a) Brightfield view of adult male *C. elegans*. The head is indicated by a dashed arrow; the tail is marked by an arrow. (b) Fluorescence image shows *nph-4::GFP* expression in the head sensory amphid neurons (dashed arrow) and the sensory ray neurons of the tail (arrow). A small amount of background fluorescence in the gut is visible. (c) DIC view of the head. AB and PB mark at 5 o’clock the rounded structures of pharynx anterior bulb and pharynx posterior bulb, respectively. (d) Arrowhead denotes the left amphid neurons; open dashed arrow labels a neuron that could be compatible with the left male-specific CEM neurons; arrow marks the right-sided outer labial neurons. Note the ciliated endings in the nosetip (dashed arrow). (e) DIC image of male tail. Arrow denotes the hook, arrowheads mark rays of a male tail, and dashed arrow labels spicule. (f) Fan autofluorescence (arrowheads), *nph-4::GFP* expression in sensory ray neurons (dashed arrow), and the cloacal ganglion (arrow) of DIC image (e) is shown. Scale bars: a = 100 μm; c = 20 μm; e = 10 μm.
Table 1. Lov efficiency of wild-type and mutant C. elegans males

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Lov Efficiency (%)</th>
<th>P Valueb</th>
<th>No. of C. elegans Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>him-5 (wild-type control)</td>
<td>313/363 (86.2)</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>nph-1; him-5 (nph-1 knockdown)</td>
<td>31/106 (29.2)</td>
<td>0.0001</td>
<td>23</td>
</tr>
<tr>
<td>nph-4; him-5 (nph-4 knockdown)</td>
<td>39/192 (20.3)</td>
<td>0.0001</td>
<td>23</td>
</tr>
<tr>
<td>nph-1; nph-4; him-5 (nph-1/nph-4 knockdown)</td>
<td>31/133 (23.3)</td>
<td>0.0001</td>
<td>23</td>
</tr>
</tbody>
</table>

aNumber of positive vulva locations within 10 minutes or at a maximum of 10 encounters are shown. Lov, location of vulva; C. elegans, Caenorhabditis elegans.

bFisher exact test determined two-tailed P values.

Mating Behavior of nph-1 and nph-4 Knockdowns in C. elegans

Mating is the most complex behavioral pattern shown by C. elegans (28). Wild-type (WT) C. elegans males (him-5) were attracted to hermaphrodites and located the vulva rapidly. The male knockdown animals showed impaired mating. This was indicated by a significantly lower number of positive vulva locations and failing to stop at the vulva (Table 1). Individualized male worms were put together with two hermaphrodites on one plate and were observed until the male responded successfully to one of the hermaphrodites by going alongside the hermaphrodite and swimming backwards. When this had occurred, the number of successful locations of the vulva (Lov) were counted. Observation lasted for a maximum of 10 min or for a maximum of 10 encounters, whichever occurred first. In WT (him-5) males, vulva location occurred successfully 313 of 363 times (86.2%; n = 69 animals). In contrast, for nph-1 knockdown males, vulva was located successfully in only 31 of 106 encounters (29.2%; n = 23 animals). Similarly, in nph-4 knockdown males, successful vulva locations occurred in only 39 of 192 encounters (20.3%; n = 23 animals). A combined knockdown experiment for nph-1 and nph-4 revealed successful vulva location in 31 of 133 encounters (23.3%; n = 23 animals; Table 1). These numbers are very similar to what has been described for lov-1 and pkd-2 (26,27). Affinity for hermaphrodites seemed to be lower, and the knockdown males often failed to respond to hermaphrodite contact. No morphologic abnormalities were visible for male nph-1 and nph-4 knockdowns. It is interesting that a certain number of male worms (44% of mating-impaired worms in nph-1 RNAi experiments and 36.8% in nph-4 RNAi experiments) showed problems in turning around the hermaphrodite, as if their tail neurons were unable to sense contact to the hermaphrodite properly. However, in some cases, knockdown males were able to find the vulva after a response was initiated. Therefore, neuromuscular control and the copulatory system seemed to be unaffected. The defect seems to lie in sensitivity of contact through mechanosensation or chemosensation, as has been described for lov-1 and pkd-2 mutants (26,27). Nph-1 and nph-4 knockdown worms showed normal responses for nose touch, mechanosensation, egg laying, and locomotion.

Discussion

The NPHP1 and NPHP4 homologs are highly conserved through evolution and can even be found in the nematode C. elegans. As this species does not have a kidney but an excretory system, we were interested in the expression pattern and function of the nephrocystin homologs in C. elegans. This animal model is advantageous, because expression for NPHP1 and NPHP4 occurs in mammalian primary cilia, and C. elegans is known to be a multicellular model organism for cilia. It is interesting that we could not detect GFP expression of the NPHP homologs in the excretory system of C. elegans, which would be the organ system closest to the human kidney. However, GFP expression of the NPHP homologs was detected in ciliated neurons. The C. elegans hermaphrodite consists of 959 somatic cells; 302 are neurons and 60 of these are ciliated. C. elegans does not have any motile cilia. The function of these immotile cilia is sensory perception, such as mechanosensation and chemosensation. The ciliated nerve endings are exposed directly to the environment or are embedded in the animal’s external cuticle (31). In the head, the outer lip neurons are suggested to function as mechanosensors (32). Also located in the head, amphid neurons consist of 12 pairs of similar neurons, each on the left and the right side of the animal, that are open to the environment at the base of the lips. Amphid neurons were found to be responsible for chemo-, thermo-, and mechanosensation (33–35). It is interesting that evolutionary conservation between two amphid neurons (ASE, AFD) and the photoreceptor cells of Drosophila melanogaster and vertebrates was found. The ASE neuron is responsible for chemosensation in C. elegans and was found to correspond to the photoreceptor cells of D. melanogaster (33). The AFD neuron senses temperature in C. elegans and is the homolog of vertebrates’ photoreceptor cells (33). This suggests that ASE and AFD neurons on the one hand and photoreceptor cells in invertebrates/vertebrates on the other hand may be counterparts in evolution (33). It is tempting to speculate that the amphid thermosensory neuron (AFD) in C. elegans (where nph-1 and nph-4 seem to be expressed) may be a homologous structure of photoreceptors of the human retina, where NPHP1 and NPHP4 are expressed (A. Mears, Kellogg Eye Center, University of Michigan, unpublished data, 2004), because 10% of all patients with nephro-ophthises types 1 and 4 develop retinitis pigmentosa (13). Male-specific CEM neurons function in chemosensation (31). Spatial
expression of *nph-1* and *nph-4* in the cloacal and lumbar ganglia of the male tail is concordant with their role in male mating behavior that we detected in this study. Male tail-ray neurons mediate contact to the hermaphrodite and are supposed to be responsible for ventral/dorsal response and turning (36). The cloacal ganglia contain the neurons and structural cells of the postcloacal sensilla and the neurons associated with the spicules (30). Within these ganglia are neurons such as the HOA, HOB, PCA, PCB, and PCC, which are responsible for the vulva location (36). The HOB hook neurons are ciliated neurons that are required for males to sense the vulva during mating, causing a defect in location of vulva, if *lov-1* (the PKD1 homolog) is knocked out (26). PHA and PHB phasmid neurons function as chemosensory cells that negatively modulate reversals to repellents (37). Antagonistic activity of head and tail sensory neurons, including PHA and PHB, help *C. elegans* to define a head-to-tail spatial map of the chemical environment.

In this study, we expressed GFP in *C. elegans* under the promoters of the NPHP1 and NPHP4 *C. elegans* homologs *nph-1* and *nph-4*, which showed expression patterns in some ciliated neurons (amphid neurons and phasmid neurons). Their expression patterns are similar to those of other *C. elegans* gene homologs, whose spatial distribution is restricted to ciliated cells (osm-5, *lov-1*, and *pkd-2*) (26,38,39). *Lov-1* (for location of vulva) and *pkd-2* are the PKD homologs in *C. elegans* (26).

Expression of *lov-1* and *pkd-2* were described in ciliated sensory neurons of the head (CEM) and in the tail in the hook neuron (HOB) and the sensory ray neurons of male animals. A similar expression pattern to *lov-1* and *pkd-2* was found for *osm-5*, the homolog of the murine gene Tgf37 encoding polaris, a protein associated with cystic kidney disease in mice, which is also expressed in *C. elegans* ciliated neurons (38). Male *C. elegans* that are deficient for *osm-5* also show impaired mating behavior. *Osm-5* is necessary for the assembly and maintenance of all sensory neurons (38). PKD1 and PKD2 interact and are expressed in primary cilia of renal epithelial cells, where they may function as mechanosensors (40). In contrast to the late GFP expression for *lov-1* and *pkd-2* at stage L4, *nph-1* and *nph-4* are already detected in embryogenesis. Earliest detection of GFP expression was noticed at the embryonic 1.5-fold stage for *nph-1* and for *nph-4*. Our GFP expression data also contradict the negative microarray mRNA expression data for *nph-1* and *nph-4* for earlier worm stages (41,42). Most probable, the microarray approach is not sensitive enough to detect gene expression in only a few cells during development. In contrast to what has been shown for *lov-1* and *pkd-2*, we show here that *nph-1* and *nph-4* expression was not restricted to males only but was also present in hermaphrodites.

It is interesting that, similar to NPHP patients, individuals with Bardet-Biedl syndrome (BBS) show renal cystic disease and retinitis pigmentosa (43). Ciliary expression for BBS4 and BBS8 genes was demonstrated recently (44). The expression patterns for four *C. elegans* homologs of BBS-causing genes (*bbs-1*, *bbs-2*, *bbs-7*, and *bbs-8*) were published (44). Expression in all four BBS homologs of *C. elegans* was similar with staining of the inner/outer labial neurons, the amphid neurons in the head, and the PHA and PHB neurons in the tail. In the male tail, the sensory ray neurons were labeled. This expression pattern is highly reminiscent of what we detect here in our *nph-1::GFP* and *nph-4::GFP* transgenic animals. Similar expression of BBS homologs and *nph-1* and *nph-4* in ciliated neurons of *C. elegans* further supports the hypothesis of participation of the encoded proteins in shared functional modules that are relevant for renal and retinal function.

*Nph-1*, *nph-4*, *lov-1*, and *pkd-2*, and the *bbs-1*, *-2*, *-7*, and *-8* genes are expressed in similar groups of ciliated neurons. However, we have not performed co-localization studies of the NPHP homologs with *lov-1* and *pkd-2*. Moreover, for all four *bbs* genes and other *C. elegans* genes expressed in ciliated neurons (*osm-1*, *osm-5*, *osm-6*, and *che-2*), the “X-box” was discovered as a common regulatory element. It represents a 14-bp repeat that is found in the 5′-UT sequence approximately 100 bp upstream of the start codon. The X-box is regulated by DAF-19, which is a member of the RFX protein family and is mandatory for cilia formation (45). Surprisingly, we could not find this element within the 200-bp sequence upstream of the *nph-1* and *nph-4* start codons, which suggests that regulatory factors other than DAF-19 may be necessary. In addition, the X-box has not been published as a regulatory element for *lov-1* and *pkd-2*, and we could not find an X-box–related sequence in these genes either. Recently, two transcription factors (*egl-44* and *egl-46*) were found to regulate *lov-1* and *pkd-2* gene expression (46).

Lov-1 and *pkd-2* knockout strains used in the mating behavior assays were created by ultraviolet trimethylpsoralen mutagenesis (UV-TMP). Recently, feeding dsRNA expressing *E. coli* to *C. elegans* was published as an efficient method to perform RNAi knockdown experiments (24,25). The location of vulva (Lov) efficiency for *lov-1* was 30% (26) and for *pkd-2* was 46% (27). We observed a similar range of Lov efficiency in our knockdown animals. Nevertheless, a genome-wide screening test performed by feeding dsRNA to *C. elegans* did not reveal any phenotype for *nph-1* and *nph-4* in former publications (47). However, mating behavior was not part of the phenotypic screening in that publication. A combined knockdown experiment for *nph-1* and *nph-4* did not result in a more severe phenotype than those of either single mutant, suggesting that *nph-1* and *nph-4* function in the same genetic pathway. By co-immunoprecipitation, it was already shown that the proteins nephrocystin-1 and nephrocystin-4 interact in humans (19). We show here evolutionary conservation of these two proteins and generate suggesting data for a common functional pathway. Because the behavior of animals knocked down for *nph-1* and *nph-4* was similar to those analyzed for *lov-1* and *pkd-2* and because of the similarity of the expression patterns of *lov-1*, *pkd-2*, *osm-5* (*Tgf37*), and *bbs* genes, we suggest that all of these cystic renal disease–causing genes may participate in a shared functional pathway that is highly conserved in evolution. The NPHP homologs may be necessary for initial assembly of the cilium, whereas the PKD homologs function as sensory transducers.
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