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

Courtney J. Haycraft1, Peter Swoboda2, Patrick D. Taulman1, James H. Thomas2 and Bradley K. Yoder1,*

1 Department of Cell Biology, University of Alabama at Birmingham Medical Center, Birmingham, AL 35294, USA
2 Department of Genetics, University of Washington, Seattle, WA 98195, USA

*Author for correspondence (e-mail: byoder@uab.edu)

Accepted 8 February; published on WWW 5 April 2001

SUMMARY

Cilia and flagella are important organelles involved in diverse functions such as fluid and cell movement, sensory perception and embryonic patterning. They are devoid of protein synthesis, thus their formation and maintenance requires the movement of protein complexes from the cytoplasm into the cilium and flagellum axoneme by intraflagellar transport (IFT), a conserved process common to all ciliated or flagellated eukaryotic cells. We report that mutations in the *Caenorhabditis elegans* gene Y41g9a.1 are responsible for the ciliary defects in *osm-5* mutant worms. This was confirmed by transgenic rescue of *osm-5(p813)* mutants using the wild-type *Y41g9a.1* gene. *osm-5* encodes a tetratricopeptide repeat (TPR)-containing protein that is the homolog of murine polaris (*Tg737*), a protein associated with cystic kidney disease and left-right axis patterning defects in the mouse. *osm-5* is expressed in ciliated sensory neurons in *C. elegans* and its expression is regulated by DAF-19, an RFX-type transcription factor that governs the expression of other genes involved in cilia formation in the worm. Similar to murine polaris, the OSM-5 protein was found to concentrate at the cilium base and within the cilium axoneme as shown by an OSM-5::GFP translational fusion and immunofluorescence. Furthermore, time-lapse imaging of OSM-5::GFP fusion protein shows fluorescent particle migration within the cilia. Overall, the data support a crucial role for *osm-5* in a conserved ciliogenic pathway, most likely as a component of the IFT process.

Movies available on-line:

Key words: Cilia, Polaris, *Tg737*, Y41G9a.1, *osm-5*, Cystic kidney disease, daf-19, Tetratricopeptide repeat (TPR), Intraflagellar transport, *C. elegans*

INTRODUCTION

Cilia and flagella are present on many eukaryotic cells where they perform a variety of functions. unicellular organisms such as *Trypanosoma brucei* and *Chlamydomonas* as well as sperm of many organisms use flagella for motility. Cilia function for fluid movement in the lung and are critical for normal function of the tissue. Motile cilia have also been implicated in embryonic patterning (Marszalek et al., 1999; Nonaka et al., 1998). In the mouse, loss of cilia caused by mutations in kinesins (Kif3a and Kif3b) leads to random left-right axis determination. According to the current model, beating of cilia on the node, a gastrulation stage organizing center, creates a leftward-extra-embryonic fluid flow required for asymmetric distribution of a morphogen (Nonaka et al., 1998; Okada et al., 1999). In addition to motile cilia and flagella, there are numerous examples of immotile cilia that function in sensory perception such as chemoreception, photoreception and mechanoreception (Bargmann and Horvitz, 1991; Dwyer et al., 1998).

Cilia and flagella are complex organelles with more than 200 peptides involved in their formation, maintenance and function (Dutcher, 1995). Studies in *Chlamydomonas* have revealed a mechanism responsible for flagellar assembly termed intraflagellar transport (IFT) and an identical mechanism has been proposed for cilia (Cole et al., 1998; Piperno and Mead, 1997; Signor et al., 1999a). Through the generation of mutants, several of the key IFT peptides are being identified (Cole et al., 1998). As protein synthesis does not occur in the cilium, proteins required for axoneme assembly concentrate at the cilium base in rafts that are transported from the basal bodies towards the distal end of the cilium (anterograde movement) by kinesins and in the reverse direction (retrograde movement) by a dynein (Signor et al., 1999a). Thus, the basal body region appears to be a holding zone for IFT particles and their motor proteins.

While cilia are found on a broad spectrum of cells in mammals, in *C. elegans* they are only present in the nervous system on 60 of the 302 neurons of the adult hermaphrodite (Ward et al., 1975; Ware et al., 1975; White et al., 1986). Cilia extend from the dendritic tips of these neurons where they function as sensory organelles. Several mutations in *C. elegans* have been identified that specifically disrupt cilia formation on all or a subset of these sensory neurons (Culotti and Russell, 1978; Dusenbery et al., 1975; Starich et al., 1995). Mutations that affect the structure of all cilia, including *che-2*, *che-3*, *osm-
Characterization of the molecular defects in these mutants with altered cilia structure has revealed that many of the proteins are homologs of IFT proteins identified in Chlamydomonas (Cole et al., 1998). The protein products of the worm osm-1 and osm-6 genes are homologs of IFT raft proteins identified in Chlamydomonas, while che-3 and osm-3 encode the dynein heavy chain DHCIb and a component of dimeric kinesin motor protein, respectively (Shakir et al., 1993; Signor et al., 1999a; Signor et al., 1999b). These genes are expressed in ciliated sensory neurons and their corresponding proteins localize to the distal end of the dendrites and within cilia. Furthermore, elegant studies by Scholey’s laboratory using GFP translational fusion proteins demonstrated that OSM-1, OSM-3 and OSM-6 migrate in the cilia axoneme indicative of the IFT process (Orozco et al., 1999; Signor et al., 1999a). Further elucidation of the ciliogenic pathway in C. elegans, as well as other ciliated and flagellated eukaryotes awaits the characterization of additional proteins involved in the IFT process.

Accordingly, we have recently described the phenotype of a mutant mouse (Tg737Δ2-3;βgal) where the function of a gene referred to as Tg737 was abolished (Murcia et al., 2000). The protein encoded by Tg737, called polaris, contains ten copies of the tetratricopeptide repeat (TPR), a motif that mediates protein-protein interactions, often as part of large complexes (Blatch and Lassle, 1999; Moyer et al., 1994). Homozygous Tg737Δ2-3;βgal mutants die during midgestation, exhibit random left-right axis determination, and lack cilia on the ventral surface of the embryonic node. This phenotype is remarkably similar to that of the kif3a and kif3b kinesin mutants, both of which encode microtubule-based motor proteins implicated in IFT (Marszalek et al., 1999; Nonaka et al., 1998). Ciliary defects are also associated with the pathology in a hypomorphic allele of Tg737 in the Oak Ridge Polycystic Kidney (Tg737nopk) mutant mouse (Pazour et al., 2000; Taulman et al., 2001). These mice develop a complex pathology consisting of cystic kidneys, biliary and bile ductule hyperplasia, acinar atrophy in the pancreas, skeletal patterning defects and hydrocephalus (Moyer et al., 1994; Yoder et al., 1997; Taulman et al., 1997; Taulman et al., 2001). The pathologies in the brain and the cystic renal lesions are associated with aberrantly formed cilia on the ependymal and the collecting duct cells, respectively. In agreement with these ciliary defects, polars concentrates near the basal bodies at the apical surface of epithelia and in the cilia axoneme (Pazour et al., 2000; Taulman et al., 2001). Together these data suggest that polaris functions in a ciliogenic pathway and that it may be a component of the IFT system.

We further our analysis of Tg737 function by characterizing its homolog in C. elegans, the gene Y41g9a.1. Our data indicate that mutations in Y41g9a.1 are responsible for the ciliary defects seen in osm-5 mutant worms. This is shown by the molecular characterization of three independent osm-5 alleles and by rescue of the osm-5 ciliary defects using the wild-type Y41g9a.1 gene. Similar to what we have determined for Tg737 and its corresponding protein polaris in the mouse, osm-5 expression is consistent with this ciliogenic role. osm-5 expression, as revealed by a GFP translational fusion with the endogenous osm-5 promotor, is detected in ciliated sensory neurons. In addition, the OSN-5 protein was found to concentrate at the distal end of the dendrites and within the cilia of the sensory neurons, as determined by immunofluorescence and in transgenic worms expressing an OSN-5::GFP translational fusion. Similar to other genes involved in the ciliogenic pathway in the worm, expression of osm-5 is regulated by the RFX-type transcription factor DAF-19 (Swoboda et al., 2000). In addition, using the OSN-5::GFP translational fusion we demonstrate that OSN-5 migrates within cilia, as has been reported for OSM-1 and OSM-6 (Orozco et al., 1999; Signor et al., 1999a). Overall, the data substantiate a ciliogenic role for polaris and OSN-5 as components of the IFT system. Finally, owing to the high sequence conservation between osm-5 and Tg737, the characterization of osm-5 in the worm will probably yield a better understanding of Tg737 function and how its disruption and subsequent ciliary defects lead to the complex pathology seen in the mouse.

MATERIALS AND METHODS

Strains and culture methods

Growth and culture of C. elegans strains were carried out as described (Brenner, 1974). All strains were grown at 20ºC, unless otherwise stated. Strains used for this study were: wild type N2 Bristol (Brenner, 1974), PR808 osm-1(l(p808), PR811 osm-6(l(p811)), CB1033 che-2(e1033), CB1124 che-3(e1124), JT204 daf-12(sa204), JT6924 daf-19(sa205), daf-19(m86), DR86 daf-19(m86), PR813 osm-5(p813), SP1412 osm-5(mn397), JT6150 daf-11(m87); osm-5(sa130), and JT8651 daf-19(m86);unC1; lin-15(n765). The following extrachromosomal arrays were used: adEx1262 (gey-5::gfp) and adEx1268 (gey-8::gfp) were employed as cell specific markers (Yu et al., 1997); saEx580-583 were used for X-box- and DAF-19-dependent osm-5::gfp expression analyses; saEx584-586 were assayed for osm-5 transgenic rescue; yhEx19 and yhEx20 were used for in vivo IFT transport assays. All osm-5 alleles used in this study have been previously described: osm-5(p813) (Culotti and Russell, 1978; Perkins et al., 1986); osm-5(mn397) (Starich et al., 1995); osm-5(sa130) (Starich et al., 1995; Vowels and Thomas, 1992).

Injection constructs, germline transformation and GFP expression analyses

The GFP transcriptional expression plasmid pCJ3 was constructed by inserting a 350 bp N2 genomic fragment containing approximately 300 bp of upstream sequence relative to the ATG and the first 16 codons of osm-5 into the GFP expression vector pPD95.75 (gift from A. Fire). The PCR rescue fragment was amplified from N2 genomic DNA using AccuTaq polymerase (Sigma, St Louis, MO) and primers flanking the osm-5 promoter and consensus polyA site (AATAAA). To generate the OSN-5::GFP translational fusion, the entire osm-5 gene, including the 300 bp upstream promoter sequence, was amplified by long-range PCR of genomic DNA using AccuTaq polymerase. The PCR product was then cloned into the SalI/XbaI site of pPD95.75. To ensure the correct translational reading frame, the junction between the vector and amplified PCR product was verified by sequencing. N2 or osm-5(p813) adult hermaphrodites were transformed using standard techniques (Mello et al., 1991). Test DNA was injected at 5-
100 ng/μl along with pRF4, which contains the rol-6(sa1006) dominant marker (Mello et al., 1991). Worms carrying the injected DNAs as stable extrachromosomal arrays were identified and maintained by their roller (Rol) phenotype. Rescue of osm-5(p813) cilia defects was assayed by analysis of amphid and phasmid neuron fluorescent dye filling, using DX-12 (Molecular Probes, Inc., Eugene, OR) as described (Fujiiwara et al., 1999).

The X-box within the osm-5 promoter region (GTGGGCT AT GTAAAC) contained on the pCJ3 expression construct was mutated by overlapping PCR primed mutagenesis replacing the X-box with nonspecific nucleotides containing indicative restriction enzyme sites (MluI and NcoIV; AGCCGTGCCGCGT). As verified by sequencing, plasmid junctions and the promoter fusion to gfp were correct and the entire promoter region contained in the pCJ3 construct was wild type, except for the intended sequence changes. The promoter:GFP fusions were introduced into worms by germline transformation typically at 100 ng/μl. The injected strain JT8651 daf-19(m86)/m1; lin-15(n765) served as wild-type background, as daf-19(m86) is fully recessive. Segregating dauers were recovered at 15°C to obtain a daf-19 homozygous background. GFP expression was analyzed in stable extrachromosomal transgenic lines at 1000× magnification (Zeiss Axioskop) by conventional fluorescence microscopy. Representative cohorts of animals of each genotype and transgenic line were examined at most developmental stages between the embryo and adult stage. Quantitative data (see Table 2) were obtained at the highest magnification on a standard stereo dissecting microscope (Wild M14Kscope) with a fluorescent light attachment (Kramer Scientific) only for the adult stage, as the transformation marker lin-15 permits unambiguous detection of transgenics only at this stage. Examinations were performed blind, except when daf-19 mutants were looked at, because the presence of dauers on the plates made blind scoring impossible. For comparisons, the relevant genotypes and X-box construct transgenics were analyzed side by side.

Neuronal cell anatomies and identities followed published descriptions (Hall and Russell, 1991; Ward et al., 1975; Ware et al., 1975; White et al., 1986).

For precise imaging of GFP expression in subcellular compartments and for in vivo intraflagellar transport (IFT) assays, a DeltaVision microscopy setup (Siemens) was used. Worms at various developmental stages were put into 0.5 μl M9 buffer on a thin 2% agarose pad containing sodium azide as an anesthetic. For IFT assays, adult worms transgenic for the OSM-5::GFP translational assay, conserved in 50 μl of antigen in phosphate buffered saline (PBS) and RNA was purified by centrifugation over a CsCl cushion. PolyA-enriched RNA was isolated using an Oligotex mRNA kit (Qiagen, Valencia, CA). Radiolabeled antisense osm-5 and act-123 (Krause et al., 1989) probes were synthesized using the Riboprobe Synthesis kit (Promega, Madison, WI). For daf-19 expression analysis, both control and experimental strains contained the daf-12(sa204) mutation to suppress the Daf-c phenotype of daf-19(m86).

**Antibody production**

A cDNA fragment corresponding to amino acids 238 through 660 of OSM-5 protein was cloned into pgEX-5x-1 (Clontech Laboratories, Palo Alto, CA). Recombinant fusion protein was purified over GST sepharose columns as previously described (Frangioni and Neel, 1993). BALB/C mice were immunized with 100 μg of OS M-5:GST fusion protein in complete Freund’s adjuvant injected subcutaneously into the hind legs. Subsequent boosts, given on days 3, 10, 15 and 20, consisted of 50 μg of antigen in phosphate buffered saline (PBS) administered in a similar manner. On day 21, cells from popliteal and inguinal lymph nodes were collected and fused with the P3X63-Ag8.653 myeloma line according to standard procedures. Fused cells were suspended in HAT media and seeded into 24-well tissue culture plates. Fourteen days after fusion, supernatants were screened for anti OSM-5 antibodies by ELISA. The ability of the antibodies to recognize an epitope contained on OSM-5 was evaluated by Western blot analysis. Antibodies were used as primary and secondary antibodies in Western blot analysis of OSM-5::GST and GST proteins purified from C. elegans using a 1:5000 dilution of 4A1 or 12B6 ascites.

**Immunofluorescence**

Whole-mount fixation and permeabilization of mixed stage larvae and adult worms were carried out as described (Miller and Shakes, 1995). Fixed and permeabilized worms were incubated with a 1:400 dilution of ascites (4A1 or 12B6), or no primary antibody as a negative control, overnight at 4°C in AbA (1% bovine serum albumin (BSA); 0.5% Triton X-100; 1x phosphate buffered saline). Following incubation, worms were washed with multiple changes of AbB (0.1% bovine serum albumin; 0.5% Triton X-100; 1x PBS) over a 3 hour period. Worms were then incubated with a 1:400 dilution of Texas Red-conjugated goat-anti mouse secondary antibody (Molecular Probes, Eugene, OR) in AbB for 2 hours at room temperature. Worms were washed extensively as described above and nuclei were stained using Hoechst No. 33528 (Sigma) diluted 1:1000. Samples were mounted on slides with mounting media (90% glycerol with 10 mg/ml p-phenylene diamine). No specific staining was detected in any secondary antibody only control. For blocking studies, antibodies

**Molecular biology**

Standard molecular biology techniques were used (Sambrook et al., 1989). The original osm-5 cDNA was isolated from a mixed stage C. elegans Uni-ZAP XR cDNA library (Stratagene, La Jolla, CA) and by RT-PCR from total worm RNA. Reverse transcription reactions were carried out using 10 μg total RNA from mixed stage worms and Moloney Murine Leukemia Virus-Reverse Transcriptase (MMLV-RT) (Promega, Madison, WI) according to the manufacturer’s instructions. PCR was performed with Taq polymerase (Eppendorf Scientific, Inc., Westbury, NY). Sequencing of all cDNAs and the initial pCJ3 injection construct was performed by the UAB Sequencing Facilities using ABI Prism model 377. BLAST searches to identify D. melanogaster and L. lycos homologs were performed using the National Center for Biotechnology Information BLAST service (http://www.ncbi.nlm.nih.gov/blast/blast.cgi). The T. brucei homolog was identified using the Sanger Center BLAST server (http://www.sanger.ac.uk/cgi-bin/blast/blast). All C. elegans genome sequence information used for this study was obtained directly from the C. elegans Genome Sequencing Centers (http://www.sanger.ac.uk/Projects/C_elegans/) and http://genomewiki.wustl.edu/gsc/C_elegans) (C. elegans Sequencing Consortium, 1998). The X-box was identified by visual inspection of the osm-5 promoter region.
were preincubated with a 10-fold molar excess of purified OSM-5::GST or GST protein. The GST proteins were then removed by incubation with two rounds of GST affinity resin and the resulting depleted antisera used in the immunofluorescence analysis as above.

For polaris localization in the mouse, tissue samples were isolated from wild-type animals, embedded in OCT, cut into 5 μm sections and fixed for 10 minutes with 4% formaldehyde, 0.2% Triton-X 100 in PBS. Sections were incubated in blocking buffer (1% BSA in PBS) for 10 minutes, followed by a 45 minute incubation with affinity-purified polyclonal anti-polaris antibody and commercial monoclonal anti-β-tubulin IV antibody (Biogenex, San Ramon, CA) diluted in blocking buffer. After three washes in PBS, slides were incubated for 45 minutes in TRITC-conjugated goat anti-rabbit and FITC-conjugated goat anti-mouse secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted in blocking buffer and mounted as described above. Specificity of the anti-polaris antibody was confirmed by Western blot analysis of protein extracts isolated from Tg737-null mutant and wild-type mice (Murcia et al., 2000; Taulman et al., 2001).

RESULTS

Cloning and structural analysis of Y41g9a.1

The Tg737 gene was first identified in the mouse through an insertional mutation that caused poly cystic kidney disease and skeletal patterning defects (Moyer et al., 1994). Polaris, the protein encoded by Tg737, contains ten copies of a degenerate motif called the tetra tricopeptide repeat (TPR) thought to mediate protein-protein interactions. While the function of polaris is not known, the localization of the protein to basal bodies and cilia axonemes along with the loss of cilia in mice mutant for Tg737 suggest that it is involved in ciliogenesis (Murcia et al., 2000; Pazour et al., 2000; Taulman et al., 2001). To facilitate further analysis of polaris function, we searched the genome databases using BLASTX to identify a potential homolog in C. elegans. The only significant homology was found in the gene Y41g9a.1, which is located on the left arm of linkage group X. Using oligonucleotide primers corresponding to the Y41g9a.1 gene, we isolated the Tg737 homolog from a mixed stage cDNA library and independently by reverse transcriptase-PCR (RT-PCR). Comparing the cDNA and genomic sequences indicates that Y41g9a.1 has 15 exons spanning over 7 kb of genomic sequence. Conceptual translation of the 2.5 kb open reading frame predicts an 820 amino acid protein. The predicted amino acid sequence of Y41G9A.1 protein shares 45% identity and 62% homology with murine polaris (Fig. 1). Like the mouse protein, Y41G9A.1 is predicted to contain ten TPR repeats, three tandem repeats toward the N terminus and seven tandem repeats near the C terminus of the protein. Additional BLASTX searches indicate that polaris sequence is also highly conserved in other organisms including Homo sapiens (Schrick et al., 1995), Gallus gallus, Xenopus laevis, Drosophila melanogaster and T. brucei (Fig. 1). A high degree of homology is present not just in the TPRs but also across much of the protein, suggesting that polaris may function in a similar pathway in all these organisms.

Mutations in the Y41g9a.1 gene are responsible for the ciliary defects in osm-5 mutant worms

Many mutations have been identified in C. elegans that disrupt cilia formation (Perkins et al., 1986; Starich et al., 1995). While several of the genes responsible for cilia morphology have been identified, the underlying genetic cause responsible for cilia defects in osm-5 mutants remained elusive. osm-5 mutants were originally isolated in an ethyl methanesulfonate (EMS) mutagenesis screen to identify genes required for normal osmotic avoidance behavior (Culotti and Russell, 1978). In addition to defects in osmotic avoidance, electron microscope analysis of osm-5(p813) mutant worms revealed morphological defects in ciliated endings of all sensory neurons (Perkins et al., 1986). We confirmed and extended these data by using GFP fusions that are expressed in specific sensory neurons throughout development (Fig. 2 and Table 1; Yu et al., 1997). ASE is a chemosensory neuron in the head with a cilium exposed to the external environment (Fig. 2A-C; Bargmann and Horvitz, 1991). We found that osm-5 mutants specifically lack the distal parts of cilia (Fig. 2E). Cilia from wild-type animals were clearly distinguishable from those in osm-5 mutants (compare Fig. 2D with 2E). As a control, we examined a GFP fusion that is expressed specifically in AFD, a thermosensory neuron in the head (Mori and Ohshima, 1997) with a rudimentary cilium and many finger-like projections (microvilli) (Fig. 2A.B.F). The finger-like microvilli at the distal end of the AFD dendrite were not visibly affected in osm-5 mutants (compare Fig. 2G with 2H). Subcellular compartments other than the cilia in both of these neurons were essentially unaffected in osm-5 mutants, demonstrating that the morphological defects in osm-5 mutants are cilium specific (Table 1). As was reported for several other cilia structure mutants (Peckol et al., 1999), we occasionally found short extraneous processes emanating from or near the cell body in osm-5 mutants (Table 1). The abnormalities in cilia structure of osm-5 mutants lead to defective osmotic avoidance (Osm), chemotaxis (Che), dauer formation (Daf-d), fluorescent dye uptake by specific amphid and phasmid neurons (Dyf), male mating ability and an increase in the normal life span (Apfeld and Kenyon, 1999).

Table 1. Morphology of two sensory neuron classes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cilium</th>
<th>Dendrite</th>
<th>Cell body, axon</th>
<th>Additional process‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>93 (full length)</td>
<td>100</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>osm-5</td>
<td>3 (full length)</td>
<td>100</td>
<td>100</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Microvilli</th>
<th>Dendrite</th>
<th>Cell body, axon</th>
<th>Additional process‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>osm-5</td>
<td>100</td>
<td>100</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

*Data are percentages: the presence and morphological appearance of different subcellular compartments were observed by conventional fluorescence microscopy. Animals at the L1 or L2 larval stage were analyzed, as the two sensory neurons ASE and AFD are born during embryogenesis. Between 55 and 65 animals were analyzed for each genotype. Wild type, N2 Bristol; osm-5, (p813).‡Additional process describes mostly short extraneous processes emanating either from the cell body or from axon bifurcations.
Fig. 1. Y41G9A.1 protein shares 45% identity and 62% homology with both murine (m) and human (h) polaris. The homology is not restricted to discrete regions, but is spread across the entire length of the protein. BLAST searches identified partial sequences of homologs in Drosophila melanogaster (predicted gene CG12548), T. brucei (sheared genomic clones 53F4.TF, 17A15.TF and 107C12.TF) and X. laevis (ESTs cm39h06.w1, b108a08.w1, b111c05.w1 and cm61b06.w1). Highlighted regions indicate similarity (gray) and identity (black). The TPR motifs predicted in mouse and human polaris and Y41G9A.1 protein are underlined in red. The locations of the mutations identified in the three osm-5 alleles described in the text are indicated with arrowheads.
associated with ciliary defects, we predicted that the \textit{Y41g9a.1} gene was responsible for the \textit{osm-5} ciliary pathology. To begin evaluating this possibility, we sequenced \textit{Y41g9a.1} cDNA from three independent \textit{osm-5} alleles. In each case, a point mutation in the \textit{Y41g9a.1} cDNA was detected and subsequently confirmed in the corresponding region of the genomic DNA, which established the gene \textit{Y41g9a.1} as \textit{osm-5}. In \textit{osm-5(p813)}, a C to T transition at position 1435 of \textit{osm-5} cDNA results in a stop codon at amino acid 473. The predicted OSM-5 protein would contain the complete N-terminal set of three TPRs but would lack the C-terminal half of the protein including six of the seven TPR motifs (Fig. 1). In the \textit{mn397} allele, a missense mutation at amino acid 229 results in a glycine to glutamate substitution (Fig. 1). This glycine is located at position 8 in the second TPR motif and is an invariant residue present in all \textit{Tg737} protein homologs analyzed to date. Furthermore, it is a crucial residue identified in other TPR-containing proteins where it is most often a glycine or small hydrophobic residue such as alanine or serine (Das et al., 1998). In the final allele analyzed, \textit{osm-5(sa130)} a point mutation was detected at the 5' donor splice site (position 214) of exon three (Fig. 1). The cDNA clone sequenced from \textit{sa130} contained a four-nucleotide insertion that resulted in a frame shift.

To confirm that the mutations identified above were directly responsible for the cilia defects in \textit{osm-5} mutants, a 7.6 kb fragment consisting of 240 base pairs upstream of the coding sequence, the exon regions, introns and consensus poly-A signal (AATAAA) was amplified from wild-type genomic DNA. The PCR fragment was introduced into \textit{osm-5(p813)} mutant worms by germline transformation. Stable transgenic lines were assayed for fluorescent dye filling of amphid and phasmid neurons using the following markers: \textit{gcy-5::gfp} and \textit{gcy-8::gfp} (Y u et al., 1997; compare with Table 2) were used to visualize cilia in wild type and in the cillum structure defective mutant \textit{osm-5}. Anterior is towards the left and dorsal is upwards. The positions in the head of bipolar ciliated sensory neurons ASE and AFD. For the bilateral pairs ASE and AFD of the amphid sensilla, only the left neurons are shown. Cell bodies are dots. The axons of AFD and ASE enter the nerve-ring between the two pharyngeal bulbs, the dendrites extend anteriorly to the tip of the nose. The sensory endings of both neurons are positioned at the tip of the dendrites and are not distinguishable in this drawing. The rectangle depicts the areas shown in B,D,E,G,H. Similar phenotypes were observed throughout development. (F) GFP expression (\textit{gcy-8::gfp}) in AFD at the L1 larval stage, illuminating the entire cell (GFP marker expression only in ASER). (D,E) The sensory ending (cilium) of ASE in L1 larvae. The cilium is full length in wild type (D) and severely reduced in length in the \textit{osm-5} mutant (E). The sensory ending (cilium) of ASE is visible as a thin projection at the tip of the dendrite (thick process to the right). The cilium is full length in wild type (D) and severely reduced in length in the \textit{osm-5} mutant (E). Similar phenotypes were observed throughout development. (F) GFP expression (\textit{gcy-8::gfp}) in AFD at the L1 larval stage, illuminating the entire cell (foreground: AFDL, partially visible in the background: AFDL). (G,H) The sensory endings (microvilli) of AFD in L1 larvae. Microvilli are clearly visible as finger-like projections at the tip of the dendrite (thick process to the right). AFD has only a rudimentary cilium, which is not visible here. Microvilli were indistinguishable between wild-type (G) and \textit{osm-5} mutant (H) backgrounds throughout development. Scale bars: 5 μm in C,F; 2 μm in D,E,G,H.
osm-5 is expressed in ciliated sensory neurons

To examine the developmental expression pattern of osm-5, Northern blot and RT-PCR analyses were performed using RNA isolated from synchronized worms. A 2.6 kb transcript was observed at highest levels during embryogenesis and early larval stages using Northern blot analysis. Expression decreased, relative to actin controls, to almost undetectable levels during L4 and adult stages (Fig. 3A). RT-PCR analysis confirmed that osm-5 is still expressed in both L4 and adult worms, albeit at very low levels (data not shown).

To determine which cells express osm-5, a transcriptional fusion was generated between GFP and the osm-5 promoter. The promoter fragment consisted of 240 base pairs upstream of the osm-5 gene and 16 codons of the first exon (pCJ3 construct). In agreement with Northern analysis, GFP expression in animals transgenic for pCJ3 was first observed around the twofold stage of embryogenesis and continued throughout development and in the adult worm. The pattern of GFP fluorescence was consistent with expression in all ciliated sensory neurons including the amphids, phasmids, and inner and outer labials (Fig. 3B,C). GFP expression was also observed in the sensory rays of the male tail (data not shown).

Thus, similar to the expression of other genes involved in ciliogenesis in the worm including che-3, daf-19, osm-1 and osm-6 (Collet et al., 1998; Fujiwara et al., 1999; Signor et al., 1999a; Swoboda et al., 2000; Wicks et al., 2000). The timing of osm-5::GFP appearance correlates well with the formation of cilia in C. elegans, which starts at the two-fold stage of embryogenesis (Fujiwara et al., 1999).

Reporter constructs that carried additional upstream sequence (roughly 3.5 kb) showed a similar pattern and level of GFP expression as the 240 bp promoter (data not shown), indicating that the 240 bp promoter is sufficient for expression of osm-5 in ciliated sensory neurons.

Localization of OSM-5 protein in wild-type worms

To evaluate the potential role for OSM-5 protein in a ciliogenic pathway and to determine its localization within C. elegans sensory neurons, we generated monoclonal antibodies against bacterially expressed recombinant OSM-5 protein (amino acids 238-660) fused to GST. Two different monoclonal antibodies were used in this study. Several experiments were conducted to evaluate their specificity. First, the ability of antibodies to recognize an epitope on the OSM-5 protein was evaluated on western blots that contained purified OSM-5::GST protein and a GST protein. Both monoclonal antibodies used in this study strongly recognize the OSM-5::GST protein but not the GST protein alone in the adjacent lane (Fig. 4). After Western analysis, it is clear that 4A1 and 12B6 recognize multiple smaller proteins. These smaller peptides are detectable upon Coomassie Blue staining and are only in the lane containing the fusion protein; thus, they probably represent degradation products of the OSM-5::GST protein that arise during purification from Escherichia coli. It is interesting that 12B6 appears to be more reactive with several of the lower molecular weight peptides than 4A1, suggesting that the antibodies may bind different epitopes within OSM-5.

As the above data suggests that the 12B6 and 4A1 recognize an epitope on OSM-5, we analyzed OSM-5 localization in wild-type N2 worms by immunofluorescence. Using either antibody, prominent OSM-5 protein staining was observed in...
the transition zones and cilia of the sensory neurons, including the amphids, phasmids, labials and rays of the male tail (Fig. 5B,C; data not shown). This localization is in agreement with osm-5::GFP expression and the ciliary defects in osm-5 mutant worms. Little or no staining was observed in the cell bodies or other areas of the worm and no specific staining was evident with the pre-immune sera or the secondary antibody-only controls.

To evaluate the specificity of the antisera further, we preblocked the antibodies with 10-fold molar excess of either OSM-5::GST or GST proteins prior to immunolocalization. Preincubation of the antibody with 10-fold molar excess of purified OSM-5::GST (E) blocks all immunostaining in the amphid and labial neurons, while preincubation of the antibody with purified GST alone (F) has no effect. (D) In the absence of DAF-19, low levels of OSM-5 protein are observed at the tips of dendrites despite the loss of cilia associated with mutations in daf-19. Scale bars: 5 µm.

Localization of OSM-5 protein in worms with ciliary defects

As we identified several mutations in osm-5 cDNAs, we wanted to determine if these mutations affect expression or localization of the OSM-5 protein. In each mutant line analyzed (p813 (the reference allele), sa130, and mn397), OSM-5 expression was still detected at the transition zones by immunolocalization (data not shown). However, it should be noted that in the sa130 allele there was a consistent reduction in the level of protein relative to the other mutants using either of the monoclonal antisera. Given that the splice site mutation in the sa130 allele there was a consistent reduction in the level of protein relative to the other mutants using either of the monoclonal antisera. Given that the splice site mutation in the sa130 allele occurs upstream of the region in osm-5 used to generate the antibodies, it was surprising that the OSM-5 protein was still detected in the truncated cilia by immunofluorescence. Thus, it is feasible that both of the OSM-5 monoclonal antibodies used in this study recognize an epitope contained on another ciliary protein. Although we were unable to directly demonstrate this, it is possible that there are alternatively spliced osm-5 mRNAs or that the mutation in the sa130 allele allows some residual product to be produced from an aberrant splice site as has been reported for similar mutations due to Tc1 insertions in the worm (Rushforth and Anderson, 1996).

In addition to the osm-5 mutant worms, several other

Fig. 5. Immunolocalization of OSM-5 protein and antibody specificity. All panels are lateral views of wild type (B,C,E,F) or daf-19(m86) (D) worms processed in parallel for immunofluorescence using the 12B6 antibody. Anterior is leftwards and dorsal is upwards. (A) A hermaphrodite worm (bottom) and a lateral view of the male tail (top). Box ‘a’ indicates the region pictured in C-F. Arrows indicate the position of the amphid cilia. In unblocked samples, immunoreactivity is observed in the rays of the male tail (B) and the ciliated amphid and labial neurons (C). Preincubation of the antibody with 10-fold molar excess of purified OSM-5::GST (E) blocks all immunostaining in the amphid and labial neurons, while preincubation of the antibody with purified GST alone (F) has no effect. (D) In the absence of DAF-19, low levels of OSM-5 protein are observed at the tips of dendrites despite the loss of cilia.

Fig. 6. Immunolocalization of polaris in the mouse. Mouse efferent duct was co-stained for polaris (red) and β-tubulin IV (green). Polaris can be observed in a structure at the base of the cilia (basal body) as well as in the cilia axoneme.

1500  C. J. Haycraft and others
mutations have been identified that disrupt formation of cilia in C. elegans (Perkins et al., 1986). We analyzed OSM-5 localization in a subset (che-2(e1033), che-3(e1124), osm-1(p808), osm-6(p811), and daf-19(m86)) of these mutations that result in similar ciliary defects as seen in osm-5 worms. In all of these mutants, OSM-5 protein was still observed at the distal tips of the dendrites at the transition zones where cilia would form (data not shown). However, in the dynein mutant che-3(e1124), the staining was generally more intense at the distal end of the dendritic tips. This possibly indicates that OSM-5 protein becomes concentrated in the electron dense material characteristically found at the distal tips of the truncated cilia in che-3 mutants (Perkins et al., 1986). The same result has been reported for OSM-6, where it was attributed to the loss of retrograde IFT raft movement in the che-3 mutants (Collet et al., 1998). Another interesting observation is that while OSM-5 protein is still found at the dendritic tips of daf-19 mutants, the level of OSM-5 protein expression was markedly reduced relative to that seen in wild-type worms (Fig. 5C,D). This result suggests that DAF-19 might be important for normal osm-5 expression as was reported for several other ciliogenic genes in the worm (Swoboda et al., 2000).

**DAF-19 regulation of osm-5 expression**

DAF-19 is a transcription factor known to regulate the expression of several genes (i.e. che-2, osm-1 and osm-6) that are required for proper cilia formation in C. elegans (Swoboda et al., 2000). DAF-19 is the only RFX-type transcription factor in C. elegans and mediates its transcriptional regulation through a conserved palindromic sequence referred to as the X-box, located approximately 100 nucleotides upstream of the translational start site (Swoboda et al., 2000). The fact that the X-box is found in the promoters of multiple ciliogenic genes that are expressed in a similar pattern as osm-5, along with the reduced levels of OSM-5 protein seen in the daf-19 background, suggested that osm-5 expression might be under DAF-19 control.

To begin evaluating the possible role of DAF-19 in the regulation of osm-5, we analyzed the 240 bp region upstream of the putative osm-5 translational start site for a potential X-box sequence. To facilitate this analysis, we compared this 240 bp region with the corresponding upstream sequence of the osm-5 homolog in the closely related nematode C. briggsae (located on cosmid G41B17), as promoter elements are typically more conserved than intergenic sequences. Interestingly, a near consensus DAF-19 binding site was identified in both species located at positions −115 in C. elegans and at −91 in C. briggsae (Fig. 7A). To explore the role of this putative X-box, we measured the relative expression levels using the pCJ3 GFP-fusion construct in wild-type and daf-19 mutants. As seen previously, the 240 base pair promoter drove moderate levels of GFP expression in ciliated sensory neurons (Table 2). Similar to what we found for the OSM-5 protein by immunofluorescence, moving this transgene into a daf-19 mutant background resulted in a significant reduction in the levels of GFP in the two independent lines tested. To further evaluate the importance of the X-box in osm-5 expression, we mutated the X-box in pCJ3 and measured GFP expression in wild-type worms. Disruption of the X-box resulted in much lower levels of GFP expression and in fewer

---

**Table 2. Expression analyses using an in vivo GFP expression assay**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wild type</th>
<th>Mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>daf-19</td>
</tr>
<tr>
<td>osm-5::gfp</td>
<td>94</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>nd</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strength of expression</th>
<th>Moderate</th>
<th>Weak/absent</th>
<th>Weak/absent</th>
</tr>
</thead>
</table>

*Data are given as percent expression in ciliated sensory neurons in the head of adult animals. osm-5::gfp is expressed in most of the 60 ciliated sensory neurons of the animal. Four independent transgenic lines were analyzed. Each number represents the data for one transgenic line. nd, experiment not done.
cells (Table 2). Finally, the GFP expression and reduced OSM-5 protein staining in *daf-19* mutants was corroborated by Northern blot analysis, where the level of *osm-5* mRNA was dramatically reduced in the absence of DAF-19 function (Fig. 7B). Together, these data show that the X-box dramatically affects the level of *osm-5* expression in a DAF-19-dependent manner, as shown for several other cilia specific genes (Swoboda et al., 2000). Thus, DAF-19 may function as a key regulator of the ciliogenic pathway suggesting that searching the *C. elegans* genome for correctly spaced X-box sequences in promoters may be a productive means of identifying other components of the pathway.

It should be noted that in the *daf-19* background *osm-5* expression was not completely abolished. This result suggests that DAF-19 may not be the sole regulator of *osm-5*. Therefore, it is noteworthy that a second highly conserved sequence was identified in the promoter region in *C. elegans* and *C. briggsae*. This sequence element is located in similar positions in the promoters in *C. elegans* (at −184) and in *C. briggsae* (at −188). The sequence element is 36 nucleotides in length and shares nearly 75% identity between the two species (Fig. 7A). Although no potential binding factor has yet been identified, the high conservation with regards to sequence and spacing suggests that it may be important for normal expression of *osm-5*.

**OSM-5 acts as a component of the IFT raft**

Characterization of several ciliary mutants with a similar phenotype as seen in *osm-5* worms has shown that the responsible proteins are components of the IFT process. The ciliary localization of OSM-5, along with the loss of cilia in mutants raises the possibility that OSM-5 is another IFT component. To address this possibility, we conducted time-lapse fluorescence imaging on the amphids and phasmids of the *OSM-5::GFP* transgenic worms using an approach similar to that reported for the analysis of *Osm-1*, *Osm-3* and *Osm-6* (Orozco et al., 1999; Signor et al., 1999a). In sequential frames, fluorescent particles can be detected migrating in both anterograde and retrograde directions within the cilium (Fig. 8). Thus, the data in this manuscript support a role for OSM-5 in a ciliogenic pathway most likely as a component of the IFT system.

**DISCUSSION**

The *Tg737* gene was originally identified because of its association with the *Oak Ridge Polycystic Kidney* mouse mutation. Although the role for the *Tg737* protein (polaris) is still uncertain, the pathology in several tissues is associated with defects in the formation or maintenance of cilia. In this report, we further the analysis of *Tg737* function by identifying and characterizing its homolog in *C. elegans*, the gene *Y41g9a.1*. The protein product of *Y41g9a.1* shares greater than 45% identity over the entire length of the mouse and human polaris sequences. We show that mutations in *Y41g9a.1* are responsible for the cilia defects in the *osm-5* mutant worms by characterizing three independent *osm-5* alleles and by rescuing these defects in *osm-5(p813)* animals with the wild-type *Y41g9a.1* gene. In agreement with a ciliogenic role, *osm-5* expression appears exclusive to ciliated sensory neurons in the worm, beginning at approximately the twofold stage of embryogenesis, coinciding with the start of cilia extension. As reported for several other genes involved in ciliogenesis in *C. elegans*, *osm-5* expression is regulated by the DAF-19 transcription factor in an X-box-dependent manner (Swoboda et al., 2000). Furthermore, the OSM-5 protein was found to concentrate at the distal end of the dendrites in the transition zone and within cilia. Finally, using an OSM-5::GFP fusion protein we are able to demonstrate that OSM-5 probably functions as a component of the IFT system, as revealed by migration of fluorescent GFP particles in the cilia of the amphids and phasmids. Overall, our analysis of the *Tg737* homolog in the worm indicates that it is a highly conserved gene present in organisms that range from the flagellated eukaryote *T. brucei* to humans, where it probably plays a common role in cilia or flagella formation.

It has previously been demonstrated that *osm-5* mutants exhibit severe truncation of all cilia on the sensory neurons (Perkins et al., 1986). In contrast to these cilia defects, mutations in *osm-5* have relatively little effect on other aspects of the morphology of the two sensory neurons (AFD and ASE) analyzed here. Specifically, cell bodies were present and positioned correctly and dendritic and axonal trajectories were grossly normal. The specificity of *osm-5* mutations on ciliary formation is further supported by the fact that another sensory specialization on the distal end of dendrites, namely the finger-like microvilli of AFD, was unaffected. However, we did observe extraneous short processes in addition to normal axons and dendrites. These structures have been reported in mutations in a diverse set of genes, including other cilium structure genes, indicating that these low penetrance abnormalities are not *osm-5* specific (Coburn and Bargmann, 1996; Coburn et al., 1998; Hobert et al., 1997; Peckol et al., 1999; Wittenburg et al.,

![Fig. 8. Movement of OSM-5::GFP in cilia of one pair of phasmid neurons. Two sequences from the same worm are shown, each composed of three sequential frames. Anterior is towards the left. The asterisk is located at the transition zones of both cilia. The arrow indicates the initial position of the fluorescent particle at time 0 in all frames. The arrowhead indicates the location of the fluorescent particle in each frame. In both sequences (A) and (B), the particle indicated moves in the anterograde direction. Scale bars: 2.5 μm.](image-url)
together on the basis of the severe cilia defects observed upon
(Swoboda et al., 2000). These genes have long been grouped
expressed in all ciliated sensory neurons of the worm,
has previously been identified in a subgroup of ciliogenic genes
elements was a near consensus X-box. This promoter element
conserved domains in the
intergenic regions. Using this approach, we identified two
promoter elements, which are more likely to be conserved than
partial genome sequences available from the closely related
elegans
example, several genes involved in cilia formation in
of a group of genes that function in the same process. For
example, several genes involved in cilia formation in
C. elegans appear to be regulated in DAF-19- and X-box-
dependent manners (Swoboda et al., 2000). Furthermore, the
partial genome sequences available from the closely related
nematode C. briggsae allows the comparison of these upstream
sequences between the two species to identify potential
promoter elements, which are more likely to be conserved than
intergenic regions. Using this approach, we identified two
conserved domains in the osm-5 promoter. One of these
elements was a near consensus X-box. This promoter element
has previously been identified in a subgroup of ciliogenic genes
expressed in all ciliated sensory neurons of the worm, including
che-2, daf-19, osm-1, osm-6 and now in osm-5
(Swoboda et al., 2000). These genes have long been grouped
together on the basis of the severe cilia defects observed upon
their mutation (Perkins et al., 1986). Mutations in che-2, osm-
1, osm-5 and osm-6 result in loss of the middle and distal
segments of cilia, while loss of functional DAF-19 also results
in loss of the transition zone located at the base of cilia. As
seen in promoters of several other ciliogenic genes, the X-box
in the osm-5 promoter appears crucial for its regulation. The
reduced expression in the daf-19 mutant background was
detected using three separate assays, including an osm-5::GFP
transcriptional construct, by northern blot analysis, and by
immunofluorescence. Overall, these data fit a model in which
DAF-19 functions as a key transcriptional regulator of the
ciliogenic pathway in the worm. Whether a similar regulatory
mechanism exists for Tg737 and other ciliogenic genes in
higher eukaryotes has yet to be determined; however, it is
interesting that a near consensus X-box sequence is also
located upstream of the Tg737 gene in the mouse (B. K. Y.,
unpublished).

Information regarding the functional domains of a protein
can often be obtained through characterization of multiple
alleles. In the case of osm-5, there are six independent alleles
(Starich et al., 1995), three of which we characterize here. The
reference allele p813 contains a nonsense mutation that
predicts a truncated protein lacking its C-terminal half. As this
truncated protein still concentrates at the distal tips of dendrites
at nearly wild-type levels, it would suggest that the N-terminal
region of OSN-5 is sufficient for its localization but not for
cilia formation. In the second allele analyzed, mn397, we
detected a missense mutation that results in the substitution of
Glu for Gly in the eighth position of the second TPR motif.
This position is an invariantly conserved Gly in all polaris
(Tg737) homologs identified to date. Furthermore, it is one of
the most crucial residues within the TPR motif in general,
where the eighth residue is normally a small nonpolar residue
such as Gly, Ala or Ser (Das et al., 1998). Similar to what we
find in the mn397 allele, functional perturbing mutations in the
eighth residue of TPRs in yeast protein cdc23 and in the human
protein p67 phox have also been identified (de Boer et al.,
1994; Sikorski et al., 1993). The effect of a mutation in the
eighth position of a TPR on these proteins can be predicted by
the crystal structure determined for the three tandem TPR
motifs in protein phosphatase 5 (PP5) (Das et al., 1998).
This structure indicates that residue 8 is one of the closest points
of contact between the two α-helices that characterize a TPR
motif. Thus, the substitution of a polar Glu for Gly at this
position would likely interfere with the hydrophobic
interactions required for proper folding of the TPR motif. One
possibility is that the mutation causes general misfolding of
OSM-5; however, the fact that the mutated protein still
localizes correctly to the distal tip of the dendrite suggests that
this may not be the case. Alternatively, this mutation could
abolish the hydrophobic cleft predicted from the crystal
structure that is thought to be required for the interaction of
PP5 with its substrates. In PP5, the cleft is generated by the
three tandem TPRs as seen in the N-terminal region of OSM-
5, thus the mutation in mn397 could specifically prevent the
interaction of OSM-5 with another protein(s) involved in
ciliogenesis.

The final allele characterized was sa130. This mutation
occurs in the 5′ splice donor site of the third osm-5 exon. Of
the three osm-5 mutations characterized, this allele is the most
likely candidate for a null. However, OSM-5 protein was still
detected by immunofluorescence at the transition zone using
two different monoclonal antibodies raised against amino acids
238 to 660, although at markedly reduced levels. Although
preincubation of the antisera with purified OSM-5::GST fusion
protein can block the immunolocalization in wild-type worms,
we cannot unequivocally exclude the possibility that there is
another protein specific to cilia that shares a common epitope
with OSM-5 and thus would still be present in the sa130
mutants. This protein would also be regulated by DAF-19, as
both antibodies show reduced expression in the daf-19 mutant
background. Alternatively, a more feasible explanation for the
continued low level of OSM-5 protein might be the use of
nonconsensus or cryptic splice donor sites, such as the AU
present in the sa130 allele. This has previously been reported
for similar splice donor mutations in other C. elegans genes
where cryptic splice sites are able to excise the Tcl insertion
(Rushforth and Anderson, 1996). In these mutations, the
resulting mRNAs contain a number of splicing variations,
some of which do not alter the reading frame, but contain small
functional perturbing insertions or deletions, owing to the use
of these cryptic donor sites.

An observation that is becoming clear from the
characterization of genes involved in cilia or flagella
formation is that they are highly conserved across a broad
range of organisms (Cole et al., 1998; Collet et al., 1998;
Signor et al., 1999a). Many of these genes are involved in
intraflagellar transport, a process required for cilia formation
and maintenance. According to this model, ciliogenic
proteins concentrate in transition zones or basal bodies near
the base of cilia where they pre-assemble into protein rafts
(Cole et al., 1998). These rafts are transported up and down
the cilium axoneme by microtubule based motor proteins such
as kinesins and dyneins. We show that the OSM-5 protein,
as previously reported for the proteins OSM-1 and OSM-6,
exhibits the localization pattern characteristic of an IFT
protein. Furthermore, mutations in osm-5 cause cilia structure
defects that are markedly similar to that seen in osm-1 and
osm-6 mutants as determined at the level of electron
microscopy (Perkins et al., 1986). Finally, using the OSM-5::GFP translational fusion we demonstrate both anterograde and retrograde movement of the fluorescent particles within the cilia with velocities similar to that reported for other IFT particles by Signor et al. (Signor et al., 1999a). From these data, we predict that OSM-5 is another core component of the IFT system. A role for OSM-5 in IFT as cargo or as part of the raft itself is yet to be determined; however, the fact that OSM-5 contains ten copies of the TPR, a motif known to mediate protein complex formation, suggests that it could serve as a scaffold upon which the other IFT raft proteins assemble.

In the mouse, mutations in Tg737 result in a complex pathology involving polycystic kidney disease, random left-right axis specification, hydrocephalus, and skeletal patterning abnormalities (Moyer et al., 1994; Murcia et al., 2000; Taulman et al., 2001). Similar to what we see in osm-5 mutants, loss of Tg737 in mice appears to be associated with ciliary abnormalities (Murcia et al., 2000; Pazour et al., 2000; Taulman et al., 2001). The common ciliary defects along with the high sequence conservation of this protein across a diverse group of ciliated and flagellated eukaryotes suggest that polaris (Tg737) and its homologs may play similar roles in all these organisms. Thus, further molecular characterization of OSM-5 and the proteins with which it interacts in C. elegans will probably yield important insight into how disruption of this protein can lead to the severe developmental abnormalities and disease states seen in Tg737 mutant mice. This is particularly relevant with regards to polycystic kidney disease (PKD). Two additional C. elegans homologs (lov-1 and pkd-2) of genes involved in mammalian PKD (pkd-1 and pkd-2) have been identified (Barr and Sternberg, 1999). Interestingly, osm-5, lov-1 and pkd-2 are expressed in ciliated sensory neurons; however, in contrast to osm-5, lov-1 appears to be expressed only in males. While cilia appear normal in lov-1 mutants, males exhibit difficulties in locating the hermaphrodite vulva, suggesting there is a defect in sensory perception similar to osm-5 mutants (Barr and Sternberg, 1999). The fact that all three genes are expressed in a common subset of ciliated sensory neurons and mutations in the homologs of these genes in mammals result in cystic kidney pathology suggests that they may be involved in a similar signaling pathway. Thus, exploring potential molecular and genetic interactions of these proteins using C. elegans as a model system will probably give crucial insight into the molecular pathology of this common human disorder.

We thank the following people for technical assistance, helpful discussions, unpublished information, C. elegans strains, cosmids and cDNA clones, and GFP vectors: K. Bubb, A. Coulson, A. Fire, R. Herman and especially members of our laboratories. We also thank D. Miller for critical reading of the manuscript; M. Accavitti and the UAB Hybridoma Core for assistance with antibody production (supported by grant 5P60-AR-20614); and A. Tousson and S. Williams of the UAB High Resolution Imaging Facility for assistance in image generation. We thank the C. elegans Genome Sequencing Consortium for providing genome sequence information and the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health, for providing some of the C. elegans strains used in this study. This work was supported in part by NIH R01 to B. K. Y. (R01 DK55007) and by a Public Health Service Grant to J. H. T. (R01 GM48700). P. S. was supported by postdoctoral fellowships from the Erwin Schrödinger Foundation (Vienna, Austria) and from the Human Frontier Science Promotion Organization (Strasbourg, France).

REFERENCES


mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc. Natl. Acad. Sci. USA* 96, 5043-5048.


