

Caenorhabditis elegans DYF-11, an orthologue of mammalian Traf3ip1/MIP-T3, is required for sensory cilia formation

Hirofumi Kunitomo¹ and Yuichi Iino^{1,2,*}

¹Molecular Genetics Research Laboratory, and

²Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan

Cilia and flagella play critical roles in cell motility, development and sensory perception in animals. Formation and maintenance of cilia require a conserved protein transport system called intraflagellar transport (IFT). Here, we show that *Caenorhabditis elegans* *dyf-11* encodes an evolutionarily conserved protein required for cilium biogenesis. *dyf-11* is expressed in most of the ciliated neurons and is regulated by DAF-19, a crucial transcription factor for ciliary genes in *C. elegans*. *dyf-11* mutants exhibit stunted cilia, fluorescent dye-filling defects (Dyf) of sensory neurons, and abnormal chemotaxis (Che). Cell- and stage-specific rescue experiments indicated that DYF-11 is required for formation and maintenance of sensory cilia in cell-autonomous manner. Fluorescent protein-tagged DYF-11 localizes to cilia and moves antero- and retrogradely via IFT. Analysis of DYF-11 movement in *bbs* mutants further suggested that DYF-11 is likely associated with IFT complex B. Domain analysis using DYF-11 deletion constructs revealed that the coiled-coil region is required for proper localization and ciliogenesis. We further show that Traf3ip1/MIP-T3, the mammalian orthologue of DYF-11, localizes to cilia in the MDCK renal epithelial cells.

Introduction

Cilia and flagella are microtubule-based cellular extensions, which play critical roles in cell motility, development and sensory perception. Assembly and maintenance of cilia depend on a process called intraflagellar transport (IFT), a bidirectional motility system that transports flagellar components from the cell body to the flagellar tip and returns turnover products back to the cell body along the axoneme (Kozminski *et al.* 1993). It has recently been reported that IFT is not only required for building cilia, but also directly involved in sensory signal transduction in *Chlamydomonas* (Wang *et al.* 2006). The IFT system consists of anterograde (from the cell body to the ciliary tip) and retrograde (from the ciliary tip to the cell body) motor complexes associated with raft-like large protein complexes called IFT particles. Genetic and biochemical analyses in model organisms such as *Chlamydomonas reinhardtii* and *Caenorhabditis elegans* have

identified IFT motor subunits as well as many of the IFT particle components (reviewed in Cole 2003; Scholey 2003).

Ciliated sensory neurons (CSNs) are the sole cell type that develops cilium structure in *C. elegans* hermaphrodites. Dendritic endings of these cells terminate in cilia, which play critical roles in perception of environmental stimuli. Morphological and functional abnormality of cilia can lead to mutant phenotypes such as chemotaxis and odorant response defects (Che and Odr), osmotic avoidance defects (Osm), dauer formation defects (Daf) and fluorescent dye-filling defects (Dyf) (Culotti & Russell 1978; Bargmann *et al.* 1993; Malone & Thomas 1994; Starich *et al.* 1995). In wild-type animals, six classes of amphid and phasmid sensory neurons absorb lipophilic fluorescent dye such as DiQ through sensory cilia that are directly exposed to environment (Hedgecock *et al.* 1985; Starich *et al.* 1995). At least 14 *dyf* loci have been genetically identified so far (Starich *et al.* 1995; Ou *et al.* 2007). Consistent with the defects in cilium structure, many genes of this class that have been characterized so far are associated with IFT (Murayama *et al.* 2005; Ou *et al.* 2005a, 2007; Bell *et al.* 2006; Efimenko *et al.* 2006).

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*Correspondence: E-mail: iino@biochem.s.u-tokyo.ac.jp

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The sensory cilia of *C. elegans*, whose structure is well defined in the amphid and phasmid channel neurons, consist of three parts: the transition zone, which is equivalent to the basal body of motile cilia; the middle; and the distal segments, which contain doublet and singlet axonemal microtubules, respectively (Perkins *et al.* 1986). Two different kinesin-2 motors, heterotrimeric kinesin-II and homodimeric kinesin, OSM-3, cooperatively work during anterograde IFT in this organism (Snow *et al.* 2004). They redundantly function to build the middle segment of the sensory cilia. Transport in the distal segment is, however, carried out solely by OSM-3 (Snow *et al.* 2004).

It has been shown that IFT particles of *Chlamydomonas* can be isolated biochemically and separated into two complexes, A and B, which collectively contain at least 16 different polypeptides (Piperno & Mead 1997; Cole *et al.* 1998; Piperno *et al.* 1998). In *C. elegans*, mutations in the components of IFT complexes A (IFT-A) and B (IFT-B) cause distinct ciliary phenotypes. That is, mutants of IFT-A (CHE-11 and DAF-10) have slightly short cilia with accumulation of ciliary components along the axoneme (Perkins *et al.* 1986; Schafer *et al.* 2003), a very similar phenotype to the mutants of retrograde motor, dynein (Signor *et al.* 1999; Schafer *et al.* 2003). In contrast, mutants of IFT-B (CHE-2, CHE-13, OSM-1, OSM-5 and OSM-6) typically show a drastic reduction of cilia length (Perkins *et al.* 1986; Fujiwara *et al.* 1999; Haycraft *et al.* 2001, 2003). These results as well as the observations in complex A mutants in *Chlamydomonas* collectively suggested that the components of IFT-A are functionally important for retrograde transport, whereas that of IFT-B are important for anterograde transport (Piperno *et al.* 1998; Cole 2003; Scholey 2003). Although previous studies have substantially clarified the mechanisms of IFT, it is not yet known how the IFT complexes are organized and regulated. Furthermore, the full complement of this process has not been identified and characterized so far.

In this study, we describe *C. elegans* DYF-11, which shares a significant homology to mammalian Traf3ip1 (tumor necrosis factor receptor-associated factor 3 interacting protein 1). The *dyf-11* gene is predominantly expressed in CSNs, and its expression is regulated by DAF-19. We defined the gene structure of *dyf-11* and characterized an available mutant allele, *mn392*, along with a newly obtained deletion allele, *pe554*. We observed severe morphological and functional defects in cilia of *dyf-11* mutants, which is consistent with a ciliogenic role of *dyf-11*. Using cell biological approaches, we show that DYF-11 is likely to be associated with IFT complex B. Protein domain analysis revealed that the coiled-coil domain is required for proper localization and function

of DYF-11. We further show that Traf3ip1 localizes to cilia in renal epithelial cells.

Results

Caenorhabditis elegans dyf-11 is predominantly expressed in ciliated sensory neurons (CSNs)

Based on a neuron-specific mRNA pull-down experiment, *C. elegans* C02H7.1 was identified as a CSN-expressed gene (Kunitomo *et al.* 2005). Through our analysis, C02H7.1 was identified as the genetically defined gene *dyf-11* (see below) and we hereafter refer to it as *dyf-11*. To reexamine the cellular expression pattern of *dyf-11*, we newly generated two transcriptional and one full-length translational fluorescent protein fusions (see Experimental procedures). The transcriptional fusions, pGdyf11p(-4.1) :: venus and pGdyf11p(-2.0) :: venus that carry 4.1- and 2.0-kb promoter, respectively, showed essentially the same expression patterns (Fig. 1A). In wild-type background, fluorescence was first detected at the 1.5-fold stage of embryogenesis and persisted throughout the lifespan. At larval and adult stages, most of the ciliated neurons, namely, all 12 classes of amphid sensory neurons, phasmid neurons PHA and PHB, the inner and outer labial neurons (IL1, IL2 and OLQ), AQR, URX, FLP, PDE, PQR and several other neurons (probably OLL, ADE and BAG) were found to express the reporter. In addition to CSNs, fluorescence was often seen in gut cells at larval stages (Fig. 1A); this intestinal expression decreased as development proceeded (see below). It was difficult to determine precise cellular expression pattern of the translational fusion, pGdyf11p(-4.1) :: venus :: dyf11, because of ciliary localization of the fusion protein VENUS :: DYF-11 expressed from this transgene (Fig. 1B and see below).

Expression of *dyf-11* is regulated by DAF-19

In *C. elegans*, DAF-19, an RFX-type transcription factor that recognizes a DNA sequence called X-box, is known to regulate the expression of many ciliary genes (Swoboda *et al.* 2000; Haycraft *et al.* 2003; Schafer *et al.* 2003; Li *et al.* 2004; Blacque *et al.* 2005; Efimenko *et al.* 2005, 2006; Murayama *et al.* 2005; Ou *et al.* 2005b). The promoter region of *dyf-11* contains a canonical X-box (Fig. 2A), suggesting that its expression may be regulated by DAF-19 (Avidor-Reiss *et al.* 2004; Blacque *et al.* 2005; Efimenko *et al.* 2005; Kunitomo *et al.* 2005). To test this possibility, the extrachromosomal array of pGdyf11p(-4.1) :: venus was transferred from *daf-19(+)* to *daf-19(m86)* mutant background and the expression

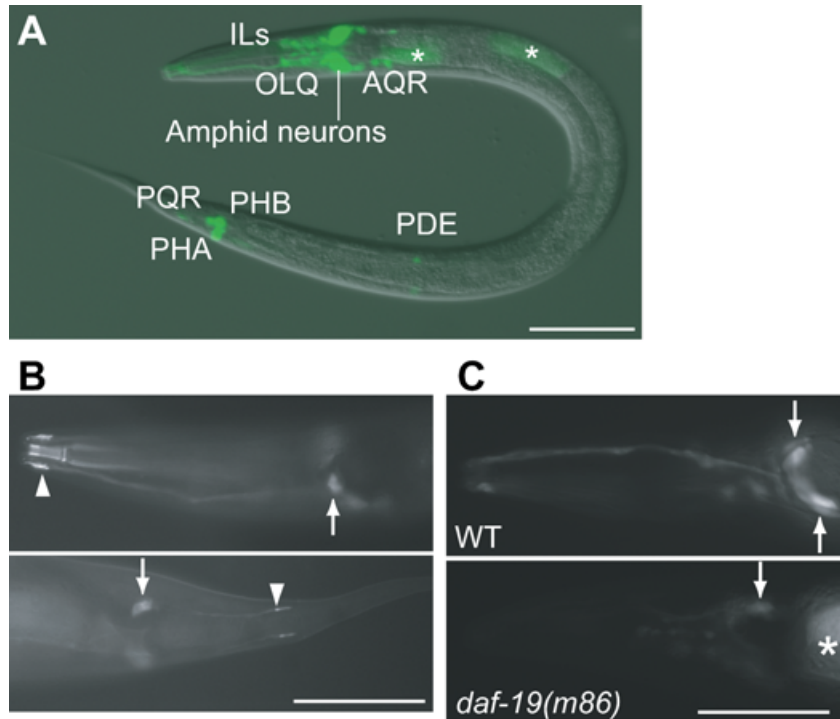


Figure 1 *C02H7.1/dyf-11* is expressed in ciliated sensory neurons and is regulated by DAF-19. (A) Expression pattern of the transcriptional reporter fusion, pGdyf11p(-4.1)::venus. Bright-field image of an L3-stage transformant was overlaid with the fluorescence image showing VENUS localization (green). Ciliated sensory neurons that express VENUS are denoted. Asterisks indicate expression in the intestine. (B) Ciliary localization of VENUS::DYF-11 in the amphid (top) and phasmid (bottom) neurons. Arrowheads indicate the brightest signal at the transition zones. Arrows indicate the cell bodies of ciliated neurons. (C) Expression of pGdyf11p(-4.1)::venus in the head of wild-type (top) and *daf-19(m86)* (bottom) adult worms. In the absence of DAF-19, expression in CSNs (arrows) is significantly reduced. Instead, strong expression in the intestine is observed (asterisk). Scale bars, 50 μ m. The anterior of the worm is toward the left (B and C). (D) Summary of the expression analyses using pGdyf11p(-4.1)::venus. Fluorescence intensity of the reporter expression was determined as described previously (Schafer *et al.* 2003).

	CSN expression			intestine expression	<i>n</i>
	strong	weak	absent		
WT	93%	7%	0%	6%	54
<i>daf-19(m86)</i>	13%	87%	0%	100%	85

level of the reporter was examined in the adult worms. Loss of DAF-19 resulted in severe reduction of the reporter expression in CSNs, whereas the expression in digestive tract still remained (Fig. 1C and D).

A deletion allele of *dyf-11/C02H7.1* causes a dye filling-defective phenotype

To elucidate the gene structure and biological function of *C02H7.1*, a full-length cDNA of this gene was isolated. The nucleotide sequence of this clone revealed an ORF of 535 amino acids consisting of seven exons (Fig. 2A). We then isolated a deletion allele of *C02H7.1*, *pe554*. Nucleotide sequence of the mutant genome revealed that *pe554* carries a 667-bp deletion in *C02H7.1* starting in the third exon and ending in the fourth intron, which was replaced by a 7-bp consecutive thymine insertion (Fig. 2A). This lesion is expected to truncate the 535-amino acid gene product at position 145. *pe554*

homozygotes are fully viable and display no obvious locomotory or fertility phenotypes (data not shown). However, the mutants are slightly shorter than wild-type animals and exhibit fluorescent dye-filling defective (Dyf) phenotype, the characteristics of ciliary abnormalities. The Dyf phenotype of the mutant animals is fully penetrant; CSNs that take up dye in wild-type animals are never stained in *pe554* mutants (Fig. 2C, compare with Fig. 2B). Expression of the VENUS::DYF-11 by its authentic promoter rescued the defects, demonstrating that the deletion in *C02H7.1* is responsible for the phenotype and this fusion protein is fully functional (Fig. 2D).

Given these phenotypes of *C02H7.1(pe554)* and the fact that this gene maps close to the genetic position of *dyf-11* (-18.27 ± 0.244) on linkage group X (Starich *et al.* 1995), we hypothesized that these two genes are identical. *dyf-11* was an uncloned locus that shows defects in dye-filling, chemotaxis, dauer formation and body length (Starich *et al.* 1995). Several lines of experimental

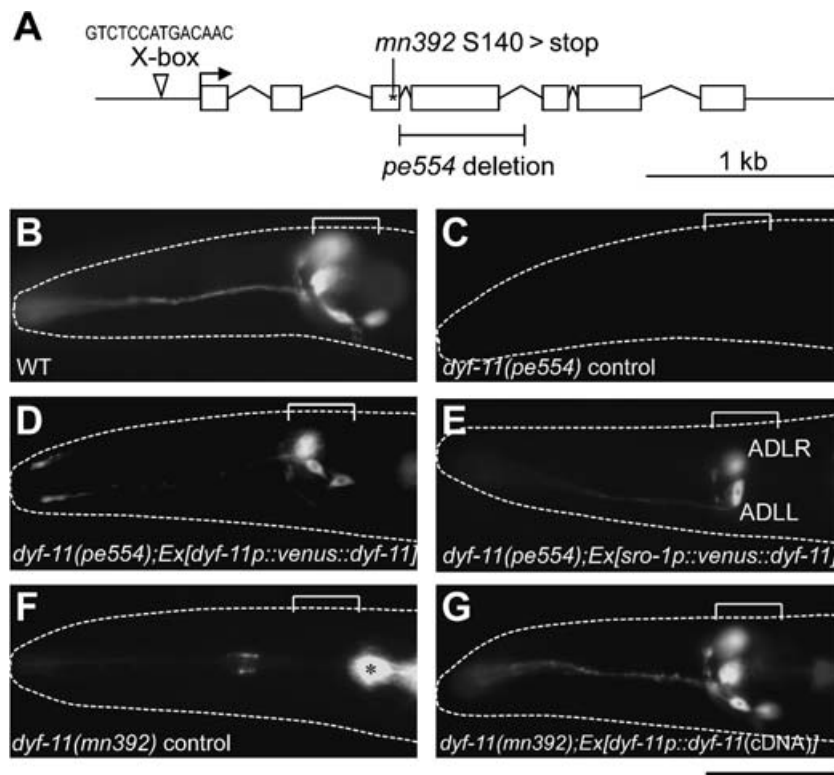


Figure 2 Molecular characterization of the *C02H7.1/dyf-11* gene. (A) Structure of the *C02H7.1/dyf-11* gene on linkage group X. The coding sequence consists of seven exons (boxes). Mutation sites of the *dyf-11* mutants used in this study are shown. *dyf-11(mn392)* carries a C to G substitution converting S140 to a stop codon (asterisk). *pe554* carries a 667 bp deletion substituted by a 7-bp insertion spanning the exons 3 and 4 (I-shaped line). The open arrowhead indicates the position of the X-box. The arrow indicates a transcription start site. (B–G) Results of dye-filling. (B) Wild type. (C–E) *dyf-11(pe554)* mutants transformed with a control vector (C), a vector carrying authentic promoter-driven VENNUS :: DYF-11 (D) and *sro-1* promoter-driven VENNUS :: DYF-11 (E). (F, G) *dyf-11(mn392)* mutants transformed with a control vector (F), and a vector carrying authentic promoter-driven *C02H7.1/dyf-11* cDNA (G). The outline of the worm head is depicted by dashed line. Parentheses indicate the positions of dye-filling neurons. The asterisk indicates DiQ-stained intestinal lumen. The anterior of the worm is toward the left. Scale bar, 50 μ m.

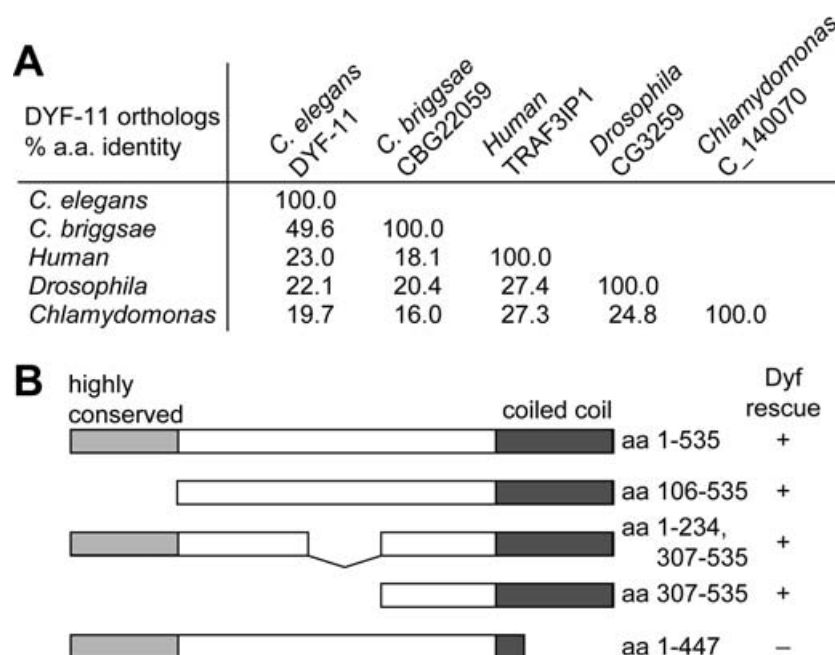
evidence were obtained to confirm this hypothesis. First, PCR-amplified DNA fragments that cover the *C02H7.1* region of wild-type genome rescued the Dyf phenotype of *dyf-11(mn392)* mutants. However, the same genome region prepared from *dyf-11(mn392)* mutants failed to do so (data not shown). Second, *C02H7.1(pe554)* phenocopied *dyf-11(mn392)* mutants in dye-filling, morphological and behavioral phenotypes (Fig. 2C, F and see below). Third, *C02H7.1(pe554)* failed to complement the Dyf phenotype of *dyf-11(mn392)* (see Experimental procedures). Fourth, *dyf-11(mn392)* genome carries a C to G substitution converting S140 of the predicted gene product of *C02H7.1* to a stop codon (Fig. 2A). And finally, the Dyf phenotype of *dyf-11(mn392)* animals was completely rescued by the expression of cDNA for *C02H7.1* (Fig. 2G). Together, we concluded that *C02H7.1* is identical to *dyf-11*.

***dyf-11* encodes a putative microtubule binding protein conserved in ciliated organisms**

In addition to the CSN-specific expression, recent studies raise a strong possibility that DYF-11 and its

orthologues are involved in ciliogenesis: identification as candidate cilium genes in comparative genomics approaches (Avidor-Reiss *et al.* 2004; Li *et al.* 2004), identification of *Chlamydomonas* orthologue, C_140070, in flagellar regeneration transcriptome analysis (Stolc *et al.* 2005) and in flagellar proteomics (Pazour *et al.* 2005), and finding X-boxes in both *C. elegans C02H7.1* and its *Drosophila* orthologue in their promoter region (Avidor-Reiss *et al.* 2004; Blacque *et al.* 2005; Efimenko *et al.* 2005; Kunitomo *et al.* 2005). The predicted gene product of *dyf-11* showed a significant similarity to the gene products evolutionarily conserved from human to *Chlamydomonas* (Fig. 3A). Among the orthologues, human TRAF3 (tumor necrosis factor receptor-associated factor 3)-interacting protein 1 (TRAF3IP1, also called MIP-T3) is the sole member whose physiological function was previously described. TRAF3IP1 is a microtubule- and β -tubulin-binding protein that recruits TRAF3 to microtubules when co-expressed in HeLa cells (Ling & Goeddel 2000). Interestingly, TRAF3IP1 also binds to the α 1 subunit of interleukin-13 (IL-13) receptor and modulates inflammatory signal transduction mediated by STAT6 (see Discussion, Niu *et al.* 2003; Low *et al.* 2006).

Figure 3 DYF-11 is a conserved protein in ciliated organisms and the coiled-coil domain is important for its ciliary function. (A) Comparison of the amino acid identity between the orthologues of DYF-11 in *C. elegans*, *C. briggsae*, *Homo sapiens*, *Drosophila melanogaster* and *Chlamydomonas reinhardtii*. (B) Properties of DYF-11 deletion constructs. The regions carried by each construct are shown in amino-acid numbers. Results of dye-filling assay of the *dyf-11* mutants transformed with each construct are indicated by plus (rescued) or minus (non-rescued).



Mutations in *dyf-11* affect structure and function of sensory cilia

Defects in dye-filling suggest that *dyf-11* mutants have abnormalities in cilium structure (Starich *et al.* 1995). To visualize the morphology of sensory cilia, we utilized fluorescent protein markers that are specifically expressed in CSNs. Gross cilium morphology of the amphid and phasmid CSNs was observed by OSM-6::GFP (Fig. 4A–D). OSM-6 is a previously characterized IFT-B protein that plays a critical role in formation of IFT-B complex (Collet *et al.* 1998; Haycraft *et al.* 2003). To characterize the morphology of cilia in detail, the ASH and ASER chemosensory neurons were visualized by *sra-6::venus* (Fig. 4E and F) and *gcy-5::gfp* (Fig. 4G and H), respectively. We found that the cilia of *dyf-11(pe554)* mutants are indeed malformed. Specifically, the length of the ciliary axoneme in *dyf-11(pe554)* mutants are short (Fig. 4B, D, F and H); the average total length of the middle and distal segments of ASER cilia was $3.1 \pm 0.4 \mu\text{m}$ ($n = 25$) in comparison to $5.3 \pm 0.5 \mu\text{m}$ in wild-type animals ($n = 27$). In addition to short cilium length, some *dyf-11(pe554)* cilia showed abnormal branching (Fig. 4F). Furthermore, aberrant posterior projection was observed in some, but not all, dendrites (Fig. 4H). These abnormalities were also observed in *dyf-11(mn392)* animals (data not shown). The phenotypes observed in *dyf-11* cilia are similar to those found in the IFT-B mutants (Perkins *et al.* 1986; Fujiwara *et al.* 1999; Haycraft

et al. 2001, 2003), suggesting that DYF-11 is associated with this function (see below).

Generally, dye-filling defects are accompanied by other sensory defects such as Osm, Che, Odr or Daf (Starich *et al.* 1995). Concerning *dyf-11(mn392)*, defective chemotaxis to NH_4Cl , reduced dauer formation on starvation, and reduced male mating efficiency have been reported by Starich *et al.* (1995). In accordance with this, *dyf-11* mutants show severe defects in chemotaxis to water-soluble attractant NaCl and in avoidance of high osmolarity. These behavioral defects of *dyf-11* animals are completely rescued by expression of VENUS::DYF-11 (Fig. 4I and J), confirming that these phenotypes are the result of *dyf-11* mutation. *dyf-11* mutants also exhibit defects in the response to AWC-sensed odorants, benzaldehyde and isoamyl alcohol (Supplementary Fig. S1). Altogether, loss of *dyf-11* leads to structural and functional abnormalities in CSNs.

DYF-11 acts cell-autonomously for formation and maintenance of sensory cilia

To examine whether *dyf-11* acts cell-autonomously in cilium biogenesis, we rescued *dyf-11(pe554)* mutants by VENUS::DYF-11 in tissue- or cell-specific manner. As mentioned above, the *dyf-11(pe554)* mutants show a severe dye filling-defective phenotype (Fig. 2C). Mutant animals expressing the fusion protein by the authentic 4.1-kb promoter restored dye-uptake in all competent

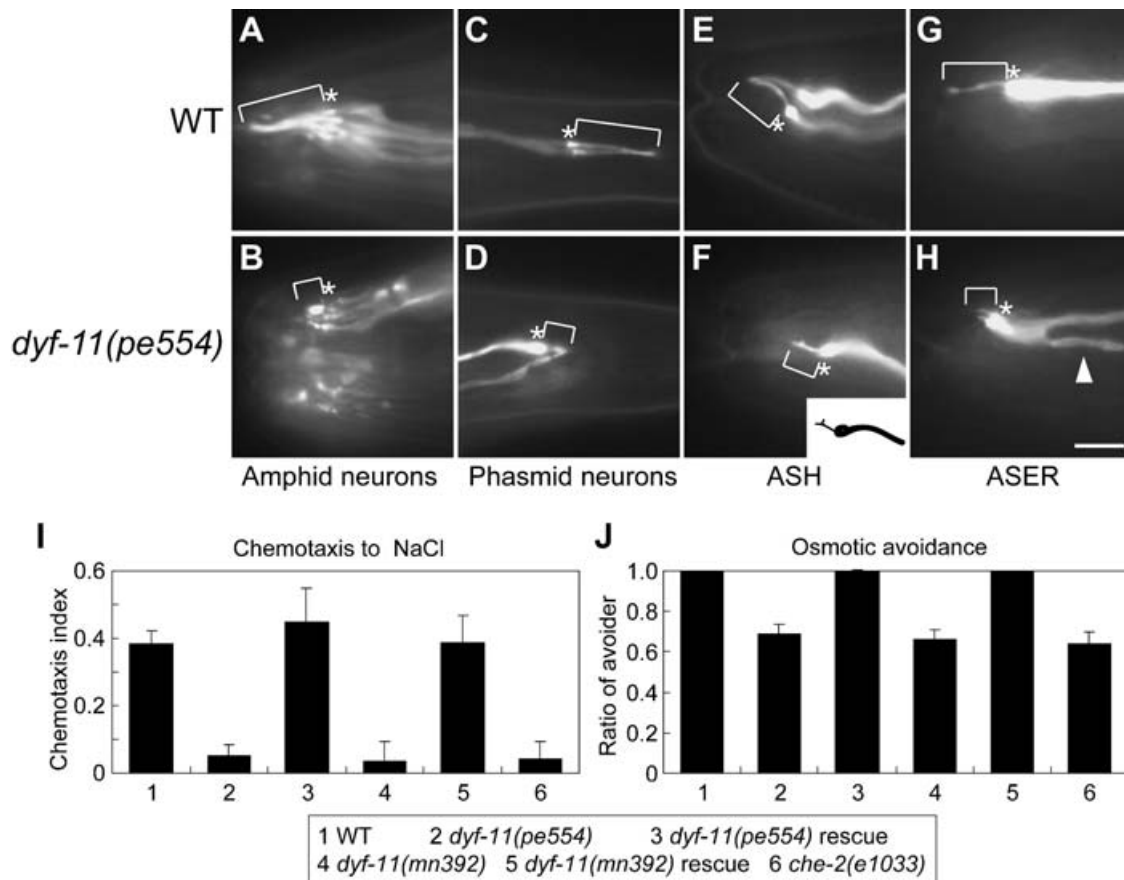


Figure 4 *dyf-11* mutants have stunted cilia and exhibit defects in the responses to environmental stimuli. (A–H) Morphology of sensory cilia in wild-type (top row) and *dyf-11(pe554)* mutants (bottom row). The amphid (A and B) and phasmid (C and D) cilia were visualized by OSM-6 :: GFP. The ASH (E and F) and ASER (G and H) sensory neurons were visualized by *sra-6* :: *venus* and *gcy-5* :: *gfp*, respectively. Asterisks indicate ciliary transition zones. Parentheses denote the middle and distal segments. The arrowhead indicates abnormal posterior projection in panel H. The anterior of the worm is toward the left in each panel. Scale bar, 5 μ m. (I and J) Behavioral defects of *dyf-11* mutants. *dyf-11* mutants have defects in chemotaxis to NaCl (I) and in avoidance of high osmolality (J) compared with wild type (column 1 for wild type). *dyf-11* mutants that carry the pGdyf-11p(-4.1) :: *venus* :: *dyf-11* transgene fully restore the normal behaviors (columns 3 and 5). The *che-2(e1033)* animals were used as a control for cilia-defective phenotype (columns 6). Error bars represent SEM.

neurons (Figs 2D and 5A). However, animals that express VENUS :: DYF-11 in muscle cells by the *myo-3* promoter did not (Fig. 5A). When VENUS :: DYF-11 was specifically expressed in ADL neurons using the *sro-1* promoter (Troemel *et al.* 1995), ADL, but not other neurons restored dye-uptake (Figs 2E and 5A). These results indicate that *dyf-11* acts cell-autonomously at least for fluorescent dye-uptake.

Cilia and flagella are known as dynamic, continuously turned-over structures (Marshall & Rosenbaum 2001). *dyf-11* is expressed in ciliated neurons from embryo to adult, during which the cilia are generated and maintained. The short and malformed cilia of *dyf-11* mutants indicate

that DYF-11 is either required to build proper cilium structure, to maintain the structure once generated during embryogenesis, or both. To examine these possibilities, we transiently expressed *dyf-11(+)* cDNA by a heat shock promoter at various developmental stages in *dyf-11(pe554)* mutants and the effect of gene expression was analyzed by prolonged observation of dye-uptake (Fig. 5B and C). Without heat-shock, a small portion of transgenic animals took up dye at any stage probably because of leaky expression of the promoter. With heat-shock at embryo or L1 stages, the animals fully restored dye-uptake if observed at 18 h after the treatment, at which time the animals have grown to L2 or L3. However,

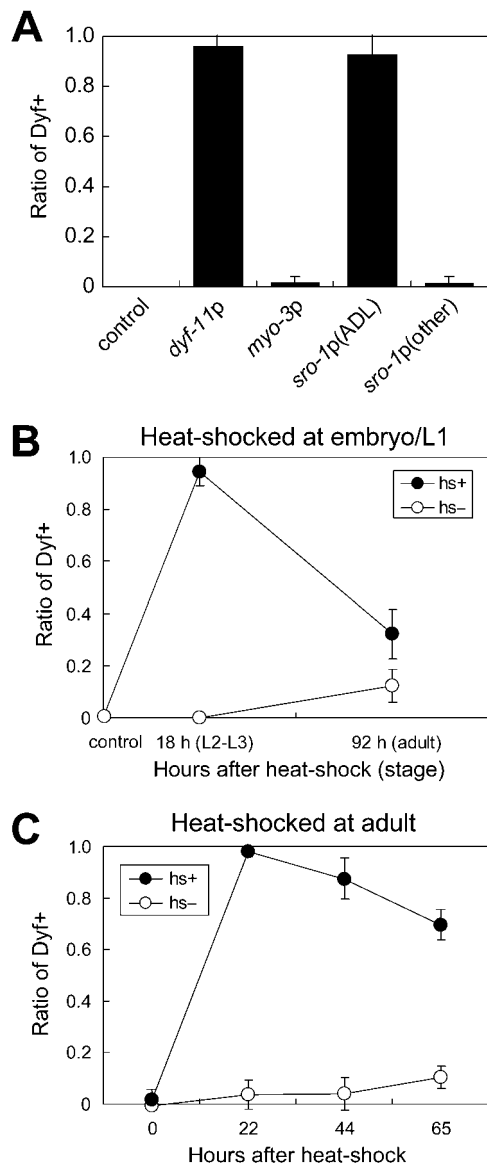


Figure 5 *dyf-11* is required for formation and maintenance of cilia in a cell-autonomous manner. (A) The ratio of dye-filling positive *dyf-11(pe554)* worms rescued by cell-specific expression of VENUS :: DYF-11. Note that the expression of VENUS :: DYF-11 with *sro-1* promoter rescued dye-uptake only in the ADL cells, in which *sro-1* promoter is active (compare the two columns from the right and also see Fig. 2E). Shown are the averages of the results from three independent transgenic lines each. (B and C) Time course dye-filling assay of *dyf-11(pe554)* animals rescued by *hsp-16.2* promoter-driven *dyf-11*. Heat-treatment was applied at embryo/L1 (B) or adult (C) followed by sampling and dye-filling assay at the times indicated. The ratio of worms that showed dye-filling at least in one neuron is presented. Each data point is the average of at least three independent assays. Error bars represent SEM.

the ratio of dye-filling competency declined at 92 h after treatment, at which time the animals have grown to adults (Fig. 5B). Heat-shock treatment at the adult stage revealed that transient expression of the wild-type gene is sufficient to correct the defective cilia in *dyf-11* mutants at least as observed by the Dyf phenotype. However, as observed in embryonic/L1 heat-shock, dye-uptake competency gradually decreased after prolonged cultivation without sustained gene expression (Fig. 5C). These results indicate that DYF-11 is a critical component of cilia and need to be continuously supplied for maintaining proper cilium structure.

DYF-11 is likely associated with IFT

To elucidate how DYF-11 is involved in ciliogenesis, we observed the localization and movement of the functional VENUS :: DYF-11 fusion proteins within cilia. Consistent with a role in cilia, the fusion protein was enriched at the transition zone and along the axoneme of ciliated neurons (Fig. 1B). Time-lapse fluorescence imaging of phasid cilia revealed that VENUS :: DYF-11 fluorescent particles are moving within the cilia in both anterograde (Fig. 6A) and retrograde (Fig. 6B) directions. Moreover, VENUS :: DYF-11 particles moved in different velocities along the middle ($0.87 \pm 0.08 \mu\text{m/s}$) and distal ($1.42 \pm 0.41 \mu\text{m/s}$) segment in the anterograde direction (Table 1). These velocities of particle movement are comparable to those for IFT proteins (Snow *et al.* 2004; Ou *et al.* 2005a; Blacque *et al.* 2006; Efimenko *et al.* 2006), suggesting that DYF-11 is moving with IFT particles carried by cooperative anterograde movement of kinesin-II and OSM-3 motors. Dependency of DYF-11 movement on IFT was further supported by the observation that VENUS :: DYF-11 accumulated at the tip of cilia in mutants of the IFT-A gene, *daf-10*, which is required for retrograde transport (Fig. 6C).

Table 1 Velocities of VENUS :: DYF-11 particles in *dyf-11* and *bbs* mutant backgrounds

Host genotype	Average velocities ($\mu\text{m/s}$)			
	Middle segment	<i>n</i>	Distal segment	<i>n</i>
<i>dyf-11(pe554)</i>	0.87 ± 0.08	11	1.42 ± 0.41	4
<i>osm-12(n1606)</i>	1.26 ± 0.19	8	ND	—
<i>bbs-8(nx77)</i>	1.03 ± 0.21	7	ND	—

n, number of particles analyzed.

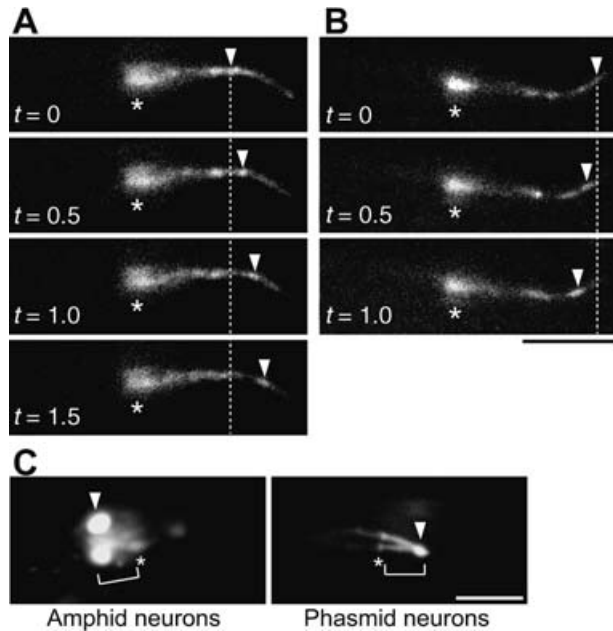


Figure 6 DYF-11 is associated with IFT. (A and B) Movement of VENUS-tagged DYF-11 in phasmid cilia. Representative sequential images of the VENUS::DYF-11 movement in phasmid neurons of the *dyf-11(pe554)* worms rescued by pGdyf11p(-4.1)::venus::dyf11. Fluorescent particles moved in both anterograde (A) and retrograde (B) directions. Arrowheads indicate the position of the same fluorescent particle at each time point. Dashed line indicates the initial position ($t = 0$) of the particle. (C) Accumulation of the VENUS::DYF-11 at the tip of cilia in *daf-10(p821)* mutants. Brackets show ciliary axonema and arrowheads denote accumulations. Compare the localization of the fusion proteins with that in wild type (Fig. 1B). Anterior is toward the left. Asterisks denote transition zones. Scale bars, 5 μ m.

It has been shown that OSM-12/BBS-7 and BBS-8 are required to stabilize interactions between IFT complexes A and B during anterograde movement; in *osm-12/bbs-7* or *bbs-8* mutants IFT particles break down into IFT-A and IFT-B subparticles, which are separately transported by kinesin-II and OSM-3 kinesin, respectively (Ou *et al.* 2005a). To gain insights into the place where DYF-11 associates in the IFT complexes, we transferred the VENUS::DYF-11 expression construct into *osm-12(n1606)* and *bbs-8(nx77)* mutant backgrounds and determined the velocities of VENUS::DYF-11 movement. In both *osm-12(n1606)* and *bbs-8(nx77)* mutants, VENUS::DYF-11 particles moved along the middle segment at velocities comparable to that of OSM-3 kinesin (Table 1). These data are similar to those observed for IFT-B proteins, suggesting that DYF-11 may associate with IFT complex B.

The coiled-coil region but not the highly conserved putative microtubule-binding region is required for the ciliary function of DYF-11

The orthologues of DYF-11 show the highest similarity with each other in their N-terminal regions; for example, the N-terminal 106 amino acids of DYF-11 show 41.1% identity to the first 112 amino acids of human TRAF3IP1 (Supplementary Fig. S2). This region was shown to be a microtubule- and β -tubulin-binding domain in TRAF3IP1 (Ling & Goeddel 2000). On the other hand, approximately 110 amino acids at the C-terminus of DYF-11 form a coiled-coil structure (Supplementary Fig. S2, aa 416–531), which is known as a protein–protein interaction domain. The equivalent region of TRAF3IP1 is also proposed to form a coiled-coil structure, which contributes to association with TRAF3 (Ling & Goeddel 2000). The amino acid identity between DYF-11 and TRAF3IP1 within the coiled-coil regions is slightly higher than that of central regions (25.0% for the coiled coil regions, and 17.7% for the central regions). To determine which regions of DYF-11 contribute to ciliary biogenesis, several DYF-11 deletion constructs were generated and tested for rescue of the Dyf phenotype of *dyf-11* worms (Fig. 3B). Interestingly, DYF-11 (aa 106–535), which lacks the N-terminal conserved region, was fully functional. Furthermore, C-terminal half of DYF-11 (aa 307–535) was sufficient for both rescue of the Dyf phenotype and localization to cilia (Supplementary Fig. S3A). In contrast, a variant that lacked the coiled-coil region (aa 1–447) failed to rescue the Dyf phenotype (Fig. 3B and Supplementary Fig. S3B). Under the wild-type background, this form of DYF-11 (aa 1–447) was uniformly distributed within the cells and hardly incorporated into IFT (Supplementary Fig. S3C). These results indicate that the coiled-coil region but not the putative microtubule-binding region is required for proper intracellular localization and function of DYF-11.

Traf3ip1 localizes to cilia in MDCK cells

Considering that DYF-11 is required for ciliogenesis in *C. elegans*, we hypothesized that mammalian Traf3ip1 may also be targeted to cilia. To examine this possibility, we determined the subcellular localization of Traf3ip1 in MDCK renal epithelial cells using anti-Traf3ip1/MIP-T3 antibodies. Cells were cultured on permeable filter supports for 7 days, during which time they polarize and develop apical primary cilia. The affinity-purified antibodies recognized a protein of approximately 90 kDa in MDCK cell lysate, which is similar to the molecular mass previously reported for Traf3ip1 (Ling & Goeddel

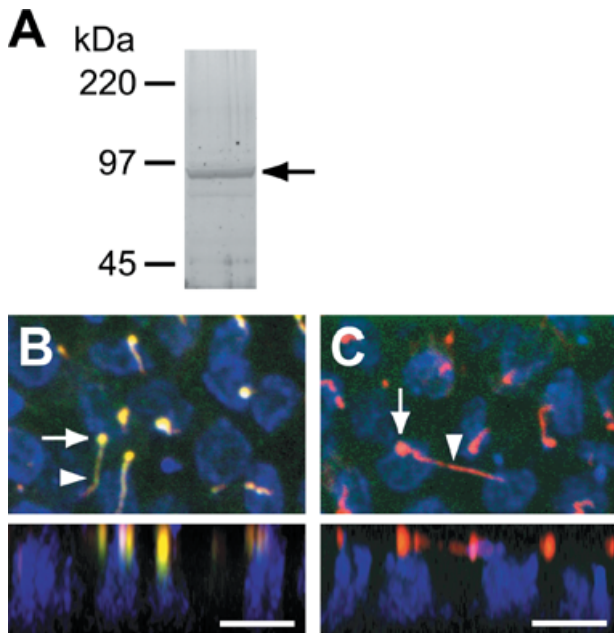


Figure 7 Traf3ip1 localizes to cilia in polarized MDCK cells. (A) The affinity-purified anti-Traf3ip1/MIP-T3 antibodies recognized a protein of approximately 90 kDa in total lysate of MDCK cells by Western blotting (arrow). (B) Staining of polarized MDCK cells with anti-Traf3ip1 (green) along with a control competitor peptide shows localization of Traf3ip1 to the base of cilia (arrow) and ciliary shaft (arrowhead) as identified by co-localization with acetylated tubulin (red). (C) Same as B except that the competitor peptides for anti-Traf3ip1 were used. No specific anti-Traf3ip1 staining was detected. Nuclei are visualized with DAPI (blue). Bottom panels show confocal x-z section views of the corresponding top images. The apical surface is up. Scale bars, 10 μm.

2000) (Fig. 7A). Immunofluorescence staining of the cells showed that Traf3ip1 co-localized with acetylated tubulin, indicating that it localizes to the basal body, from which ciliary axoneme extends, and to the ciliary shaft (Fig. 7B). Preincubation of the antibodies with excess competing peptide blocked the immunofluorescence signal in cilia, verifying that the ciliary localization pattern was specific for Traf3ip1 antigen (Fig. 7C). These results indicate that ciliary localization of the orthologues of DYF-11 is conserved in higher eukaryotes.

Discussion

Defects or absence of cilia have been associated with a variety of human genetic disorders such as polycystic kidney disease (PKD) and Bardet-Biedl syndrome (BBS) (Snell *et al.* 2004; Badano *et al.* 2006). Because of such an

importance, significant efforts have been made to identify molecules involved in ciliogenesis (Inglis *et al.* 2006). Here we described a new member of such molecules, *C. elegans* DYF-11, which shares a significant similarity to mammalian Traf3ip1.

The expression pattern of *dyf-11* fits the model in which ciliary genes are classified into two groups based on the property of the X-box motifs (Efimenko *et al.* 2005). In this model, genes that carry symmetric X-boxes (group 1) are expressed in most or all CSNs and required for general aspects of cilia formation, whereas genes that carry asymmetric X-boxes (group 2) are required in more specialized subsets of ciliated neurons (Efimenko *et al.* 2005). The promoter region of *dyf-11* contains a symmetric X-box sequence motif (GTCTCCAT-GACAAC, in which the underlined residues are critical for symmetrical X-box), which meets the signature of group 1 genes. In accordance with this, *dyf-11* mutants showed highly penetrant dye-filling defects. Moreover, they showed various behavioral abnormalities such as osmotic avoidance defects and chemotaxis defects to NaCl and odors, demonstrating that at least several classes of sensory neurons are affected. Together, these results strongly indicate that *dyf-11* is required for general aspects of ciliogenesis and ciliary functions.

It has recently been proposed that *C. elegans* IFT machinery has a modular structure consisting of IFT complex A, IFT complex B and BBS proteins that may function interdependently, as well as motor and cargo modules. An IFT-associated molecule can be assigned into distinct modules based on its transport and mutant profiles (Ou *et al.* 2007). We showed that *dyf-11* mutants have short and occasionally malformed cilia and that fluorescent protein-tagged DYF-11 are transported at velocities comparable to that of OSM-3 kinesin in the *bbs* mutants. These results are consistent with the idea that DYF-11 is associated with IFT complex B. The results of cell- and stage-specific rescue experiments also fit this idea because a previously known IFT-B protein CHE-2/IFT80 shows similar properties (Fujiwara *et al.* 1999). DYF-11 may be an integral component of the IFT-B particle. Alternatively, DYF-11 may be a cargo protein carried by the OSM-3/IFT-B complex that is critically required for ciliogenesis. Binding assay or purification of IFT particles is required to further attribute DYF-11 to any of the modular components.

Tubulins are the fundamental structural component of cilia. *tbb-4*, which encodes one of the six β -tubulin isoforms in *C. elegans*, is selectively expressed in CSNs. Furthermore, the promoter region of *tbb-4* contains a symmetric X-box. These observations raised a possibility that TBB-4 is a component of ciliary microtubules (Portman

& Emmons 2004). Because DYF-11 is an orthologue of β -tubulin-binding protein, we hypothesized that it may be involved in ciliary localization of TBB-4. To examine this possibility, we observed the intracellular localization of TBB-4 :: GFP in wild-type and *dyf-11* mutant backgrounds. The fusion proteins localized to the cell bodies, axons, dendrites and cilia of CSNs in wild-type animals. The intracellular localization pattern of TBB-4 :: GFP in *dyf-11* mutants was mostly similar to that of wild-type animals; in the cilia of *dyf-11(pe554)* mutants, GFP-tagged TBB-4 still entered the residual cilia (our unpublished results). Together with the observation that N-terminal tubulin-binding domain of DYF-11 is not required for ciliogenesis, it seems unlikely that DYF-11 is involved in the transport of TBB-4. Our results showed that the C-terminal coiled-coil domain of DYF-11 is critical for its ciliogenic function. Many of IFT-associated proteins are known to contain protein-protein interaction domains such as WD40 repeats, TPR motifs or coiled-coil domains. These domains are considered to be involved in formation of the IFT particles and interaction with cargo proteins. The coiled-coil domain of DYF-11 is likely to serve similar functions for its interaction with other IFT particle components or cargo proteins.

IL-13 signaling is critical in type 2 helper T cell-induced inflammation and therefore considered to be an important target of therapies for allergic disorders (Wynn 2003). Recent studies have shown that the mammalian orthologue of DYF-11, Traf3ip1/MIP-T3 binds to the $\alpha 1$ subunit of IL-13 receptor (IL-13R $\alpha 1$) and modulates the activation of STAT6. STAT6 is also known to localize to cilia and bind to polycystin-1 (PC1, the autosomal-dominant PKD-associated ciliary protein) in renal cells (Niu *et al.* 2003; Low *et al.* 2006). Here, we showed that Traf3ip1 localizes to cilia in polarized MDCK cells. Considering that DYF-11 is involved in IFT, DYF-11/Traf3ip1 may function as an adaptor that carries some classes of receptors to cilia and further might be involved in signal transduction by modulating signal transducers; in mammalian cells, we expect that IL-13R $\alpha 1$ is targeted to cilia by IFT in association with Traf3ip1, although it needs to be verified experimentally.

Experimental procedures

Strains, culture and genetics of *Caenorhabditis elegans*

Growth and culture of *C. elegans* was carried out by standard methods (Brenner 1974), except that the *Escherichia coli* strain NA22 was used as food source. Standard mating procedures were used to transfer reporter constructs from the original transgenic worms to various mutant backgrounds. Although *dyf-11* mutants

show reduced male mating, which is 10%–30% efficient as wild type (Starich *et al.* 1995), cross-progenies were successfully obtained throughout this study. Genotypes of the resulting strains were determined by reporter expression and fluorescent dye-filling (Dyf) phenotype. The Bristol N2 was used as wild type. Following alleles were used in this study: linkage group (LG) II: *daf-19(m86)*; LG III: *osm-12(n1606)*; LG IV: *daf-10(p821)*; LG V: *bbs-8(nx77)*, *odr-3(n2150)*; LG X: *che-2(e1033)*, *dyf-11(mn392)*, *dyf-11(pe554)*. Transgenes *mnIs17[osm-6 :: gfp]*, *ntIs1[gcy-5 :: gfp]*, and *Ex[sra-6 :: venus]* were used for visualizing sensory cilia. All strains used and construction details are available on request.

Assays

Staining of living nematode with lipophilic fluorescent dye was carried out essentially as described (Hedgecock *et al.* 1985), except that 4-(*p*-Dihexadecylaminostyryl)-*N*-methylquinolinium iodide (DiQ, Sigma, St Louis, MO) was used at the concentration of 10 μ g/mL in M9 buffer. Chemotaxis assays were performed as described (Bargmann *et al.* 1993; Saeki *et al.* 2001) with modifications (Matsuki *et al.* 2006). Osmotic avoidance assays were carried out as described (Culotti & Russell 1978) with minor modifications (Fujiwara *et al.* 1999). Behavioral assays were performed at least 6 times in each experiment. Heat-shock experiments were performed as described (Fujiwara *et al.* 1999).

Isolation of *C02H7.1/dyf-11(pe554)* deletion allele

The deletion allele *pe554* was obtained from a UV/TMP-induced deletion library as described previously (Gengyo-Ando & Mitani 2000). The original mutated strain was outcrossed 4 times with N2, resulting in the homozygous strain JN554: *dyf-11(pe554)* X. This strain was then used in further characterization and genetic crosses. Fluorescent dye-filling assay revealed that Dyf phenotype is linked to linkage group X and that *dyf-11(pe554)* is fully recessive. Nested primer pairs used for the PCR screening of the *dyf-11* deletion mutations were 5'-TAAAAGTGCGATGAGCGTTG-3' and 5'-GGGCATTTCCAAGTTTTC-3' for the first round, and 5'-TTTCACGACCACCATTCAAA-3' and 5'-TGATC-CTGTCTCCTCCATC-3' for the second round reaction, respectively.

Complementation test

Complementation between *C02H7.1(pe554)* and *dyf-11(mn392)* was performed as follows. Males of *C02H7.1(pe554)* were cultured with hermaphrodites of *dyf-11(mn392)* overnight. The mated *mn392* hermaphrodites were separated and individually cultured for 3 days to obtain their progeny. Hermaphrodites were picked from the progeny that contained significant ratio of males and tested for dye-filling. Heterozygosity of the tested animals was confirmed by single-worm PCR. We examined 38 *pe554/mn392* heterozygotes that resulted from three independent crosses and found that all of them exhibited defective dye-filling.

Cloning and molecular characterization of the *C02H7.1/dyf-11* gene

A 5-kb genome DNA fragment, which contains the entire *C02H7.1* coding region, was amplified from *dyf-11(mn392)* worms using the primers 5'-AGCGTGGCTGCTCATTA-3' and 5'-AGTGTGGACCTAAACAAA-3', and cloned into pBluescript II vector. The nucleotide sequence of all exons was determined and compared with that of wild-type worms to determine the mutation point. The result was confirmed by sequencing two independently amplified PCR fragments. The same genome region obtained from both wild-type and *dyf-11(mn392)* animals was used to rescue *dyf-11(mn392)* (see text). A cDNA clone for *dyf-11*, yk91d12, was provided by the *C. elegans* cDNA project led by Yuji Kohara. Nucleotide sequence of this clone verified the predicted exon-intron structure (The WormBase: <<http://www.wormbase.org/>>, Chen *et al.* 2005), whereas it just lacked initiation codon. We obtained the missing 5' part of cDNA by RT-PCR with a trans-splicing leader sequence SL1 as the forward primer and 5'-GTTCCCAACATTTGCAGCA-3' as the reverse primer. The full-length cDNA thus obtained was cloned into a Gateway destination vector (Invitrogen, Carlsbad, CA) and used to generate rescue constructs in combination with various promoters.

Plasmid construction and generation of transgenic worms

The Gateway cloning technology (Invitrogen) was used to generate plasmids. Two different lengths (4.1 and 2.0-kb) of promoter region of *dyf-11* were subcloned into pENTR1A entry vector. Promoters were then transferred to a destination vector, pDEST-venus, which carries the coding sequence for a variant of GFP, VENUS. Thus transcriptional reporter fusions, pGdyf11p(-4.1) :: venus and pGdyf11p(-2.0) :: venus were generated. Similarly, authentic promoter-driven and heat shock-inducible *dyf-11(+)* expression vectors used in Figs 2G, and 5B and C were generated by combining the *dyf-11* full-length cDNA with the *dyf-11* (4.1-kb) promoter or the heat-inducible *hsp-16.2* promoter, respectively. A destination vector for expressing VENUS :: DYF-11 fusion protein was generated based on the full-length cDNA construct. The coding sequence for VENUS without a termination codon (gift of T. Ishihara) was inserted in frame into the translation start site of *dyf-11*. The resulting plasmid was then recombined with the entry clones that harbor the 4.1-kb *dyf-11* promoter, generating pGdyf11p(-4.1) :: venus :: dyf11. Tissue specific VENUS :: DYF-11 expressing constructs used in Figs 2D, E and 5A were generated in a similar fashion. DYF-11 deletion constructs used in the functional domain analyses were generated by PCR to remove particular *dyf-11* region from the pGdyf11p(-4.1) :: venus :: dyf11.

Reporter constructs were introduced into worms by germ-line transformation with standard method (Mello *et al.* 1991). For expression analyses, wild-type animals were transformed with 25 ng/μL of test DNA with 50 ng/μL pRF4, a transformation marker carrying the dominant *rol-6(su1006)* gene (Mello *et al.* 1991). In rescue experiments, test DNA was injected at a concentration of 5 ng/μL. The *myo-3 :: venus* transcriptional fusion,

which enables the transformants recognizable under dissecting fluorescence microscope, was used as a transformation marker in behavioral assays.

For localization studies of VENUS :: DYF-11, wild-type animals were transformed with pGdyf11p(-4.1) :: venus :: dyf11 at 5 ng/μL along with 50 ng/μL pRF4. To obtain the transgenic mutant strains used in motility assays, adult Rol males carrying the extra-chromosomal array were mated to *dyf-11(pe554)*, *osm-12(n1606)* or *bbs-8(nx77)* hermaphrodites. F1 hermaphrodites were screened for Rol phenotype and allowed to self-fertilize. Homozygous transgenic strains were screened by the presence of Rol phenotype and defects in dye-filling.

Cell culture and immunofluorescence

MDCK cells were grown in MEM Eagle's medium supplemented with 10% fetal bovine serum and 0.1 mM MEM non-essential amino acids (Gibco, Carlsbad, CA). Cells were allowed to polarize on Transwell (Corning, NY) for 7 days with daily media change. Affinity-purified anti-MIP-T3/Traf3ip1 antibodies were obtained from Santa Cruz Biotechnology. Immunofluorescence staining was performed as described (Hirabayashi *et al.* 2003). Anti-MIP-T3 antibodies diluted 1 : 100 were preincubated with 50 mg/mL of either control (3×FLAG, Sigma) or competing (MIP-T3, Santa Cruz, Santa Cruz, CA) peptide prior to application to fixed cells along with anti-acetylated tubulin (Sigma). Images were obtained with a 40× objective on a Zeiss Axioplan2 microscope equipped with CSU21 confocal scanning unit (Yokogawa, Musashino, Tokyo, Japan). Three-dimensional reconstruction of the serial sections was processed by Metamorph software (Molecular Devices, Downingtown, PA). Western blotting was performed using the anti-MIP-T3 antibodies diluted 1 : 100 as primary antibodies.

Imaging and IFT motility assay

Cellular expression patterns of the reporter constructs were determined as described (Kunitomo *et al.* 2005). For IFT motility assays, worms were anesthetized and mounted as described (Orozco *et al.* 1999; Signor *et al.* 1999; Zhou *et al.* 2001). Time-lapse images of the sensory cilia expressing VENUS :: DYF-11 was obtained using a 100× objective on a Zeiss Axioplan2 fluorescence microscope equipped with ORCA-ER CCD camera (Hamamatsu, Shizuoka, Japan). Images were acquired at two frames per second for 15 s and processed by Metamorph software. Velocities of particle movement were determined as described (Haycraft *et al.* 2003).

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References

- Avidor-Reiss, T., Maer, A.M., Koundakjian, E., Polyanovsky, A., Keil, T., Subramaniam, S. & Zuker, C.S. (2004) Decoding cilia function: defining specialized genes required for compartmentalized cilia biogenesis. *Cell* **117**, 527–539.
- Badano, J.L., Mitsuma, N., Beales, P.L. & Katsanis, N. (2006) The ciliopathies: an emerging class of human genetic disorders. *Annu. Rev. Genomics Hum. Genet.* **7**, 125–148.
- Bargmann, C.I., Hartwig, E. & Horvitz, H.R. (1993) Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* **74**, 515–527.
- Bell, L.R., Stone, S., Yochem, J., Shaw, J.E. & Herman, R.K. (2006) The molecular identities of the *Caenorhabditis elegans* intraflagellar transport genes *dyf-6*, *daf-10* and *osm-1*. *Genetics* **173**, 1275–1286.
- Blacque, O.E., Li, C., Inglis, P.N., Esmail, M.A., Ou, G., Mah, A.K., Baillie, D.L., Scholey, J.M. & Leroux, M.R. (2006) The WD repeat-containing protein IFTA-1 is required for retrograde intraflagellar transport. *Mol. Biol. Cell* **17**, 5053–5062.
- Blacque, O.E., Perens, E.A., Boroevich, K.A., et al. (2005) Functional genomics of the cilium, a sensory organelle. *Curr. Biol.* **15**, 935–941.
- Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.
- Chen, N., Harris, T.W., Antoshechkin, I., et al. (2005) WormBase: a comprehensive data resource for *Caenorhabditis* biology and genomics. *Nucleic Acids Res.* **33**, D383–D389.
- Cole, D.G. (2003) The intraflagellar transport machinery of *Chlamydomonas reinhardtii*. *Traffic* **4**, 435–442.
- Cole, D.G., Diener, D.R., Himelblau, A.L., Beech, P.L., Fuster, J.C. & Rosenbaum, J.L. (1998) *Chlamydomonas* kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. *J. Cell Biol.* **141**, 993–1008.
- Collet, J., Spike, C.A., Lundquist, E.A., Shaw, J.E. & Herman, R.K. (1998) Analysis of *osm-6*, a gene that affects sensory cilium structure and sensory neuron function in *Caenorhabditis elegans*. *Genetics* **148**, 187–200.
- Culotti, J.G. & Russell, R.L. (1978) Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* **90**, 243–256.
- Efimenko, E., Blacque, O.E., Ou, G., Haycraft, C.J., Yoder, B.K., Scholey, J.M., Leroux, M.R. & Swoboda, P. (2006) *Caenorhabditis elegans* DYF-2, an orthologue of human WDR19, is a component of the intraflagellar transport machinery in sensory cilia. *Mol. Biol. Cell* **17**, 4801–4811.
- Efimenko, E., Bubb, K., Mak, H.Y., Holzman, T., Leroux, M.R., Ruvkun, G., Thomas, J.H. & Swoboda, P. (2005) Analysis of *xbx* genes in *C. elegans*. *Development* **132**, 1923–1934.
- Fujiwara, M., Ishihara, T. & Katsura, I. (1999) A novel WD40 protein, CHE-2, acts cell-autonomously in the formation of *C. elegans* sensory cilia. *Development* **126**, 4839–4848.
- Gengyo-Ando, K. & Mitani, S. (2000) Characterization of mutations induced by ethyl methanesulfonate, UV, and trimethylpsoralen in the nematode *Caenorhabditis elegans*. *Biochem. Biophys. Res. Commun.* **269**, 64–69.
- Haycraft, C.J., Schafer, J.C., Zhang, Q., Taulman, P.D. & Yoder, B.K. (2003) Identification of CHE-13, a novel intra-flagellar transport protein required for cilia formation. *Exp. Cell Res.* **284**, 251–263.
- Haycraft, C.J., Swoboda, P., Taulman, P.D., Thomas, J.H. & Yoder, B.K. (2001) The *C. elegans* homolog of the murine cystic kidney disease gene *Tg737* functions in a ciliogenic pathway and is disrupted in *osm-5* mutant worms. *Development* **128**, 1493–1505.
- Hedgecock, E.M., Culotti, J.G., Thomson, J.N. & Perkins, L.A. (1985) Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. *Dev. Biol.* **111**, 158–170.
- Hirabayashi, S., Tajima, M., Yao, I., Nishimura, W., Mori, H. & Hata, Y. (2003) JAM4, a junctional cell adhesion molecule interacting with a tight junction protein, MAGI-1. *Mol. Cell. Biol.* **23**, 4267–4282.
- Inglis, P.N., Boroevich, K.A. & Leroux, M.R. (2006) Piecing together a ciliome. *Trends Genet.* **22**, 491–500.
- Kozminski, K.G., Johnson, K.A., Forscher, P. & Rosenbaum, J.L. (1993) A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc. Natl. Acad. Sci. USA* **90**, 5519–5523.
- Kunitomo, H., Uesugi, H., Kohara, Y. & Iino, Y. (2005) Identification of ciliated sensory neuron-expressed genes in *Caenorhabditis elegans* using targeted pull-down of poly(A) tails. *Genome Biol.* **6**, R17.
- Li, J.B., Gerdes, J.M., Haycraft, C.J., et al. (2004) Comparative genomics identifies a flagellar and basal body proteome that includes the *BBS5* human disease gene. *Cell* **117**, 541–552.
- Ling, L. & Goeddel, D.V. (2000) MIP-T3, a novel protein linking tumor necrosis factor receptor-associated factor 3 to the microtubule network. *J. Biol. Chem.* **275**, 23852–23860.
- Low, S.H., Vasanth, S., Larson, C.H., Mukherjee, S., Sharma, N., Kinter, M.T., Kane, M.E., Obara, T. & Weimbs, T. (2006) Polycystin-1, STAT6, and P100 function in a pathway that transduces ciliary mechanosensation and is activated in polycystic kidney disease. *Dev. Cell* **10**, 57–69.
- Malone, E.A. & Thomas, J.H. (1994) A screen for nonconditional dauer-constitutive mutations in *Caenorhabditis elegans*. *Genetics* **136**, 879–886.
- Marshall, W.F. & Rosenbaum, J.L. (2001) Intraflagellar transport balances continuous turnover of outer doublet microtubules: implications for flagellar length control. *J. Cell Biol.* **155**, 405–414.
- Matsuki, M., Kunitomo, H. & Iino, Y. (2006) Go α regulates olfactory adaptation by antagonizing Gq α -DAG signaling in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **103**, 1112–1117.
- Mello, C.C., Kramer, J.M., Stinchcomb, D. & Ambros, V. (1991) Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**, 3959–3970.
- Murayama, T., Toh, Y., Ohshima, Y. & Koga, M. (2005) The *dyf-3* gene encodes a novel protein required for sensory cilium formation in *Caenorhabditis elegans*. *J. Mol. Biol.* **346**, 677–687.

- Niu, Y., Murata, T., Watanabe, K., Kawakami, K., Yoshimura, A., Inoue, J., Puri, R.K. & Kobayashi, N. (2003) MIP-T3 associates with IL-13R α 1 and suppresses STAT6 activation in response to IL-13 stimulation. *FEBS Lett.* **550**, 139–143.
- Orozco, J.T., Wedaman, K.P., Signor, D., Brown, H., Rose, L. & Scholey, J.M. (1999) Movement of motor and cargo along cilia. *Nature* **398**, 674.
- Ou, G., Blacque, O.E., Snow, J.J., Leroux, M.R. & Scholey, J.M. (2005a) Functional coordination of intraflagellar transport motors. *Nature* **436**, 583–587.
- Ou, G., Koga, M., Blacque, O.E., Murayama, T., Ohshima, Y., Schafer, J.C., Li, C., Yoder, B.K., Leroux, M.R. & Scholey, J.M. (2007) Sensory ciliogenesis in *Caenorhabditis elegans*: assignment of IFT components into distinct modules based on transport and phenotypic profiles. *Mol. Biol. Cell* **18**, 1554–1569.
- Ou, G., Qin, H., Rosenbaum, J.L. & Scholey, J.M. (2005b) The PKD protein qilin undergoes intraflagellar transport. *Curr. Biol.* **15**, R410–R411.
- Pazour, G.J., Agrin, N., Leszyk, J. & Witman, G.B. (2005) Proteomic analysis of a eukaryotic cilium. *J. Cell Biol.* **170**, 103–113.
- Perkins, L.A., Hedgecock, E.M., Thomson, J.N. & Culotti, J.G. (1986) Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev. Biol.* **117**, 456–487.
- Piperno, G. & Mead, K. (1997) Transport of a novel complex in the cytoplasmic matrix of *Chlamydomonas* flagella. *Proc. Natl. Acad. Sci. USA* **94**, 4457–4462.
- Piperno, G., Siuda, E., Henderson, S., Segil, M., Vaananen, H. & Sassaroli, M. (1998) Distinct mutants of retrograde intraflagellar transport (IFT) share similar morphological and molecular defects. *J. Cell Biol.* **143**, 1591–1601.
- Portman, D.S. & Emmons, S.W. (2004) Identification of *C. elegans* sensory ray genes using whole-genome expression profiling. *Dev. Biol.* **270**, 499–512.
- Saeki, S., Yamamoto, M. & Iino, Y. (2001) Plasticity of chemotaxis revealed by paired presentation of a chemoattractant and starvation in the nematode *Caenorhabditis elegans*. *J. Exp. Biol.* **204**, 1757–1764.
- Schafer, J.C., Haycraft, C.J., Thomas, J.H., Yoder, B.K. & Swoboda, P. (2003) XBX-1 encodes a dynein light intermediate chain required for retrograde intraflagellar transport and cilia assembly in *Caenorhabditis elegans*. *Mol. Biol. Cell* **14**, 2057–2070.
- Scholey, J.M. (2003) Intraflagellar transport. *Annu. Rev. Cell Dev. Biol.* **19**, 423–443.
- Signor, D., Wedaman, K.P., Orozco, J.T., Dwyer, N.D., Bargmann, C.I., Rose, L.S. & Scholey, J.M. (1999) Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*. *J. Cell Biol.* **147**, 519–530.
- Snell, W.J., Pan, J. & Wang, Q. (2004) Cilia and flagella revealed: from flagellar assembly in *Chlamydomonas* to human obesity disorders. *Cell* **117**, 693–697.
- Snow, J.J., Ou, G., Gunnarson, A.L., Walker, M.R., Zhou, H.M., Brust-Mascher, I. & Scholey, J.M. (2004) Two anterograde intraflagellar transport motors cooperate to build sensory cilia on *C. elegans* neurons. *Nat. Cell Biol.* **6**, 1109–1113.
- Starich, T.A., Herman, R.K., Kari, C.K., Yeh, W.H., Schackwitz, W.S., Schuyler, M.W., Collet, J., Thomas, J.H. & Riddle, D.L. (1995) Mutations affecting the chemosensory neurons of *Caenorhabditis elegans*. *Genetics* **139**, 171–188.
- Stolc, V., Samanta, M.P., Tongprasit, W. & Marshall, W.F. (2005) Genome-wide transcriptional analysis of flagellar regeneration in *Chlamydomonas reinhardtii* identifies orthologs of ciliary disease genes. *Proc. Natl. Acad. Sci. USA* **102**, 3703–3707.
- Swoboda, P., Adler, H.T. & Thomas, J.H. (2000) The RFX-type transcription factor DAF-19 regulates sensory neuron cilium formation in *C. elegans*. *Mol. Cell* **5**, 411–421.
- Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A. & Bargmann, C.I. (1995) Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* **83**, 207–218.
- Wang, Q., Pan, J. & Snell, W.J. (2006) Intraflagellar transport particles participate directly in cilium-generated signaling in *Chlamydomonas*. *Cell* **125**, 549–562.
- Wynn, T.A. (2003) IL-13 effector functions. *Annu. Rev. Immunol.* **21**, 425–456.
- Zhou, H.M., Brust-Mascher, I. & Scholey, J.M. (2001) Direct visualization of the movement of the monomeric axonal transport motor UNC-104 along neuronal processes in living *Caenorhabditis elegans*. *J. Neurosci.* **21**, 3749–3755.

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Supplementary material

The following supplementary materials are available for this article online:

Figure S1 *dyf-11* mutants show defects in chemotaxis to AWC-sensed odorants.

Figure S2 Alignment of the predicted amino acid sequences of *C. elegans* DYF-11 and human TRAF3IP1.

Figure S3 Characterization of the DYF-11 deletion constructs.

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