Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella

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Et al.
**Chlamydomonas IFT88 and Its Mouse Homologue, Polycystic Kidney Disease Gene Tg737, Are Required for Assembly of Cilia and Flagella**

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**Abstract.** Intraflagellar transport (IFT) is a rapid movement of multi-subunit protein particles along flagellar microtubules and is required for assembly and maintenance of eukaryotic flagella. We cloned and sequenced a *Chlamydomonas* cDNA encoding the IFT88 subunit of the IFT particle and identified a *Chlamydomonas* insertional mutant that is missing this gene. The phenotype of this mutant is normal except for the complete absence of flagella. IFT88 is homologous to mouse and human genes called Tg737. Mice with defects in Tg737 die shortly after birth from polycystic kidney disease. We show that the primary cilia in the kidney of Tg737 mutant mice are shorter than normal. This indicates that IFT is important for primary cilium assembly in mammals. It is likely that primary cilium have an important function in the kidney and that defects in their assembly can lead to polycystic kidney disease.

Key words: orpk • intraflagellar transport • primary cilia • kinesin-II • cytoplasmic dynein

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**Introduction**

Defects in the Tg737 gene cause kidney and liver defects in mice that are very similar to those seen in humans with autosomal recessive polycystic kidney disease (Moyer et al., 1994). This disease affects ∼1 in 10,000 children born each year and may be responsible for a much higher proportion of stillbirths and prenatal deaths (Blyth and Ockenden, 1971; Cole et al., 1987). The function of the Tg737 protein is unknown. Here we identify a protein in *Chlamydomonas* that is homologous to Tg737 and show that it is required for assembly of flagella.

The epithelial cells lining the collecting tubules of the kidney have very well developed primary cilia (Andrews and Porter, 1974). The role of these cilia is unknown; however, they extend into the lumen of the tubule and may serve as sensory appendages. Precedence for primary cilia serving a sensory role is well established in vision and olfaction, as the outer segments of the rod and cone cells of the eye and the olfactory cilia of the nose have evolved to serve as sensory appendages. Precedence for primary cilia also serve a sensory role (White et al., 1976; Perkins et al., 1986). Eukaryotic cilia and flagella are built and maintained by a process called intraflagellar transport (IFT) (Rosenbaum et al., 1999). Most well characterized in *Chlamydomonas*, IFT is a rapid movement of particles along the axonemal microtubules of cilia and flagella. The outward movement of these particles from the cell body to the tip of the flagellum is driven by FLA10 kinesin-II (Kozminski et al., 1993, 1995), whereas the transport of the particles from the tip back to the cell body is driven by DHC1b/DHC2 cytoplasmic dynein (Pazour et al., 1998, 1999; Porter et al., 1999). The particles that are transported by IFT are composed of at least 17 protein subunits (Piperno and Mead, 1997; Cole et al., 1998). The functions of the individual subunits are not known, but the proteins are conserved between green algae, nematodes, and vertebrates (Cole et al., 1998; Rosenbaum et al., 1999). In this manuscript, we describe the cloning of the IFT88 subunit of the *Chlamydomonas* IFT particle and show that cells missing this gene do not assemble flagella. We further show that IFT88 is homologous to the polycystic kidney disease gene Tg737 and that mice with mutations in this gene have shorter than normal primary cilia in their kidney.
Figure 1. Sequence and structure of the Chlamydomonas IFT88 protein. (a) Chlamydomonas IFT88 is homologous to the mouse and human Tg737 proteins. The sequences of the Chlamydomonas (Cr_IFT88, accession number AF298884), mouse (Mm_Tg737, accession number AAB59705), and human (Hs_Tg737, accession number AAA86720) proteins were aligned using ClustalW (Thompson et al., 1994). (*) Complete conservation; : , highly conserved substitutions; and (.) semiconservative substitutions (below the alignment). (b) IFT88 contains 10 TPR repeats. Residues matching the TPR consensus sequence (bottom) are indicated by bold font. (c) The 10 TPR repeats (shaded boxes) are organized in a group of three in the NH2-terminal half of the protein and a group of seven in the COOH-terminal half of the protein.
The motility/flagellar defect in V79 was rescued by transforming with BAC clones carrying the IFT88 gene. Transformation was performed by the glass bead method of Kindle (1990), and rescued cell lines were identified by restoration of their ability to swim. One rescued cell line was crossed to wild-type CC124 cells. Tetrad from this cross were dissected and analyzed by southern procedures (Levine and Ebersold, 1960; Harris, 1989) as described in Pazour et al. (1998). The flagellar phenotype was scored by light microscopy when the cells were in the early log phase of growth.

### Electron Microscopy

**Chlamydomonas** cells were fixed in glutaraldehyde for EM (Hoops and Witman, 1983) and processed as described in Wilkerson et al. (1995). Tissues of anesthetized mice were fixed in situ by brief cardiac perfusion with 5% glutaraldehyde in 100 mM cacodylate buffer. The kidneys were removed and a small amount of additional fixative was injected under the capsule of the kidney. The kidneys were placed in additional fixative for 1 h. At that time, the kidneys were sliced in half and further fixed for 2 d. The tissue was freeze fractured and metal impregnated as described in McManus et al. (1993).

### Western Blotting

Whole cell extracts of wild-type and mutant cells were made by suspending log-phase cells in SDS-sample buffer, heating at 50°C for 10 min, and repeatedly drawing the sample through a 26-gauge needle to shear the DNA. Proteins were separated by SDS-PAGE, blotted onto polyvinylidene difluoride membranes, and probed with antibodies as described in Pazour et al. (1998). Antibodies used included mAb57.1, mAb81.1, mAb139.1, and mAb172.1, which are mAbs against IFT particle proteins (Cole et al., 1998); FLA10N, which is specific for a kinesin-II motor subunit (Cole et al., 1998); DHC1b, which is specific for the heavy chain of DHC1b/DHC2 cytoplasmic dynein (Pazour et al., 1999); and B-S-1, which is specific for alpha tubulin (Piperno and Fuller, 1985).

### Mouse Genotyping

**Chlamydomonas** strains used in this work included: g1 (nit1, aggl, mt+) (Pazour et al., 1995), CC124 (nit1, mt2, mt+), and CC390 (flu2, mt+). The latter two strains are available from the Chlamydomonas Genetics Center (Duke University, Durham, NC). Strains generated in the course of this work included: V79 ([IFT88-1::NIT1, mt+], which was generated by random insertional mutagenesis of g1, V79/40-B3#2.5 ([IFT88-1::NIT1, IFT88, mt+]), generated by transformation of V79 with BAC clone 40-B3, and 3276.2 ([IFT88-1::NIT1]), which is a progeny from a cross between V79/40-B3#2.5 and CC124.

# Materials and Methods

## Purification and Microsequencing of Chlamydomonas IFT88

168 IFT particles were purified from *Chlamydomonas* flagella as described in Cole et al. (1998). The IFT88 subunit was further purified by two-dimensional gel electrophoresis and transferred to ImmobilonP (Millipore) as described previously (Cole et al., 1998). The spot corresponding to IFT88 was excised and digested with trypsin. Tryptic peptides were eluted from the membrane and fractionated by high performance liquid chromatography. Pure peptides, identified by mass spectrometry, were subjected to microsequence analysis in the UMMS Protein Sequencing Facility.

## Cloning IFT88

Portions of the IFT88 peptide sequence (LEGETDQA and GIDPYCVE) were used to design two degenerate oligonucleotide PCR primers (GA[A/G][A/G][C/T][G/C][T][G/C][T] A[A/G] TA and GC[C/T][C][A/G][G][A/G][A/G]TA [A/G]GG [A/G][C/T][G/A][AT]). These primers amplified a 365-bp fragment of genomic DNA that contained parts of two exons and a 132-bp intron. This fragment of genomic DNA was used to screen a *C. reinhardtii* genomic DNA library (Genome Systems; McManus et al., 1993).

Four independent BAC clones (40-B3, 11-O21, 24-F2, and 27-M3) were identified and sequenced by the Genome Systems Genomics Unit (Duke University, Durham, NC). Strains generated in the course of this work included: V79 (nit1, aggl, mt+), generated by transformation of V79 with BAC clone 40-B3, and 3276.2 ([IFT88-1::NIT1]), which is a progeny from a cross between V79/40-B3#2.5 and CC124.

## Mouse Genotyping

DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by digesting mouse tails with proteinase K, extracting with 50% phenol / 50% chloroform, once with chloroform, and then DNA was purified by dig...
The IFT88 protein sequences and used to amplify a fragment of genomic DNA. Degenerate PCR primers were designed from these sequences, and the resulting clones were sequenced by primer walking. A cDNA library was screened with the genomic fragment of IFT88 genomic DNA as a probe. The single band in wild-type cells was split into two bands in the mutant indicating that the selectable marker integrated into the gene (Fig. 2 a). The fact that the single hybridizing band in wild-type cells was split into two bands in the mutant indicated that the selectable marker integrated into the gene within the region covered by the probe and did not result in a large deletion of the genome at the site of integration. DNA was isolated from ~400 insertional mutants having behavioral or motility defects and was screened by Southern blotting using a fragment of IFT88 genomic DNA as a probe. One cell line (V79) was identified that had an insertion in the IFT88 gene (Fig. 2 a). The fact that the single hybridizing band in wild-type cells was split into two bands in the mutant indicated that the selectable marker integrated into the gene within the region covered by the probe and did not result in a large deletion of the genome at the site of integration. The mutant allele was termed ift88-1.

The ift88-1 cells grew at the same rate as wild-type cells, indicating that IFT88 is not required for processes essential for growth or cell division (Fig. 2 b). However, in contrast to wild-type cells that normally have two ~10-μm-long flagella, flagella are not formed on ift88-1 cells. Cells were recorded by differential interference contrast microscopy as described in Pazour et al. (1998).

Sequence analysis showed that the IFT88 cDNA contains a 2,346-nucleotide open reading frame that is predicted to encode an 86.3-kD protein (Fig. 1 a) with a pI of 5.87. Perfect matches to both IFT88 tryptic peptides are found in the open reading frame of this cDNA, rigorously confirming that these clones encode the Chlamydomonas IFT88 protein. No discernible motifs were identified within the sequence except for the presence of 10 tetra-tripeptide repeats (TPR) (Fig. 1 b). TPRs are degenerate 34–amino acid repeats (Lamb et al., 1995), present in tandem arrays of 3–16 U that are predicted to form amphipathic helices (Hirano et al., 1990). The first three TPR motifs are found closely spaced between amino acid residues 185–294. The other seven TPR motifs occur without spacing between amino acid residues 441–676 (Fig. 1 c).

Chlamydomonas IFT88 Is Homologous to a Mouse Polycystic Kidney Disease Gene

Blast searches with the Chlamydomonas IFT88 protein sequence indicate that it is very similar to the mouse (41% identical; BLAST E=e-148) and human Tg737 (40% identical; BLAST E=e-146) proteins. Mice with defects in Tg737 have severe polycystic kidney disease and die within a few weeks of birth. The protein also is homologous to proteins predicted by ESTs from zebra fish and swine and fragments of preliminary C. elegans and Drosophila melanogaster genomic sequence. IFT88 and Tg737 are likely to be functionally equivalent orthologs as the similarity between the Chlamydomonas and mammalian proteins is robust and distributed over the entire coding region and not just within the TPR domains (Fig. 1 a). 40% identity is very typical of the amount of similarity seen between other Chlamydomonas and mammalian orthologs that encode flagellar proteins (Pazour, G.J., B.L. Dickert, and G.B. Witman, manuscript in preparation).

IFT88 Is Required for Flagellar Assembly

To learn more about the function of IFT88 in cells, we searched our collection of Chlamydomonas insertional mutants (Pazour et al., 1995, 1998; Pazour and Witman, 2000) for a cell line with a defect in this gene. The insertional mutants were made by transforming cells with DNA carrying a selectable marker. In Chlamydomonas, transforming DNA is integrated randomly throughout the genome and disrupts genes at the site of integration. DNA was isolated from ~400 insertional mutants having behavioral or motility defects and was screened by Southern blotting using a fragment of IFT88 genomic DNA as a probe. One cell line (V79) was identified that had an insertion in the IFT88 gene (Fig. 2 a). The fact that the single hybridizing band in wild-type cells was split into two bands in the mutant indicated that the selectable marker integrated into the gene within the region covered by the probe and did not result in a large deletion of the genome at the site of integration. The mutant allele was termed ift88-1.

The ift88-1 cells grew at the same rate as wild-type cells, indicating that IFT88 is not required for processes essential for growth or cell division (Fig. 2 b). However, in contrast to wild-type cells that normally have two ~10-μm-long flagella extending from the anterior end of the cell body, the ift88-1 cells completely lack flagella (Fig. 2 c). Electron microscopic analysis of these cells showed that the basal bodies were structurally normal but the flagella did not extend beyond the transition zone (Fig. 3, arrows). In some cells, the membrane covering the flagellar tips was tightly apposed to the microtubules with no material between them and the membrane. In other cells, the flagellar stubs were slightly swollen and contained fragments of microtubules in random orientations. However, in contrast to the IFT mutants fla14 (Pazour et al., 1998) and dhc1b
(Pazour et al., 1999), no accumulation of IFT particles was observed in any of the flagellar stubs.

To determine the effect of the lack of IFT88 on the IFT particle and the IFT motors, we examined whole-cell extracts by Western blotting (Fig. 4). The IFT particle is made up of two large complexes. Complex A is composed of four to five proteins and includes IFT139, shown in Fig. 4. Complex B is composed of IFT88 and 10 other proteins including IFT172, IFT81, and IFT57, shown in Fig. 4. The complex A protein IFT139 is enriched in the mutant, suggesting that the gene may be upregulated in the mutant cells. The mutation has little or no effect on the levels of complex B proteins IFT172 and IFT81, but causes a significant decrease in IFT57, another complex B protein. The cellular levels of the IFT motors FLA10 kinesin-II and DHC1b are not affected by the ift88 mutation.

To be certain that the flagellar assembly defect is caused by the mutation in IFT88 and is not the result of another mutation elsewhere in the genome, we transformed the mutant cells with BAC clones carrying the IFT88 gene.

Three independent BAC clones (40-B3, 24-F2, and 27-M3) complemented the flagellar defect. The complemented cell lines swam like wild-type cells and had IFT. One of the rescued cell lines was crossed to a wild-type cell line and 26 tetrads were dissected. All four products of one tetrad and a single product of the remaining 25 tetrads were analyzed by Southern blotting. Because the transformed copy of the IFT88 gene inserted at a site unlinked to the original locus, the inserted DNA segregated independently from the original gene, allowing offspring to carry zero, one, or two copies of the wild-type gene. Cells that carry at least one copy of wild-type IFT88 have normal flagella and motility, whereas those that carry no copies of wild-type IFT88 lack flagella and are nonmotile (Fig. 5). These data indicate that the flagellar defect is tightly linked to the ift88 mutation and is almost certainly the result of it.

**Primary Cilia in the Kidney of Tg737 Mice Are Shorter than Normal**

Primary cilia are present on many cells in the mammalian body (Wheatley, 1995; Wheatley et al., 1996), and are particularly well developed in the kidney (Andrews and Porter, 1974). Inasmuch as Chlamydomonas IFT88 is necessary for assembly of flagella and is homologous to mammalian Tg737, and because a defect in mouse Tg737 leads to kidney
disease, it was of great interest to determine whether the defect in Tg737 affected formation of the primary cilia in the kidney. In wild-type rats, the cilia are 2.5–3.5 µm long and are found in the proximal tubule, the loop of Henle, the distal tubules, and the collecting ducts (Andrews and Porter, 1974). In wild-type mice, these cilia are 5–6 µm long and similarly distributed (Flood and Totland, 1977).

We obtained the hypomorphic Tg737-mutant mice from Oak Ridge National Laboratory and examined the kidneys of 4- and 7-d–old pups by scanning electron microscopy. Numerous monociliated cells were observed in the kidneys of both wild-type (Fig. 6 a, 1/1) and homozygous mutant (Fig. 6 a, 2/2) mice, but the cilia in the mutant kidneys were much shorter. To quantify this difference, the cilia lengths were measured from scanning electron micrographs taken from the tubules distal to the proximal tubule (Fig. 6, b and c). The proximal tubule was avoided because it contains a thick brush border that can obscure a micron or more of cilia length. The tubules distal to the proximal tubule have only sparse microvilli that do not obscure cilia, and the cilia in these regions are uniform in length (Andrews and Porter, 1974). These cilia in wild-type mice were 3.1 ± 1.4 and 3.5 ± 1.7 µm at 4 and 7 d, respectively, whereas these cilia in the mutant mice were 1.0 ± 0.6 and 1.3 ± 0.6 µm at 4 and 7 d, respectively. These values represent minimum lengths as it is difficult to accurately measure cilia that are lying at different angles in the tubules. However, the differences are quite large and are significant at the >99% level. Thus, Tg737 plays an essential role in assembly of the primary cilia in the mouse, just as IFT88 is essential for flagellar assembly in Chlamydomonas.

**Discussion**

**The IFT88 Gene Is Required for Flagellar Assembly in Chlamydomonas**

Chlamydomonas cells lacking the IFT88 gene do not assemble flagella, indicating that the IFT88 protein is required for flagellar assembly. This is the first Chlamydomonas IFT particle subunit to be shown to be required for ciliary assembly. Loss of IFT88 causes a substantial decrease in the amount of IFT57 relative to other IFT particle proteins in the cytoplasm, suggesting that IFT88 is important for assembly of at least a portion of the IFT particle. Thus, IFT may be blocked at a very early stage in the ift88-1 mutant. Consistent with this, IFT particles do not accumulate in the flagellar stubs of the ift88 mutant, in sharp contrast to the dramatic accumulation of apparently intact IFT particles in the flagella of mutants with defects in cytoplasmic dynein DHC1b/DHC2 (Pazour et al., 1999) or the dynein light chain LC8 (Pazour et al., 1998). Alternatively, IFT88 may have a vital role in the attachment of the IFT particle to its cargo or to the anterograde IFT motor FLA10-kinesin-II. In either case, loss of IFT88 would preclude flagellar assembly. It is also possible that IFT88 is essential for transduction of a signal that is necessary for flagellar assembly.

**Tg737, the Mouse IFT88 Homologue, Is Required for Assembly of the Primary Cilia in Kidney**

We have shown that IFT88 is highly similar to the mouse and human Tg737 proteins and that mice with defects in Tg737 have defective cilia in their kidneys. Tg737 was
identified at Oak Ridge National Laboratory by random insertional mutagenesis of mice. Hypomorphic mutations in Tg737 cause kidney disease and death within a few weeks of birth. The phenotype of this mutation closely resembles human autosomal recessive polycystic kidney disease (ARPKD) in that the mice develop cystic kidneys and have hepatic biliary disease, which is also common in human patients with ARPKD (Moyer et al., 1994). The mice develop large cysts in their collecting ducts and are unable to concentrate urine (Yoder et al., 1996, 1997). Null alleles of Tg737 have a more severe phenotype and cause the mice to die during midgestation (Murcia et al., 2000). The phenotype caused by the null Tg737 mutation closely resembles the phenotype of kinesin-II knockout mice (Nonaka et al., 1998; Marszalek et al., 1999, Takeda et al., 1999). Both the kinesin-II and Tg737 null mice have left-right asymmetry defects, lack cilia on the embryonic node, and die during midgestation. Our finding that IFT88 is required for ciliary assembly provides the first evidence that the lack of nodal cilia on embryos of Tg737 null mutant mice is a direct result of a defect in IFT.

Primary cilia are extremely widely dispersed throughout the mammalian body. The only cells that are known not to contain primary cilia are hepatocytes and differentiated cells of myeloid or lymphoid origin (Wheatley, 1995; Wheatley et al., 1996). The primary cilia in kidney tubules and ducts (Andrews and Porter, 1974) and hepatic biliary ducts (Motta and Fumagalli, 1974) are unusually long and project into the lumens of these structures. The role of the primary cilia in the kidney or hepatic ducts is not known, but has been suggested to be sensory (Roth et al., 1988).

Figure 6. Primary cilia in the kidney of Tg737 mutant mice are shorter than normal. (a) Kidneys from 4-d-old pups were fixed with glutaraldehyde, freeze fractured, metal impregnated, and examined by scanning electron microscopy. Numerous cilia were found on the epithelial cells in the tubules and collecting ducts of the wild-type mice (+/+). Cilia were also found in the homozygous mutant (−/−) pups, but they were usually <2-μm long and most were only short stubs. (b) Cilia length in kidneys of 4-d-old mice. Cilia were measured in scanning electron micrographs of tubules located distal to the proximal tubule. Wild-type cilia averaged 3.1 ± 1.4 μm (n = 50), whereas the mutant cilia averaged 1.0 ± 0.6 μm (n = 50). (c) Cilia length in kidneys of 7-d-old mice. The cilia lengths were measured in scanning electron micrographs of tubules located distal to the proximal tubule. Wild-type cilia averaged 3.5 ± 1.7 μm (n = 50), whereas the mutant cilia averaged 1.3 ± 0.6 μm (n = 50).
cilia is clearly to serve as an appendage to concentrate sensory machinery. *C. elegans* also makes extensive use of primary cilia to detect osmolarity gradients and chemical signals (White et al., 1976; Lewis and Hodgkin, 1977). Primary cilia on other cells may similarly have a sensory role. Supporting this idea, the somatostatin 3 receptor has recently been localized to primary cilia in the brain (Handel et al., 1999). Kidney epithelial cells sense multiple extracellular signals, including peptide hormones such as angiotensin and ions such as chloride (reviewed in Gunning et al., 1996). Whether any of these sensory receptors are localized to the primary cilia of the vertebrate kidney remains to be determined.

*C. elegans* homologues of the human polycystic kidney disease genes, PKD1 and PKD2, are localized to sensory cilia (Barr and Sternberg, 1999). Humans with mutations in PKD1 and PKD2 develop kidney disease similar to that caused by Tg737 mutations in mice. PKD1 and PKD2 are transmembrane proteins that interact with each other (Qian et al., 1997; Tsiokas et al., 1997). PKD1 has a large extracellular domain that is thought to bind an unknown ligand (Hughes et al., 1995; The International Polycystic Kidney Disease Consortium, 1995). PKD2 is homologous to calcium-regulated cation channels, suggesting that PKD2 is a cation channel (Chen et al., 1999). Further work will be necessary to determine if PKD1 or PKD2 are ever found on mammalian primary cilia.

**TPR Repeats in IFT88 and Tg737 Suggest that These Proteins Are Involved in Protein–Protein Interactions**

TPR repeats are degenerate 34 amino acid motifs (Lamb et al., 1995) that are present in tandem arrays in proteins. These arrays are predicted to form super helices (Hirano et al., 1990) with amphipathic grooves responsible for binding specific target proteins (Das et al., 1998). TPR domains have been found to mediate multiple simultaneous protein–protein interactions in such multiprotein complexes as molecular chaperones and the anaphase-promoting complex (reviewed in Blatch and Lassle, 1999). In IFT88 and Tg737, there are three closely spaced TPR repeats in the amino-terminal half of the protein and another seven TPR repeats in the carboxyl-terminal half of the protein. These two separate TPR domains might serve to bind simultaneously to two separate target proteins. These target proteins could be axonemal subunits that are transported via IFT to the flagellar tip, where they are assembled (Piperno et al., 1996). The targets also could be membrane proteins such as receptors and channels, as IFT particles are tightly associated with the flagellar membrane (Kozkinski et al., 1995; Pazour et al., 1998). Alternatively, IFT88 could be binding to other subunits of the IFT particle and holding it together. IFT57 is likely to be an interacting protein because it is de-stabilized in the absence of IFT88.

**IFT Is a Conserved Mechanism in the Assembly and Maintenance of Cilia and Flagella**

A strong body of evidence indicates that IFT is necessary for assembly and maintenance of all eukaryotic motile and sensory cilia. Previous work has shown that the antero-grade motor, kinesin-II, is necessary for assembly and maintenance of cilia and flagella in diverse organisms that include green algae, ciliated protozoa, nematodes, echinoderms, and vertebrates (reviewed in Cole, 1999; Marszalek and Goldstein, 2000). The retrograde motor, cytoplasmic dynein DHC1b/DHC2, also has been shown to be required for assembly of *Chlamydomonas* flagella (Pazour et al., 1999; Porter et al., 1999) and *Caenorhabditis* sensory cilia (Signor et al., 1999; Wicks et al., 2000). Our initial report on the composition of the *Chlamydomonas* IFT particle proteins showed that IFT52 was homologous to *C. elegans* OSM-6 and that IFT172 was homologous to *C. elegans* OSM-1 (Cole et al., 1998). OSM-1 and OSM-6 are required for assembly of sensory cilia in worms (Perkins et al., 1986; Collet et al., 1998). The involvement of these two nematode proteins in IFT was recently confirmed when GFP-labeled OSM-6 and OSM-1 were both shown to undergo IFT in transformed *C. elegans* (Orozco et al., 1999; Signor et al., 1999). The work in this paper shows that the IFT particle protein IFT88 is required for ciliary assembly in *Chlamydomonas* and that the IFT88 homologue, Tg737, is required for assembly of primary cilia in the kidney of mice. Thus, evidence from a diverse group of eukaryotes shows that both the IFT motors and the IFT particle proteins are required for assembly and maintenance of cilia and flagella. This indicates that IFT is an ancient and conserved mechanism by which eukaryotic cilia and flagella are built and maintained.

**IFT Is Likely to Play Important Roles in Many Disease States**

In addition to polycystic kidney disease, discussed above, there are many other diseases that may involve IFT. This includes retinitis pigmentosa, which is a genetic disorder that causes destruction of photoreceptor cells, resulting in progressive vision loss. Transport of opsin and other components of the rod outer segment is very important in photoreceptor cells, as ~10% of the outer segment is turned over each day. Transport from the inner segment to the outer segment occurs through the connecting cilium (reviewed in Besharse and Horst, 1989). Kinesin-II and several IFT particle proteins are located in the connecting cilium of photoreceptor cells (Beech et al., 1996; Muresan et al., 1999; Whitehead et al., 1999; Pazour, G.J., D.G. Cole, J.A. Deane, S.A. Baker, B.L. Dickert, J.L. Rosenbaum, G.B. Witman, and J.C. Besharse, manuscript submitted for publication). Moreover, Marszalek et al. (2000) showed that photoreceptor cells lacking kinesin-II accumulate opsin and arrestin in the inner segment, indicating that kinesin-II is involved in transport in photoreceptor cells. Therefore, IFT is likely to be an important transport mechanism in vertebrate photoreceptor cells, and mutations in IFT particle proteins are likely to cause vision defects.

Defects in IFT also are likely to affect motile cilia and flagella and be a cause of primary ciliary dyskinesia (PCD). PCD is a syndrome caused by defects in motile cilia and is characterized by male infertility, respiratory disease, and situs inversus. It is well known that defects in axonemal components cause PCD (Afzelius, 1979; Pen-narun et al., 1999). However, IFT particle proteins are highly expressed in the testis and lung, suggesting that they are involved in the assembly of motile sperm flagella and respiratory tract cilia (Pazour, G.J., unpublished results). Thus, a mutation in an IFT particle protein could lead to
defects in sperm flagellar assembly and as result in sperm with short disorganized tails, as have been described in some infertile human males (Baccetti et al., 1993). It is possible that mutations that prevent assembly of both motile and sensory cilia are so severe that the embryos terminate during gestation, as has been observed with mutations in the mouse kinesin-II motor subunits kif3a (Marszalek et al., 1999; Takeda et al., 1999) and kif3b (Nonaka et al., 1998).

We thank Ruth Hesselton, Greg Hendricks, Dennis Diener, Matthew Whitacre, Ben Lucker, John Leszky, and Diane Casey for valuable assistance during this project.

This work was supported by National Institutes of Health grants GM50626 to G.B. Witman and GM14642 to J.L. Rosenbaum, by a grant from the University of Idaho Agricultural Experiment Station to D.G. Cole, by a Diabetes and Endocrinology Research Center Grant (DK 32520) at the University of Massachusetts Medical School, by an American Association of University Women International Fellowship (Y. Vucica), and by the Robert W. Booth Fund at the Greater Worcester Community Foundation (G.B. Witman).

Submitted: 22 May 2000
Revised: 28 August 2000
Accepted: 7 September 2000

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