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Dynein and Intraflagellar Transport

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Intraflagellar transport

Intraflagellar transport (IFT) is the bi-directional movement of particles along the length of cilia/flagella (terms used interchangeably here) (reviewed in Rosenbaum and Witman, 2002; Baldari and Rosenbaum, 2010). This movement was first observed in *Chlamydomonas reinhardtii* by differential interference contrast microscopy (DIC) (Kozminski et al., 1993). Later, fluorescence microscopy (especially total internal reflection fluorescence [TIRF] microscopy) was widely used to observe the movement of individual proteins during IFT (Engel et al., 2009). Shortly after the discovery of IFT, the motor protein that powers the movement from the base of the flagellum to the tip (anterograde IFT) was found to be heterotrimeric kinesin 2 (composed of FLA10, FLA8, and FLA3 in *Chlamydomonas*; KIF3A, KIF3B, and KAP in mammals) (Walther et al., 1994; Kozminski et al., 1995; Morris and Scholey, 1997; Cole et al., 1998; Nonaka et al., 1998). Over the years, additional kinesins were found to be involved in anterograde IFT in *Caenorhabditis elegans* and mammals. It appears that the heterotrimeric kinesin-2 motor is part of the core IFT machinery that functions wherever IFT occurs, whereas accessory kinesins function in a cell-specific manner to generate diversity in ciliary shape and function (Verhey et al., 2011). By taking advantage of the *Chlamydomonas fla10* temperature-sensitive mutant, two groups initially isolated at least 15 proteins that compose the particles moving during IFT (Piperno and Mead, 1997; Cole, et al., 1998). To date, at least 22 IFT-particle proteins have been identified (Ou et al., 2005; Taschner et al., 2012; Ishikawa et al., 2014); these proteins form two different complexes, termed IFT-A and IFT-B. The motor protein that powers the movement of the particles from the tip of the flagellum to the base (retrograde IFT) is called cytoplasmic dynein 1b in *Chlamydomonas* and cytoplasmic dynein 2 in vertebrates. Here, for simplicity, we collectively refer to the dyneins that power retrograde IFT as IFT dyneins.

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The subunits of IFT dyneins in *Chlamydomonas*, *C. elegans*, and humans are listed in Table 1.

Composition of the mainstream IFT dynein – cytoplasmic dynein 1b/2

Heavy chain

The dynein 1b/2 heavy chain was first identified, independently, in the sea urchin (Gibbons et al., 1994) and in the rat (Tanaka et al., 1995). In phylogenetic trees, this subspecies of dynein heavy chain diverges from the trunk at a position nearly equidistant between the cytoplasmic and axonemal forms. The expression of DHC1b is upregulated during cilia regeneration in sea urchin embryos, which is similar to that of the axonemal dyneins, but differs from that of the conventional cytoplasmic dynein. However, DYNC2H1, the mammalian homolog, was proposed to be a cytoplasmic dynein that participates in intracellular trafficking in polarized cells based on its ubiquitous expression in all rat tissues examined and its localization in the apical regions of the cytoplasm of ciliated rat tracheal epithelial cells but not in cilia (Criswell et al., 1996). DYNC2H1 was proposed by another group to play a role in the organization and/or function of the Golgi apparatus since it was found to localize predominantly to the Golgi apparatus and microinjection of an antibody to DYNC2H1 caused dispersion of the Golgi complex (Vaisberg et al., 1996).

Conclusive evidence that cytoplasmic dynein 1b/2 is responsible for retrograde IFT came from studies on *Chlamydomonas* DHC1b and *C. elegans* CHE-3 mutants. The *Chlamydomonas dhc1b* null mutant grows normally and appears to have a normal Golgi apparatus, but has very short flagella. Loss of DHC1b also results in a massive redistribution of IFT-particle proteins from a peri-basal body pool to the flagella as shown by electron microscopy and immunofluorescence microscopy. It was proposed that DHC1b is an essential part of the motor for retrograde IFT and that the redistribution of IFT-particle proteins occurred due to a defect in retrograde IFT. Consistent with this, western blots indicated that DHC1b is present in the wild-type flagellum, predominantly in the detergent- and ATP-soluble fractions (Pazour et al., 1999). Independently, another group also cloned *Chlamydomonas* DHC1b and identified *dhc1b* null mutants, which have flagella assembly defects and accumulate particles in their extremely short flagella (Porter et al., 1999). In addition to null mutants in DHC1b, three *Chlamydomonas* temperature-sensitive *dhc1b* mutants have been identified (Iomini et al., 2001; Engel et al., 2012; Lin et al., 2013). While one of them, *dhc1b-2*, has half-length flagella at permissive temperature, two of them, *dhc1b-3* and *fla24*, have normal-length flagella at permissive temperature yet have a reduced level of DHC1b in the flagella and reduced retrograde IFT. These results showed that *Chlamydomonas* flagellar assembly requires retrograde IFT yet a reduced level of retrograde IFT is still sufficient for flagellar assembly and maintenance. Mutant analysis in *C. elegans* similarly showed that CHE-3, the *C. elegans* homologue of DHC1b, is specifically responsible for the retrograde transport of the anterograde motor, kinesin 2, and its cargo within sensory cilia (Signor et al., 1999) and is required for sensory cilia structure and function (Wicks et al., 2000).

Mouse null mutants for DYNC2H1 are embryonic lethal (Huangfu et al., 2005; May et al., 2005; Ocbina et al., 2008) and, like *Chlamydomonas* DHC1b null mutants, have short cilia

with bulges along the axoneme. Although the short and bloated nodal cilia in the *Dync2h1* mutant could not be detected by acetylated tubulin staining, they were clearly observed by scanning electron microscopy (May et al., 2005). Thus, loss of this dynein heavy chain homologue has a remarkably consistent effect on ciliary structure in organisms ranging from *Chlamydomonas* to mammals, indicating that the function of cytoplasmic dynein 1b/2 as a retrograde IFT motor and its importance in flagellar assembly has been highly conserved throughout evolution. In addition, Hedgehog signaling, which in vertebrates is dependent on cilia, is disrupted in the mouse mutants. These data supported the idea that primary cilia act as specialized signal transduction organelles required for coupling Smo activity to the biochemical processing of Gli3 protein. Interestingly, when DYNC2H1 was knocked down by siRNA, human telomerase immortalized retinal pigment epithelial cells (hTERT-RPE1) either had no cilia, short cilia and normal-length cilia, or abnormally long cilia depending on the siRNA constructs. It was concluded that highly effective suppression of DYNC2H1 leads to a failure to produce cilia in hTERT-RPE1 cells whereas partial suppression results in increased cilia length (Palmer et al., 2011). In the absence of DHC1b/DYNC2H1, the other subunits of dynein 1b/2 are usually unstable and redistribute from basal body region (Perrone et al., 2003; Hou et al., 2004; Rompolas et al., 2007).

The prevailing model is that dynein 1b/2 is composed of a homodimer of DHC1b/DYNC2H1 plus intermediate, light-intermediate, and light chain subunits (see following sections). However, genomes of *Leishmania mexicana*, *Leishmania major*, *Leishmania infantum*, and *Trypanosoma brucei* contain two dynein 1b/2 heavy chain isoforms. In *L. mexicana*, one of the heavy chains, *Lmx*DHC2.2, is required for flagellar assembly and also participates in the maintenance of promastigote cell shape while the other heavy chain, *LmxDHC2.1*, could be an essential gene because no homologous disruptions could be obtained (Adhiambo et al., 2005). In *T. brucei*, the two distinct dynein heavy chains DHC2.1 and DHC2.2 (~40% identity) are both essential for retrograde IFT and were co-immuniprecipitated, showing that in this organism the heavy chains form a heterodimer (Blisnick et al., 2014).

Light-intermediate chain

Unlike cytoplasmic dynein 1, which has two light-intermediate chain genes, so far only one light-intermediate chain has been identified for cytoplasmic dynein 1b/2. This protein is termed DYNC2LI1 in mammals and D1bLIC in *Chlamydomonas*. DYNC2LI1 was initially cloned independently by two groups (Grissom et al., 2002; Mikami et al., 2002). Grissom et al. immunoprecipitated DYNC2H1 and identified DYNC2LI1 as an interacting protein. Mikami et al. cloned and sequenced a cDNA encoding this protein and found that the predicted protein sequence was closely related to that of the previously characterized cytoplasmic dynein 1 light-intermediate chains 1 and 2. Both studies showed that DYNC2LI1 is associated with DYNC2H1. Mikami et al. further showed that dynein 2 was most abundant in ciliated epithelia and in the connecting cilia of photoreceptor cells. Immunocytochemistry of cultured cells revealed a clear staining of primary cilia, but no specific association with the Golgi apparatus. These data favored a predominant role for dynein 2 in transport within ciliated structures in the brain and elsewhere.

Subsequently, it was shown that *Chlamydomonas* D1bLIC is in the same complex as DHC1b, that mammalian DYNC2H1 and DYNC2LI1 co-localize in the apical cytoplasm and axonemes of ciliated epithelia in the lung, brain, and efferent duct, and that a *C. elegans* mutant in this dynein light intermediate chain has disrupted retrograde IFT (Perrone et al., 2003; Schafer et al., 2003). A *Chlamydomonas* D1bLIC mutant has variable length flagella that accumulate IFT-particle proteins, indicative of a defect in retrograde IFT (Hou et al., 2004). DHC1b levels are reduced in the mutant, indicating that D1bLIC is needed to stabilize dynein 1b, but the remaining DHC1b is normally distributed in the mutant flagella, strongly suggesting that the defect impairs binding of cargo to the retrograde motor rather than motor activity *per se*. Cell growth and Golgi apparatus morphology are normal in the mutant, indicating that D1bLIC is involved mainly in retrograde IFT. D1bLIC has a phosphate-binding domain (P-loop) at its N-terminus, as do the mammalian light-intermediate chains. To investigate the function of this conserved domain, *d1blic* mutant *Chlamydomonas* cells were transformed with constructs designed to express D1bLIC proteins with mutated P-loops. The constructs rescued the mutant cells to a wild-type phenotype, indicating that the function of D1bLIC in IFT is independent of its P-loop.

As is the case for the IFT dynein heavy chain, a mouse homozygote null mutation of DYNC2LI1 is embryonic lethal (Rana et al., 2004), and the first detectable defect in mouse embryos lacking DYNC2LI1 is a failure of cilium formation in the node. Thus, a dynein light-intermediate chain is an essential, evolutionarily conserved component of IFT dynein.

Intermediate chains

So far two intermediate chains have been identified for cytoplasmic dynein 1b/2. FAP133 (now termed D1bIC2, encoded by *DIC5*) was first shown in *Chlamydomonas* to be a subunit of cytoplasmic dynein 1b. FAP is an acronym for “flagellar associated protein” applied to novel, previously uncharacterized proteins found in the *Chlamydomonas* flagellar proteome (Pazour et al., 2005). This protein is homologous to dynein 1 intermediate chains and was co-purified with retrograde IFT dynein subunits from flagella (Rompolas et al., 2007). A *Chlamydomonas dic5* mutant has flagellar assembly defects and accumulates IFT-particle proteins in its flagella, which is typical for retrograde IFT defects (Hou et al., unpublished). WDR34, which is the human homologue of FAP133, was identified as a TAK1- interacting protein in yeast two-hybrid screens (Gao et al., 2009). Overexpression and knockdown analysis of WDR34 suggested that WDR34 is a TAK1-associated inhibitor of the IL-1R/TLR3/TLR4-induced NF-κB activation pathway. This and later findings led to the recent proposal that the cilium is a locality for regulation of the molecular events defining NFκB signalling events, tuning signalling as appropriate (Wann et al., 2014). Knockdown of the zebrafish WDR34 homologue confirmed the involvement of this protein in ciliary assembly (Krock et al., 2009). This study showed that zebrafish in which DYNC2H1, DYNC2LI1, or WDR34 homologues were knocked down by morpholino oligonucleotides had similar phenotypes, with small eyes, kidney cysts, and in some cases ventral curvature of the body axis. These phenotypes are generally associated with defects in ciliary assembly or function and are consistent with dynein 2’s role in retrograde IFT. Primary cilia in fibroblasts from human patients with WDR34 mutations were significantly shorter than normal and had a bulbous tip (Huber et al., 2013). Knockdown of WDR34 in hTERT-RPE1 cells also showed

that this protein is required for ciliary assembly (Asante et al., 2013). Highly effective depletion of the Golgi protein giantin or WDR34 led to an inability of cells to form primary cilia, whereas partial depletion of giantin or of WDR34 led to an increase in ciliary length. Since the typical pericentrosomal accumulation of WDR34 is lost on suppression of giantin, it was proposed that giantin acts through dynein 2 to regulate cilia assembly. In human hTERT-RPE1 cells, GFP-tagged WDR34 was found localized at the base of the cilium with known components of the basal body (γ- tubulin, ODF2 and OFD1) and within the cilia (Asante et al., 2014); this distribution is typical for proteins associated with IFT dynein. However, a 70–80% reduction in WDR34 in a murine chondrocyte precursor cell line was not sufficient to cause any cilia assembly or length defects (Schmidts et al., 2013).

The sequence of a second dynein intermediate chain (D1bIC1/FAP163, encoded by *DIC6*) is closely related to that of D1bIC2 (Patel-King et al., 2013). Biochemical analysis showed that it is in the same complex as D1bIC2 and LC8 and is transported by IFT in *Chlamydomonas*. In addition, when the D1bIC1 homologue was depleted by RNAi in planaria, cilia were present as short stubs in which large quantities of material resembling IFT particles had accumulated between the doublet microtubules and the membrane, supporting the idea that this protein is a dynein intermediate chain that is absolutely required for retrograde IFT and ciliary assembly. WDR60, the human homologue of D1bIC1, localizes at the base of the primary cilium in wild-type cultured human chondrocytes. McInerney-Leo et al. (2013) examined fibroblasts from individuals with mutations in WDR60 and found that the percent of ciliated cells was drastically reduced compared to control fibroblasts. Knockdown of WDR60 in hTERT-RPE1 cells also resulted in a significant reduction in the proportion of ciliated cells, with a clear increase in length of the remaining cilia. As was observed for WDR34, suppression of giantin resulted in mislocalization of WDR60 (Asante et al., 2014). It is noteworthy that siRNA-mediated suppression of dynein 2 subunits in human cells typically has resulted in the assembly of either fewer cilia or longer cilia (Palmer et al., 2011; Asante et al., 2013; Asante et al., 2014). Asante et al. (2014) interpreted these results as a threshold effect: partial loss of function compromises retrograde IFT and results in longer cilia (as anterograde IFT continues to deliver axonemal and other components to the tip), while more effective depletion results in an inability of cells to form cilia, with limited or no extension of the axoneme. However, longer cilia typically have not been observed on cells of patients with cytoplasmic dynein 2 mutations (Merrill et al., 2009; Huber et al., 2013; McInerney-Leo et al., 2013; Schmidts, Hou et al., submitted). It is possible that the human mutations that have been characterized all cause a greater loss of dynein function than would result in longer cilia, or that this difference reflects differences between the two systems.

Light chains

Like cytoplasmic dynein 1, cytoplasmic dynein 1b/2 contains multiple light chains. Dynein light chains can be placed into one of three major groups: the DYNLL/LC8 group, the DYNLT/Tctex group, and the DYNLRB/LC7 group. LC8 was originally identified and cloned as a light chain of *Chlamydomonas* outer arm axonemal dynein (Piperno and Luck, 1979; Pfister et al., 1982; King and Patel-King, 1995). It was later shown to be associated with many different complexes, including cytoplasmic dynein 1 (King et al., 1996; Dick et

al, 1996), the inner arm dynein I1 (Harrison et al., 1998), and myosin V (Espindola, et al., 2000). A *Chlamydomonas* LC8 null mutant has short, immotile flagella with deficiencies in radial spokes, in inner and outer arms, and in the beak-like projections in the lumens of the B tubules of the outer doublet microtubules (Pazour et al., 1998). Most dramatically, the space between the doublet microtubules and the flagellar membrane contains an unusual accumulation of electron-dense material. Western blotting showed that these flagella accumulate IFT-particle proteins. DIC microscopy revealed that anterograde IFT is normal but retrograde IFT is absent. It was concluded that LC8 was a component of the retrograde IFT motor, the other subunits of which were unknown at that time (Pazour et al., 1998). The other structural abnormalities in the mutant axoneme may reflect a direct requirement for LC8 in the assembly of the affected components, or in the transport of the components to the flagellum or within the flagellar shaft. In protein-interacting networks, LC8 is a hub protein with diverse interacting partners; it is believed that it functions to promote dimerization of its otherwise monomeric partners (Rapali et al., 2011; Barbar and Nyarko, 2014).

The mouse mutant *Dynll1*$^{GT/GT}$, which is functionally null for DYNLL1 (mammalian homologue of LC8) exhibits embryonic lethality with exencephaly, abnormal retinal epithelium distribution, and very small, mispatterned lungs (Goggolidou et al., 2014). *Dynll1*$^{GT/GT}$ embryonic cilia showed shortening and bulging, a phenotype similar to that of the *Chlamydomonas* LC8 mutant and generally associated with defects in retrograde IFT. Goggolidou et al. (2014) also found that DYNLL1 and DYNLL2 interact with WDR34 and proposed that the protein ATMIN promotes ciliogenesis by regulating *Dynll1* expression.

The first hint that light chains of the other two groups might be associated with IFT dynein came from analysis of candidate dynein genes predicted to be present in the *C. elegans* genome (Hao et al., 2011) and from analysis of DYNLT1 in mammals (Li et al., 2011; Palmer et al., 2011). Hao et al showed that *C. elegans* homologues of Tctex1/DYNLT1, Tctex2b/TCTEX1D2, and LC7b/DYNLRB1 move bidirectionally along the worm's sensory cilia at the anterograde and retrograde rates characteristic of IFT in this system, suggesting that they are components of IFT dynein in the worm. However, available mutants of two of these genes (*dylt-1* and *dylt-2*, encoding homologues of DYNLT1 and TCTEX1D2, respectively) apparently have no defects in sensory cilia. This study did not report if the mutations affected the rate of retrograde IFT, and the mutants were not examined for IFT-dynein stability. Li et al. found that although DYNLT1 suppression did not affect cilia assembly or ciliary length in human hTERTRPE1 cells, serum-induced disassembly of the cilium was significantly blocked, suggesting that Tctex-1 is not required for ciliogenesis, but is critical for cilium disassembly. Palmer et al. found that siRNA knockdown of DYNLT1 in human hTERT-RPE1 cells resulted in longer cilia, a result similar to that discussed above for the cytoplasmic dynein 2 heavy chain and intermediate chains. In addition, depletion of DYNC2H1 caused loss of DYNLT1. These data suggested that DYNLT1 most likely functions as part of cytoplasmic dynein 2.

In an effort to further define the subunit composition of the human cytoplasmic dynein 2, Asante et al. (2014) tagged WDR34 in hTERT-RPE1 cells with GFP and characterized WDR34's interactome by immunoprecipitation and mass spectrometry. Their study confirmed that WDR34 and WDR60 are dynein 2 subunits and further suggested that

DYNLL1 and DYNLL2, DYNLT1 and DYNLT3, DYNLRB1 and DYNLRB2, and TCTEX1D2 are associated with dynein 2. Although the ability of the cells to form primary cilia was not obviously affected following TCTEX1D2 depletion, there was a statistically significant increase in ciliary length.

In *Chlamydomonas*, Tctex1, Tctex2b, and LC7b are specifically pulled down by HA-tagged D1bIC2 (Schmidts, Hou et al., under review). In a *Chlamydomonas tctex2b* null mutant, retrograde IFT is dramatically reduced, and although Tctex2b is not required for flagellar assembly and maintenance, cells lacking Tctex2b assemble flagella more slowly than wild-type cells. This probably reflects a reduced level of dynein 1b, as the dynein is unstable in the absence of Tctex2b and the normal amount of dynein 1b in the flagellum is reduced.

Loss of TCTEX1D2 in human patient skin fibroblasts did not affect cilium length (Schmidts, Hou et al., under review). However, there was marked accumulation of an IFT-particle protein in approximately 35% of the ciliary tips of TCTEX1D2-deficient fibroblasts, compared to <10% of control fibroblasts, consistent with a defect in retrograde IFT. Affinity purification of SF-TAP-tagged TCTEX1D2 from HEK293T cells pulled down WDR34 and WDR60 and the dynein light chains DYNLT1, DYNLT3, and DYNLRB1. Taken together, these data indicate that Tctex2b/TCTEX1D2 is a conserved subunit of retrograde IFT dynein. Functional data are still required to confirm the involvement of DYNLT1, DYNLT3, DYNLRB1, and DYNLRB2 in retrograde IFT.

IFT dynein interacting proteins

It is known that cytoplasmic dynein 1 associates with multiple accessory proteins (Allan, 2011). Evidence that IFT dynein also has accessory proteins is emerging. Asante et al. (2014) reported that NudCD3 associates with dynein 2 as it does with dynein 1. In contrast, the common dynein 1 regulators dynactin, LIS1, and BICD2 were not found in association with dynein 2.

Two dyneins or no dynein needed to build cilia

Interestingly, Hao et al. (2011) found that a novel dynein heavy chain (DHC-3) predicted by the *C. elegans* genome was expressed specifically in outer labial quadrant ciliated neurons (OLQ-type neurons) among all the ciliated sensory neurons. Mutation of CHE3, the *C. elegans* homologue of DHC1b/DYNC2H1, affects development and structure of the sensory cilia on most of the worm's neurons but has no obvious effect on the OLQ neurons, raising the possibility that DHC-3 might substitute for CHE-3 in these neurons. Two dynein light chains, DYRB-1 (an LC7b/DYNLRB1 homologue) and DYLA-1 (a homologue of p28, a *Chlamydomonas* axonemal inner arm dynein subunit), also are expressed in OLQ neurons and may be associated with DHC-3. A mutation in *dyla-1* caused no noticeable ciliary defects in OLQ cilia, but this is consistent with findings that some dynein light chains are not critical for ciliary assembly (see "Light chains" subsection above).

In *Tetrahymena*, the expression of the *DYH2* (homologue of DHC1b/2 DYNC2H1) and *D2LIC* genes increases during re-ciliation, consistent with their expected roles in IFT (Rajagopalan et al., 2009). However, the targeted elimination of either *DYH2* or *D2LIC*

resulted in only a mild phenotype. Both knockout cell lines assembled motile cilia, but the cilia were more variable in length and less numerous than wild-type controls. Electron microscopy revealed normally shaped cilia with no swelling and no obvious accumulation of material in the distal ciliary tip. Rajagopalan and colleagues concluded that dynein 2 contributes to the regulation of ciliary length but is not critical for ciliogenesis in *Tetrahymena*. When the D2LIC knockout cells were de-ciliated and then allowed to re-ciliate under conditions in which the cells did not divide, they regrew their cilia more slowly than wild-type cells but eventually the average ciliary length reached nearly the average length on wild-type cells. However, the low cilia density persisted and the variability in cilia length increased over time (Asai et al., 2009). These results indicate that *Tetrahymena* ciliogenesis is less dependent on dynein-2 than *Chlamydomonas*, *C. elegans*, or mammals. Further studies are needed to determine if another motor is compensating for the loss of conventional dynein 2 in retrograde IFT, or if retrograde IFT simply is less important in *Tetrahymena* than in *Chlamydomonas*, *C. elegans*, and mammals.

Consistent with the idea that dynein 1b/2 functions primarily, if not solely, as a retrograde IFT motor, dynein 1b/2 is absent from all organisms that do not build cilia at some stage in their life cycle. However, it is also absent from *Plasmodium falciparum*, *Toxoplasma gondii*, and *Thalassiosira pseudonana*, all of which have flagellated gametes (Wickstead and Gull, 2007). *P. falciparum* builds its flagellum in the cytoplasm and thus has no need for IFT to move flagellar precursors to the tip of the assembling flagellum; it also lacks kinesin-2 and IFT-particle proteins. *T. gondii* and *T. pseudonana* possess at least some of the central components of IFT, including the kinesin-2 motor. It is possible that another motor functions in retrograde IFT in these organisms; alternatively gametic flagella may undergo little or no turnover of axonemal and membrane proteins and thus do not need retrograde IFT.

In vitro studies on IFT dynein

Recombinant human DYNC2H1 has been expressed in HEK-293 cells and purified (Ichikawa et al., 2011); the purified heavy chain was associated with DYNC2LI1 but association with any other subunit was not detected. Electron microscopy of the purified molecules revealed a two-headed structure composed of characteristic dynein motor domains. However, the tail domain was not easily visualized. This was in contrast to similarly purified dynein 1, which had a clear tail domain. An in vitro microtubule-gliding assay confirmed that dynein 1b/2 is a minus-end directed motor. However, the microtubule movement was very slow for dynein 2 compared with dynein 1. Further analysis needs to be done to determine if the in vitro structural and motility differences between dynein 1 and dynein 2 are real or caused by loss of other dynein 2 subunits in the purified DYNC2H1/DYNC2LI1 samples. A crystal structure of the DYNC2H1 motor domain with ADP˙vanadate has revealed details of how ATP hydrolysis leads to remodeling of the linker domain and regulation of microtubule affinity (Schmidt et al., 2014).

IFT dynein and human diseases

Many human cells have cilia; as a result, defects in cilia cause a wide range of diseases. Such diseases are called ciliopathies (reviewed in Yuan and Sun, 2013; Brown and Witman, 2014).

The first dynein 2 subunit to be associated with human disease was DYNC2H1. Mutations in DYNC2H1 cause Jeune asphyxiating thoracic dystrophy (JATD) and short rib polydactyly syndrome (SRP) type III, both of which are characterized by short ribs and shortened tubular bones. In one family, SRP individuals were homozygous for an exon 12 missense mutation that predicted the amino acid substitution R587C. Compound heterozygosity for one missense and one null mutation was identified in two additional nonconsanguineous SRP families. Cultured chondrocytes from affected individuals showed morphologically abnormal, shortened cilia. In addition, the chondrocytes had abnormal cytoskeletal microtubule architecture, implicating an altered microtubule network as part of the disease process. These findings established SRP as a ciliopathy and demonstrated that DYNC2H1 is essential for skeletogenesis (Merrill et al., 2009). Studies on a consanguineous family from Morocco also identified homozygous mutations in the *DYNC2H1* gene in siblings with SRP. Compound heterozygosity for *DYNC2H1* mutations was observed in four additional families. Among the five families, children in three were diagnosed with JATD, and in two of the families pregnancies were terminated for SRP type III. These data showed that JATD and SRP type III are variants of a single disorder belonging to the ciliopathy group (Dagoneau et al., 2009). Subsequently, additional DYNC2H1 mutations were identified as a common cause of JATD without major polydactyly, renal, or retinal involvement (Schmidts et al., 2013) and one more case of compound heterozygous mutations in DYNC2H1 was shown to cause the typical SRP III phenotype (Okamoto et al., 2014).

Two groups independently identified mutations in WDR34 as the cause of JATD and SPR diseases (Huber et al., 2013; Schmidts et al., 2013). Huber and colleagues found homozygosity for three missense mutations in *WDR34* in three independent families, as well as compound heterozygosity for mutations in one family. By structural modeling, two of the three mutations were predicted to alter specific structural domains of WDR34. Schmidts and colleagues, using exome sequencing and a targeted next-generation sequencing panel, identified a total of 11 mutations in WDR34 in 9 families with the clinical diagnosis of JATD. Three-dimensional protein modeling suggested that the identified mutations all affect residues critical for WDR34 protein-protein interactions.

WDR34 was identified by cDNA microarray as one of the 30 genes that have the highest difference between urinary bladder carcinoma patients with and without recurrence (Mares, et al., 2013). More work will be necessary to understand the significance of this finding.

WDR60 mutations also cause skeletal ciliopathies (McInerney-Leo et al., 2013). By using whole-exome capture and massive parallel sequencing of DNA from an affected Australian individual with SRP type III, Mclnerney-Leo et al. detected two novel heterozygous mutations in WDR60. These mutations segregated appropriately in the unaffected parents

and another affected family member, confirming compound heterozygosity, and both were predicted to have a damaging effect on the protein. Analysis of an additional 54 skeletal ciliopathy exomes identified compound heterozygous mutations in WDR60 in a Spanish individual with JATD of relatively mild presentation. Interestingly, these two families share one novel WDR60 missense mutation, although haplotype analysis suggested no shared ancestry. In another study, it was found that duplication of the subtelomeric region of chromosome 7q containing functional genes (FAM62B, WDR60, and VIPR2) can be tolerated without phenotypic consequences (Bartsch et al., 2007). Lack of one allele of WDR60 is also asymptomatic (Perche et al., 2013). Genomewide association studies for bipolar disease cases versus controls using single nucleotide polymorphism microarray data implied that WDR60 is one of the associated genes (Xu et al., 2014). WDR60 also was identified as one of the many genes associated with autism spectrum disorders based on exome sequencing (Cukier et al., 2014).

Mutations in TCTEX1D2 also cause skeletal ciliopathy (Schmidts, Hou et al., submitted). The JATD patients all carry biallelic null alleles in TCTEX1D2. The disease phenotype appears to be incompletely penetrant, indicating that TCTEX1D2 plays a less important role in retrograde IFT than do other IFT dynein subunits. Consistent with this, a *Chlamydomonas* mutant null for Tctex2b can still assemble flagella, albeit more slowly than wild type (Schmidts, Hou et al., submitted).

Thus, it is well established that cytoplasmic dynein 2 mutations in humans cause skeletal ciliopathy – specifically JATD and SRP type III. Although human mutations in DYNC2LI1 have not been reported, DYNC2LI1 also should be considered a candidate gene for causing skeletal ciliopathy. The possibility that cytoplasmic dynein 2 is involved in cancer and psychological diseases needs to be further tested.

Future directions

It is likely that most, if not all, of the components of IFT dynein have been identified. However, their specific functions within the complex have not been well studied, although generally the subunits need each other to be stable as shown by *Chlamydomonas* mutants in DHC1b, D1bLIC, FAP133, and Tctex2b (Perrone et al., 2003; Hou et al., 2004; Schmidts, Hou, et al., submitted; Hou et al., manuscript in preparation). Axonemal dyneins undergo a complex assembly process, involving multiple assembly factors, in the cytoplasm before being transported into the flagellum (Fowkes and Mitchell, 1998; Omran et al., 2008; Duquesnoy et al., 2009; Mitchison et al., 2012); it remains to be determined if IFT dynein is dependent on similar cytoplasmic assembly factors. It will be important to determine if this dynein is regulated during IFT. Is it inactivated for anterograde IFT when it is a cargo of kinesin? If so, how is this achieved, and how is it then turned on at the tip of the flagellum when its own motor activity is required for retrograde IFT? There also is still much to be learned about how this dynein interacts with other proteins, including those of the IFT machinery necessary for its import into the flagellum, and the cargos that it exports from the flagellum. It will be of interest to determine the subset of proteins that are transported retrogradely in flagella by dynein 1b/2. It would be naive to think that every protein that is cycled through the flagellum is transported back to the cell body by IFT dynein. For

example, this does not appear to be the case for kinesin-2 in *Chlamydomonas*. In contrast to the IFTparticle proteins, kinesin-2 does not undergo a massive redistribution into *dhc1b* flagella (Pazour et al., 1999), which implies that its exit out of the flagellum is independent of dynein 1b. Moreover, TIRF microscopy of fluorescently tagged KAP failed to detect retrograde tracks, which is in contrast to the clear tracks observed for retrograde IFT-particle proteins (Engel et al., 2009). It also remains to be determined if dynein 1b/2 has a function in the cytoplasm, perhaps related to ciliary assembly. Finally, mutations in IFT dynein in humans cause skeletal ciliopathies, but only rarely are associated with the kidney or retinal disorders caused by defects in other IFT proteins; it will be important to understand why certain tissues are so much more susceptible than others to these mutations.

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Table 1

IFT dynein components

		C. reinhardtii			*C. elegans*	*H. sapiens*	
		Gene	**Protein**	**Alias**	**Protein**	**Protein**	**Alias**
Heavy chain		*DHC16*	DHC1b		CHE-3 DHC-3[#1]	DYNC2H1	ATD3; DHC2; DHC1b; DNCH2; DYH1B; SRTD3; SRPS2B; hdhc11
Light intermediate chain		*DLI1*	D1bLIC		XBX-1	DYNC2LI1	LIC3; D2LIC; CGI-60
Intermediate chain		*DIC5*	D1bIC2	FAP133	Not identified	WDR34	DIC5; FAP133; SRTD11; bA216B9.3
		DIC6	D1bIC1	FAP163	Not identified	WDR60	SRPS6; SRTD8; FAP163
Light chains	DYNLL/LC8 group	*DLL1*	LC8		DLC-2	DYNLL1	LC8; PIN; DLC1; DLC8; LC8a; DNCL1; hdlc1; DNCLC1
						DYNLL2	Dlc2; DNCL1B; RSPH22
	DYNLT/Tctex group	*DLT3*	Tctex1		DYLT-1	DYNLT1	CW-1; TCTEL1; tctex-1
						DYNLT3	RP3; TCTE1L; TCTEX1L
		DLT4	Tctex2b		DYLT-2	TCTEX1D2	
	DYNLRB/LC7 group	*DLR2*	LC7b		DYRB-1[#]	DYNLRB1	BLP; BITH; DNCL2A; DNLC2A; ROBLD1
						DYNLRB2	DNCL2B; DNLC2B; ROBLD2
					DYLA-1[#2]		

[#]Proposed to compose a novel IFT dynein that is required to form the cilia in *C. elegans* OLQ neurons (Hao et al., 2011).

[1]Closest *C. reinhardtii* homolog is axonemal dynein heavy chain 6. However, *C. reinhardtii* axonemal dynein heavy chain 6 is closer to *C. elegans* cytoplasmic dynein 1 (E=0) and CHE-3 (E=5e-159) than to DHC-3 (E=1e-70). Similar results were obtained when blasting CHE-3 against human proteins. This result could be because DHC-3 in the database is a partial sequence.

[2]*C. reinhardtii* homolog is p28, which is an inner arm dynein component and was not co-immunoprecipitaed with D1bIC2-HA (Schmidts, Hou et al., submitted). There is also no evidence that the human homolog, DNALI1, is a dynein 2 component.