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Cytoplasmic dynein-2 at a glance

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ABSTRACT

Cytoplasmic dynein-2 is a motor protein complex that drives the movement of cargoes along microtubules within cilia, facilitating the assembly of these organelles on the surface of nearly all mammalian cells. Dynein-2 is crucial for ciliary function, as evidenced by deleterious mutations in patients with skeletal abnormalities. Long-standing questions include how the dynein-2 complex is assembled, regulated, and switched between active and inactive states. A combination of model organisms, *in vitro* cell biology, live-cell imaging, structural biology and biochemistry has advanced our understanding of the dynein-2 motor. In this Cell Science at a Glance article and the accompanying poster, we discuss the current understanding of dynein-2 and its roles in ciliary assembly and function.

KEY WORDS: Dynein-2, Cilia, Intraflagellar transport, Microtubule motors

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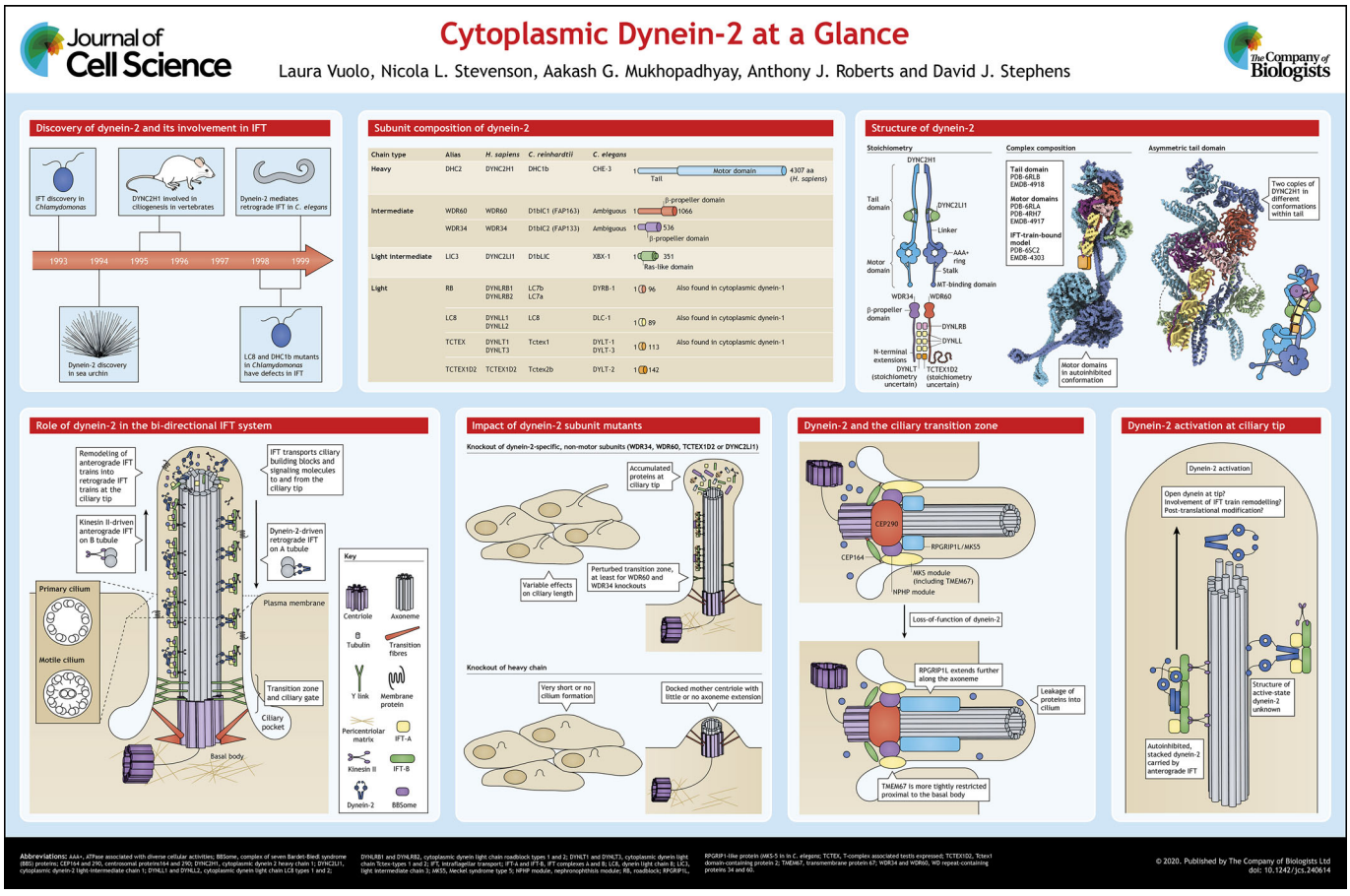
Introduction

Cytoplasmic dynein-2 (hereafter referred to as dynein-2) is an ATP-dependent motor protein that steps along microtubules to transport cargoes within cilia and flagella (Box 1). It is related to cytoplasmic dynein-1 (hereafter referred to as dynein-1), which is involved in the transport of cargos within the cytoplasm, in organelle dynamics (Reck-Peterson et al., 2018) and in mitotic spindle organisation during mitosis (Raaijmakers and Medema, 2014). By contrast, dynein-2 does not act in canonical membrane traffic (Palmer et al., 2009) but functions primarily, if not exclusively, within the intraflagellar transport (IFT) system (Box 2). Here, dynein-2 assembles with kinesin-2, and IFT-A and IFT-B complexes to form polymeric IFT 'trains', which move cargoes to the ciliary tip (mediated kinesin-2) and back to the cell



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Box 1. Primary and motile cilia

Cilia are microtubule-based structures, with an axoneme based on nine cylindrically arranged microtubule (MT) doublets. Primary – also known as sensory – cilia are solitary structures on the cell surface and function as 'antenna' that transduce signals from the extracellular environment. Motile cilia are present on specialised cell types and function to drive the movement of fluids in multiciliated epithelia in vertebrates, the locomotion of sperm as well as the motility of many unicellular organisms. In addition to the nine MT doublets, motile cilia usually feature an additional central pair of MTs in the axoneme lumen (Mirvis et al., 2018). Axonemal dyneins generate the force to bend the axoneme in motile cilia (King and Sale, 2018). In all cilia and flagella, each MT doublet consists of A and B tubules, with the A tubule formed by 13 protofilaments and the B tubule formed by ten protofilaments. Although motile cilia typically present a 9+2 structure along the length of the axoneme, the structure of primary cilia is more variable. Recent electron tomography data indicate that, in the primary cilium of several kidney cell lines, two of the MT doublets progressively shift towards the core of the axoneme at the region where the primary cilium starts to extend into the extracellular space, forming a 7+2 arrangement (Sun et al., 2019). The structure of cilia includes a series of evolutionarily conserved subdomains that are each defined by a specific cohort of proteins. The cilium extends from the basal body, formed by the mother centriole together with subdistal and distal appendages proteins. Transition fibres connect the basal body to the plasma membrane. Distal to the basal body is the transition zone, characterised by membrane-associated Y-shaped links. Transition fibres and the transition zone compartment form a permeability barrier called the 'ciliary gate' that regulate the composition of ciliary proteins (Jensen and Leroux, 2017; see poster).

Box 2. The bi-directional intraflagellar transport system

Intraflagellar transport (IFT) was first described in *Chlamydomonas reinhardtii*, where large particles moving in both directions along the length of the flagella were observed by using differential interference contrast (DIC) microscopy (Kozminski et al., 1993). Subsequently, using time-lapse imaging of specifically-labelled proteins, IFT has been described in many model systems, including *Caenorhabditis elegans* (Orozco et al., 1999), *Tetrahymena thermophila* (Brown et al., 1999), *Trypanosoma brucei* (Absalon et al., 2008) and vertebrate cells (Follit et al., 2006; Pazour et al., 2002, 2000). IFT-trafficking complexes called 'trains' comprise IFT-A and IFT-B subcomplexes that mediate the interactions between the ciliary motors and cargo (see poster). The IFT-B complex is generally associated with anterograde trafficking; it is formed of a core subcomplex of ten subunits (IFT88, -81, -74, -70, –56, 52, -46, -27, -25 and -22), a peripheral complex of six subunits (IFT172, -80, -57, -54, -38 and -20), and associates with the small GTPase RabL2 (Kanie et al., 2017). IFT-A, which is generally required for retrograde transport as well as the ciliary import of a variety of membrane proteins, includes IFT144, -140, -139, -122, -121 and -43 (Taschner and Lorentzen, 2016), and associates with the cargo adapter TULP3 (Mukhopadhyay 2010). A further complex, the BBSome – a complex of seven Bardet–Biedl syndrome (BBS) proteins – associates with IFT trains to stabilise their assembly (Wei et al., 2012), and mediates retrograde membrane protein trafficking (Nachury and Mick, 2019). In *Chlamydomonas*, anterograde and retrograde IFT trains have been defined to move on B and A tubules of the axonemal microtubule doublets, respectively (Stepanek and Pigino, 2016). Although there are several common features of IFT between model organisms, there are also key differences. In *Chlamydomonas*, kinesin-2 appears to mainly diffuse back to the ciliary base (Engel et al., 2012), whereas, in metazoans, kinesin-2 motors appear to be recycled to the ciliary base predominantly through retrograde IFT (Mijalkovic et al., 2017; Signor et al., 1999; Vuolo et al., 2018; Williams et al., 2014). Interestingly, in *C. elegans*, an additional dynein heavy chain, i.e. DHC-3, has been implicated in the formation of a subset of cilia, and DHC-3 was identified – together with the dynein-2 heavy chain – in genetic screens for anti-helminth resistance (Page, 2018). The deposited protein sequence for DHC-3 suggests it to be a highly divergent dynein heavy chain that lacks ATP-binding sites is, thus, unlikely to function as a conventional motor.

body (mediated dynein-2). Dynein-2-driven transport occurs in the confined space between the ciliary microtubule doublets and the ciliary membrane (Roberts, 2018). There is some evidence for dynein-2 functioning outside of cilia. In *Chlamydomonas*, for example, which lacks dynein-1, dynein-2 is implicated in cytoplasmic trafficking to the base of cilia (Cao et al., 2015).

Dynein-1 and dynein-2 are distantly related to their axonemal cousins (Kollmar, 2016; Wickstead and Gull, 2007) that drive the beating of motile cilia and flagella (Box 1). Below, and in the accompanying poster, we provide an overview of dynein-2 discovery, subunit composition, structure and regulation. We also discuss new insights into the functions of dynein-2 in maintaining the ciliary transition zone – the gatekeeper between the cilium and the cytoplasm (Box 1) – as well as the connection between dynein-2 and human disease.

Discovery of dynein-2 and its role in IFT

Dynein-2 was first identified in sea urchin (Gibbons et al., 1994) and rat (Tanaka et al., 1995), based on sequence similarity to dynein-1 in mammals. It was described as a cytoplasmic dynein and shown to be upregulated prior to ciliogenesis in sea urchin embryos (Gibbons et al., 1994) and mammalian cells (Criswell et al., 1996). Retrograde IFT was first linked to a cytoplasmic dynein motor in *Chlamydomonas* (Pazour et al., 1998). Further work revealed that mutations of dynein-2 resulted in cells with short flagella that accumulated IFT proteins at their tip (Pazour et al., 1998, 1999b; Porter et al., 1999), and also perturbed retrograde transport of kinesin-2 in *C. elegans* (Signor et al., 1999).

Structure and composition of dynein-2

Dynein-2 is a large multiprotein complex, composed of 16 copies of at least eight different proteins in humans (see poster). Insights into dynein-2 subunit composition have come from a variety of cell biology, genetic and biochemical studies (see below), and a recent cryo-electron microscopy (EM) structure of the dynein-2 complex (Toropova et al., 2019). Like other dyneins, the subunits of dynein-2 are classified as heavy, intermediate, light-intermediate and light chains depending on their mass. Most subunits in the dynein-2 complex are unique to dynein-2 but a subset of the light chains are also found in dynein-1 (Asante et al., 2014). Naming of dynein-2 subunits varies (see poster) and, here, we use the human nomenclature unless specified.

Dynein-2 is built around two copies of cytoplasmic dynein 2 heavy chain 1 (DYNC2H1) (Criswell et al., 1996; Mikami et al., 2002). The C-terminal region forms the motor domain, which converts the energy from ATP hydrolysis into movement (Schmidt et al., 2015). Its N-terminal region forms the tail, an extended structure that binds the other subunits (Hamada et al., 2018) and holds the two heavy chains in a homodimer (Toropova et al., 2017, 2019). In an interesting variation compared to other organisms, trypanosomatids possess two distinct dynein-2 heavy chains that form a heterodimer (Adhiambo et al., 2005; Blisnick et al., 2014).

The cytoplasmic dynein-2 light-intermediate chain 1 (DYNC2LI1) (Grissom et al., 2002; Hao et al., 2011; Hou et al., 2004; Li et al., 2015; Mikami et al., 2002), binds directly to the tail of each heavy chain and is important for stabilising its structure (Hou et al., 2004; Reck et al., 2016; Toropova et al., 2017). The light-intermediate chain has a Ras-like fold and appears to bind to nucleotides (Schroeder

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et al., 2014; Toropova et al., 2019). Although nucleotide-binding by the light-intermediate chain does not seem essential for dynein-2 function (Hou et al., 2004), whether it serves a structural role or has a minor regulatory function remains unclear.

The other dynein-2 subunits – namely, the intermediate and light chains – form a stoichiometrically unusual subcomplex at the core of the tail of dynein-2, which makes its structure highly asymmetric (Toropova et al., 2019). While dynein-1 is composed of homodimeric subunits, including its intermediate chain, dynein-2 differs notably in that it contains two different intermediate chains. Originally defined as FAP133 (Rompolas et al., 2007) and FAP163 (Patel-King et al., 2013) in *Chlamydomonas*, these subunits have been validated as bona fide mammalian dynein-2 subunits named WD repeat-containing protein 34 (WDR34) (Asante et al., 2013, 2014; Huber et al., 2013; Schmidts et al., 2013b) and WD repeat-containing protein 60 (WDR60) (Asante et al., 2014; McInerney-Leo et al., 2013).

WDR34 and WDR60 form a heterodimer (Asante et al., 2014; Hamada et al., 2018; Toropova et al., 2019; Vuolo et al., 2018) (see poster). Their C-terminal β-propeller domains each bind a copy of the heavy chain, and their extended N-terminal regions are held together by an array of light chain dimers (Toropova et al., 2019). These comprise one dimer of dynein light chain roadblock (DYNLRB) proteins that binds proximal to the β-propellers, followed by three dynein light chain LC8 (DYNLL) dimers, and a putative heterodimer between a dynein light-chain Tctex-type protein (DYNLT) and Tctex1 domain-containing protein 2 (TCTEX1D2) (Asante et al., 2014; Hamada et al., 2018; Kanie et al., 2017; Toropova et al., 2019; Tsurumi et al., 2019). Coexpression studies indicate that WDR34 preferentially interacts with DYNLL and DYNLRB, whereas WDR60 preferentially interacts with the DYNLT–TCTEX1D2 dimer (Hamada et al., 2018). Among the light chains, TCTEX1D2 is specific to dynein-2 (Asante et al., 2014; Gholkar et al., 2015; Schmidts et al., 2015). The other light chains (DYNLRB, DYNLL, and DYNLT) are also found in dynein-1 (Asante et al., 2014), and each has two orthologs in mammals (e.g. DYNLRB1 and DYNLRB2). The orthologs appear to play interchangeable roles (Hamada et al., 2018) but may have subtly different biochemical properties or generate tissue-specific expression patterns (King et al., 1998). In summary, the unusual stoichiometry of dynein-2's intermediate and light chains is a distinctive feature of the complex; as described below, it has important roles in dynein-2 motility regulation and attachment to IFT trains.

Regulation and motility

Dynein-2 motility is tightly regulated to enable its functions in IFT. The dynein-2 motor domain contains a ring of six ATPases associated with diverse cellular activities (AAA+) modules, of which the N-proximal module (AAA1) is the main ATPase site (Schmidt et al., 2015). N-terminal to AAA1 is a rod-like 'linker' domain that amplifies conformational changes. The microtubule-binding domain of dynein-2 is at the tip of a coiled-coil stalk (see poster).

The current generally accepted model is that dynein-2 is transported passively from the ciliary base to tip by kinesin-2 (Hao et al., 2011; Rosenbaum and Witman, 2002). Following activation, it then actively transports the IFT machinery and cargoes from tip to base during retrograde IFT. The motile properties of the human dynein-2 motor domain have been recently described from *in vitro* assays (Toropova et al., 2017). Interestingly, monomeric constructs moved significantly faster (~500 nm/s) than dimers, as the motor domains in the dimer stack against one another to give rise to an autoinhibited conformation (Toropova et al., 2017, 2019). Accordingly, disruption of the stacking interface induced a significant increase in velocity. These results suggested that the dynein-2 motor domains intrinsically exist in an autoinhibited stacked conformation that facilitates transport of dynein-2 to the ciliary tip by kinesin-2 (Toropova et al., 2017). Supporting this model, motility assays in which both kinesin-2 and dynein-2 were used showed that the velocity of kinesin-2 was only minimally affected by inactive dynein-2, whereas an unstacked active dynein-2 mutant conferred resistance against kinesin-2 (Toropova et al., 2017). *In vivo* support for dynein-2 autoinhibition came from an analysis of IFT trains using cryo-electron tomography in *Chlamydomonas* (Jordan et al., 2018). In this study, the anterograde trains were observed as densely packed and ordered structures composed of three repeats with approximate lengths of 6, 11 and 18 nm, which were assigned to IFT-B, IFT-A and dynein-2, respectively. Notably, dynein-2 appeared in a stacked, autoinhibited, conformation when interacting with anterograde trains, with its stalks oriented away from the microtubule.

Recent cryo-EM and cryo-electron tomography studies shed light on how the subunits of dynein-2 enable it to associate with anterograde IFT trains to travel to the ciliary tip. In particular, the dynein-2 subcomplex of intermediate and light chains has at least two important roles. First, it brings two copies of the heavy chain together into a stable dimer with autoinhibited motors domains (Toropova et al., 2019). This is likely to be a suitable state for loading onto anterograde trains at the ciliary base (Wingfield et al., 2017). Second, the intermediate and light chains contort the two copies of the heavy chain into different conformations within the tail (Toropova et al., 2019). This asymmetric architecture is tailored to the repeating structure of the anterograde IFT-B train: each dynein-2 complex spreads out over seven to eight IFT-B repeats, and is tightly packed with the neighbouring dynein-2 complexes along the train (Jordan et al., 2018; Toropova et al., 2019). An important question for future studies is to determine which subunits of the IFT-B complex interact with dynein-2 on the anterograde train. However, molecular genetic studies have implicated IFT172 as being important for dynein-2 targeting or turnaround at the ciliary tip (Pedersen et al., 2005; Tsao and Gorovsky, 2008; Williamson et al., 2012).

The mechanism by which dynein-2 is repositioned to bind to the axoneme and switched to an active conformation at the tip remains one of the most intriguing questions in the field. Biochemical and genetic studies suggest that classic dynein-1 accessory factors, such as dynactin (Reck-Peterson et al., 2018), are not involved in dynein-2 regulation (Asante et al., 2014; Roberts, 2018). One possibility is that IFT-A and IFT-B themselves regulate dynein-2 activity, and that the rearrangement of these large complexes during train disassembly and reassembly facilitates a conformational switch within dynein-2 to form an active complex at the ciliary tip (Yi et al., 2017). Because the intermediate and light chains stabilise the autoinhibited conformation of dynein-2, they must either rearrange or dissociate to activate the motor at the ciliary tip (Pazour et al., 2000; Toropova et al., 2019). Post-translational modifications of dynein-2 of the IFT subunits might have a role in dynein-2 activation but these are not yet well described. It is also possible that other, thus far unknown, regulators are involved in this process.

Ciliogenesis and cilia function in dynein-2 mutants

Mutants of the dynein-2 heavy chain in many model organisms, including *Chlamydomonas*, *C. elegans*, mouse and zebrafish, as well as cultured mammalian cells, present similar phenotypes with short cilia and bulbous ciliary tips (Adhiambo et al., 2005; May et al., 2005; Pazour et al., 1999a; Porter et al., 1999; Wicks et al.,

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2000). In both mouse (Wu et al., 2017) and cultured human cells (Vuolo et al., 2018), loss of WDR34 is associated with severe ciliogenesis defects. Other studies, however, have shown that ciliogenesis is only moderately impaired in WDR34 knockout (KO) cells (Tsurumi et al., 2019). By contrast, WDR60 is required for correct retrograde trafficking but dispensable for extending the ciliary axoneme in cultured human cells (Asante et al., 2014; Hamada et al., 2018; Vuolo et al., 2018). Moreover, fibroblasts from affected individuals carrying mutations in WDR60 still extend the ciliary axoneme but the percentage of ciliated cells is variable (McInerney-Leo et al., 2013). Similar phenotypes showing normal cilia length and a moderate reduction in cilia number were observed in TCTEX1D2-mutant fibroblasts from individuals affected by short rib–polydactyly syndromes (SRPSs) (Schmidts et al., 2015) or in TCTEX1D2 KO cells (Hamada et al., 2018).

Although defects in DYNC2LI1 do not completely abolish cilia extension, its mutation is associated with a ciliary accumulation of IFT proteins and defects in the regulation of cilia length, as observed in fibroblasts of human patients (Kessler et al., 2015; Taylor et al., 2015). Moreover, DYNC2LI1 appears to play a crucial role in the stability of the dynein-2 complex in *Chlamydomonas* (Hou et al., 2004; Reck et al., 2016). These variations in phenotype could result from low-level expression or, in some cases of genome engineering, expression of truncated proteins, leading to retention of partial function. Furthermore, loss of one subunit may affect the overall stability of the complex as seen for WDR34 and WDR60 KOs. This outcome has also been clearly described for mice lacking the transcription factor ATM interactor (ATMIN), as they have a severely reduced expression of the dynein light chain LC8 type 1 (DYNLL1), which results in partial depletion of other dynein-2 subunits (King et al., 2019). Overall, full dynein-2 function does not appear to be absolutely required for ciliogenesis per se but is needed to maintain the overall structure, including length control, and for core ciliary signalling functions.

Dynein-2 and the ciliary transition zone

Recently, new insights into IFT trafficking revealed an unexpected role for IFT-A and dynein-2 in maintaining compartmentalisation of the transition zone and, thus, of the ciliary structure in *C. elegans* and human cells (Jensen et al., 2018; Vuolo et al., 2018; Scheidel and Blacque, 2018). The transition zone consists of a densely packed domain containing multiple proteins that are assembled in a tightly regulated process (see Box 1 and poster). The hierarchy of transition zone assembly has been extensively described for several organisms and presents some common features in different models (reviewed by Gonçalves and Pelletier, 2017). Super-resolution imaging and EM have resolved a map that defines the localisation of distinct modules of the transition zone (see poster). Centrosomal protein of 290 kDa (CEP290) lies at the core of the base of the transition zone and facilitates the assembly of other transition zone components (Yang et al., 2015). The RPGRIP1-like protein (RPGRIP1L; known in *C. elegans* as MKS-5) is a core component of transition zones in *C. elegans* and vertebrates (Li et al., 2016; Wiegering et al., 2018). It localises distally to CEP290 and adjacent to microtubules within the transition zone. The nephronophthisis (NPHP) module links the CEP290 core to the Meckel syndrome (MKS) module that includes Meckel syndrome type 1 protein (MKS1), tectonic-1 and tectonic-2 (TCTN1 and TCTN2, respectively) as well as several membrane proteins, including transmembrane protein 67 (TMEM67) (Awata et al., 2014; Dean et al., 2016; Goncalves and Pelletier, 2017; Schouteden et al., 2015; Wang et al., 2013). This organisation has also been supported by proteomic mapping of the base of the cilium (Gupta et al., 2015). The transition zone links the axonemal microtubules to the ciliary membrane and acts to gate entry and exit of proteins and lipids to the cilium. As such, it serves a vital function in the compartmentalisation of ciliary signalling.

Recent data showed that dynein-2 is important to maintain the structure and integrity of the transition zone. Loss of the dynein-2 intermediate chains WDR34 and WDR60 disrupts the composition of the transition zone in cultured human cells (Jensen et al., 2018; Vuolo et al., 2018), and a temperature-sensitive mutant showed that dynein-2 is required for transition zone assembly and gating function in *C. elegans* (Jensen et al., 2018). In particular, the studies in human cells showed a distal extension of the RPGRIP1L domain of the transition zone and a reduction of the TMEM67 area, whereas other transition zone components, such as TCTN1 and CEP290, were not affected (see poster). Interestingly, KO of WDR34 or WDR60 is also associated with mislocalisation of several ciliary membrane proteins and IFT components, suggesting a defect in the entry and/or export mechanism that is regulated by the transition zone (Vuolo et al., 2018). Consistent with these data, the temperature-sensitive mutation in the dynein-2 heavy chain resulted in a defective transition zone composition in *C. elegans* (Jensen et al., 2018). In these worms, at the restrictive temperature, some transition zone components, such as nephrocystin-4 (NPHP-4), centrosomal protein of 290 kDa (CEP290) and MKS-6 (known as CC2D2A in mammals), notably mislocalised to a more distal region of the cilium. Furthermore, disruption of the transition zone resulted in the ectopic localisation of two different basal body proteins, i.e. translocating chain-associated membrane protein 1 (TRAM-1) and human retinitis pigmentosa-2 ortholog RPI-2 in the ciliary axoneme (Jensen et al., 2018), suggesting a defect in the ‘ciliary gate’ formed by the transition zone. Interestingly, correct transition zone organisation was restored at permissive temperature, indicating that maintenance of transition zone integrity is an active process that requires dynein-2.

It is uncertain how dynein-2 mediates transition zone assembly but it might involve its association with the IFT-A cargo adapter complex (Scheidel and Blacque, 2018). In *C. elegans*, analysis of IFT-A mutants indicated that IFT-A subunits play different roles in cilia entry and/or export of transition zone components in the cilia. According to this model, core subunits of IFT-A (i.e. IFT140) promote entry of transition zone proteins into cilia, whereas its non-core subunits (i.e. IFT121, IFT139, IFT43) regulate ciliary export. Consistent with observations in dynein-2 KO cells (Vuolo et al., 2018), the key transition zone component RPGRIPL1 is mislocalised in IFT-A mutants. Although the cilia from both IFT-A and dynein-2 mutants show a mislocalisation of several transition zone proteins, no major defects are observed in the overall architecture of the transition zone, as determined by using EM (Jensen et al., 2018). High-resolution views of the structure and dynamics of the components of the transition zone may help to elucidate its gating function and dependence on IFT-A and dynein-2.

Human diseases associated with defects in dynein-2 function

Defects in cilia formation and function lead to human pathologies that, collectively, are termed ciliopathies (Reiter and Leroux, 2017). Mutations in dynein-2 are associated with a group of ciliopathies called ‘skeletal ciliopathies’ that are described as short-rib thoracic dysplasia (SRTD) with or without polydactyly (Huber and Cormier-Daire, 2012). The phenotypes related to skeletal ciliopathies include craniofacial abnormalities, short stature, shortened ribs, brachydactyly

and polydactyly. The skeletal phenotype can appear in association with abnormalities in other organs, with retinal and kidney abnormalities as the most-common ones observed outside the skeletal system (Huber and Cormier-Daire, 2012). The skeletal abnormalities observed in some SRTD patients are most likely to be related to defects in signalling pathways during embryonic development, including hedgehog (Hh) signalling, that requires cilia (Huangfu et al., 2003). In this context, cilia are particularly important to ensure correct Hh signalling during bone formation, and defects in dynein-2 result in the mislocalisation of Smoothened, a key component of Hh signalling, to cilia (May et al., 2005; Tsurumi et al., 2019; Vuolo et al., 2018; Wu et al., 2017). In recent years, whole exome-sequencing has enabled the identification of new mutations involved in skeletal ciliopathies, with the most common mutations affecting DYNC2H1 (Badiner et al., 2017; Cossu et al., 2016; Dagoneau et al., 2009; Merrill et al., 2009; Schmidts et al., 2013a). Moreover, mutations in WDR34 (Huber et al., 2013; Schmidts et al., 2013b), WDR60 (Cossu et al., 2016; McInerney-Leo et al., 2013), DYNC2LI1 (Kessler et al., 2015; Taylor et al., 2015) and TCTEX1D2 (Gholkar et al., 2015; Schmidts et al., 2015) have also been associated with SRTD, and a conditional knockout of DYNLL1 in mouse limb mesoderm resulted in bone shortening, similar to that observed in SRTD patients (King et al., 2019). A comprehensive review of dynein-2 genes associated with skeletal ciliopathies has been recently published (Schmidts and Mitchison, 2018).

Conclusions

Although we know much about the composition of the dynein-2 motor, its interactions and now even have a structure of the dynein-2 complex, there is still much to be determined. A question for both mechanistic and clinical studies is how defects in dynein-2 relate to anterograde and retrograde trafficking. The tight co-assembly of dynein-2 with IFT-B trains defines the crucial position of dynein-2 in anterograde IFT trains (Jordan et al., 2018; Toropova et al., 2019). Understanding the role of dynein-2 in maintaining a functional cilium and coordinating different signalling pathways, notably Hh, is likely to help us to understand the contributions of dynein-2 and cilia in skeletogenesis. Open questions include how, at the atomic level, dynein-2 co-assembles with IFT complexes at the ciliary base, and how its entry into the cilium is gated. It is also unclear what triggers the disassembly of anterograde kinesin-2-driven IFT trains at the ciliary tip, how retrograde trains – driven by active dynein-2 – are formed, or why dynein-2 is used to actively transport kinesin-2 to the ciliary base in vertebrate cilia (Broekhuis et al., 2014; Williams et al., 2014), when diffusion appears to be sufficient in *Chlamydomonas* (Chien et al., 2017; Engel et al., 2012).

Intensive and integrated efforts that combine biochemistry, structural biology, clinical genetics, cell and developmental biology are required to address these challenges, giving an opportunity to fully understand the mechanism and functions of dynein-2 in cilia biology and to apply this knowledge to improve human health.

Competing interests

The authors declare no competing or financial interests.

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A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.240614.supplemental

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