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REVIEW

Cytoplasmic dynein-2: from molecules to human diseases

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Abstract **The dynein motor protein family is involved in a wide variety of functions in eukaryotic cells. The axonemal dynein class and cytoplasmic dynein-1 subclass have been well characterized. However, the cytoplasmic dynein-2 subclass of the family has only recently begun to be understood. We describe the entire dynein family but focus on cytoplasmic dynein-2. Dynein-2 consists of a heavy, an intermediate, a light intermediate, and a light chain. The complex appears to function primarily as the retrograde motor for intraflagellar transport. This process is important for the formation and maintenance of cilia and flagella. Additionally, dynein-2 has roles in the control of ciliary length and in non-ciliary functions. Mutations in the human dynein-2 heavy chain lead to cilia-related diseases.**

Keywords cilia, dynein, flagella, intraflagellar transport, microtubule

Introduction

Dyneins are minus-end directed microtubule motors that are involved in a variety of activities in eukaryotic cells. Each dynein is a complex of multiple subunits including heavy chains containing the motor domains. Smaller subunits include intermediate, light intermediate, and light chains that are involved in regulation and targeting of the complexes (Sakato and King, 2004; Pfister et al., 2006). Dyneins comprise a family well conserved across eukaryotic species. The family can be divided into two classes: (1) axonemal dyneins and (2) non-axonemal, or cytoplasmic, dyneins.We will review the entire dynein family but focus on one specific cytoplasmic dynein, the dynein-2 complex. Dynein-2 has been characterized far less than the other dyneins but recent results have added greatly to our understanding of this motor.

Axonemal dyneins

Dynein was first isolated as an ATPase containing component from the cilia of *Tetrahymena* (Gibbons, 1963; Gibbons and Rowe, 1965). Subsequently several different dyneins were found to exist as parts of the axoneme (i.e., the cilium without the cell membrane). These axonemal dyneins are the molecular motors that power the movement of cilia and eukaryotic flagella (Porter, 1996). The axonemal dyneins include two subclasses: (1) outer arm dyneins and (2) inner arm dyneins. Each subclass is anchored to the outer doublet A tubules and produces shear forces between the adjacent outer doublet microtubules. Change in shear as a function of position along the axoneme produces ciliary/flagellar bending (Brokaw, 1994). The outer arm dyneins are composed of two or three heavy chains depending on the organism. In metazoans the outer arm is two-headed, corresponding to the α and β heavy chains, while in protozoans (e.g., *Tetrahymena*, *Chlamydomonas)* the outer arm is a three-headed structure comprising α, β, and γ heavy chains. Identical outer arms are located along the entire length of an axoneme with a 24 nm spacing. Outer arms are required for establishing the high beat frequencies of flagella (Gibbons and Gibbons, 1973, 1976; Brokaw and Kamiya, 1987). Inner arm dyneins are more diverse than outer arm dyneins. Along the length of an axoneme one- and two-headed inner arm dyneins are organized in 96 nm repeating units in which each unit contains one each of an I1, I2, and I3 sub-region (Dutcher, 1995). The I1 region contains a two-headed dynein while the I2 and I3 regions each have two one-headed dyneins. The inner arms are involved in regulating the amplitude of axonemal bending (Brokaw and Kamiya, 1987).

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Cytoplasmic dynein-1

About 20 years after the discovery of axonemal dyneins, a non-axonemal component was shown to have dynein characteristics (Paschal et al., 1987). Two subclasses of these non-axonemal or cytoplasmic dyneins exist (Vale, 2003; Pfister et al., 2006). Dynein-1 is found in all eukaryotes except higher plants and is the only dynein present in species without cilia or flagella. Dynein-2 is found only in organisms that have cilia or eukaryotic flagella. Dynein-1 is a homodimer of two identical heavy chains (DYH1) and several smaller accessory proteins (Fig. 1; King et al., 2002; Pfister et al., 2006). These accessory proteins include two molecules each of an intermediate chain (often referred to as IC74) and a light intermediate chain (D1LIC). In addition, the dynein-1 complex contains three types of light chains: (1) LC7/roadblock, (2) LC8, and (3) Tctex1/rp3.

Unlike the axonemal dyneins that all move the same cargo, sliding one outer doublet microtubule with respect to another, cytoplasmic dynein-1 performs many distinct tasks. DYH1 is involved in axonal retrograde transport (Schnapp and Reese, 1989; Hirokawa et al., 1990), the transport of endosomal and Golgi-destined vesicles (Burkhardt et al., 1997; Harada et al., 1998; Itin et al., 1999), nuclear migration in multiple species (Xiang et al., 1994; Bruno et al., 1996; Fridolfsson and Starr, 2010), nuclear envelope breakdown (Salina et al., 2002), and the formation, positioning, and function of the mitotic spindle (Echeverri et al., 1996; Lee et al., 1999; Ma et al., 1999). In *Dictyostelium*, dynein-1 is important for microtubule (Koonce and Samsó, 1996) and Golgi (Ma et al., 1999) organization. In neuronal cells, dynein-1 is required for cell migration (Willemsen et al., 2012) and the proper organization of both microtubules and organelles (Zheng et al., 2008). Dynein-1 is essential for the asymmetric localization of mRNA in *Drosophila* embroyos (Wilkie and Davis, 2001). Organization of the endoplasmic reticulum in mammalian cells is dependent on cytoplasmic dynein-1 (Woźniak et al., 2009).

Cytoplasmic dynein-2

Dynein-2 heavy chain sequence

The first suggestion that there was a second "cytoplasmic" dynein came from sequencing results of sea urchin dynein heavy chain (DYH) genes (Gibbons et al., 1994). The sequences near the catalytic ATP binding portions of the motor domains of most of the heavy chain sequences were found to be similar to the axonemal outer arm β heavy chain, and so were deduced to be axonemal. The sequence of the dynein-2 heavy chain, originally called DYH1b, is more similar to the cytoplasmic dynein-1 sequence. Although the motor domains of the dynein heavy chains are highly conserved, early sequencing results demonstrated that there were differences that could be used to distinguish between axonemal and cytoplasmic sequences (Asai and Brokaw, 1993; Asai et al., 1994). In addition, the two cytoplasmic dynein subclasses can be defined by a sequence located nearby. All dynein heavy chains across all species have an absolutely conserved Walker A motif that is required for the hydrolysis of ATP (Fig. 2). Five residues downstream of the Walker A motif all cytoplasmic DYHs have an alanine while the axonemal DYHs have an aspartic acid at this position. The two cytoplasmic DYHs can be distinguished by the sequence approximately 100 amino acids downstream of the Walker A motif (FVTMNPGY in the *Tetrahymena* DYH1 sequence; Fig. 2). All cytoplasmic dynein-2 heavy chains contain an insertion of AGK within this region. Thus, with knowledge of

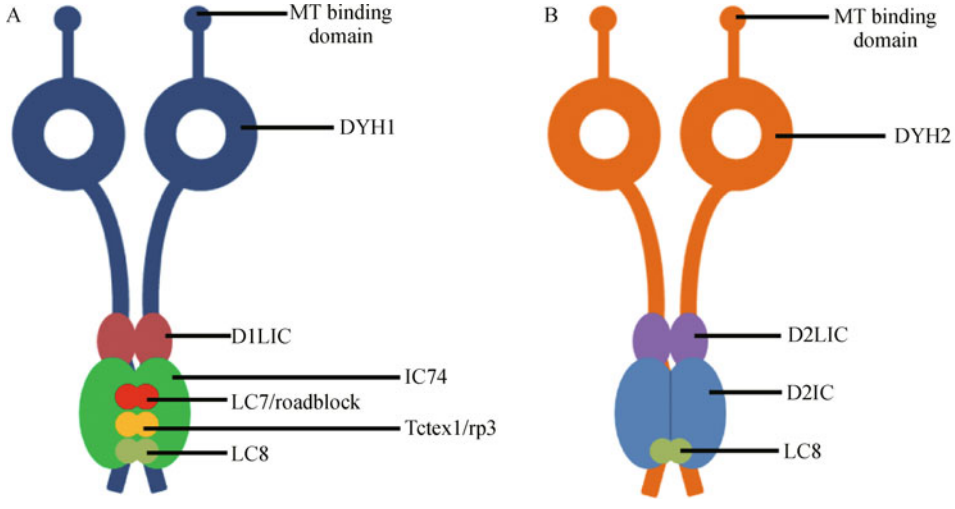

Figure 1 Organization of the cytoplasmic dynein complexes. (A) Dynein-1 is composed of a homodimer of the DYH1 heavy chain (blue) that contains the motor activity. The smaller accessory subunits include two copies each of an intermediate chain (IC74), light intermediate chain (D1LIC), and three different light chains (LC7/roadblock, LC8, and Tctex1/rp3). (B) Dynein-2 consists of a homodimer of the DYH2 heavy chain (orange) and two copies each of an intermediate chain (D2IC), light intermediate chain (D2LIC), and light chain (LC8).

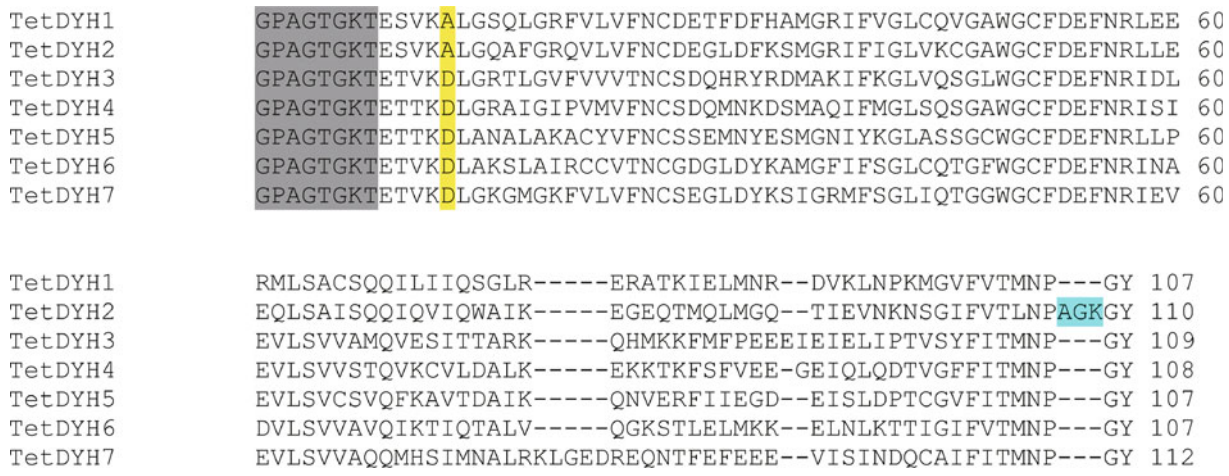

Figure 2 Dynein heavy chain sequence motifs define classes and subclasses of the family. Sequences near the ATP binding site of seven of the *Tetrahymena* dynein heavy chains are aligned. The Walker A motif is absolutely conserved (gray box). Cytoplasmic (DYH1and DYH2) heavy chains are distinguished from axonemal (DYHs3-7) heavy chains by an alanine instead of an aspartic acid located five residues (yellow) from the end of the Walker A motif. Dynein-2 heavy chain sequences across species contain an AGK (blue) insertion approximately 100 residues from the Walker A motif.

just a small region of a dynein heavy chain sequence a gene can be defined as an axonemal dynein, cytoplasmic dynein-1, or cytoplasmic dynein-2.

The dynein-2 complex

Overall the dynein-2 complex is similar to that of dynein-1 (Fig. 1). Including the heavy chain, the dynein-2 complex consists of four different components. The heavy chain (DYH2) appears to form a homodimer within the complex. This organization was first suggested by sedimentation assays (Mikami et al., 2002) and was supported recently by electron microscopic images of the purified complex from human cells (Ichikawa et al., 2011). The EM images of the dynein-2 heavy chains appeared similar to those of dynein-1 with pairs of circular motor domains. However, the tails of the two heavy chains appeared to differ significantly. Dynein-1 complexes had relatively large and complex-looking tail domains. In contrast, the tail domain of human dynein-2 appeared very simple and was difficult to discern in the EM images. This difference may be due to the dynein-2 complex containing fewer small accessory proteins than the dynein-1 complex and to some of the accessory proteins being absent from the preparation.

The first of the smaller accessory proteins found in the dynein-2 complex was a light intermediate chain, D2LIC, of mammalian cells (Grissom et al., 2002). D2LIC was later shown to be a component of the dynein-2 complex in *Chlamydomonas* (Perrone et al., 2003; Hou et al., 2004) and rat (Mikami et al., 2002). This subunit associates with DYH2 in both sedimentation and immunoprecipitation assays (Grissom et al., 2002; Mikami et al., 2002; Perrone et al., 2003; Hou et al.,2004). A D2LIC gene is present in all genomes examined that also have DYH2. Several years later two more subunits were characterized as components of the dynein-2 complex. From *Chlamydomonas* extracts, an intermediate chain, FAP133, and a light chain, LC8, were found to co-sediment and co-immunoprecipitate with the dynein-2 heavy chain (Rompolas et al., 2007). FAP133 and LC8 readily dissociate from the complex; thus great care was required during the fractionation. It appears that both D2LIC and FAP133 (also referred to as D2IC) bind directly to the heavy chain. LC8 binds to the intermediate chain within the complex. The discovery of LC8 as a subunit of dynein-2 explained previously confusing results from a *Chlamydomonas* mutant. Almost a decade earlier, Pazour et al. (1998) had shown that LC8 was required for retrograde intraflagellar transport in *Chlamydomonas* (see below). This movement is powered by the dynein-2 motor. However, LC8 was never found as a component of dynein-2 until the study by Rompolas et al. (2007). The current model for the dynein-2 complex consists of homodimers of the heavy chain, intermediate chain, light intermediate chain and light chain. Dynein-1 differs in this basic organization only in having three types of light chain.

Dynein-2 functions

A ciliary function for dynein-2?

The same investigation by Gibbons et al. (1994) that examined the sequences of the heavy chains also provided the first evidence for the function of dynein-2. RNA isolated from sea urchin embryos before deciliation and after one and two rounds of deciliation was used to determine the expression levels of each of the dynein heavy chain genes. Consistent with its role outside of the axoneme, the expression of cytoplasmic dynein-1 heavy chain (called DYH1a by Gibbons et al.) did not respond to deciliation. As

expected, expression of the axonemal outer arm β heavy chain was shown to increase after each deciliation. The dynein genes with sequences similar to the β sequence were also induced during reciliation, suggesting that their gene products are axonemal dyneins. Surprisingly, dynein-2 heavy chain expression was also induced during reciliation despite the similarity of its sequence to that of DYH1a. Gibbons et al. (1994) proposed an explanation that eventually proved correct: "One possibility is that DYH1b encodes a different cytoplasmic dynein that plays a role in the transport of materials required for ciliary regeneration…" Further support for a role for dynein-2 in cilia came from a study on rat trachea epithelial cells (Criswell et al., 1996). Without stimulation these cells do not possess cilia; however, they can be induced to differentiate into a ciliated mucociliary epithelium. DYH2 expression was low in unstimulated cells but increased with either introduction of an air-liquid interface or the removal of growth factors (both treatments stimulate ciliogenesis). In immunofluorescence assays, the DYH2 protein localized to the apical end of the epithelial cells at the base of the cilia. These initial studies with sea urchins and rat epithelial cells laid the groundwork for what appears to be a well conserved function for dynein-2 in cilia.

Retrograde motor for intraflagellar transport

Studies mainly in *Chlamydomonas* and *Caenorhabditis elegans* led to the current view of the role of dynein-2 in intraflagellar transport (IFT; Fig. 3). IFT is the process by which cilia and flagella are built and maintained (Rosenbaum and Witman, 2002; Scholey, 2003; Blacque et al., 2008). All of the hundreds of different components that comprise an axoneme are synthesized in the cell body. These components are then incorporated at the distal tip of the axoneme where all of the microtubule (+) ends are located. Anterograde IFT, powered by the (+)-end-directed microtubule motor kinesin-II, carries materials from the cell body along the outer doublet microtubules to the tip. Dynein-2 is the motor for retrograde IFT and brings the anterograde machinery back to the base of the cilium so that it can be reused for further ciliary growth.

Functional disruptions of the DYH2 (Pazour et al., 1999; Porter et al., 1999) and LC8 (Pazour et al., 1998) genes in *Chlamydomonas* provided the textbook phenotype for defects in retrograde IFT. Disruption of either dynein-2 component resulted in short immotile flagella filled with large amounts of electron dense material between the outer doublet microtubules and the flagellar membrane. In addition, microtubules, radial spokes, and other axonemal components are disorganized. The accumulation of excess material is due to fully functional anterograde IFT continuing to carry components into the flagella but no retrograde transport of the IFT machinery back to the cell body.

Although *C. elegans* does not have motile cilia it does have several types of sensory cilia. DYH2 mutants do not show retrograde movement within either amphid or phasmid sensory cilia when observed by time-lapse microscopy (Signor et al., 1999). Additionally, components of kinesin-II and IFT trains accumulate at the distal tips of the cilia. In a separate study of *C. elegans* DYH2 mutants, the sensory cilia were malformed and shorter than normal (Wicks et al., 2000). These worms also showed defects in chemotaxis due to non-functional sensory cilia. Mutations of the D2LIC homolog, xbx-1, in *C. elegans* result in phenotypes similar to those seen for heavy chain mutations. These mutations result in short sensory cilia with bulb-like tips (Schafer et al., 2003; Bae et al., 2008). In addition, these mutations cause defects in behavioral phenotypes such as dye filling, osmotic avoidance, and mating.

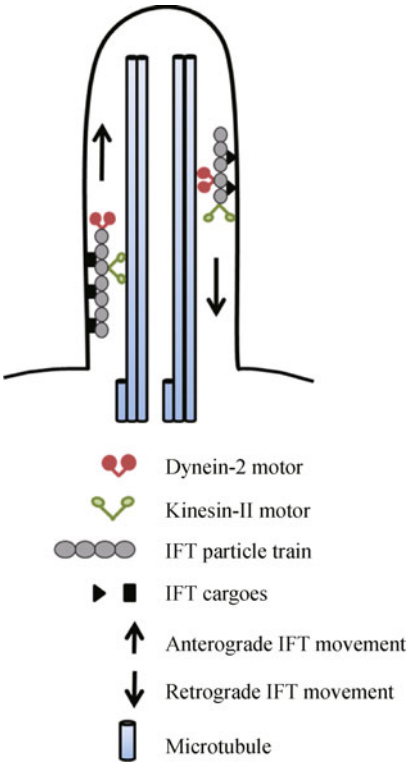

Figure 3 Basic model of intraflagellar transport. Ciliary component proteins are synthesized in the cell body. These cargoes are attached to IFT particle trains at the base of the cilium and are transported to the growing tip, their site of incorporation. Kinesin-II drives anterograde movement along the outer doublet microtubules. Inactive dynein-2 is also carried as a cargo by the kinesin-II motor in the anterograde direction. Dynein-2 carries the inactive kinesin-II and turnover products in the retrograde direction back to the cell body to be recycled.

Chlamydomonas and *C. elegans* were key organisms in defining the function of dynein-2 in the process of IFT. Disruptions of dynein-2 components in other organisms have yielded similar results. The parasite *Leishmania* expresses two genes coding for dynein-2 heavy chains. Disruption of one of the genes resulted in immotile cells lacking flagella (Adhiambo et al., 2005). This phenotype was more severe than that seen in *Chlamydomonas* mutants that do form short

stumpy flagella. The other *Leishmania* DYH2 was essential and mutant cell lines could not be analyzed. In *Drosophila* the dynein-2 heavy chain is coded for by the *btv* gene. Mutants in *btv* resulted in abnormal accumulation of vacuoles in the sensory cilia of Johnston's organ (Eberl et al., 2000). IFT particles also showed incorrect localization in *Drosophila btv* mutants supporting a role for dynein-2 in IFT movement (Lee et al., 2008). Krock et al. (2009) investigated the role of dynein-2 in zebrafish by morpholino knockdown of the heavy chain, intermediate chain, or light intermediate chain. The zebrafish morphants showed developmental defects in eyes and kidneys most likely caused by ciliary abnormalities. Both pronephros and nasal cilia were short and swollen in the zebrafish morphants. Dynein-2 knockout mice also have developmental defects probably due to abnormal cilia. D2LIC knockout mice had left-right asymmetry and neural tube closure defects (Rana et al., 2004). Also in these mice the cells on the ventral node had either short or missing cilia. Knockout of mouse DYH2 resulted in polydactyly and abnormal brain and heart loop morphology (Huangfu and Anderson, 2005). The nodal cilia of these mice were shorter than normal with bulges near their tips. Mouse neural tube primary cilia also become swollen in DYH2 knockout mice (Ocbina et al., 2011). Disruption of DYH2 in mammalian tissue culture cells affects cilia. A DYH2 mutation in mouse embryonic fibroblasts resulted in short cilia (Ocbina and Anderson, 2008). Disruption of DYH2 by shRNA in NIH 3T3 cells led to excess accumulation of the hedgehog pathway proteins, Smo and Gli-2, in primary cilia (Kim et al., 2009). Thus, in a wide variety of species dynein-2 appears to be involved in retrograde movement within cilia.

Control of ciliary length

Although a role for dynein-2 as the retrograde IFT motor appears conserved, dynein-2 may have a more complicated function than simply recycling components back to the cell body. One of the first exceptions to the model for dynein-2 being required for ciliogenesis came from DYH2 knockdown in *Tetrahymena* (Lee et al., 1999).In these cells no wild type copies of the DYH2 gene were detected but cells still possessed cilia that appeared morphologically normal. The knockdown cells were also able to regenerate cilia after deciliation. Follow up studies have further defined the role of dynein-2 in *Tetrahymena*. Rajagopalan et al. (2009) created *Tetrahymena* knockout cell lines of either the DYH2 or D2LIC. Both cell lines behaved similarly. In the mutants cilia were shorter on average than for wild type control cells. This result is consistent with the phenotypes seen for other organisms such as *Chlamydomonas* and *C. elegans*. However, the *Tetrahymena* mutant cilia did not accumulate excess electron dense material and, surprisingly, many cilia were much longer than normal. The *Tetrahymena* D2LIC knockouts can regrow cilia after deciliation but they reciliate at a slower rate than normal (Asai et al., 2009). D2LIC mutants in *Chlamydomonas* also show a range of flagellar lengths including near full length (Hou et al., 2004). Knockdown of DYH2 expression by siRNA in human RPE1 cells resulted in primary cilia that were much longer than seen in control cells (Palmer et al., 2011). Thus, several studies suggest that dynein-2 plays an active role in the regulation of ciliary length.

Human ciliopathies

Defects in IFT components can cause an array of human diseases such as primary ciliary dyskinesia, Bardet-Biedl syndrome, retinitis pigmentosa, and polycystic kidney disease (Hildebrandt et al., 2011; Ferkol and Leigh, 2012). Three recent studies have identified a direct correlation between the occurrence of the skeletal developmental disorders short rib polydactyly type III (SRP III) and Jeune asphyxiating thoracic dystrophy (ATD) to mutations in the human dynein-2 heavy chain gene. SRP III is characterized by extreme shortening of limbs, severely narrowed thorax, polydactyly, and renal dysplasia. ATD symptoms include narrow thorax, renal fibrocystic disease, dwarfism, retinitis pigmentosa, and polydactyly. Dagoneau et al. (2009) studied a large family with two children affected with ATD. They found two homozygous missense mutations in the conserved motordomain of the DYH2 gene. Examination of four other families identified 3 premature stop codons and 7 missense heterozygous mutations in the DYH2 gene of other individuals with either ATD or SRP III. In a similar study, Merrill et al. (2009) also found a connection between DYH2 defects and SRP III. Affected individuals in a consanguineous family had a homozygous missense mutation in the dynein-2 heavy chain gene coding for the heavy chain tail. The cilia on primary chondrocytes fromone of the affected individuals were shorter than normal (affected = 1.95 ± 0.44 μm vs. control = 3.25 ± 0.57 μm). These cilia also had bulbous distal ends indicating a possible accumulation of IFT material as seen with dynein-2 heavy chain mutations in *Chlamydomonas* and *C. elegans* (see above). Merrill et al. (2009) also identified compound heterozygous mutations in the DYH2 gene in two non-consanguineous SRP III families. These mutations included both missense and nonsense varieties. Thiel et al. (2011) suggest a digenic diallelic inheritance of SRP Majewski type in a non-consanguineous family with heterozygous mutations in both the Nim-A related kinase (NEK1) and DYH2 genes. The NEK1 gene in this individual had a heterozygous insertion producing a premature stop codon and a heterozygous mutation in the DYH2 gene which affected the splice-acceptor site in exon 82. The importance of dynein-2 in human health is clear.

Non-ciliary functions

The first evidence that dynein-2 is involved in processes that are not related to cilia came from investigations of mammalian tissue culture cells. The dynein-2 heavy chain

co-localized with Golgi apparatus markers in NRK, COS-7, HeLa, 3T3, and BHK cells (Vaisberg et al., 1996). This co-localization remained even after fragmentation of the Golgi by pharmacological treatment. Vaisberg et al. (1996) micro-injected anti-dynein-2 heavy chain antibodies into NRK cells to disrupt the function of the heavy chain. This disruption resulted in an increase in the number of cells with the Golgi dispersed into fragments. A similar study by Grissom et al. (2002) showed that D2LIC co-localized with both the dynein-2 heavy chain and Golgi compartments in COS-7 cells. Microinjection of anti-D2LIC antibodies into these cells did not result in Golgi dispersal. Knockdown of DYH2 or D2LIC expression in HeLa cells by siRNA did not cause any abnormal phenotypes in Golgi or endosomal trafficking (Palmer et al., 2009). In *Chlamydomonas* dynein-2 heavy chain mutants the Golgi appeared normal (Porter et al., 1999). However, the *Chlamydomonas* mutants did have fewer and shorter than normal cytoplasmic microtubule arrays. In a screen for genes involved in lipid storage, Söhle et al. (2012) found that knockdown of DYH2 in human (pre)adipocytes increased lipid accumulation. Secondary screening with new siRNA sequences and new cell lines verified the results. Although cytoplasmic dynein-1 is known to be the motor for transport of lipid droplets toward the (–) ends of microtubules (Gross et al., 2000), no direct connection between dynein-2 and lipid droplet movement has been shown. Thus, a number of studies have demonstrated that cytoplasmic dynein-2 is important for non-ciliary functions, but a conserved role is not clear.

Dynein-2 motor activity

Although much is known about the mechanism of dynein-1 motility (Burgess et al., 2003; Nishiura et al., 2004; Reck-Peterson et al., 2006), only recently has the movement of dynein-2 been demonstrated. Ichikawa et al. (2011) completed the first study showing the *in vitro* motility of dynein-2. They expressed tagged versions of human dynein-1 and dynein-2 heavy chains in human embryonic kidney 293 cells and purified each complex separately. The purified dynein-1 complex consisted of recombinant dynein-1 heavy chains associated with endogenous dynein-1 intermediate and light intermediate chains. The purified dynein-2 complex consisted of dynein-2 heavy chains associated with endogenous dynein-2 light intermediate chains.

For the *in vitro* gliding assay of the dynein-1 complex 97.2% of the microtubules were observed to glide across the surface, with an average speed of 905 nm/s. The speeds observed were much slower for dynein-2 than for dynein-1, with an average of 69.6 nm/s. The dynein-2 speeds were also much slower than those observed for IFT retrograde movement of approximately 1 μm/s (Signor et al., 1999). However, the presence of dynein-2 intermediate chain could not be confirmed. It is possible that the missing intermediate chains could at least partially account for the significantly slower speeds observed in dynein-2 versus dynein-1 in the *in vitro* sliding assay. In addition, only 43.3% of the microtubules were observed to glide across the surface with the dynein-2 complex. The *in vitro* motility assay was also performed using polarity-marked microtubules, which moved with their (–) ends trailing demonstrating that the dynein-2 complex is a minus-end directed motor as is true for all previously characterized dyneins.

Summary

Although cytoplasmic dynein-2 was discovered less than 20 years ago, we are beginning to build an understanding of what its roles are and how it functions in these roles. Dynein-2 is a complex of at least four different subunits and is a (–) end directed microtubule motor. The complex functions primarily as the retrograde motor for IFT but may perform other roles. Its importance to human health is clear with the recent characterization of inherited dynein-2 heavy chain mutations that result in ciliopathies. Future prospects include determination if additional components are present in the dynein-2 complex, how the complex is regulated, and defining non-ciliary functions.

Abbreviations

DYH, dynein heavy chain; IFT, intraflagellar transport.

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