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Asymmetries in the cilia of *Chlamydomonas*

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The generation of ciliary waveforms requires the spatial and temporal regulation of dyneins. This review catalogues many of the asymmetric structures and proteins in the cilia of *Chlamydomonas*, a unicellular alga with two cilia that are used for motility in liquid medium. These asymmetries, which have been identified through mutant analysis, cryo-EM tomography and proteomics, provide a wealth of information to use for modelling how waveforms are generated and propagated.

This article is part of the Theo Murphy meeting issue 'Unity and diversity of cilia in locomotion and transport'.

1. Introduction

Cilia and flagella are often described as microtubule-based structures with nine-fold symmetry. In this review, a discussion of the structural and biochemical asymmetries in *Chlamydomonas* cilia and their potential roles will be presented. It is likely that similar asymmetries are present in motile cilia in other organisms. Cilia have nine doublet microtubules (DMTs) that surround two singlet microtubules. These organelles and their associated structures are able to generate both asymmetric and symmetric waveforms that move single cells or move fluid in multi-ciliated tissues.

Motile cilia are highly conserved organelles composed of over 500 proteins that assemble into the 9 + 2 structure, which is the canonical structure associated with these organelles [1–3] (figure 1*a*). The nine outer DMTs each consist of one complete microtubule, the A-tubule, with 13 protofilaments, and an incomplete microtubule, the B-tubule, with 10 protofilaments and an inner junction filament composed of two proteins, FAP20/BUG22 and Parkin coregulated gene protein (PACRG) [4–6]. Axonemal complexes attach to the outer DMT to form the structural feature of the axoneme, termed the 96 nm repeat. The 96 nm repeat contains multiple structures; they are the four outer dynein arms, two complete radial spokes (S1 and S2) and a third vestigial spoke (S3), the Modifier of Inner Arms (MIA) complex, the nexin–dynein regulatory complex (N-DRC), the calmodulin- and spoke-associated complex (CSC) and multiple inner dynein arms [7–10]. A molecular ruler composed of CCDC39 and CCDC40 is important for the docking of several structures [11]. Mutations in *CCDC39* or *CCDC40* result in the loss of the N-DRC and misplacement of the radial spokes [11,12]. Additional filaments are observed on the surface of the A-tubule using the hybrid Tygress method, which achieves 12 Å resolution [13]. These filaments may provide additional rulers for other complexes. In the centre of the cylinder of nine outer doublets is the central pair complex (CPC), composed of singlet microtubules, C1 and C2, and their associated appendages. These microtubules are more similar in their structure and stability to the cytoplasmic microtubules.

Chlamydomonas cilia exhibit two different waveforms. One is termed asymmetric and is characterized by a whip-like, or breaststroke, motion. The whip-like movement can be broken into two phases. The power stroke begins with the cilium fully extended. Then, the cilium moves through the liquid to lie next to the cell body. The recovery stroke involves an unfolding of the cilium to return to the fully extended starting position (figure 2*a*). This waveform is often referred to as the ciliary waveform as it is used by the lateral cilia of the clam gill

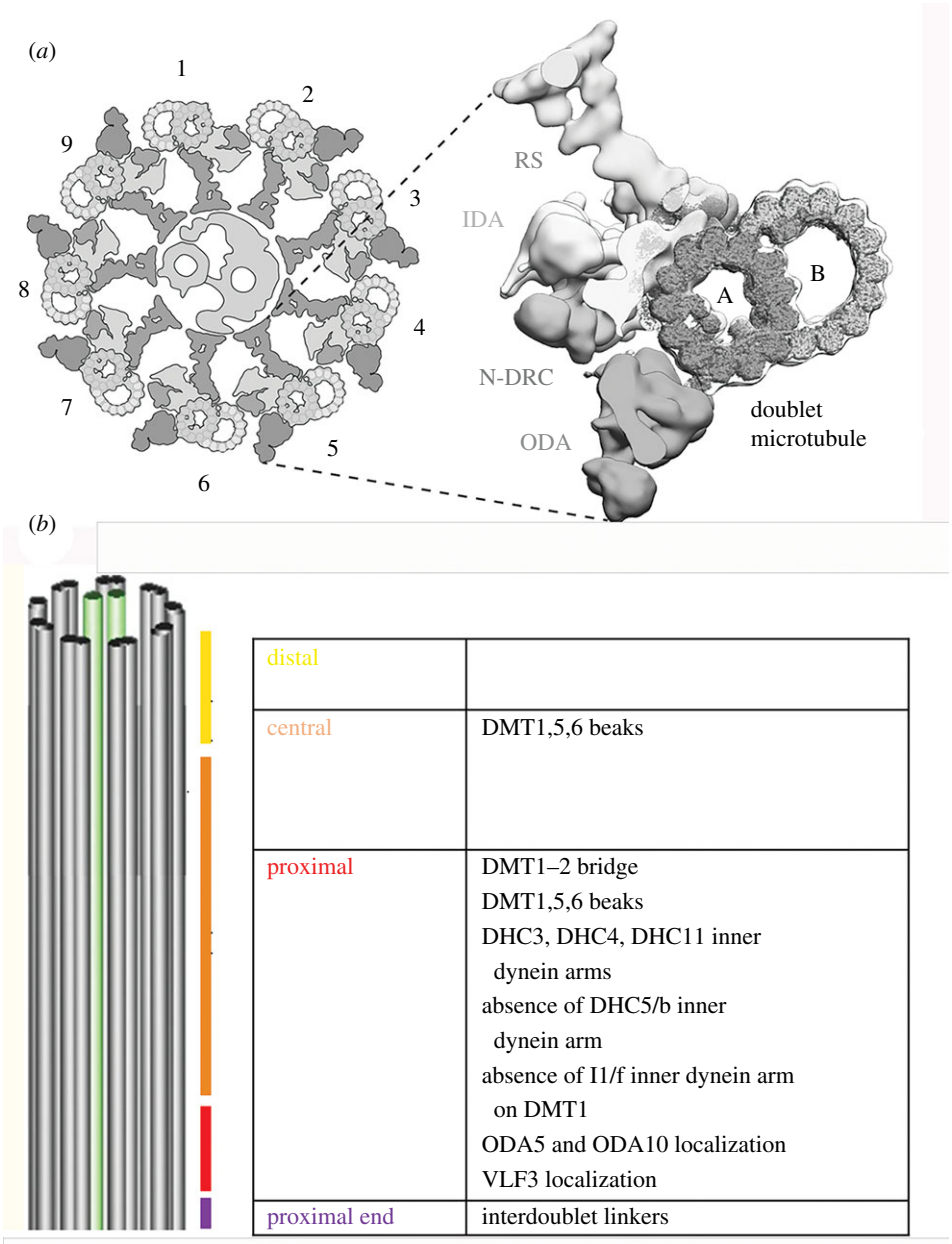

Figure 1. Diagrams of the *Chlamydomonas* cilium. (*a*) Cross-sectional view of the cilium with one doublet microtubule enlarged to show a radial spoke (RS), an inner dynein arm (IDA), the N-DRC and an outer dynein arms (ODAs) as well as the A and B microtubules of the doublet microtubule. The DMTs are labelled 1–9. On doublet microtubule 1 (DMT1), there is no outer arm dynein. The central pair microtubules reside in the middle. (*b*) A longitudinal view to see the four regions and the unique features of these regions along the length the *Chlamydomonas* axoneme.

or mammalian respiratory cilia. The waveform results in the *Chlamydomonas* cell moving forward with the cilia leading the way. In a wild-type *Chlamydomonas* cell, the beat frequency is generally about 60 Hz and the swimming velocity is about 150 µm s^{-1}. A sinusoidal, or flagellar waveform as it is often called, is used by both vertebrate and invertebrate sperm tails. The waveform is initiated at the base of the cilium and propagated along the cilium to the tip, resulting in the backward motion of a *Chlamydomonas* cell with the cilia trailing (figure 2*b*).

Ciliary movement is generated by the controlled sliding of adjacent outer DMTs in the axoneme. The dynein motors use the energy derived from adenosine triphosphate hydrolysis to generate the sliding movement, which is converted into bending to create the waveform. If all of the ciliary dyneins were active at one time, the cilia would be in a rigor state or tug-of-war, which results in no net movement or bending. In order to generate an effective bend, dynein motor function must be controlled both along the length of the axoneme and around the circumference of the axoneme across a defined axis. In elegant cryo electron microscopy (cryo-EM) tomography studies, Lin & Nicastro unexpectedly found that most dyneins are in an active state conformation, with a smaller population of inactive dyneins [14]. The locations of the inactive dyneins are asymmetric and bend-dependent. They propose a switch-inhibition mechanism in which the bend is generated by inhibiting, rather than activating, dyneins on one side of the cilium. The data suggest that the initiation of a bend starts with the inhibition of the inner dynein arms (a, d and g) on specific DMTs. Events on DMT 2 to 4 are needed for initiating the asymmetric waveform and events on DMT 7–9 are needed for initiating the symmetrical waveform. Once enough dyneins are inhibited, the dyneins on the other site help to start a bend. This bend is propagated until an unknown signal reactivates these dyneins and the axoneme straightens. In paralysed mutants (*pf*), all the dyneins are active and in a tug-of-war with each other [14].

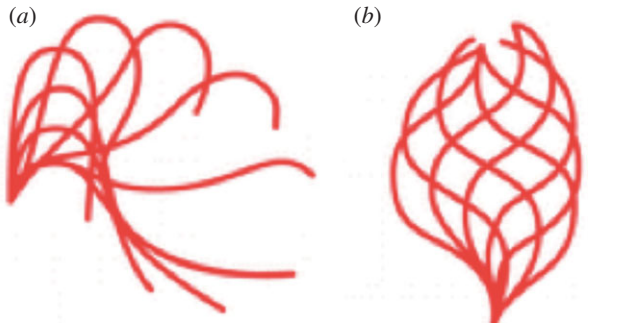

Figure 2. The two waveforms of *Chlamydomonas*. (*a*) The asymmetric waveform with the power and recovery stroke. (*b*) The symmetric waveform, which moves the cells backwards.

The asymmetrical structural features described in this review are likely to be key for generating the waveforms. This review discusses proximal/distal differences, radial/doublet specific differences and *cis*/*trans* differences. The *Chlamydomonas* cilium has been divided into four parts: the very proximal portion of the axoneme (0.3 µm), the proximal portion (2 µm), the central portion (7 µm) and the distal portion (2–3 µm) [10] (figure 1*b*). Part of the distal segment is marked by an extension of the A-tubule [10]. The DMTs are numbered DMT1–9. Two cilia are indicated as *cis* and *trans* based on their position relative to the single eyespot.

(a) The dynein arms

The outer dynein arms are positioned on the outer circumference of eight of the nine outer DMTs every 24 nm. The outer dynein arm attachment is stabilized by a complex of three proteins [15]. Multiple mutations fail to assemble all, or part of the outer dynein arms, as monitored by electron microscopy and by biochemical analysis of salt-extracted mutant axonemes [16–23]. These loci encode most of the 16 structural proteins of the outer dynein arm (figure 3). The role of the outer dynein arms in ciliary movement has been determined from analysis of the mutant phenotypes. While the ciliary waveform is normal, *oda*$^{-}$ mutants display significantly reduced ciliary beat frequencies. The outer dynein arms are needed for increasing the power of the ciliary stroke. In these mutant strains, the ciliary beat is reduced by more than one-half and results in a reduced forward swimming speed [24–26].

Inner dynein arms are found along the inner circumference of the nine outer DMTs and function to generate the ciliary waveforms. Inner dynein arm (*ida*$^{-}$) mutations result in a shallow ciliary waveform with reduced amplitude that produces reduced forward swimming speeds [27]. Unlike the outer dynein arms, there are at least 10 biochemically distinct inner arm structures in the 96 nm repeat [28,29]. One of the inner dynein arms (I1/f) is a two-headed structure with 10 subunits [30]. The remaining six inner dynein arms are single-headed motors with only one heavy chain; they are biochemically defined as dyneins a, b, c, d, e and g [28]. In addition, there are three minor dynein heavy chains. Inner arm mutants in nine genes have been isolated that affect their assembly [30–40], and these mutant strains show altered waveforms. Additional proteins associated with specific inner arms have been identified using biochemical analyses [41–44].

2. Proximal differences

(a) The inner dynein arms

Three (*DHC3*, *DHC4* and *DHC11*) of 14 dynein heavy chain genes in *Chlamydomonas* produce proteins that are found at low abundance. Immunofluorescence microscopy revealed that DHC11 localizes exclusively to the proximal approximately 2 µm portion, and DHC3 and DHC4 are likely to be localized to this proximal portion [45]. This conclusion is supported by proteomic data of isolated axonemes. DHC3, DHC4 and DHC11 peptides are present at about 12% of the single-headed inner arm dynein proteins and at about 6% of the I1/f inner dynein arm. DHC11 may replace inner dynein arm a (IAa) in the proximal portion [10]. By contrast, inner dynein b (DHC5/IAb) shows an inverse distribution pattern in which it is missing from the proximal 2 µm, but is present along the rest of the axoneme.

(b) Doublet microtubule bridge

In the proximal axoneme, doublet microtubule 1 (DMT1) has a bridge with DMT2 [46–48]. This bridge is not unique to *Chlamydomonas*. In sea urchins, two of the doublets are attached by a bridge (DMT5–DMT6) with a 96 nm periodicity [48]. The bridge in sea urchins is made up of two parts called the inner and the outer parts. The inner part of the bridge, i-SUB5–6, is present along the entire length of a DMT in both *Strongylocentrotus* (DMT5) and *Chlamydomonas* (DMT1) cilia. It localizes between the I1/f dynein and the N-DRC (figure 3).

(c) Polyglutamylation of tubulin

Axonemal microtubules contain post-translational modifications on tubulin that include acetylation, phosphorylation, polyglycylation and polyglutamylation [49]. The functional importance for ciliary function is not clear for all of the modifications. Tubulin polyglutamylation adds multiple glutamates onto the gamma carboxyl group of any of several glutamine residues in the carboxyl terminus of either α- or β-tubulin. Different proteins are required for polyglutamylation (PolyE) along the length of the axoneme in *Chlamydomonas*. PolyE staining appears to be restricted to the B-tubule and is found along the length of the axoneme expect for the distal end [50]. In *Chlamydomonas*, mutations in the genes *TTLL9* or *FAP234*, encoding either the tubulin tyrosine ligase-like enzyme or the subunit needed for its localization, [51] affect polyglutamylation of α-tubulin [12,52]. The proximal end of the axoneme (approx. 1.5 µm) remains polyglutamylated in these mutants [53]. Double mutant analysis suggests that loss of *ttll9* activity regulates inner dynein arms [54]. In mutants of the molecular ruler (CCDC39/40), there is a reduction in the signal intensity of polyglutamylated α-tubulin relative to polyglutamylated β-tubulin. The PolyE signal in the proximal region requires CCDC39 [12]. CCDC39 appears to be required for the transport or docking of a different TTLL enzyme, which acts at the proximal end.

The purpose of having different enzymes for polyglutamylation at the proximal and central regions is not known. It is interesting to speculate that the length or spacing of the polyglutamate tails may be different and allow different proteins to bind and interact at the proximal and distal ends [55]. If B-tubules are highly modified by polyglutamylation, this

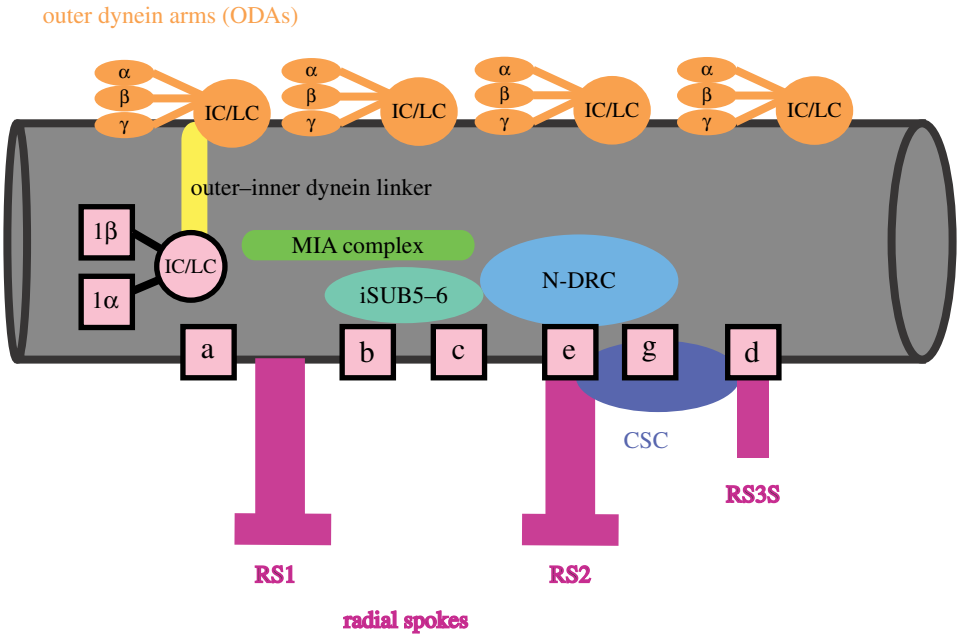

Figure 3. The 96 nm repeat found along the central region of a doublet microtubule. The outer dynein arms are shown in orange and have the three heavy chains and the intermediate and light chains (IC/LC). The inner dynein arms are in pink. The I1/f dynein, which is two-headed, is indicated by its heavy chains (1α and 1β) as well as the IC/LC proteins. The six other inner dynein arm complexes are shown as boxes. The regulatory MIA complex is shown in green, the outer-inner dynein linker (OIDL) complex is in yellow, the iSUB5–6 complex is in teal, the N-DRC complex is shown in light blue, and the CSC complex is in dark blue. The radial spokes are in magenta.

modification might participate in multiple functions, which include stabilizing DMTs and regulating dynein motor activity.

(d) LF5 and length control

Recent work showed that length affects the motility properties of the cilium. Mechanical metrics (force, torque and power) all increased in proportion to the ciliary length. The mechanical efficiency of beating appeared to be maximal at the normal wild-type length of 10–12 µm [56]. In *lf*$^-$ (long flagella) mutants, the cilia are often two to three times longer than in wild-type cells and are unable to generate an effective waveform. Length control is regulated by the LF5 kinase, which is a homologue of CDKL5, and localizes to the proximal 1 µm of the cilia. This localization requires a complex of the LF1, LF2 and LF3 proteins [57]. The role of LF5 at the proximal end of the axoneme is not clear but could affect entry or exit of intraflagellar transport trains.

3. Proximal and radial asymmetries

(a) The outer–inner dynein linker

The outer–inner dynein linker (OIDL) connects the I1/f dynein arm to the outer dynein arms [10]. OIDLs in the central and distal regions are similar among all of the DMTs. However, at the proximal end, more OIDL densities are found on DMT2, DMT6, DMT7 and DMT8 than on the other DMTs. The OIDL contains the intermediate chain (IC2) of the outer dynein arm and DRC4 of the N-DRC [58]. It plays a role in regulating both inner and outer dynein arms.

(b) The ODA5/ODA10 protein complex

ODA5 and ODA10 play two roles. They are required for outer dynein arm assembly in the cytoplasm [59], and they are assembled into the cilia as well [60]. Using tagged transgenes, ODA10 and ODA5 were found to localize to the proximal 2–3 µm of the cilia (figure 4) and only to DMT1, with a 24 nm spacing, as assayed by immuno-EM [59]. *Chlamydomonas* has paralogues of ODA5 (ODA1/DC3) and of ODA10 (ODA3/DC1) which generate a separate docking complex (DC1/DC3) that assembles along most of the cilium and is associated with the stabilization of the outer dynein arms [15]. In humans, there are only two proteins; they are CCDC151 and CCDC114. They play a role in placement of the outer dynein arms in humans and are found along the entire length of the ciliary axoneme [61,62]. It is not known what restrains the ODA5/ODA10 complex to one microtubule in the proximal axoneme and what its role is in the proximal cilium.

Unlike in *Chlamydomonas*, the trypanosome waveform can start at the tip or the base of the axoneme. The initiation of the waveform is based on a proximal/distal asymmetry of the outer arm docking complexes. The trypanosomes have two paralogues of DC1 and two paralogues of DC3, which form a proximal docking complex and a distal docking complex. This asymmetry is set up by a gradient of proximal docking complexes using the intraflagellar transport machinery [63].

(c) VFL3/CCDC61 plays another role

The VFL3 protein has been suggested to play a role in segregation of the basal bodies in *Chlamydomonas* by assembling the distal striated fibres that hold the mature basal bodies together [64]. In addition, VLF3 has been postulated to have a role in the templating of new basal bodies [65]. Recently, we have found that the VFL3 protein also localizes to the proximal region of the cilia [66] (figure 4).

Analysis of the waveform of cilia in the *vfl3* mutant using high-speed filming revealed that the flagellar waveform and frequency are similar to those of wild-type cells [67]. Wan & Goldstein showed that the two cilia in the *vlf3* mutant display markedly different synchronization from the wild-type pattern. They used micromanipulation of flagella and

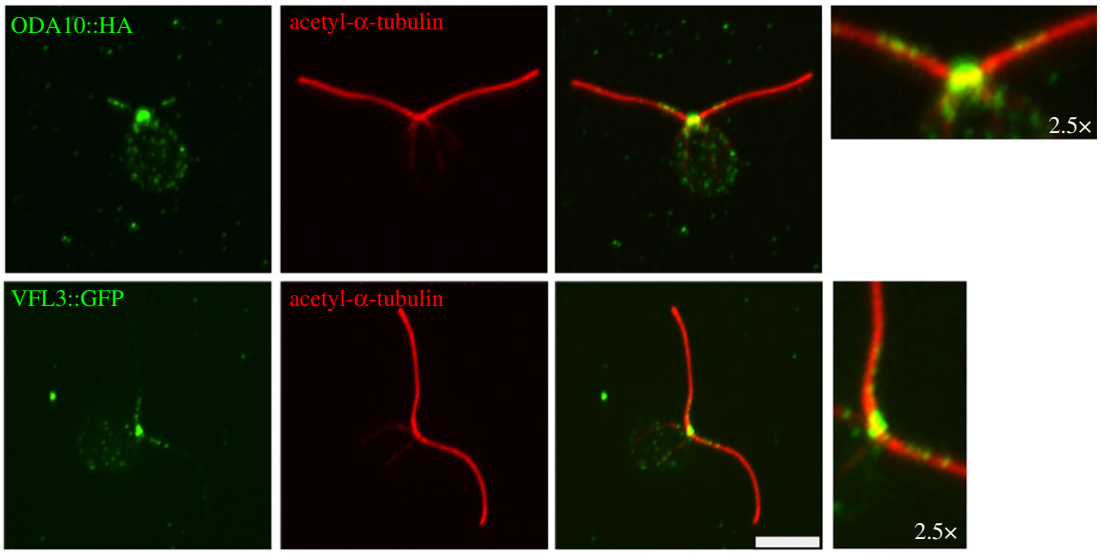

Figure 4. Localization of ODA10 and VFL3. Tagged transgenes (HA or GFP) for ODA10 and VFL3 show localization to the proximal region of the cilia. Antibodies to acetylated α-tubulin. The HA and GFP signals are shown in green and the acetylated α-tubulin is shown in red. (Online version in colour.)

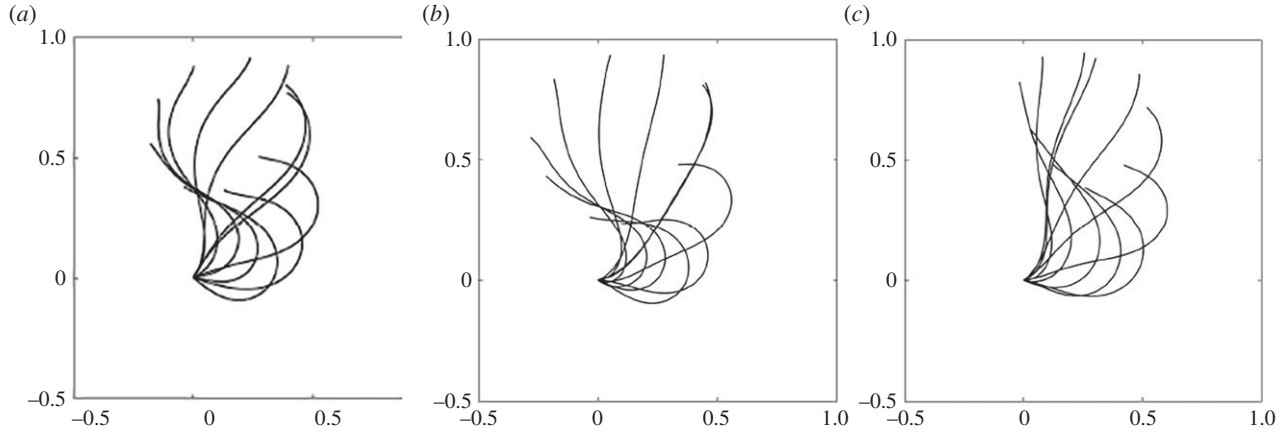

Figure 5. Waveforms of cilia from biciliated *vfl3-2* cells are unchanged from those of uniciliated cells. (*a*) Uniciliated *vfl3-2* cell, (*b*) biciliated *vfl3-2* cell and (*c*) uniciliated *uni1-2* cell.

concluded that a mechanism, internal to the cell, must provide additional flagellar coupling [68]. The waveform of biciliated cells in the *vfl3-2* mutant is unchanged but the two cilia from the *vfl3* mutant move independently (figure 5). We do not know if coupling offered by the striated fibres between the two basal bodies or the role of the VLF3 protein in the proximal cilia is responsible for the lack of synchrony between the two cilia [49]. As in wild-type cells, the cilia of *vfl3* coordinately switch to a symmetrical, ciliary-type waveform during the shock response, which shows that the VFL3 protein is not needed for photoshock response.

(d) B-tubule projections or beaks show proximal and radial asymmetries

Structural analyses of axonemes revealed the presence of projections in the centre of the B-tubules. These projections are only in the proximal half of the axoneme and in only three outer doublet microtubules (DMT1, 5 and 6) [46]. They are referred to as beaks [46]. Mutant strains with defects in the *MBO1* and *MBO2* genes lack beaks in DMT5 and DMT6, but retain the beak in DMT1 [69]. The *mbo*$^{-}$ mutant cells fail to generate the asymmetric waveform required for normal forward swimming. As a result, the mutant cells *m*ove *b*ackwards *o*nly (*mbo*) using the symmetrical waveform. The *MBO2* gene encodes a coiled-coil protein (CCDC146) [70] that localizes along the length of the ciliary axoneme. Proteomic analysis of *mbo1* and *mbo2* mutants revealed that the mutant axonemes are missing many more proteins than shown originally by two-dimensional electrophoresis ([69], M. Porter 2019, personal communication). The composition of the beaks remains unknown. The BUG22/FAP20 protein, which is part of the inner junction, plays a role in the placement of all the three beaks, and *fap20/bug22* mutants show a symmetrical waveform that generates backwards swimming [4]. The role of MBO2 or FAP20 in beak assembly is not known. Loss of BUG22 in *Drosophila* sperm affects polyglycylation of tubulin [71], but polyglycylation has not been examined in the *Chlamydomonas* mutants.

4. Radial asymmetries

(a) The central pair complex

Cilia in all studied metazoans have a fixed CPC orientation in which the plane through the two CPC microtubules is perpendicular to the bend plane. By contrast, the CPC has a variable orientation in *Chlamydomonas*. This is related to an overall helical twist of the CPC structure and a change in the orientation of the

CPC during bending. The plane through the two CPC microtubules is parallel to the bend plane [72].

The central pair microtubules are asymmetric. The two microtubules, called C1 and C2, have differential stability [73]. The two central microtubules have different appendages based on cryo-EM and analysis of mutants [74–82]. Recent cryo-EM tomography shows that the C1 microtubule has six projections while the C2 microtubule has five projections [83]. The two central pair microtubules are connected by a bridge as well that has at least three appendages. The known mutants affect specific appendages. *CPC1* and hydin mutations affect the appendages on the C2 microtubule (2b-2d), *PF16* (*SPAG6*) mutations affect the C1 tubule, and *PF6* mutations affect the appendage 1a. The *PF20* mutation affects the bridge between the two microtubules [83]. Recent proteomics on the *pf18* mutant, which lacks a CPC, suggests that it is composed of at least 44 proteins [84]. The CPC together with the radial spokes are needed for the ciliary waveform. Mutants affecting different appendages show a range of phenotypes. The *fap47* mutant is intriguing in that it is defective in phototaxis [84].

(b) DMT1 and DMT9

As mentioned previously, DMT1 shows proximal differences. In addition, DMT1 and DMT9 are missing several single-headed IDAs (IAb, IAc and IAe); these are missing from the entire length of these two DMTs [48].

(c) The MIA complex and outer dynein arms are needed for assembly of I1/f dynein

Chlamydomonas has two responses to light: phototaxis and photoshock. Chlamyrhodopsin is the photoreceptor [85] and the signal transduction process involves transmembrane Ca^{2+} fluxes. The cell responds by changes in ciliary behaviour. The *MIA1* and *MIA2* genes were identified among mutants that showed reduced positive phototaxis and resulted in increased phosphorylation of the IC138 subunit of the I1/f dynein [86]. These genes encode FAP73 and FAP100, and they localize near I1/f dynein and may connect with the N-DRC. The assembly of the I1/f dynein arm requires both the MIA1 and ODA complexes [87]. Consequently, in the *mia1* or *mia2* mutants, the I1/f dynein arms are missing from DMT1, which lacks outer dynein arms. In a *mia1*; *oda6* double mutant, the I1/f dynein is completely missing from the axoneme [87].

(d) Mutations that affect dynein docking on a subset of doublet microtubules

The *ODA2* gene encodes the gamma-dynein heavy chain of the outer dynein arms. Some alleles cause loss of the entire outer arm complex while others show a partial reduction. Surprisingly, the alleles isolated as suppressors of central pair mutations show a partial reduction of outer dynein arms on a subset of doublets (DMT3,6,7,8,9) [88]. The *bop2* mutation was identified as a suppressor that affects the assembly of inner dynein arms on only a subset of the DMTs [89,90]. By electron microscopy tomography, doublets 5, 6 and 8 show the most severe deficiency, doublet 9 has an intermediate phenotype, and doublets 2, 3, 4 and 7 show the least severe phenotype. A more recent examination of *bop2* mutants using cryo-EM tomography shows that a subset of inner dynein arms is missing on DMT5–9 [91]. *BOP2* encodes a WDR protein (FAP57), and a patient with a mutation in *CFAP57* show primary ciliary dyskinesia [92].

5. *cis* and *trans* asymmetries

The two cilia in *Chlamydomonas* are called the *cis* and the *trans* cilia. The *cis* cilium is nearest to the eyespot, which lies on the equator of the cell. The *cis* cilium is templated by the daughter basal body and the *trans* cilium is templated by the older or mother basal body [93]. Differences in the behaviour of the *cis* and *trans* cilia are thought to play a key role in orientation of the cell to light and calcium signals [94].

Differences between the *cis* and *trans* cilia have been studied using detergent-extracted and demembranated cells. At lower calcium concentrations, the *trans* axoneme is inactivated but at higher calcium concentrations, the *cis* axoneme is inactivated. These intrinsic differences between the two cilia are thought to be important for phototaxis [95]. In living cells with one cilium, the ciliary beat frequency of the *trans* cilium is about 30% higher than that of the *cis* cilium. But the cilia generally beat with the same frequency when both are present [96]. More recently, high-speed, high-resolution imaging of pipette-held cells with two cilia again has shown synchronized breaststrokes. At a low rate, there is a 'slip' and the *trans* cilium shows a faster beat frequency by about 30% while the *cis* cilium retains the original beat frequency [97].

This difference between the *cis* and *trans* cilia beat frequencies is not observed in mutants lacking the outer arm docking complex (*oda1* and *oda3*) or in the *oda11* mutant, which lacks the α heavy chain, but remains in the other outer dynein arm mutants [98,99]. How the docking complex and the α heavy chain but not the loss of the entire outer dynein arm influences these intrinsic differences is not known. A protein with four EF-hand domains (*ODA14*/DC3) requires DC1 and DC3 for its assembly [100]. DC3 plays a role in sensing calcium and redox poise that could be involved in these intrinsic differences between the two cilia [21]. In the non-phototactic mutant, *ptx1*, both cilia show the *trans* beat frequency [101], which suggests that PTX1 may define the *trans* cilium behaviour. The *PTX1* gene has not been identified as of yet. In another non-phototactic mutant, *lsp1*, both cilia show the *cis* beat frequency [102].

Mutants that assemble primarily the *trans* cilium are available [103]. These uniciliated cells facilitate recording of the waveform, although cilia behaviour may differ somewhat in uniciliated compared with biciliated cells [104]. Estimates of the 'equivalent' swimming speeds of uniciliated cells are much lower than the known swimming speeds of wild-type biciliated cells [56]. The reasons for these differences are not yet resolved. Biciliated *Chlamydomonas* may synchronize the two cilia by 'cell body rocking' with minimal direct hydrodynamic interactions between the two cilia [104], by hydrodynamic coupling [105] or by coupling of the basal bodies via the striated fibres [68]. Proteomics of cilia from the *uni1* mutant [103] will help to determine the protein composition of the *cis* and *trans* cilia, and will help us to understand the intrinsic differences.

6. Why have asymmetries in the cilium?

Regulation of ciliary and/or flagellar motility requires the coordinated spatial control of dynein-driven microtubule sliding

[106]. However, the mechanisms for regulating the location and symmetry of dynein activity are still not understood completely. It seems very likely that the asymmetries observed in the cilium of *Chlamydomonas* are critically important for the initiation and regulation of the waveform. This regulation will require proximal asymmetries as the initiation of the waveform occurs in the proximal region as well as radial asymmetries as proposed by Lin & Nicastro [14]. The presence of different dynein arms as well as the alterations in DMT1 and DMT2 in the proximal axoneme could have a role in initiating the waveform. DMT1–2 in *Chlamydomonas* are located in a plane almost perpendicular to the bending plane and show little or no interdoublet sliding [107,108].

The role of the central pair and radial spokes in regulating the dyneins is also demonstrated by the isolation of suppressor mutations that restore some motility to paralysed mutants that arise from defects in the CPC/RS and radial spokes [2,19,33,88,109,110]. These mutations affect both outer and inner dynein arms as well as the N-DRC, which modulates dynein activity. The loss of the N-DRC is able to turn-on dynein activity in the absence of CPC/RS signals. An alternative model suggests that interactions between dynein and the passive components of the axoneme can produce coordinated, propulsive oscillations like the fluttering of a flag. Steady, distributed axial forces, acting in opposite directions on coupled beams in viscous fluid, lead to dynamic structural instability and oscillatory, wave-like motion [111].

In wild-type cells, the symmetrical waveform (backwards swimming) is initiated when cells are exposed to intense light signals, which cause an influx of calcium ions into the cells [112]. Several lines of evidence suggest that the outer dynein arms are potential targets of the central pair–radial spoke control system for generating the symmetrical waveform [113,114].

The continued analysis of these asymmetries in *Chlamydomonas* and other organisms will be key to understanding the generation of the asymmetric and symmetric waveforms. Further biophysical, microscopic and modelling studies of the many mutants affecting these asymmetrically localized proteins will be key to understanding the waveforms and the large molecular ciliary machine.

Data accessibility. This article has no additional data.

Competing interests. I declare I have no competing interests.

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