Evidence for Axonemal Distortion During the Flagellar Beat of *Chlamydomonas*

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In order to understand the working mechanism that governs the flagellar beat it is essential to know if the axoneme undergoes distortion during the course of the beat cycle. The rapid fixation method employed by Mitchell was able to preserve the waveform of *Chlamydomonas* flagella much as it appears during normal flagellar beating [Mitchell, Cell Motil Cytoskeleton 2003;56:120–129]. This conservation of the waveform suggests that the stress responsible for the production of bending is also trapped by the fixation procedure. Longitudinal sections of these well-preserved flagella were used to document variations in the relative axonemal diameter. Sections aligned to the plane of bending, showing both the central pair microtubules and outer doublets, were examined for this purpose. Micrographs were selected that continuously showed both the outer doublets and the central pair from a straight region to a curved region of the flagellum. Axoneme diameters measured from these select micrographs showed an increase in relative diameter that averaged 39 nm greater at the crest of the bent region. This constituted a 24% increase in the axoneme diameter in the bends. The transverse stress acting across the axoneme during bending was calculated from the Geometric Clutch computer model for a simulated *Chlamydomonas*-like flagellar beat. If we assume that this is representative of the transverse stress acting in a real flagellum, then the Young’s modulus of the intact axoneme is ~0.02 MPa. The possibility that the distortion of the axoneme during the beat could play a significant role in regulating dynein function is discussed. Cell Motil. Cytoskeleton 64:580–589, 2007.

Key words: cilia; dynein; central pair; radial spokes; Geometric Clutch; t-force

INTRODUCTION

The mechanism that coordinates the beat in eukaryotic flagella is incompletely understood. Two rather dissimilar viewpoints coexist, each having a fairly substantial base of experimental support. One view holds that the coordination of the flagellar beat is regulated through the rotation of the central pair, which selectively triggers activation of specific dyneins through an enzymatic cascade in the central pair-spoke-dynein regulatory complex. The second view is that the mechanical properties of the axoneme provide a feedback control that works by the action of accumulated tension to change the interdoublet distance and regulate the dynein motor proteins. Both of these viewpoints have been reviewed in detail [Cosson, 1996; Lindemann and Kanous, 1997; Omoto et al., 1999; Kamiya, 2002].

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In the flagella of *Chlamydomonas* it has been observed that the central pair rotates as it is extruded from disintegrating axonemes concurrent with the loss of flagellar motility [Kamiya, 1982; Kamiya et al., 1982]. Furthermore, the orientation of the central pair seems to correlate with the doublets that slide apart in the disintegration of *Chlamydomonas* axonemes [Wargo and Smith, 2003]. Recently, the orientation of the central pair was observed in *Chlamydomonas* flagella that were rapidly fixed using a method that preserved the waveform of the beat [Mitchell, 2003b]. This study confirmed that during the beat the central pair orientation changed coincident with the bending pattern. Transmission electron microscopy (TEM) of longitudinal sections through the bending waves showed that the central pair was flat to the plane of the section in the curved regions and perpendicular to the plane of the sections in the interbend regions.

These observations are generally consistent with the viewpoint that the central pair orientation controls the beat cycle in *Chlamydomonas* flagella. The accumulated evidence supporting a role for central pair rotation in the beat cycle is perhaps stronger in *Chlamydomonas* than in any other flagellar system. Nonetheless, there is also considerable evidence that mechanical feedback also plays a role in the beat cycle of *Chlamydomonas*. Based on the accumulated experimental evidence on *Chlamydomonas* motility, Hayashibe et al. [1997] concluded that both central-pair rotation and mechanical feedback are needed to fully explain all of the observed behaviors of the *Chlamydomonas* flagellum.

There is also the question of causality. A recent study provides evidence that the central pair is in isolation is a helical structure, and that its shape may cause it to rotate and thus follow the beating pattern [Mitchell and Nakatsugawa, 2004]. In this way the rotation may be driven by the beat, rather than driving the beat.

The Geometric Clutch hypothesis is a mechanical coordination scheme that can successfully simulate flagellar beating in a computed model [Lindemann, 1994a,b]. The working principle of the Geometric Clutch mechanism utilizes the transverse force (t-force) that develops across the axoneme to coordinate the action of the dynein motors. The t-force can push doublets together to activate dynein engagement and can also force the doublets apart to terminate the action of the dynein motors. The Geometric Clutch hypothesis is only viable as a coordination mechanism if the t-force acting on a real axoneme generates a significant distortion of the axoneme during the beat cycle. The distortion should be present over the course of a cycle of flagellar bending and should result in an increase in the flagellar diameter at the crest of the flagellar bending waves [Lindemann, 2003]. The extent of the distortion would be determined by the mechanical resistance to stress of the structures that compose the axoneme, essentially the stress to strain ratio or Young’s modulus.

The TEM technique that was used by Mitchell [2003b] to verify the rotation of the central pair in the *Chlamydomonas* beat cycle is ideally suited to look for axoneme diameter distortion in the plane of the beat. The fixation procedure developed for that study preserves the waveform of the beat and therefore, it is a reasonable expectation that the stresses and strains on the protein matrix of the axoneme are also locked in by the technique. In this report, the collection of longitudinal sections produced for the Mitchell [2003b] study was examined to see if a consistent pattern of axonemal distortion could be detected coincident with the cycle of flagellar bending.

The results are considered in the context of the magnitude of the t-force that is present in a *Chlamydomonas*-like beat as calculated by the Geometric Clutch model. From the t-force and the measured distortion we derive a first estimate of the trans-axonemal Young’s modulus.

**MATERIALS AND METHODS**

Complete details for culture and fixation of *Chlamydomonas* for transmission electron micrographs used in this study were given in Mitchell [2003b]. Wild type cells of the 137c+ strain were used for the forward swimming micrographs, which constituted all but one of the analyzed flagella. The remaining analyzed flagellum was a wt flagellum in the reverse, backward swimming mode.

Optimal preservation of waveform was achieved by rapid addition of one volume of 6% glutaraldehyde, 50 mM cacodylate, pH 7.5, to an equal volume of cells during continuous ambient room illumination. After 5 min, 4 vol of 50 mM cacodylate, pH 7.5, was added to reduce the glutaraldehyde to 1%. The flagella were fixed while the cells were still in suspension. Samples were transferred to clean glass coverslips where settling and fixation continued for 1 h at RT. This resulted in a high number of cells at the glass surface with their flagella extending parallel to the coverslip surface. The cells were subsequently embedded on the coverslip by substituting a 50 mM cacodylate buffer that had 1% low melt agarose. Subsequent removal of the glass coverslips and sectioning parallel to the flat surface yielded a number of sections aligned through the flagellar bend plane that are the basis of this study.

TEM negatives were scanned on an Epson Perfection 4990 photo scanner and enlarged to ~9 \times 11 in. and printed on photographic quality paper. The enlargements were distorted by less than 1% in the two image dimensions as determined by the ratio of the length to width of the enlargements as compared to the negatives. Magnification of the final images was found from the
ratio of the edge-to-edge dimension of the print number on the original negative and the positive enlargement. Points along the axoneme were selected for measurement on the basis of the visibility of the central-pair and the two outermost doublets. A protractor was used to define a line across the axoneme image orthogonal to the doublet axis at the points of measurement. For a flagellar image to be included in the data, a valid measurement point had to be present in a bend and a straight region on the same flagellum. When possible (three cases), an intermediate point in the transition region that also fulfilled the inclusion criteria was measured as well.

All measurements were made with a vernier caliper with a resolution of 0.05 mm. At every point of measurement, an outer diameter was measured from the outer edge to outer edge distance of the two outermost doublets, and inner diameter was also measured from the inner edge to the inner edge of the outermost doublets. The average of these two diameter determinations was taken to be the center-to-center diameter of the axoneme at the point of measurement. Comparison of the inner and outer diameter was also used to verify that variations in relative diameter were not systematically resulting from size variations in the longitudinal images of the outer doublets.

When adjacent sections were available, the appearance of the doublet microtubules confirmed that the doublets do not twist, and the section appears not to ‘wander’ from side to side. Maintaining the central pair (CP) within the section was the only way to monitor the section plane when adjacent sections were not preserved.

**Computer Simulations**

The Geometric Clutch computer model version 3 as published by Lindemann [2002] was used to set up a simulation of a *Chlamydomonas* flagellum in both the forward and the reverse beating mode. The force-velocity kinetics for dynein that yielded the best lung cilium simulations in the earlier report was used [Fig. 2d of Lindemann, 2002]. Table I gives the modeling parameters that were used in the computer model to produce a *Chlamydomonas*-like beating pattern. The principal differences in the modeling parameters that were required to produce a *Chlamydomonas*-like beat are a length of 12 μm, combined with a somewhat more flexible passive flagellar stiffness (0.7 × 10^{-22} N m^2 vs. 1.3 × 10^{-22} N m^2) than was used to simulate a lung cilium. The value we used for the nexin link elasticity (2 × 10^{-5} N/m) is the same as that measured by Yagi and Kamiya [1995] for the passive *Chlamydomonas* axoneme.

The transition from the forward to the reverse type of waveform was effected by a change in a single parameter. The probability of spontaneously forming a dynein cross-bridge on the P-bend forming side of the axoneme was changed from 0.08 to 0.02. Such a change in the base level probability could easily be accomplished in a real flagellum by a calcium-mediated change in the dynein orientation on one side of the axoneme. The reduced stiffness of a *Chlamydomonas* axoneme, as compared with a lung cilium, is predicted by the model but is unconfirmed.

For this study, the model was used as a simulacrum of the real flagellum but one that has accessible internal t-force data. The t-force acting in the model at a similar frequency and waveform should be a fairly good indicator of the t-force acting in the real *Chlamydomonas* flagellum.

**RESULTS**

Figure 1 shows the change in the diameter of a *Chlamydomonas* flagellum in the forward beating mode. The lower panel of the figure places slices of the upper image side by side at higher magnification to facilitate visual comparison of the differences in diameter. Figure 2 shows the changes in the flagellar diameter of a *Chlamydomonas* flagellum in the reverse beating mode. The lower panel of the figure places slices of the flagellum image from the two bends side by side with a slice of the flagellum from the connecting straight region. The variation in the relative diameter is easily seen in the side-by-side comparisons. Table II summarizes the diameter measurements made from TEM micrographs of *Chlamydomonas* flagella, which showed good waveform preservation. Six different bends were measured in a total of

### TABLE I. Modeling Parameters—*Chlamydomonas* Simulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
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<tr>
<td>Number of segments</td>
<td>30</td>
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<td>Functional diameter(^a)</td>
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<td>Drag coefficient</td>
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<td>Passive stiffness (IE)</td>
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<td>Elastic constant per nexin link(^b)</td>
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<td>Attachment</td>
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<td>Principal bend bridges</td>
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<td>Reverse bend bridges</td>
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<tr>
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<td>Adhesion scaling factor(^d)</td>
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<tr>
<td>Dynein heads per length</td>
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</tr>
<tr>
<td>In outer arm file</td>
<td>125/μm</td>
</tr>
<tr>
<td>In inner arm file</td>
<td>73/μm</td>
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</table>

\(^a\)Functional diameter for bending torque calculation based on the spacing between doublets 2–4 and 7–9.
\(^b\)Based on 100 nm nexin spacing.
\(^c\)Scales t-force acting in the real flagellum with accessible internal t-force data.
\(^d\)Scales dynein force to bridge attachment probability.
five different flagella. All but one of the six bends showed a significant increase in the internal and external diameters as compared to a straight region of the same flagella. The one non-conforming bend was the most distal of the bends included in the analysis and showed very similar diameters in the bent and straight region. This is important to the extent that it demonstrates that it is possible to have nearly identical diameters in the straight and bent regions even with the central pair in different orientations.

The average diameter distortion at maximum bending is 24% for all six determinations. This represents a 39 nm average distortion or stretching of the axoneme in the beat plane during bending. The greatest distortion of 48% was exhibited by the most basal bend (which also had the greatest curvature) and the least distortion was
7% for the most distal bend. The flagellar diameters were measured from the reverse mode beat shown in Fig. 2. The distal and the proximal bends were both measured and their diameters compared to the inter-bend region of the same flagellum. The proximal bend showed a 27% distortion (49 nm greater diameter than the straight region) and the distal bend showed a 17% distortion (32 nm) change in diameter. Although our sample is too small to establish such a relationship with certainty, the data hint that the distortion may be greatest near the flagellar base and decreases as bends propagate distally.

An issue that requires some consideration is whether the observed difference in diameter we see is in fact the result of distention in the bent regions or is instead the result of compression (flattening) in the straight regions. Measurements on images of Chlamydomonas flagellar cross-section yield a somewhat variable estimate of the axoneme diameter probably due to differences in TEM magnification calibrations. Measurements were taken from high quality published TEM images of wild type Chlamydomonas flagella [Hoops and Witman, 1983, Fig. 3c; Piperno et al., 1990, Fig. 7]. Flagellar cross sections from these sources yielded 183 and 195 nm respectively for the outer diameter and 130 and 133 nm inner diameter. This gives center-to-center distances of 157 and 164 nm for the two sources respectively, which can be compared to the values in Table II. The images of Chlamydomonas flagellar cross sections in Mitchell [2003a] produced on the same electron microscope as the plates examined in this report, give an average outer diameter of 178 nm, an inner diameter of 130 nm and a center to center spacing of 154 nm. While those cross-sectional images are not of wild type flagella, the dimensions are again more consistent with the dimensions we measure in the straight flagella, not in the bent regions. Therefore, measurements from all three

<table>
<thead>
<tr>
<th>Swimming Direction</th>
<th>Bent (nm)</th>
<th>Straight (nm)</th>
<th>Δ (nm)</th>
<th>%</th>
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<tr>
<td>Forward</td>
<td>189</td>
<td>160</td>
<td>29</td>
<td>18</td>
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<tr>
<td>Forward</td>
<td>224</td>
<td>151</td>
<td>73</td>
<td>48</td>
</tr>
<tr>
<td>Forward</td>
<td>189</td>
<td>177</td>
<td>12</td>
<td>07</td>
</tr>
<tr>
<td>Forward</td>
<td>198</td>
<td>157</td>
<td>41</td>
<td>26</td>
</tr>
<tr>
<td>Reverse proximal</td>
<td>233</td>
<td>184</td>
<td>49</td>
<td>27</td>
</tr>
<tr>
<td>Reverse distal</td>
<td>216</td>
<td>184</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td>Averages</td>
<td>208</td>
<td>168</td>
<td>39</td>
<td>24</td>
</tr>
</tbody>
</table>

TABLE II. Axoneme Diameter Distortion

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sources suggest that the diameters we measure in the bent regions represent distention and the diameters in the straight areas are approximately normal cross-sectional diameters.

The size of the transverse force acting in a beating sea urchin flagellum is estimated to be \( \sim 0.25–1.0 \, \text{nN/\mu m} \) [Lindemann, 2003]. The Geometric Clutch simulation of a Chlamydomonas beat in both the forward and reverse mode is shown in Fig. 3. Figure 4 shows the total trans-axoneme t-force downloaded from the Geometric Clutch computer simulation. The t-force in a flagellum propagating bends in both the forward and reverse beating modes is shown in the figure. The flagellar position of the simulation at the point selected for the t-force plot, as well as three earlier positions to provide some context of motion is shown in an inset. The printout positions were selected for their resemblance to the configurations of the real flagella in Figs. 1 and 2.

The total t-force that is plotted in Fig. 4 is the numerical sum of the t-force contributed by the two opposite sides of the axoneme acting in the beat plane. This is the model-derived equivalent of the t-force that acts on the doublets at the inner (concave) surface and outer (convex) surface of the flagellum, and would be the t-force responsible for distorting the axoneme during the beat. In the computed simulations of a Chlamydomonas flagellum the total trans-axoneme t-force is between 0.3 and 1.2 \( \text{nN/\mu m} \) at the crest of the propagating bends.

The model-derived estimates of t-force do depend to some extent on our assumptions about the interdoublet nexin link elasticity. However, based on the currently available experimental estimate of interdoublet elasticity in Chlamydomonas [Yagi and Kamiya, 1995] the contribution of the nexin links will be small compared to the contribution from the active dyneins. This issue was examined in Lindemann [2003] and in an active bend the contribution of the interdoublet links to the t-force was estimated to be about 0.01 of the t-force contributed by the real flagellum in Fig. 1 (forward) and a reverse bend similar to the real flagellum in Fig. 2 (reverse). The inset of each graph shows four flagellar positions of the model (at 2 ms intervals) ending with the one corresponding to the t-force plot (thickest line). Note the negative spikes of t-force that correspond to the propagating bends.

![Fig. 3. Simulations of the Chlamydomonas beating pattern with the Geometric Clutch computer model.](image)

![Fig. 4. The t-force acting on the Chlamydomonas flagellum as found from the Geometric Clutch simulation.](image)
the dyneins. Consequently, the exact value used for the nexin elasticity will result in only a minor adjustment to the magnitude of the t-force.

Young’s modulus (Y) can be found by applying the classical stress to strain relationship, \( Y = \Delta F/m^2 \), where \( \Delta F \) is the t-force, \( m \) is the rest diameter and the strain is the change in diameter in the beat plane with the application of force. To estimate the effective area of a cylinder, the average diameter is the long axis and in the plane of the beat is 1.7 nm. Curiously, the spokes are most tilted in curved regions, where interdoublet shear is changing, as is also evident in the micrographs of Warner and Satir [1974]. The spokes are most tilted in curved cilia that exhibit the greatest spoke tilting in the Warner and Satir [1974] report.

The problem for this explanation is that the central pair apparatus including accessory proteins (i.e. the central pair projections), is remarkably close to circular as seen in cross section [Mitchell and Sale, 1999; Mitchell, 2003a; Yokoyama et al., 2004]. Furthermore, the positioning of the spoke heads in cross sections of cilia and flagella are arranged in an almost perfect circle, as seen in high quality superimposed images of many axonemes [Afzelius et al., 1995]. These facts would argue that the intact central pair apparatus with accessory proteins is basically cylindrical. Consequently, rotation of the entire apparatus, including the central pair projections, should be expected to cause no net distortion of the outer doublet positions.

The measurements reported here do not suggest that the observed distortion of the axoneme is a direct consequence of the central pair orientation. The central pair orientation was always flat to the plane of the bend in the curved sections we analyzed, but the amount of diameter distortion was not uniform. The most basal bend showed the greatest distortion and the most distal bend showed the smallest, indeed negligible, distortion. The diameter change from base to tip would indicate that the amount of distortion in each bend varied independent of the central pair orientation.

Another issue that should be considered is the effect of radial spoke tilt on the distortion. If the radial spokes are of fixed length they should reduce the diameter of the axoneme when they are in a tilted configuration. Warner and Satir [1974] showed that the radial spokes respond to microtubule sliding by first tilting to an angle of about 33° before translocating to a new position on the central pair (cp) apparatus. Assuming the spokes do not elongate, this is enough of a change in geometry to produce a 17% reduction in the axoneme diameter. Curiously, the spokes are most tilted in curved cilia where interdoublet shear is changing, as is also evident in the micrographs of Warner and Satir [1974]. The spokes are most tilted in curved cilia that exhibit the greatest spoke tilting in the Warner and Satir [1974] report.

The method of rapid fixation and post fixation that was used in the Mitchell [2003b] study was able to preserve the waveform of the beat that Chlamydomonas flagella exhibit in vivo. The waveform in a living flagellum is the result of stresses created by the cumulative action of the dynein motors on a flexible structure moving in a viscous medium. As shown in Fig. 5a, when a bend is created by this motor process, the doublets on the outside of the bend are shifted base-ward by sliding and the doublets on the inside of the bend are shifted tip-ward in what is commonly called the inter-doublet shear. When the flagellum bends due to the action of the dynein motor proteins, tension accumulates on the doublets on the inside (concave side) of a bend and compression accumulates on the doublets on the outside (convex) side.
of each bend as illustrated in Fig. 5a. This tension and compression generates the force couplet that creates a torque across the diameter of the axoneme and this is also what causes the axoneme to bend and to move through the external fluid.

In a real axoneme when the dyneins on one side are actively working to produce a bend, the tension produced by the dyneins is ultimately distributed across the entire axoneme diameter because the dyneins on the active side of the axoneme act in series, as illustrated in Fig. 5b. Therefore, the force produced by the dyneins on each doublet is transmitted to the doublets at each end of the active group, namely doublet 1 or doublets 5–6. These doublets experience the resulting tension and compression, and consequently, these doublets also experience the full outwardly directed t-force, as illustrated in Fig. 5c. This is the source of the t-force that could be acting to increase the diameter of the axoneme in the areas where a bending wave is present.

If a large number of active dyneins are permanently and rapidly cross-linked to their adjacent doublet by the action of a strong fixative, it should presumably lock the inter-doublet shear in place. The outer doublets have been shown to be relatively inextensible during the course of the beat cycle [Brokaw, 1989, 1990]. Due to this property of the doublets, there is a well-defined geometric relationship between the waveform and amount of inter-doublet shear. Consequently, preservation of the waveform of the beat is visible evidence that the fixation procedure successfully locked-in most of the inter-doublet shear. That the geometry of the beat is preserved by fixation also necessitates that at least a portion of the axonemal stress must be locked in place as well, since torque is required to bend a straight flagellum into a bent configuration. This can be understood from the perspective that structures that were distorted by stress and strain during the beat, such as the nexin links, spokes and dyneins, would not be able to relax fully to their unstressed configuration. The structural geometry of the preserved bends does not permit relaxation of the internal components to the configurations they would assume if the flagellum were straight.

Evaluating the amount of axonemal stress that is captured in the fixation process is more problematic. Gibbons [1975] reported that sea urchin sperm under similar fixation conditions underwent a gradual distortion of the preserved bending waves. In Chlamydomonas, prolonged fixation at the high concentrations of glutaraldehyde required to preserve bends also leads to a relaxation of the bends, but can be avoided by dilution of the glutaraldehyde within the first 5 min as described in the methods section. In this study, sectioned flagella with abnormal curves were encountered but were discarded from the analysis.

Since all motion is arrested by fixation, the component of the active torque that was driving the motion through the fluid is gone. Therefore, it is likely that some of the interdoublet stress would also dissipate during fixation. Consequently, the distortion that we see may not be as large as is present in a beating flagellum, but is unlikely to be larger than the distortion in a moving flagellum.

Fig. 5. Stress and strain in a beating flagellum. When a flagellum is bent by the action of the dynein motors, the doublets on the outside of the bend are under compression and those on the inside of the bend experience tension as shown in (a). The result is an outwardly directed t-force that will tend to increase the axoneme diameter as shown. In a real flagellum the circular arrangement of the doublets dictates that the force developed by each set of dyneins in the active series is relayed to the elements at the beginning and end of the series. Consequently, when the dyneins on the doublets 6 through 9 are active, as is shown in (b) the resulting tension and compression is delivered to doublets 1 and 5–6 on opposite sides of the axoneme. This results in the t-force acting across the axoneme as shown in (c) and increasing the diameter of the axoneme in the plane of the t-force, which is also the bending plane of the beat. Reproduced from Lindemann [2003], with permission from the Biophysical Journal.
Another uncertainty is the effect of fixation on the elastic properties of the interdoublet connections, which include the dyneins, spokes and nexin links. These structures must resist the action of the t-force; their elastic response will determine the stress to strain ratio, which determines the extent of the resulting distortion. Fixation usually alters proteins by the introduction of cross-linkages. While the most likely result of fixation is to stiffen protein connections, cross-linking could also reduce their elastic tendency to recoil when stress is released. Therefore, this factor could either be reducing the distortion that remains after fixation, or helping to capture the distortion that was present during active beating. In either case, the observed distortion would still be a directly observable result of the t-force.

As is seen in Figs. 5b and 5c, there are four interdoublet spaces separating doublets 1 and 5–6 of the axoneme. If the 39 nm average increase in the axonemal diameter that we measure is evenly distributed across each of the interdoublet spaces, the distortion is large enough to alter the interdoublet spacing in the beat plane by an average of 10 nm over the course of the complete beat cycle. This is a significant distance when the size of the dynein molecule is taken into consideration. The length of the B-link of dynein, which is thought to be the main structure that forms the dynein-microtubule bridge, is 15 nm. The diameter of the globular dynein head is on the order of 10 nm. Lindemann and Hunt [2003] did an analysis of the stiffness of the dynein molecule using the data of Burgess et al. [2003] and found that the dynein stem is very flexible; therefore, the dynein head and B-link would not be able to transmit force between doublets without a mechanism to securely anchor the head to the A-subtubule. This puts constraints on the length of the dynein bridge during the force transmission stage of the power stroke. An increase in doublet separation of 10 nm would decrease, or prevent, effective force transmission between the doublets and therefore, could potentially terminate the action of the dyneins. This undocked condition of the dynein is visible in the electron micrographs of Goodenough and Heuser [1984, Figs. 13–17] and Burgess et al. [1991, Figs. 11 and 12]. It was initially thought to be an anomaly due to damage, but it may, in fact, be of central importance to the mechanism of dynein functioning!

There is preexisting experimental evidence that the state of the dynein bridges between the doublets can influence the axonemal diameter. It has been noted that the diameter of the axoneme in rigor is considerably reduced from the relaxed condition [Gibbons, 1975]. Warner [1978] also showed that divalent cations could change the state of the dynein bridges and alter the axoneme diameter. Most recently, Sakakibara et al. [2004] showed that the axoneme diameter can exhibit dynein-dependent oscillations. This is strong evidence that the cycle of attachment and detachment of dynein bridges can alter the interdoublet spacing. Viewed in this context, the axoneme may actually experience a reduced diameter when dyneins are actively engaged, and the increase in diameter that we observe in the bends may correspond to the expansion that occurs when the dyneins are triggered to release due to the t-force.

The present study can only address the diameter of the axoneme in the plane perpendicular to the beat plane. It is possible that the diameter of the axoneme in the plane perpendicular to the beat also changes in a compensatory ratio, so that the axoneme in cross-section becomes oval in the bends and the interdoublet distance does not change in proportion to the long-axis diameter. We do not consider this alternative to be as likely because of the presence of the radial spokes. The spokes of doublets 3, 7, and 8 are aligned perpendicularly to the beat plane and therefore should experience very little spoke tilting, since there is very little shear across the axoneme in this direction. These spokes abut against the cp apparatus and should act as spacers to maintain the axoneme diameter in this plane. In turn, the central pair apparatus, when the central pair projections and sheath are included, is cylindrical (as noted above). In order to reduce the diameter of the axoneme in the plane perpendicular to the beat, the spokes would either have to shorten, or the central apparatus would have to flatten, to accommodate a reduction in cross section. This is within the realm of possibility, since the cp projections 1a and 1b will be facing these spoke heads in the curved regions [Mitchell, 2003a,b]. If these cp projections are flexible, it is possible that the distance across the axoneme could simultaneously be expanded in the plane of the beat and reduced perpendicular to the beat plane. Unfortunately, this possibility cannot be evaluated at the current time. It would be interesting to see if a similar diameter distortion can be observed in flagella such as sea urchin where the cp orientation appears to be fixed [Gibbons, 1975]. Newer imaging techniques such as cryo-electron tomography, which can image the axoneme in three dimensions [Nicastro et al., 2005, 2006], should be capable of resolving this issue in the near future.

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REFERENCES

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