

Orientation of the Central Pair Complex During Flagellar Bend Formation in *Chlamydomonas*

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Thin section electron micrographs of rapidly fixed *Chlamydomonas* cells were used to establish a relationship between flagellar bends and orientation of the central pair microtubule complex. Using conditions that preserve flagellar waveforms during both forward swimming (asymmetric bends) and backward swimming (symmetric bends), we found that central pair orientation differs in bent regions and straight regions. During forward swimming, a plane through the two central pair microtubules is parallel to the bend plane throughout principal bends, in both effective stroke and recovery stroke phases of the beat cycle. In these curved segments, the C1 microtubule always faces the outer edge of the curve. This parallel orientation twists in straight regions both proximal and distal to bends. During backward swimming episodes induced by photoshock, when *Chlamydomonas* flagella beat with principal and reverse bends of similar magnitude, the central pair twists by 180 degrees between successive bends. These observations support a model in which central pair orientation in *Chlamydomonas* is linked to doublet-specific dynein activation, and bend propagation is linked to rotation of the central pair complex. *Cell Motil. Cytoskeleton* 56:120–129, 2003.

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INTRODUCTION

The two microtubules of the central pair complex are highly conserved structural elements of typical “9+2” eukaryotic cilia and flagella, but their function is still poorly understood [Smith and Lefebvre, 1997]. The central pair is essential for motility, as evidenced by the total lack of motility associated with mutations that disrupt central pair assembly in *Chlamydomonas* [Witman et al., 1978] and in humans [Baccetti et al., 1979], but some organisms have evolved motile flagella that lack a central pair [Gibbons et al., 1983]. Suggested roles for the central pair complex, including bend initiation [Hayashibe et al., 1997] and waveform regulation [Brokaw and Luck, 1985], have been contradicted by the discovery of experimental conditions that allow initiation and propagation of planar bending waves in reactivated central pairless *Chlamydomonas* flagellar axonemes [Wakabayashi et al., 1997]. The central pair, therefore, provides

important but at present uncharacterized functions to most cilia and flagella.

Current models postulate that interactions of central pair projections with adjacent radial spoke heads regulate spoke-associated kinases, which in turn modulate dynein activity along outer doublet microtubules [Porter and Sale, 2000]. Evidence in support of this model includes the observation that doublet sliding rates

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in protease-treated *Chlamydomonas* axonemes are modulated by radial spokes in a kinase-dependent pathway [Smith and Sale, 1992; Smith, 2002], and that sliding occurs preferentially along doublets closest to the C1 central pair microtubule [Wargo and Smith, 2003]. Understanding how such doublet-specific activation functions during bend propagation requires more detailed information on spoke-central pair interactions in actively beating flagella, and on the relationship between regions of activity and central pair orientation.

Two conflicting views of central pair orientation relative to bend formation have emerged from published studies [Omoto et al., 1999]. In echinoderm spermatozoa, which have planar and primarily symmetric beat patterns, the central pair appears to maintain a fixed orientation in which the flagellar bend plane passes between the two central pair microtubules, i.e., the central pair is perpendicular to the bend plane [Sale, 1986]. A similar orientation occurs in molluscan gill cilia [Gibbons, 1961], which have an asymmetric planar effective stroke and a three-dimensional recovery stroke, and in comb plate cilia of a ctenophore [Tamm and Tamm, 1981]. In these metazoans, the central pair plane is apparently fixed perpendicular to the plane of the effective stroke at all times. In the ciliate *Opalina*, which also has a three-dimensional recovery stroke, rotation of the central pair apparently keeps the central pair orientation perpendicular to the bend plane during each beat cycle, even when the effective stroke direction changes [Tamm and Horridge, 1970]. These observations led to models in which central pair orientation is always perpendicular to the bend plane and controls the direction of bend formation.

In contrast, during the three-dimensional beat pattern of *Paramecium* cilia, the central pair apparatus both rotates through 360° during each beat cycle, and twists so that the central pair is parallel to the bend plane in curved regions, but perpendicular in straight regions on either side of a bend [Omoto and Kung, 1979, 1980]. Rotation of the central pair complex during active beating has been observed in several other unicellular organisms, including *Chlamydomonas* [Kamiya, 1982], but a functional relationship between bend formation and central pair orientation has only been established for *Paramecium*. The non-planar recovery stroke in *Paramecium* cilia has hampered detailed analysis of central pair orientation during all phases of the beat cycle, but published results show that interbend twists are always left-handed [Omoto and Kung, 1980]. Based on the hypothesis that each bend is associated with a twist in the central pair, bend propagation should result in central pair rotation. To determine whether such a mechanism could explain the observed rotation of the central pair apparatus in a system where the entire beat cycle is planar, central pair structure is well-characterized, and signal transduction

pathways between dyneins and the central pair complex have been identified, we examined central pair orientation in *Chlamydomonas* flagella. Cells rapidly fixed during normal forward swimming (asymmetric planar bends) or during transient reversals (symmetric planar bends) reveal twists in the central pair that correlate with bend direction. Significantly, the central pair is parallel to the bend plane in both principal and reverse bends, suggesting a specific relationship between bend formation and/or propagation, and central pair complex orientation.

MATERIALS AND METHODS

Wild-type cells (strain 137c⁺), grown in liquid minimal medium on a 14h/10h light/dark cycle to mid-log phase, were used for most experiments. For photography of backward swimming waveforms, cells of the *uni1* strain [Huang et al., 1982] were grown to early log phase. Waveforms of live cells were photographed under stroboscopic darkfield illumination as previously described [Mitchell and Rosenbaum, 1985].

Cells were harvested by centrifugation and resuspended in fresh medium to a final concentration of about 5×10^6 ml⁻¹ prior to fixation. Optimal fixation of waveforms during forward swimming 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. To preserve waveforms during reversal episodes, cells were dark-adapted for 5 min, then exposed to a high-intensity illumination source for 2–5 sec before addition of buffered glutaraldehyde as above. After 5 min, 4 volumes of 50 mM cacodylate, pH 7.5, was added to reduce glutaraldehyde concentration to 1%. Samples were transferred to clean glass coverslips and fixation was continued for 1 h at RT while cells settled onto the cover slip; most cells settled on coverslips with their flagella extended approximately parallel to the surface.

Glutaraldehyde fixative was gently removed and replaced with 50 mM cacodylate, pH 7.5, containing 1% low melt agarose. After the agarose had solidified, coverslips were rinsed by immersion and processed through standard post-fixation (1% OsO₄), dehydration, and embedding in epoxy resin. After curing, the coverslips were removed and small areas of the embedded material were remounted and sectioned parallel to the coverslip surface to obtain longitudinal sections through the flagellar bend plane. Sections were stained with uranyl acetate and lead citrate, and examined in a Jeol 100CX II electron microscope.

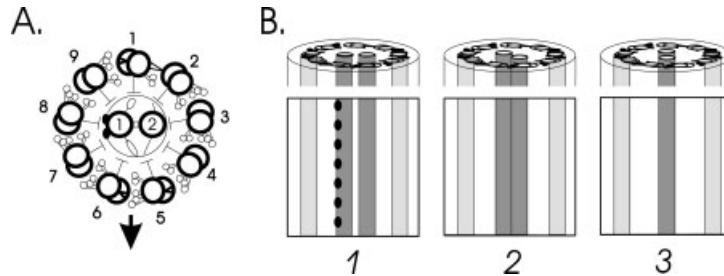


Fig. 1. Diagram of structural landmarks used in this report. **A:** Features in cross-sectional images that provide orientation markers include modifications to doublets 1, 5, and 6, absence of outer row dyneins on doublet 1, and asymmetric projections from the two central pair microtubules. Doublets are numbered around the outside, central pair microtubules in the center of each tubule. *Arrow* indicates the direction of the principal bend; *small dark ovals* represent structures

with a known 32-nm repeat interval along the edge of C1. **B:** In longitudinal images, the central pair microtubules appear as one of three easily distinguished orientations, parallel to the section (1), 3/4 view (2), and perpendicular to the section (3). In parallel views, C1 can sometimes be distinguished from C2 by short projections (dark ovals) that repeat at 32 nm along the outer margin of C1.

RESULTS

Forward-Swimming (Asymmetric) Waveforms and Doublet Orientation

Chlamydomonas reinhardtii swim with two flagella that beat with near mirror-image symmetry. Each flagellum is fixed in orientation by its association with basal bodies that form extensive attachments to each other, to a specialized microtubule cytoskeleton, and to other cytoplasmic anchoring structures [Ringo, 1967]. This orientation can be recognized in flagellar cross-sections based on distinguishing morphological landmarks associated with specific doublets [Hoops and Witman, 1983]. By convention, doublet number one of each flagellum is the doublet that lacks outer row dyneins and always faces toward the midline of the cell. Doublets five and six face in the effective stroke principal bend direction (Fig. 1A). To determine central pair complex orientation relative to flagellar shape, we examined thin sections that passed longitudinally through the cell body and one or both flagella of cells that were fixed while swimming. Three central pair microtubule orientations could be routinely recognized in these images (Fig. 1B): parallel to the bend plane, perpendicular to the bend plane, or intermediate (3/4 view). In about a third (9 out of 28) of the images with parallel orientations, projections from the lateral margin of the C1 central pair microtubule, which repeat at a 32-nm interval [Mitchell, 2003], provided a marker to determine absolute central pair orientation. In the remaining images, features that unequivocally distinguished C1 from C2 were not evident.

Radial spokes in *Chlamydomonas* project from each doublet in pairs with a 32-nm spacing between members of each pair, and a 64-nm spacing between pairs [Goodenough and Heuser, 1985]. We believe the densities spaced at 32 nm are contributed by C1 central pair-associated structures rather than radial spokes for

several reasons. First, because radial spokes from one doublet occur in pairs, they would not be expected to generate a continuous row of particles with 32-nm spacing. However, Goodenough and Heuser also determined that spokes on adjacent doublets are offset by 32 nm so that if two rows of spokes were imaged in one section, they might combine to form a single row of densities with a 32-nm period. Because spoke heads from adjacent rows of spokes are closer together on the surface of the central pair complex than the thickness of the typical approximately 80-nm thin section used for this study, we might expect some images to contain densities with 32-nm spacing that were generated by spoke heads from two rows of spokes. In that case, every other density would be reinforced, which is not a characteristic of the densities that we associate here with the C1 central pair microtubule. In addition, a row of densities produced by radial spokes would continue as long as the doublets remained parallel to the section plane, regardless of central pair orientation. The densities that we consider diagnostic of C1 instead are only visible for limited regions in which the central pair itself is parallel to the section plane, and are not seen in regions where the central pair twists into other orientations even though the doublet microtubules continue in the same plane.

Central Pair Orientation in Principal Bends of the Effective Stroke

To assure that images of fixed cells were representative of *in vivo* waveforms, thin sections were compared to images of living cells. As illustrated in Figure 2A, forward swimming in *Chlamydomonas* is characterized by highly asymmetric waveforms [Brokaw and Luck, 1983]. The effective stroke is generated by formation of a principal bend at the flagellar base, with little change in flagellar shape distal to the bend. Distal propagation of



Fig. 2. Darkfield images of live, swimming cells document normal in vivo waveforms during forward (A) and reverse (B) swimming patterns. The cell in B is a *unil* mutant and has only one flagellum. Waveforms during forward swimming are highly asymmetric, whereas those during reversals are symmetric. Visibility of the complete length of a flagellum in one focal plane, regardless of the phase of beat, indicates that waveforms are essentially planar. Arrows in A indicate principal (p) and reverse (r) bends. Images are printed in negative contrast. Bar = 5 μm .

this principal bend constitutes the recovery stroke. Shallow reverse bends are sometimes evident distal to the principal bend at the end of an effective stroke, and in early stages of recovery strokes. During backward swimming, an avoidance response in *Chlamydomonas*, flagella beat essentially symmetrically by generating principal bends and reverse bends of similar size and curvature (Fig. 2B). The two flagella on a cell do not collide with each other, even during backward swimming, because their basal bodies are slightly offset from each other [Ringo, 1967] and they therefore do not beat in the same plane. As a consequence, individual thin sections rarely pass through the midline of both flagella.

When cells were fixed during an effective stroke, the entire length of a flagellum sometimes appeared in one section, along with the cell and basal body, confirming the planarity of this stage of the *Chlamydomonas* waveform. In sections that revealed the central pair microtubules, as in Figure 3, both central pair microtubules were parallel to the bend plane (side by side) throughout the bend. This orientation is the same as that seen in bent regions of *Paramecium* cilia [Omoto and Kung, 1980], and is opposite to that in metazoan cilia [Gibbons, 1961] and flagella [Sale, 1986].

Central Pair Orientation in Principal Bends of the Recovery Stroke

Sections of cells fixed during the recovery phase were encountered more often than those in the effective stroke of the bend cycle, most likely because of the relatively slower shear rate during the recovery phase [Brokaw and Luck, 1983]. A total of 16 cells fixed during the recovery phase were analyzed for central pair orientation within the principal bend, as opposed to only 3 judged to be fixed during the effective stroke. In every case, the central pair microtubules were parallel to the bend plane within each bend (Fig. 4). These images were further analyzed to determine whether the C1 or C2 microtubule was consistently on the inside of the curve, closest to doublets 5–6, or the outside, closest to doublet

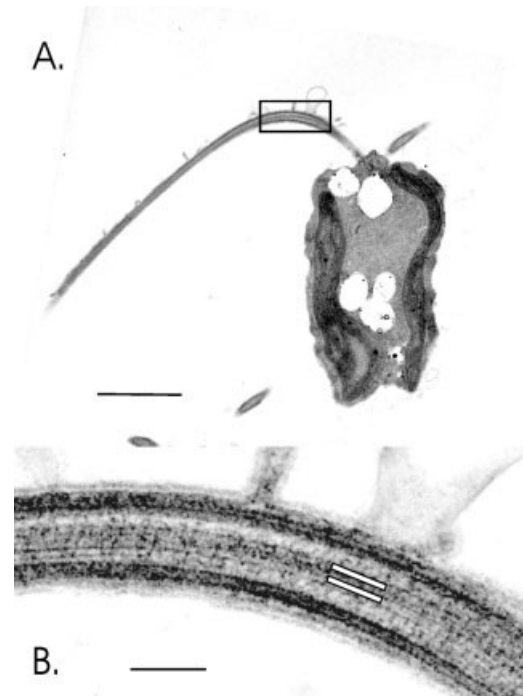


Fig. 3. Central pair orientation in an effective stroke principal bend. A low-magnification image (A) shows the location and overall orientation of the enlarged region (B). This cell is at a similar stage in the beat cycle as the third image in Figure 2A, except that no reverse bend is evident in this example. Central pair orientation is parallel to the bend plane throughout the bend, as highlighted by parallel white rectangles in B. Bar = 5 μm in A, 0.2 μm in B.

one. In all cases where the 32 nm C1-associated repeat was visible (8 of the 16 cells analyzed), it lay along the edge of the outer central pair microtubule (arrows in Fig. 4B,D,F). The central pair, therefore, maintains a fixed orientation in bends, with C1 closest to doublet one.

In favorable sections, central pair orientation could be followed into straight regions proximal or distal to bends. In both directions, the central pair complex gradually twists. Twists distal to the principal bend were not only observed in cells fixed early in the recovery stroke (Fig. 5), but also in one cell fixed at the end of the effective stroke and several cells captured midway through the recovery stroke (not shown). As seen in Figure 5, regions distal to principal bends have twists of as much as 180° when a shallow reverse bend is present. Twist handedness could not be established in these images but several examples supported a continuous, unidirectional twist. The central pair did not twist by 90° and then untwist back to a parallel orientation, but rather twisted through a full 180° between principal and reverse bends. As a consequence, the C1 microtubule resided on the outside of reverse as well as principal bends in these forward-swimming cells.

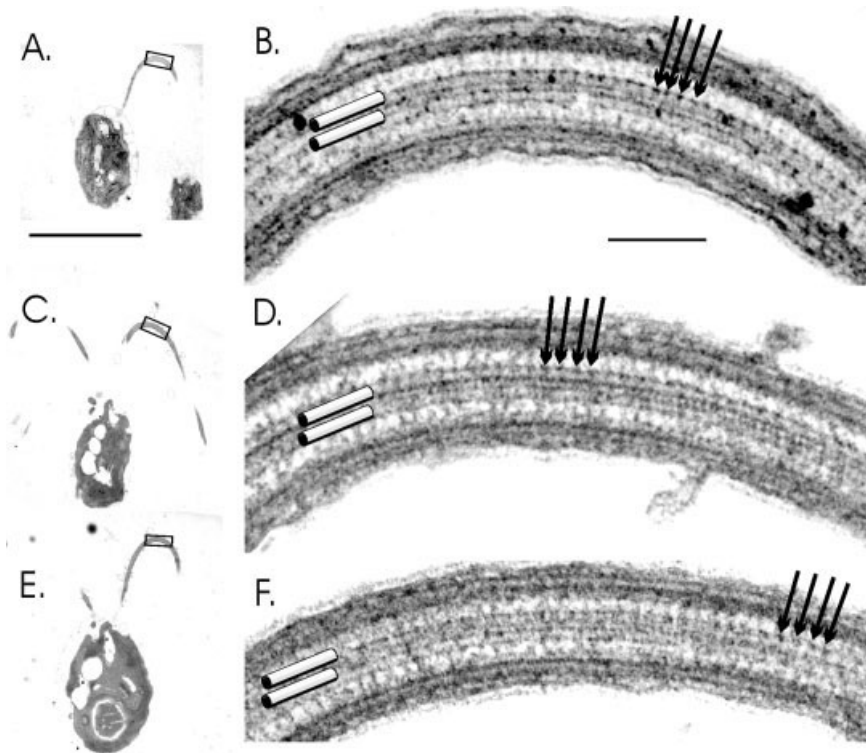


Fig. 4. Central pair orientation remains parallel to the bend plane as the principal bend propagates during the recovery stroke. **A,C,E:** Low-magnification views of three cells fixed during forward swimming show the regions selected for analysis of central pair orientation. All three cells are in early to middle stages of the recovery stroke. **B,D,F:** Enlargements show the parallel orientation of central pair microtubules throughout each bend. *Arrows* indicate a prominent density with a 32-nm repeat period associated with the outer central pair microtubule. Bar = 5 μm in A, C, and E, 0.2 μm in B, D, and F.

The cell shown in Figure 6 was fixed late during a recovery stroke, and central pair orientation can be determined both within the principal bend and in part of the straight segment proximal to the bend. Orientation changes gradually from a 3/4 view in the most proximal region exposed in this thin section, to a parallel view throughout the bend, i.e., the total twist visible in this section is less than 90° . Proximal straight regions with twists greater than 90° were not seen in the samples available for this study.

One possible cause of a twist in the central pair would be a twist in the entire flagellum [Gibbons, 1975]. However, although we did not routinely collect serial sections, adjacent sections were available for several cells in which central pair twists could be seen, and in every case the surrounding outer doublets remained untwisted and could be followed continuously as parallel lines through both curved and straight regions.

Reverse-Swimming (Symmetric) Waveforms

Although phase contrast examination of fixed cell populations confirmed that fixation after photoshock preserved many cells with backward swimming waveforms, orientation of those fixed cells was rarely optimal for obtaining thin sections in the flagellar bend plane. However, several examples were obtained in which central pair orientation could be scored in at least one principal or reverse bend, and in every case the central pair com-

plex was parallel to the bend plane in curved regions (Fig. 7). Similar to the case with forward swimming cells, the central pair complex in flagella of backward swimming cells twists in interbend regions between principal and reverse bends (Fig. 8) so that the same central pair microtubule faces the inside of the curve in both bends. None of our examples of backward swimming cells provided unequivocal identification of C1 as opposed to C2, so that an absolute central pair complex orientation could not be determined.

DISCUSSION

Our results provide the first direct confirmation that conclusions based on prior analysis of *Paramecium* cilia are broadly significant for developing models of eukaryotic ciliary and flagellar motility. We show that central pair complex orientation in *Chlamydomonas* is also parallel to the bend plane in bent regions, and twists both proximal and distal to bends. In addition, we determined that the absolute orientation of the central pair complex in principal bends is always the same, with the C1 microtubule facing doublet number one along the outer edge of each bend (summarized in Fig. 9), and that the same relative orientation occurs in reverse bends as a consequence of twists of 180° between each principal and reverse bend. Although we did not identify an absolute central pair orientation in principal bends of flagella

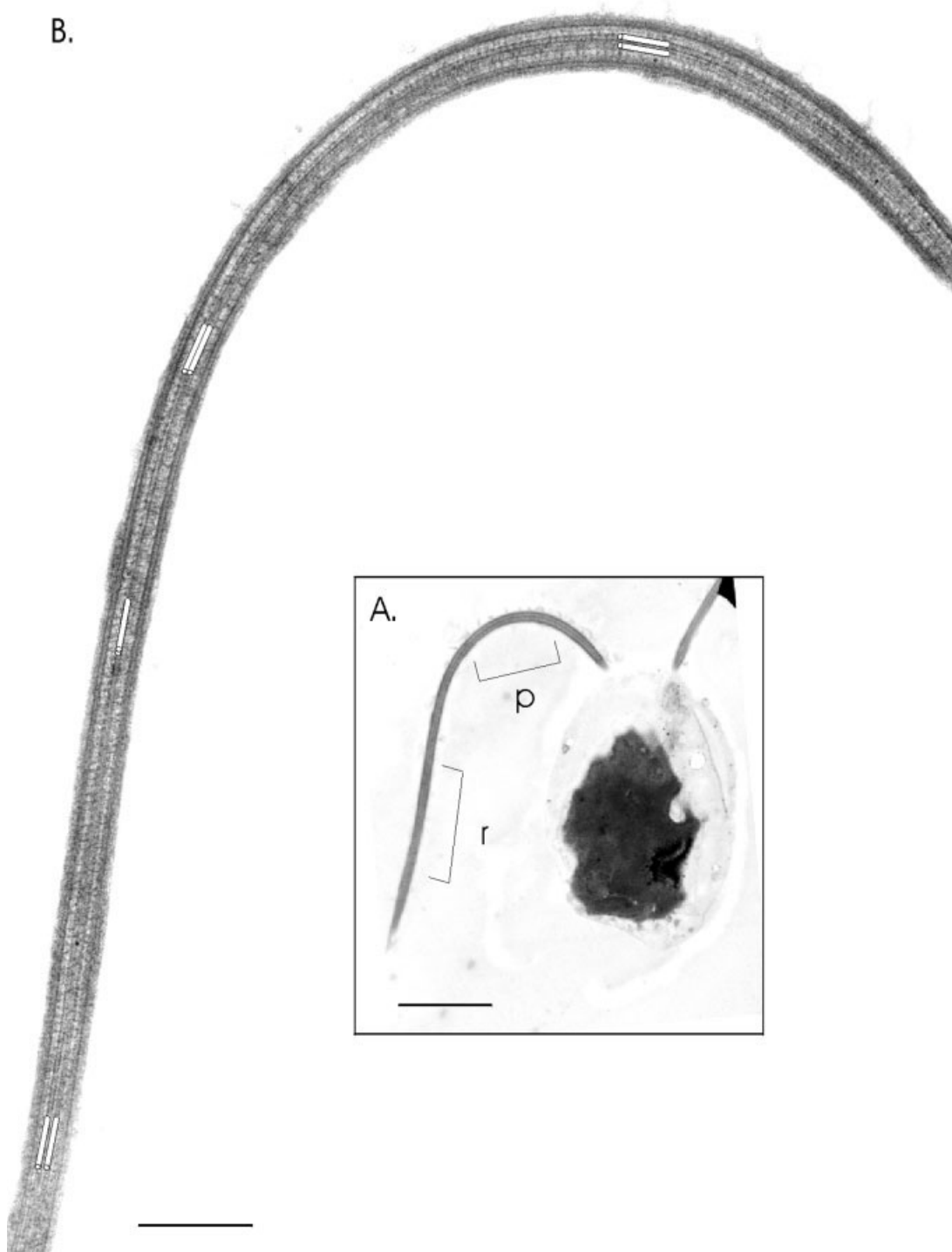


Fig. 5. The central pair complex twists between bends. **A:** Low-magnification image shows that this flagellum was fixed early during a recovery stroke, preserving a prominent principal bend (p) and a shallow reverse bend (r). **B:** Higher magnification reveals that central pair orientation is parallel to the bend plane within the principal bend, then gradually twists through 180° until once again parallel to the bend plane within the reverse bend. Bar = $2\ \mu\text{m}$ in A, $0.5\ \mu\text{m}$ in B.

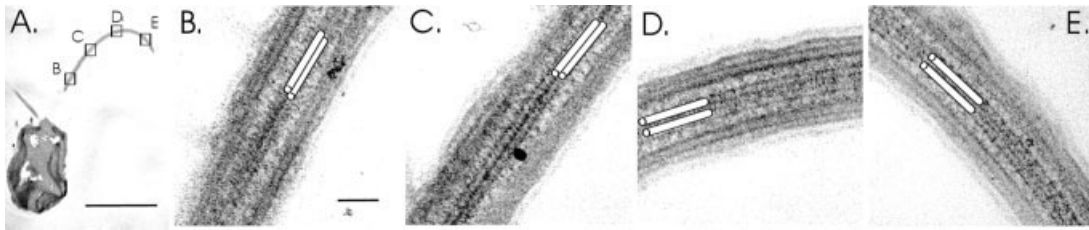


Fig. 6. The central pair complex twists in straight regions proximal to bends. **A**: Low-magnification view shows that this flagellum was fixed late in the recovery stroke. The central pair complex appears in 3/4 view in proximal regions (**B**, **C**) and twists gradually until parallel to the bend plane in the principal bend (**D**, **E**). Bar = 5 μm in **A**, 0.1 μm in **B**–**E**.

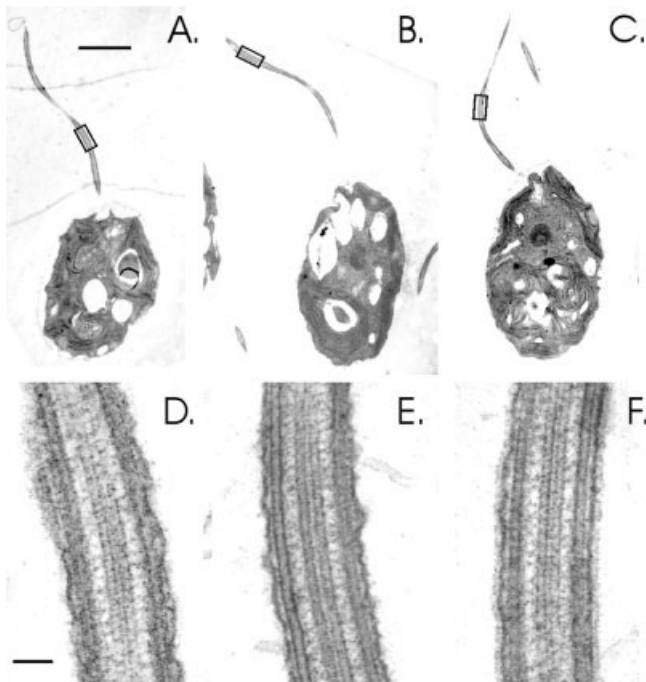


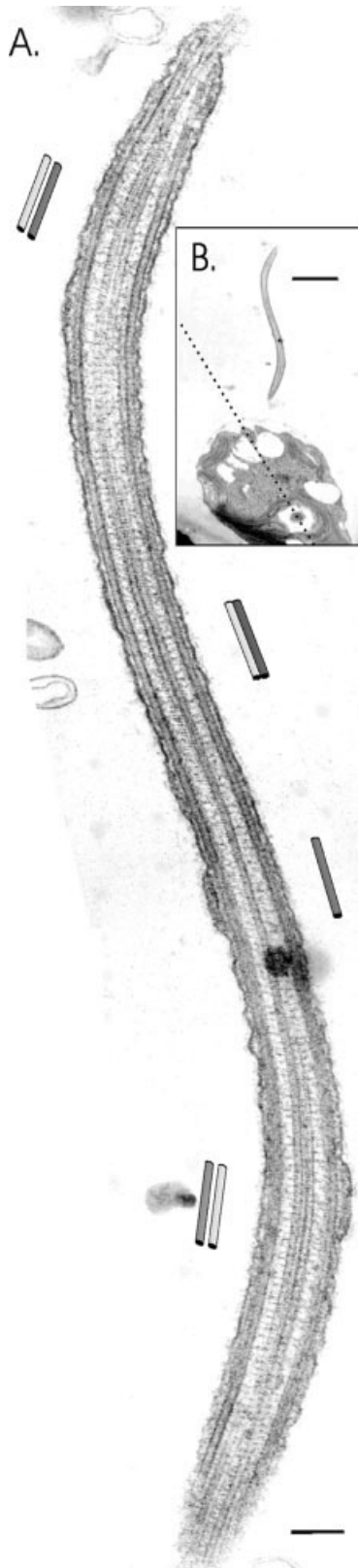
Fig. 7. Symmetrical waveforms are preserved in cells fixed after a photoshock. **A**–**C**: Low-magnification views show the portion of each flagellum enlarged to reveal central pair complex orientations (**D**–**F**). CP orientation is parallel to the bend plane in both principal bends (**D**) and reverse bends (**E**, **F**). Bar = 2 μm in **A**–**C**, 0.1 μm in **D**–**F**.

fixed during the effective stroke, C1 is likely to face doublet number one during formation as well as propagation of principal bends as it would be difficult to accommodate an additional 180° twist to the central pair at the time that principal bend propagation begins, especially as the principal bend may begin to propagate before it has attained maximum curvature [Brokaw and Luck, 1983].

The observed tight correlation between central pair orientation and bend direction supports models in which a direct link exists between dynein activation and interaction of adjacent radial spokes with specific central pair projections, but falls short of the information needed to

establish a causal relationship. Central pair orientation may influence dynein activation patterns (and thereby regulate beat parameters), but it is equally possible that central pair orientation is itself determined by regional dynein activation patterns. These are not mutually exclusive possibilities. A central pair orientation generated in response to a standard dynein activation pattern could provide precise alignment of specific central pair complex projections with subsets of radial spokes. Signals received by the central pair complex, such as changes in calcium ion concentration, could then be efficiently transferred to a unique set of dynein motors in order to modulate beat frequency and/or waveform. Details of this mechanism must differ between organisms in which a rotating central pair is parallel to the bend plane, as in *Paramecium* [Omoto and Kung, 1980] and *Chlamydomonas* (this study), and perpendicular to the bend plane, as in *Opalina* [Tamm and Horridge, 1970].

Precise regulation of central pair complex orientation in *Chlamydomonas* could occur through several possible mechanisms, but some features are common to all of them. First, a twisting force at the proximal end of the central pair complex could induce central pair twist in straight proximal regions of the flagellum. This active force could be distributed along the entire straight distal segment of the flagellum, but is unlikely to be localized to the very distal end of the central pair [Omoto et al., 1999]. No specialized structural modifications have been reported for the distal end of the central pair in *Chlamydomonas* [Jarvik and Suhan, 1991], and rotation continues after partial central pair extrusion [Kamiya, 1982]. During proximal bend formation, a constraint could prevent accumulation of additional force-generated twist and lead to the parallel central pair orientation seen throughout bends. This constraint could take the form of a temporary and localized cessation of the twisting force, a physical impediment to further twisting, or activation of a counteracting twisting force in the opposing direction. Once bend formation stops and bend propagation begins, a new straight axonemal region will form at the flagellar base and allow further active central pair twist-



ing to proceed. Formation of a reverse bend would coincide with twists of 180° , formation of a new principal bend with a full 360° twist.

Alternatively, if the central pair were an inherently twisted structure, then a twisting force need not exist. Unconstrained proximal segments would form twists passively. Development of each new bend at the base of a flagellum would incrementally straighten (untwist) a proximal segment of the central pair and over-twist the distal inter-bend region. Electron microscopic studies show no passive twisting of the central pair in axonemes that have undergone active sliding [Wargo and Smith, 2003], or in intact isolated axonemes fixed in the absence of ATP (D.R.M., unpublished observations), but a more careful evaluation of whether the central pair complex twists over extensive lengths in quiescent, straight flagella is warranted. After extrusion from axonemes, isolated *Chlamydomonas* central pair complexes do tend to curve such that the C1 microtubule always forms the outer edge of the curve (similar to the curves seen in bent regions of rapidly fixed flagella as reported here) and to take the form of left-handed helices [Kamiya et al., 1982]. Straightening of such a helix would introduce twists, as anyone knows who has attempted to pull straight a coiled garden hose. This tendency to form a helix with the C1 microtubule outermost extends to central pair complexes from *Tetrahymena* cilia (D.R.M., unpublished observations), and is thus likely to be true of related ciliates such as *Paramecium* in which central pair rotation and twist have been documented. Serial thin sections and stereo images of thick sections revealed that twists in the *Paramecium* central pair complex are left-handed in vivo [Omoto and Kung, 1980]. We were unable to determine the handedness of central pair twist in our longitudinal thin section images, but hypothesize that the *Chlamydomonas* central pair also has a left-handed twist. In *Chlamydomonas*, as in *Paramecium* and *Micromonas*, the central pair rotates in a counter-clockwise (CCW) fashion as viewed from outside the cell [Omoto et al., 1999]. If the central pair maintains a fixed orientation within each bend, but twists between bends, then bend propagation will induce rotation of the central pair. A right-handed twist in interbend regions would lead to CW rotation, a left-handed twist to the CCW rotation observed in *Chlamydomonas*.

Fig. 8. Longitudinal thin section through the flagellum of a photo-shocked cell reveals central pair twist in interbend regions (A). Central pair orientation changes by 180° between principal and reverse bends, as indicated by the cartoons in which one CP microtubule is shaded. Based on the location of this flagellum relative to a midline through cellular landmarks (pyrenoid, nucleus, and vacuoles, marked by a dashed line in B), the principal bend is the more distal of the two bends visible on this flagellum. Bar = $0.2 \mu\text{m}$ in A, $2 \mu\text{m}$ in B.

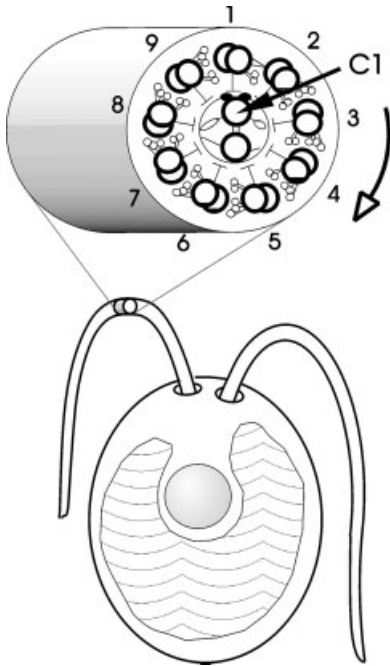


Fig. 9. Cartoon of the relationship between doublet position and central pair orientation in a principal bend during the recovery stroke. The central pair is depicted as parallel to the bend plane within the bend. Two structures on the C1 microtubule (black ovals) that repeat at 32 nm were used to identify C1 in longitudinal thin sections. A curved arrow indicates the direction of central pair rotation, as reported by others (CW as viewed from inside the cell) [Kamiya et al., 1982].

Tilted configurations of radial spokes in curved segments of gill cilia support models of dynein regulation in which radial spoke-central pair interactions report accumulated sliding displacement [Warner and Satir, 1974]. In *Chlamydomonas*, maximal sliding displacement (and therefore maximal expected spoke tilt) occurs in developing principal bends between doublet one and the C1 microtubule, and between doublets 5–6 and the C2 microtubule. Maximal displacement continues distal to the bend, but the central pair rotates to present other surface structures to these potentially tilted spokes. Preliminary examination of our preparations has not revealed spoke tilt within bends, perhaps reflecting a difference imposed by the 90° difference in central pair orientation between *Chlamydomonas* flagella and mussel gill cilia. Our fixation and staining methods were not optimized for spoke analysis, but preliminary observations suggest that, in *Chlamydomonas* flagella, spoke tilt may accumulate in straight segments distal to bends, rather than within bends (see Fig. 8 for an example).

Electron microscopy of *Chlamydomonas* axonemes that had been induced to slide with ATP and protease treatment showed that the C1 microtubule was usually oriented toward the gap where doublets had been dis-

placed by sliding, and led to a model in which central pair orientation, and more specifically the C1 microtubule, determine regions of active sliding [Wargo and Smith, 2003]. It should be evident from structural considerations, however, that the central pair orientation reported here does not fit well with some aspects of that model. Dynein activity during principal bend formation should be limited to dyneins along doublets 2-3-4 in the region of the developing bend (perhaps limited to a small segment at the leading edge of the bend) [Sale, 1986]. When the bend reaches its maximum size and bend propagation begins (recovery stroke), this pattern should switch to dyneins along doublets 6-7-8. As summarized in Figure 9, the C1 microtubule interacts with spokes projecting from doublet 1, rather than doublets 2-3-4 or doublets 6-7-8, in bent regions, and thus does not point toward regions of expected dynein activity. If central pair rotation is an active process dependent on interaction between radial spokes and the C1 microtubule, and can continue in the absence of bend formation, then a gap in the cage of doublets produced by sliding in Wargo and Smith's experiments could prevent further rotation, and would generate the central pair orientations seen in that study but would not reveal a specific relationship between central pair orientation and dynein activation. If central pair rotation is a passive response to bend propagation, then other explanations must be sought for the apparent correlation between the C1 microtubule and active sliding in Wargo and Smith's data, and between the C1 microtubule and inactive doublets 1 (principal bends) or 5–6 (reverse bends) in beating flagella as reported here.

Chlamydomonas is the best-characterized model system in which flagellar motility is approachable with genetic and molecular tools [Mitchell, 2000; Silflow and Lefebvre, 2001], but are results obtained in this system, where the central pair rotates during the beat cycle, applicable to an understanding of metazoan flagella whose central pair complex maintains a fixed orientation? Although the fundamental reasons for this dichotomy between a fixed vs. a rotating central pair remain unresolved, rotation is most common among organelles that show the greatest variability in beat parameters. Flagellar motility in *Chlamydomonas* fits this pattern as it must be highly regulated to provide the organism with phototactic, chemotactic, and avoidance responses, and to shut motility on and off completely as cells switch from swimming in a liquid to gliding along a surface (air/water or solid/water interface). Because organelles with rotating central pair complexes have likely retained a regulatory mechanism that evolved early in the history of eukaryotes, and was subsequently modified in some metazoan lineages, the *Chlamydomonas* flagellum should

remain a useful example of the entire range of regulation available in this organelle.

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