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# Regulation of Eukaryotic Flagellar Motility

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**Abstract.** The central apparatus is essential for normal eukaryotic flagellar bend propagation as evidenced by the paralysis associated with mutations that prevent central pair (CP) assembly. Interactions between doublet-associated radial spokes and CP projections are thought to modulate spoke-regulated protein kinases and phosphatases on outer doublets, and these enzymes in turn modulate dynein activity. To better understand CP control mechanisms, we determined the three-dimensional structure of the *Chlamydomonas reinhardtii* CP complex and analyzed CP orientation during formation and propagation of flagellar bending waves. We show that a single CP microtubule, C1, is near the outermost doublet in curved regions of the flagellum, and this orientation is maintained by twists between successive principal and reverse bends. The *Chlamydomonas* CP is inherently twisted; twists are not induced by bend formation, and do not depend on forces or signals transmitted through spoke-central pair interactions. We hypothesize that CP orientation passively responds to bend formation, and that bend propagation drives rotation of the CP and maintains a constant CP orientation in bends, which in turn permits signal transduction between specific CP projections and specific doublet-associated dyneins through radial spokes. The central pair kinesin, Klp1, although essential for normal motility, is therefore not the motor that drives CP rotation. The CP also acts as a scaffold for enzymes that maintain normal intraflagellar ATP concentration.

**Keywords:** Central Pair, Cilia, Radial Spoke, Chlamydomonas, Flagella.
**PACS:** 87.16.Ka; 87.16.Nn; 87.17.Jj

## INTRODUCTION

As motile organelles, cilia and flagella are highly conserved across most eukaryotic phyla, with only minor structural variations seen between ciliates and humans [*1*]. They are important organelles during the life cycle of such human parasites as *Plasmodium, Giardia*, *Trypanosoma*, and *Leishmania.* In vertebrates, ciliated epithelia provide essential transport functions in respiratory airways, brain ventricles, and fallopian tubes, and power spermatozoan motility. Defects in ciliary motility cause a high incidence of upper respiratory infections, and may be linked to hydrocephaly. Ciliary motility in the early embryo is also essential for normal left-right asymmetry during organogenesis [*2*]. The absence of sperm motility causes sterility in males, and recent evidence shows that regulation of sperm motility (capacitation and chemotactic attraction to the egg) is also important for successful transmission of the germ line in mammals [*3*]. Thus regulation of ciliary and flagellar motility has broad significance for eukaryotic organisms.

In mammalian systems, ciliary motility regulation is manifested as an increase in beat frequency to increase mucociliary clearance rates, and as changes in both

frequency and waveform of sperm flagella during activation, capacitation, and chemotaxis. In the organism used most extensively for both genetic and biochemical dissection of flagellar motility, the single-celled alga *Chlamydomonas reinhardtii*, regulation of beat frequency, changes in waveform, and reversible quiescence are normal physiologic responses to environmental variables such as light intensity. Through the analysis of mutations that disrupt flagellar structures we and others have developed a detailed understanding of the *Chlamydomonas* flagellar structure and the contribution of many structural elements, including the central pair, to motility [*4-8*]. In this review we focus on recent advances in our understanding of the regulation of ciliary and flagellar motility by the central pair apparatus.

## CENTRAL PAIR REGULATION

Although central pair microtubules are not part of the oscillatory bending mechanism and are even missing from some simplified "9+0" motile organelles, they contribute an essential regulatory function in typical cilia and flagella. Mutations that block central pair assembly in *Chlamydomonas* cause a complete lack of flagellar motility, and constitute a subset of paralyzed flagella (*pf*) mutations in the alga [*9-11*]. Human mutations that disrupt central pair structures, like those that disrupt dyneins, are associated with upper respiratory infections (due to lack of mucociliary clearance) and infertility (due to immotile sperm) [*12-14*], and mice with central pair defects have also been diagnosed with hydrocephaly from lack of brain ventricle ciliary activity [*15*].

### Central Pair-Radial Spoke Interactions

Hypotheses about the mechanism of central pair regulation have focused on kinase-phosphatase cascades that require radial spokes and that regulate dynein-dependent microtubule sliding rates in simplified flagellar models (protease-treated axonemes) [*16-18*]. Based on these hypotheses dyneins are inhibited by the activity of a kinase or kinases associated with outer doublet microtubules, and activated when protein phosphatases dominate and kinase affects are reversed [*5*]. Each of the nine outer doublet microtubules has a row of radial spokes that are arranged as pairs within a 96 nm repeat. The two spokes in each pair are uniquely positioned relative to inner row dyneins and to a dynein regulatory complex [*19*]. In thin sections of rapidly fixed, actively beating cilia, radial spokes appear to attach to central pair projections and tilt as a result of dynein-induced sliding displacement of the doublet microtubules [*20*]. Because radial spokes are associated with all nine outer doublets, but dynein activity must be limited to a subset of doublets at any one location along a flagellum, specific rows of central pair projections are thought to interact with overlying radial spokes in a doublet-specific pattern to modulate spoke regulation of these kinases and phosphatases. Doublet microtubule sliding patterns in protease-treated *Chlamydomonas* axonemes support a model in which spokes that interact with the C1 central pair microtubule activate adjacent dyneins [*21*].

In *Chlamydomonas*, phototaxis requires the presence of inner row dynein I1, and has been linked to radial spoke-regulated phosphorylation of a 138 kD intermediate chain I1 subunit (IC138) [*22*]. Sale and colleagues have shown that IC138 phosphorylation is regulated by the activity of a cAMP-dependent protein kinase (PKA) and a PP1 isozyme, and that regulation of these enzymes in turn depends upon an intact radial spoke/central pair system [*23*]. At present, however, we still know little about the specific radial spoke proteins that interact with the central pair, or the specific central pair structures involved in those interactions. We recently completed a detailed structural analysis of the central pair [*24*] and a study of central pair orientation during the flagellar beat cycle [*25*] which formed the basis for experiments (summarized below) that clarified the relationship between dynein activity patterns and orientation of the central pair [*26*].

## Central Pair Rotation and Twist

Although general features of central pair structure have been described for organelles from several different organisms, the most detailed studies have relied upon mutational analysis of *Chlamydomonas* flagella (Fig. 1). Mutations that partially disrupted CP structure, such as *pf6* [*10*], *pf16* [*10*], and *cpc1* [*27*], together with biochemical extractions that selectively solubilized portions of the CP complex [*9*], showed that the two CP microtubules are dissimilar and that each is associated with a unique set of associated proteins. To extend these structural studies, we analyzed longitudinal thin sections, transverse thin sections, and quick-freeze, deep-etch images to build a three-dimensional view of the *Chlamydomonas* CP [*24,27*]. We concluded that all projections directly associated with the C2 microtubule have a 16 nm periodicity, while several of the C1-associated projections have a 32 nm repeat period. We also found that many of the projections seen in transverse images serve as supports for elements that form an apparently smooth cylindrical surface apposed to radial spoke heads. Departures from these smooth surfaces occur primarily along the CP microtubule surfaces closest to radial spoke heads, such as the region to which the Klp1 kinesin has been localized. The recent cloning of several central pair proteins, including PF16p, essential for stability of the C1 microtubule [*28*], and the products of the *PF6* [*29*], *PF15* [*30*], *PF20* [*31*], and *CPC1* [*32*]genes, is gradually filling the blank spaces on our CP map.

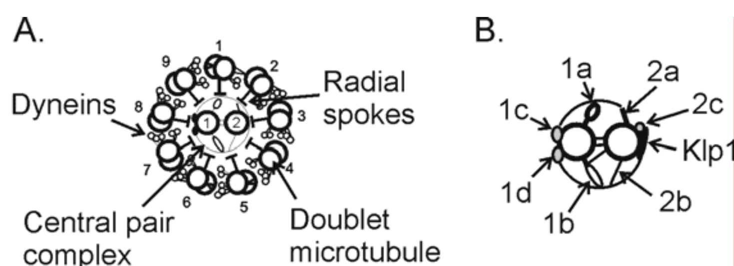


**FIGURE 1.** A, Diagram of a transverse section through a *Chlamydomonas* flagellar axoneme as viewed from inside the cell. Adapted from [*25*] by copyright permission of John Wiley and Sons, Inc. B, Diagram of the central pair apparatus with major densities labeled. The region labeled Klp1 is specifically depleted in axonemes from Klp1 knockdown strains [*33*].

## *Central Pair Kinesins*

The central pair complex rotates during the bending cycle in the cilia of some organisms [*34*], including *Chlamydomonas* [*35,36*], but retains a fixed orientation in others [*37*]. Difficulty observing and recording central pair rotation has prevented a careful analysis of this phenomenon, which likely plays a significant role in the central pair regulatory process. The only motor proteins known to be in the central pair complex itself are kinesin-like proteins [*38-40*], so if rotation is driven by spoke-central pair interaction, kinesins are the likely motors. Although most kinesins act as translocating motor proteins, the functional capacity of central pair kinesins has not been determined and as yet no mutations have been characterized that selectively disrupt their function or prevent their assembly.

Thus far only two flagellar kinesin genes have been characterized in *Chlamydomonas*, *FLA10* which encodes one heavy chain of the kinesin for intraflagellar transport (IFT) [*41-43*], and *KLP1* which encodes a kinesin that has been localized to the C2 central pair microtubule [*38*]. As an initial step toward testing hypotheses about central pair kinesin activity, we characterized the phenotype that results from RNAi knockdown of Klp1 [*33*]. Previous immunolocalization showed that Klp1 is associated with the C2 central pair microtubule, but could not further determine which C2 structures might contain Klp1. Our thin section electron micrographs show that the only structure not seen in any of the klp1 knockdown flagella was a density associated with the C2 microtubule, part of projection 2c. A second structure, projection 2b, was also depleted in isolated, demembranated knockdown axonemes. For more detailed analysis of structural changes associated with knockdown of Klp1, cross section images of wild type and knockdown axonemes were analyzed by image averaging. These images confirmed that part of 2c is the only density completely missing from knockdown axonemes, and place Klp1 precisely on the surface of C2 in a position where it could interact with radial spoke heads (Fig. 1B).

Knockdown strains swim with reduced velocity as the result of an altered flagellar motility pattern. Preliminary analysis shows a reduction in beat frequency as the primary motility defect, with a small fraction of the population completely paralyzed. Therefore, Klp1 may be essential for flagellar motility. However (as summarized below) we have now shown that central pair rotation and twist in *Chlamydomonas* do not depend on interactions between radial spoke heads and central pair projections [*26*], so the role of central pair kinesins remains completely unknown. We hypothesize that CP kinesins act as mechanochemical switches, rather than as actual motors. Much like G proteins, they could undergo conformational changes based upon the presence of ATP vs ADP at the active site that resulted not in net movement, but in a change in conformation that alters the interaction between the kinesin and another flagellar component. ATP hydrolysis rates could respond to changes in flagellar bend angle or changes in the state of a chemical signaling pathway (e.g. phosphorylation) and each conformational state could in turn correspond to a different interaction between radial spokes and central pair projections.

## *Central Pair Orientation*

In organisms with a fixed CP orientation the two central pair microtubules remain perpendicular to the bend plane, whereas one published report [*44*] suggested that, at least in bent regions, the central pair twists to become parallel to the bend plane. Resolving this puzzle, at least for *Chlamydomonas*, should help determine which central pair projections are in contact with radial spokes along active doublets, and which ones are in contact with spokes on inactive doublets, during formation and propagation of bends. To find out, we developed methods to fix actively swimming cells such that their waveforms were preserved, and then to embed and section those cells to reveal the orientation of the central pair in both straight and bent regions (Fig. 2). One tremendous advantage of the *Chlamydomonas* system for this study is the nearly planar shape of the waveform during both asymmetric, ciliary bends and symmetric, flagellar-style bends, and the added ability to induce cells to switch between these two bending modes by a simple shift in light intensity (photoshock).

Our conclusion, consistent with previous observations from other labs [*34*], is that the central pair in *Chlamydomonas* is twisted whereas surrounding doublet microtubules are not. In addition, the position of the twist propagates along with each bend, so that the *Chlamydomonas* central pair is always parallel to the bend plane within bent regions (Fig. 2B and 2D), but twists until it is perpendicular to the bend plane in straight regions (Fig. 2C) [*25*]. Propagation of a twist can account for the appearance of central pair rotation during each beat cycle. This rotation mechanism has interesting consequences for hypotheses about central pair-radial spoke interactions.

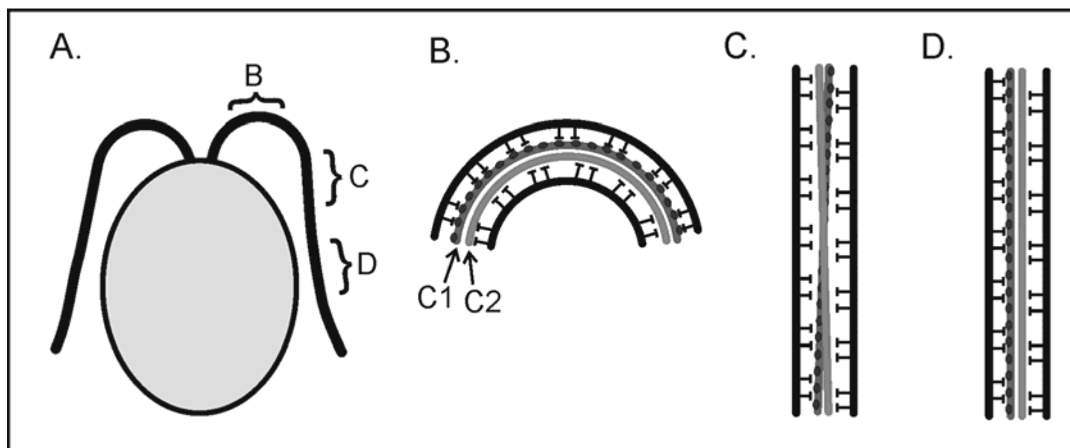


**FIGURE 2.** A, diagram of a *Chlamydomonas* cell during forward swimming. Brackets indicate the principal bend (B), the interbend region (C) and the reverse bend (D) and correspond to the three diagrams. B, CP orientation seen in thin sections through principal bends. The C1 CP microtubule is distinguished by a row of projections (dark ovals) repeating every 32 nm. C, central pair twist in an interbend region. D, CP orientation in a reverse bend region.

### *Forces Generating Central Pair Rotation and Twist*

We recently determined that the central pair is inherently twisted and that neither twist nor bend-specific orientation depend on central pair-radial spoke interactions [*26*]. These conclusions are based on two types of experiments. First, quiescent (non-beating), straight flagella adhering to a flat surface (a coverglass) still have twists in their CPs, whether the cells assemble wild type radial spokes, or radial spokes that lack spoke heads. Second, when a mutation that blocks spoke head assembly is combined with a suppressor mutation that permits motility (bend propagation) in the absence of spoke heads, CP orientation in bent and straight regions follows the same pattern as seen in wild type flagella. This leads to the conclusion that CP orientation conforms to the bend, regardless of the bend plane. We hypothesize that central pair function evolved to regulate beat frequency and waveform independently of effective stroke orientation [*1,26*]. According to this hypothesis, in organelles that need to change effective stroke direction, the CP can passively re-orient to the beat direction. In organelles that always beat in the same plane, evolutionary selection for a system with fewer moving parts has resulted in a CP that maintains a fixed orientation, perhaps through semi-permanent attachments of modified spokes on opposite sides of the axoneme (doublets 3 and 8) to each CP microtubule.

## CONCLUSIONS

In the single-celled alga *Chlamydomonas reinhardtii*, flagella beat with a nearly planar waveform and with a central apparatus that twists, although the surrounding doublet microtubules do not. We conclude that twist results from an underlying helical shape of the central apparatus, and that the orientation of the central pair within *Chlamydomonas* flagella depends upon the formation and propagation of bends. This orientation is independent of interactions between radial spoke heads and central pair projections, and therefore central pair rotation is not driven by these interactions but rather by the propagation of bends through forces generated by doublet-associated dynein motors. Central pair kinesins are important for normal motility, but cannot be essential motors for central pair rotation.

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## REFERENCES

1. D. R. Mitchell, *Biol. Cell* **96**, 691-696 (2004).
2. S. Nonaka, Y. Tanaka, Y. Okada, S. Takeda, A. Harada, Y. Kanai, M. Kido, and N. Hirokawa, *Cell* **95**, 829-837 (1998).
3. M. Eisenbach, *Dev Genet* **25**, 87-94 (1999).
4. D. R. Mitchell, *J. Phycol.* **36**, 261-273 (2000).

5. M. E. Porter and W. S. Sale, *J. Cell Biol.* **151**, F37-F42 (2000).
6. E. F. Smith and P. Yang, *Cell Motil. Cytoskeleton* **57**, 8-17 (2004).
7. M. E. Porter, *Curr. Opin. Cell Biol.* **8**, 10-17 (1996).
8. S. K. Dutcher, *Trends Genet.* **11**, 398-404 (1995).
9. G. M. W. Adams, B. Huang, G. Piperno, and D. J. L. Luck, *J. Cell Biol.* **91**, 69-76 (1981).
10. S. K. Dutcher, B. Huang, and D. J. L. Luck, *J. Cell Biol.* **98**, 229-236 (1984).
11. G. B. Witman, J. Plummer, and G. Sander, *J. Cell Biol.* **76**, 729-747 (1978).
12. C. Chapelin, A. Coste, P. Reinert, M. Boucherat, M. C. Millepied, F. Poron, and E. Escudier, *Ann. Otol. Rhinol. Laryngol.* **106**, 854-858 (1997).
13. H. Okada, H. Fujioka, N. Tatsumi, M. Fujisawa, K. Gohji, S. Arakawa, H. Kato, S. I. Kobayashi, S. Isojima, and S. Kamidono, *Hum. Reprod.* **14**, 110-113 (1999).
14. B. Baccetti, A. G. Burrini, A. Maver, V. Pallini, and T. Renieri, *Andrologia* **11**, 437-443 (1979).
15. R. Sapiro, I. Kostetskii, P. Olds-Clarke, G. L. Gerton, G. L. Radice, and I. I. I. Strauss, *Mol Cell Biol* **22**, 6298-6305 (2002).
16. A. R. Gaillard, D. R. Diener, J. L. Rosenbaum, and W. S. Sale, *J. Cell Biol.* **153**, 443-448 (2001).
17. E. F. Smith and W. S. Sale, *Science* **257**, 1557-1559 (1992).
18. P. Yang, D. R. Diener, J. L. Rosenbaum, and W. S. Sale, *J Cell Biol* **153**, 1315-1326 (2001).
19. L. C. Gardner, E. O'Toole, C. A. Perrone, T. Giddings, and M. E. Porter, *J. Cell Biol.* **127**, 1311-1325 (1994).
20. F. D. Warner and P. Satir, *J. Cell Biol.* **63**, 35-63 (1974).
21. M. J. Wargo and E. F. Smith, *Proc. Natl. Acad. Sci. USA* **100**, 137-142 (2003).
22. S. J. King and S. K. Dutcher, *J. Cell Biol.* **136**, 177-191 (1997).
23. G. Habermacher and W. S. Sale, *J. Cell Biol.* **136**, 167-176 (1997).
24. D. R. Mitchell, *Cell Motil. Cytoskeleton* **55**, 188-199 (2003).
25. D. R. Mitchell, *Cell Motil. Cytoskeleton* **56**, 120-129 (2003).
26. D. R. Mitchell and M. Nakatsugawa, *J Cell Biol* **166**, 709-715 (2004).
27. D. R. Mitchell and W. S. Sale, *J. Cell Biol.* **144**, 293-304 (1999).
28. E. F. Smith and P. A. Lefebvre, *J. Cell Biol.* **132**, 359-370 (1996).
29. G. Rupp, E. O'Toole, and M. E. Porter, *Mol. Biol. Cell* **12**, 739-751 (2001).
30. E. E. Dymek, P. A. Lefebvre, and E. F. Smith, *Eukaryot. Cell* **3**, 870-879 (2004).
31. E. F. Smith and P. A. Lefebvre, *Mol. Biol. Cell* **8**, 455-467 (1997).
32. H. Zhang and D. R. Mitchell, *J Cell Sci* **117**, 4179-4188 (2004).
33. R. Yokoyama, E. O'Toole, S. Ghosh, and D. R. Mitchell, *Proc Natl Acad Sci U S A* **101**, 17398-17403 (2004).
34. C. K. Omoto, I. R. Gibbons, R. Kamiya, C. Shingyoji, K. Takahashi, and G. B. Witman, *Mol. Biol. Cell* **10**, 1-4 (1999).
35. R. Kamiya, *Cell Motility [Suppl. ]* **1**, 169-173 (1982).
36. H. J. Hoops and G. B. Witman, *J. Cell Biol.* **97**, 902-908 (1983).
37. S. L. Tamm and S. Tamm, *J. Cell Biol.* **89**, 495-509 (1981).
38. M. Bernstein, P. L. Beech, S. G. Katz, and J. L. Rosenbaum, *J. Cell Biol.* **125**, 1313-1326 (1994).
39. L. A. Fox, K. E. Sawin, and W. S. Sale, *J. Cell Sci.* **107**, 1545-1550 (1994).
40. K. A. Johnson, M. A. Haas, and J. L. Rosenbaum, *J. Cell Sci.* **107**, 1551-1556 (1994).
41. Z. Walther, M. Vashishtha, and J. L. Hall, *J. Cell Biol.* **126**, 175-188 (1994).
42. K. G. Kozminski, P. L. Beech, and J. L. Rosenbaum, *J. Cell Biol.* **131**, 1517-1527 (1995).
43. J. Rosenbaum, *Curr. Biol.* **12**, R125 (2002).
44. C. K. Omoto and C. Kung, *J Cell Biol* **87**, 33-46 (1980).