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Video Essay

# Rotation of the Central Pair Microtubules in Eukaryotic Flagella[V]

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## INTRODUCTION

The typical structure of the eukaryotic flagellum consists of a central pair of singlet microtubules surrounded by nine doublet microtubules, called the axoneme. Much has been discovered regarding the mechanism by which axonemes produce motion: ATP is used by dynein arms found on the A tubules of the doublet microtubules to produce shear force against the B tubules. These shear forces are then converted to bending. However, if all the dynein arms along the length of the axoneme and on all doublets attempted to produce shear simultaneously, no effective movement would result. Thus, regulation of active shear force is required. Evidence suggests that the central pair–radial spoke complex is involved in this regulation. The first evidence came from an electron micrograph study in which the central pair microtubules of *Paramecium*, "instantaneously fixed" and serially sectioned, appeared to be oriented in systematically changing angles. This was interpreted as rotation of the central pair with respect to the nine outer doublets per beat cycle (Omoto and Kung, 1979, 1980). It was suggested that the central pair may act as a "distributor" to regulate the activity of dyneins.

### *Rotation of the Central Pair Microtubules of Micromonas pusilla*

To test the hypothesis that the central pair microtubules rotate, an organism with the central pair extending well beyond the 9 + 2 region was used to try to directly visualize movement of the central pair. Such an organism is the small marine alga, *M. pusilla*. The central pair of the single flagellum of this alga extends 4–5 $\mu$m beyond an extremely short (<1 $\mu$m) 9 + 2 region. The central pair of this flagellum is similar to that of other cilia and flagella in that it is helical (Omoto and Witman, 1981). Movie 1 shows *M. pusilla* swimming with what appears to be the helical central pair rotating and pushing the cell (Figure 1A). The basal 9 + 2 region is also beating; however, it is obscured by the glare from the cell body in these dark-field images. Note that the videos were made from negatives of original dark-field cinemicrographs, so the cell body and flagella appear dark against a light background. An appearance of rotation can result from propagation of a helical wave along a nonrotating central pair. To distinguish between this and true central pair rotation, an experiment analogous to that used to demonstrate rotation of the bacterial flagellum (Silverman and Simon, 1974) was used. If the movement is true rotation rather than helical wave propagation, then a cell attached to the slide by its flagellum should rotate. Movie 2 shows such an experiment (Figure 1B); the flagellum is clearly visible and unmoving, and the cell body rotates. These images clearly and directly demonstrate that the central pair of microtubules of the 9 + 2 flagellum of *M. pusilla* rotate. The direction of central pair rotation in *M. pusilla* is clockwise as viewed from base to tip. This is the same as that for central pair rotation in *Paramecium* inferred from the electron microscopic observations.

### *Extrusion and Rotation of the Central Pair of Chlamydomonas reinhardtii*

When *Chlamydomonas* cell models are kept in the presence of 1 mM ATP, they can beat for >30 min. With time, however, the axonemes tend to disintegrate, frequently accompanied by extrusion of the central pair

[V] Online version of this essay contains video material for Figures 1–4. Online version available at www.molbiolcell.org.

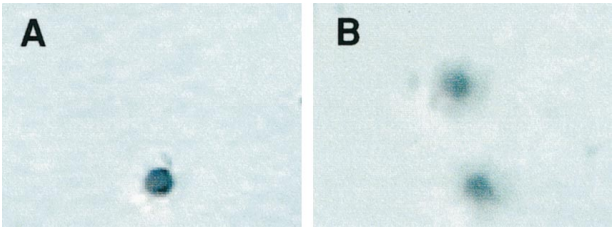


**Figure 1.** Dark-field images of *M. pusilla*, a small alga that swims using an unusual flagellum with a very short 9 + 2 region and a long extension of the central pair microtubules. These movies were originally taken on 16-mm film using dark-field optics. The negatives were converted to video format; thus the cell appears dark against a light background. (A) Movie 1 shows a free-swimming *M. pusilla* being propelled by a rotating helical central pair of microtubules (~600-kb QuickTime movie). (B) Movie 2 shows a *M. pusilla* stuck by its flagellum and the cell body rotating (~1-Mb QuickTime movie).

of microtubules (Kamiya, 1982). Movie 3 shows a demembranated cell model with one of the two flagella extruding the central pair and rotation of that central pair (Figure 2). This phenomenon is facilitated by a mild elastase treatment of axonemes to the point that >90% of the axonemes extrude the central pair (Hosokawa and Miki-Noumura, 1987). This central pair extrusion and rotation can be seen in isolated axonemes (Movies 4 and 5 [Figure 3]). The helical twist of the central pair and the direction of rotation correspond to propagation of helical waves distally. Although these videos show the movement after much of the central pair has extruded, upon initial

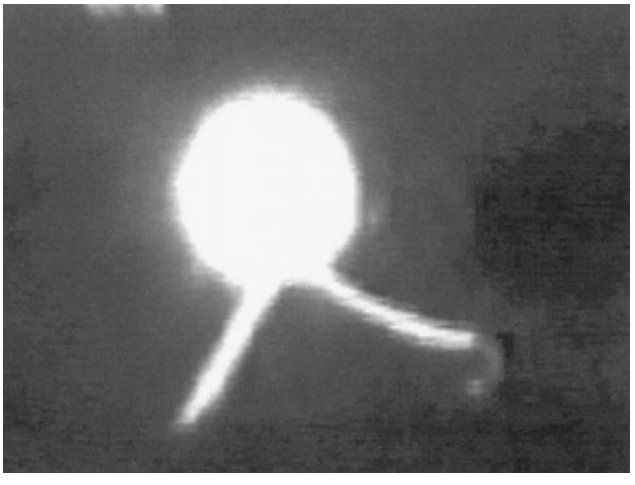

**Figure 2.** Movie 3 shows central pair rotation in a demembranated *Chlamydomonas* cell model at pCa6 with the central pair rotating and extruding. Dark-field images were taken with a silicon intensified target camera. Because a mirror was inserted between the ocular and camera, the image is a mirror image. Note that the cell body is rotated by the frictional force between the rotating central pair and the surface of the glass slide (~430-kb QuickTime movie).

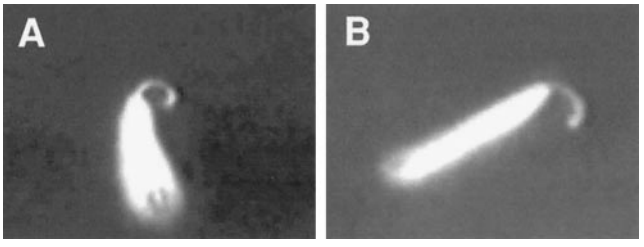


**Figure 3.** Movies 4 (A) and 5 (B) show detached *Chlamydomonas* axonemes reactivated at pCa4. Note the extrusion and rotation of the central pair microtubules (~610- and ~530-kb QuickTime movies).

extrusion of the central pair, the bend amplitude greatly decreases. This suggests that the mechanism that causes the central pair extrusion and rotation may be coupled to the mechanism of bend formation and propagation. The clockwise rotation, as viewed from the base to the tip, and the left-handed helix of the central pair are the same as those in *M. pusilla*.

Although it remains to be determined whether the central pair rotates in intact *Chlamydomonas* axonemes, electron microscopic observations of central pair orientation in the basal portion of the two axonemes suggest that the plane of the central pair is not fixed within the cylinder of nine outer doublets (Kamiya *et al*., 1982). Therefore, the central pair may rotate in beating *Chlamydomonas* flagella in vivo. Such a rotation may facilitate propagation of bending waves in the axoneme.

### *Mechanism and Force for Central Pair Rotation*

What drives the central pair rotation? Central pair rotation does not require the basal body, because central tubules can rotate and extrude out from the distal end of detached flagella, as seen above. By the same argument, it seems that the force for rotation cannot be localized at the base. There are then two general possibilities left for what drives the rotation. One is that some enzymes, possibly kinesins, which have recently been found to be associated with the central pair (Bernstein *et al*., 1994; Fox *et al*., 1994), are actively rotating the central microtubules. Alternatively, the helical central pair is passively rotated by the bending of the axoneme. The central pair free in solution takes on a helical conformation (Kamiya *et al*., 1982). When such a helical shape is confined within the cylinder of the nine doublets, the helical shape may orient itself to conform to the bend. When the bend propagates distally, the helical shape will rotate to place the helix in the lowest energy position to conform to the bend. This type of mechanism is consistent with the left-handed helix of the central pair and the clockwise rotation of the central pair as viewed from the base. It is also consistent with the following observation in

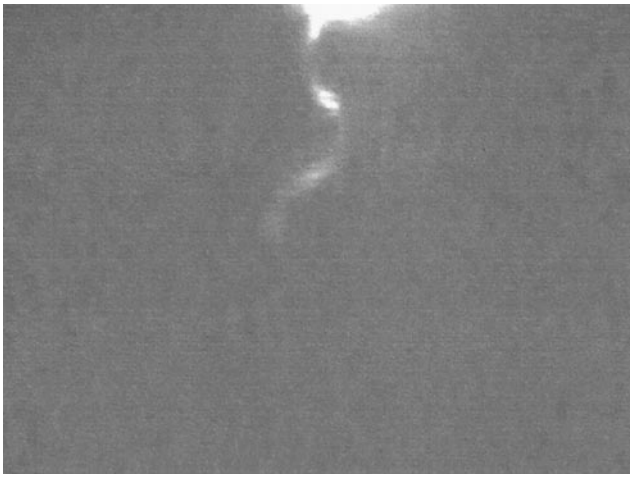

**Figure 4.** Movie 6 shows a sea urchin *Hemicentrotus pulcherrimus* sperm with its head held in the tip of a vibrating micropipette (top center) while the plane of pipette vibration is rotated. The first part (~1 s) shows the planar bending of the sperm flagellum before vibration begins. The second part (~1 min 20 s) shows the pipette tip vibrating; the bending plane of the flagellum rotates along with the plane of pipette vibration as the latter is rotated several complete revolutions. In the third part (~15 s), the vibration is stopped abruptly; the flagellar bend plane then rotates spontaneously back to its original orientation. This rotation is fast at first and slows down for the last rotations (~5.9-Mb QuickTime movie).

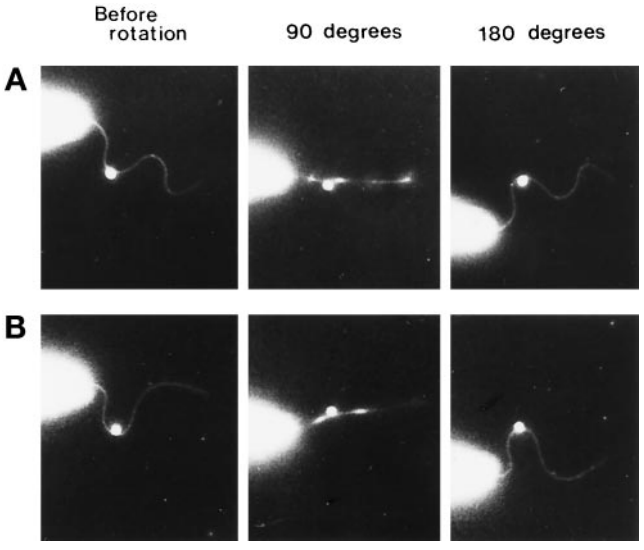


**Figure 5.** Images of demembranated sea urchin sperm with a bead attached on one side of the flagellar axoneme. Two different sperm are shown in A and B, before rotation (left panel) and after imposed vibration that rotates the bending plane 90° (middle panel) and 180° (right panel). The bead remains below or above the axoneme in A and B, respectively, regardless of the orientation of the bending plane. This demonstrates that the cage of nine doublet microtubules forming the outer cylinder of the axoneme remains untwisted during the rotation of the bend plane.

experiments manipulating the beat plane of sea urchin sperm.

### *Rotation of Plane of Bend of Sea Urchin Sperm*

Sea urchin sperm flagella normally beat in a plane (Gray, 1955). However, this beat plane can be manipulated by holding the sperm head in a micropipette and vibrating it in a plane (Gibbons *et al.*, 1987; Shingyoji *et al.*, 1991; Takahashi *et al.*, 1991). When the plane of imposed vibration was gradually rotated, the flagellar bend plane rotated along with it, up to 11 complete revolutions. The surprising observation shown in Movie 6 is that when that imposed vibration was stopped, the sperm flagellum spontaneously "unwound" its bend plane back to the original orientation (Figure 4). By imposing rotation on an axoneme that had a marker bead stuck to the outer doublet microtubules on one side, it was possible to show that the imposed rotation of the bend plane involves a rotation in the coordinated pattern of sliding between the microtubules, rather than a twisting of the whole flagellar structure (Figure 5). It is hypothesized that the rotating pattern of sliding or the resultant bending forces the rotation of the central pair microtubules. This rotation would cause a twisting of the central pair if the basal end is firmly anchored. When the imposed vibration is stopped, the central pair presumably untwists elastically back to its original orientation, in the process regulating the pattern of sliding of the outer doublets which is manifest as rotation of the bending plane. In organisms with flagella that beat only in one plane, such as these sea urchin sperm, the central pair may be firmly anchored at its basal end. Only with such a firm anchoring can we explain the twisting and untwisting observed here.

### *Function of the Central Pair*

What might be the function of central pair? It is clear that the central pair is not needed for bending per se, because there are naturally occurring motile flagella that lack the central microtubules (Schrevel and Besse, 1975; Prensier *et al.*, 1980; Goldstein and Schrevel, 1982; Gibbons *et al.*, 1985; Ishijima *et al.*, 1988). Although the central pair–deficient mutants of *Chlamydomonas* are paralyzed, they can move in the presence of extragenic suppressor mutations (Huang *et al.*, 1982; Brokaw and Luck, 1985), under altered nucleotide conditions in reactivation (Omoto *et al.*, 1996; Frey *et al.*, 1997), or under mechanical stimulation (Hayashibe *et al.*, 1997). Yet a great majority of axonemal structures possess the central pair, and mutants defective in them are paralyzed under physiological conditions (Witman *et al.*, 1978; Afzelius, 1985). Thus we propose that the nine outer doublets exhibit a default movement in the absence of central pair–radial spoke com-

plex. The presence and activity of the central pair and radial spokes imposes a higher-order regulation on this default movement to enable a more complex three-dimensional waveform or ciliary-type waveform.

## SUMMARY

It has been 20 years since the phenomenon of central pair rotation in eukaryotic flagella was reported (Omoto, and Kung, 1979). At that time, a model was proposed in which the central pair functions as a distributor to regulate dynein activity among the outer doublet microtubules. The video evidence obtained since then and gathered together in this essay is consistent with this model. Geometric arguments indicate that there must be circumferential and longitudinal regulation of shear forces to produce effective bending motion of an axoneme. Central pair microtubules are ideally situated to perform this regulatory function. The regulation of outer doublet sliding by the central pair, together with the rotation of the latter, where this occurs, may explain the wide diversity of two- and three-dimensional flagellar and ciliary waveforms that is found in organisms using the same basic 9 + 2 structure.

## ACKNOWLEDGMENTS

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