Flagellar Waveform and Rotational Orientation in a *Chlamydomonas* Mutant Lacking Normal Striated Fibers

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ABSTRACT The *Chlamydomonas* mutant *vfl-3* lacks normal striated fibers and microtubular rootlets. Although the flagella beat vigorously, the cells rarely display effective forward swimming. High speed cinephotomicrography reveals that flagellar waveform, frequency, and beat synchrony are similar to those of wild-type cells, indicating that neither striated fibers nor microtubular rootlets are required for initiation or synchronization of flagellar motion. However, in contrast to wild type, the effective strokes of the flagella of *vfl-3* may occur in virtually any direction. Although the direction of beat varies between cells, it was not observed to vary for a given flagellum during periods of filming lasting up to several thousand beat cycles, indicating that the flagella are not free to rotate in the mature cell. Structural polarity markers in the proximal portion of each flagellum show that the flagella of the mutant have an altered rotational orientation consistent with their altered direction of beat. This implies that the variable direction of beat is not due to a defect in the intrinsic polarity of the axoneme, and that in wild-type cells the striated fibers and/or associated structures are important in establishing or maintaining the correct rotational orientation of the basal bodies to ensure that the inherent functional polarity of the flagellum results in effective cellular movement. As in wild type, the flagella of *vfl-3* coordinately switch to a symmetrical, flagellar-type waveform during the shock response (induced by a sudden increase in illumination), indicating that the striated fibers are not directly involved in this process.

Striated fibers are associated with most cilia and flagella (26, 36), including the modified cilia of sense organs (3). Their ubiquitous presence suggests that these fibers play an important role or roles in flagellar and ciliary morphogenesis or function. Several possible functions have been proposed for the striated fibers, including (*a*) an active role in the initiation or coordination of flagellar movement (7, 15, 19, 31, 32, 42), (*b*) a passive role in anchoring the cilia and flagella to resist the forces and moments resulting from ciliary or flagellar beating (1, 25, 26, 37, 38), and (*c*) a role in the development or maintenance of the proper positioning of the basal bodies (9, 11, 25, 26). However, there has previously been no experimental evidence for any of these functions.

Recently, a mutant of *Chlamydomonas* was described that is defective in striated fibers and microtubular rootlets (43). This mutant provides an opportunity for learning more about the roles of these structures. We studied flagellar waveform and orientation in forward and reverse swimming cells of this mutant; the flagella have a waveform similar to that of wild type in both forward and reverse beating modes. However, the direction of beat is highly variable, and thin-section analysis shows that the mutant's flagella are present in abnormal rotational orientations. These results suggest that the striated fibers of *Chlamydomonas* are not required for initiation or control of flagellar motion, but are important in determining the rotational orientation of the basal bodies.

MATERIALS AND METHODS

Chlamydomonas reinhardtii mutant strain *vfl-3-207* (43) and its parental wild-type strain *NO* were cultured in 125 ml of medium bubbled continuously with 5% CO_2 as previously described (13). Cells were gently concentrated with a clinical centrifuge at ~100 *g* for 2 min, and observed in their conditioned medium using a Zeiss Universal microscope with phase-contrast optics. Cells were photographed at 64-256 frames/s on 16 mm Tri-X reversal film using a Redlake Locam Model 51 high speed motion camera (Redlake Corp., Photo Instrument Division, Campbell, CA) synchronized with a Chadwick-Helmuth Strobex power supply and lamp (Chadwick-Helmuth Co. Inc., Monrovia, CA).

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Ciliary-type beating was filmed in mutant and wild-type cells that had been handled normally in room-light. Flagellar-type beating was filmed in cells from cultures that had been kept in the dark for ~30 min before filming. A Corning No. 2404 red filter (Corning Glassworks, Corning, NY) was placed between the microscope light source and condenser during focusing and removed by hand during filming; the sudden increase in light intensity caused the cells to undergo the shock response characterized by flagellar-type beating.

Fixation, embedding, and sectioning was as described previously (13).

RESULTS

As previously reported (43), the mutant *vfl-3* lacks normal striated fibers and microtubular rootlets (Fig. 1). In some cells striated fibers are associated with the flagellar bases, but these usually do not connect the basal bodies as in wild-type cells. Microtubules are often present near the bases of the flagella, but are rarely arranged into microtubular rootlets. When microtubular rootlets are present, they are usually abnormal in position or morphology.

Flagellar Waveform and Direction of Beat in "Forward Swimming" vfl-3

Cells of *vfl-3* have 0-4 flagella per cell (43). The mutant's flagella usually beat with a ciliary-type motion consisting of an effective stroke and a recovery stroke; this is readily recorded in uniflagellated cells (Fig. 2), where the flagellar beat causes the majority of cells to rotate in a counterclockwise direction when the cells are in contact with the slide and in the opposite direction when the cells are in contact with the coverslip. Typically, a cell rotates ~0.34 radians (19.5°) per beat cycle. Flagellar waveform in uniflagellated *vfl-3* appears identical to that of wild-type (15, 16, 27, 28, 33) and the *uni-1* mutant (5, 6).

The flagella of biflagellated cells of *vfl-3* also have a waveform virtually identical to that of wild type. However, in biflagellated cells of *vfl-3*, the effective strokes of the two flagella are usually not directed to opposite sides as in wild type, but may have virtually any orientation. As a result, the cells generally do not display effective forward motion. In some cells the effective strokes of the two flagella are in the *same* direction, causing the biflagellated cells to spin in place like uniflagellated cells (Fig. 3). In biflagellated cells in which the effective strokes have other abnormal orientations, the cells display different swimming patterns, usually consisting of rolling or tumbling motions. Cells with this type of swimming behavior are not easily filmed while swimming freely, so such cells were trapped by gentle suction on a micropipette and held steady, away from the slide and coverslip, while filming (Fig. 4). In the cell illustrated, it can be seen that the form of the beat is normal in each flagellum, but the right flagellum has an effective stroke to the left, and the left flagellum has an effective stroke to the right.

Although the direction of the effective stroke differs for flagella of different cells, the direction of the effective stroke was not observed to change for a given flagellum over several minutes of filming corresponding to many thousand beat cycles. This indicates that the flagella of *vfl-3* are not free to rotate.

Beat Frequency in vfl-3

Under our filming conditions, the beat frequency of *vfl-3* is generally between 40 and 60 Hz, although in certain cases the frequency was higher or lower than these values. This is similar to the beat frequency of the *NO* wild-type cells from

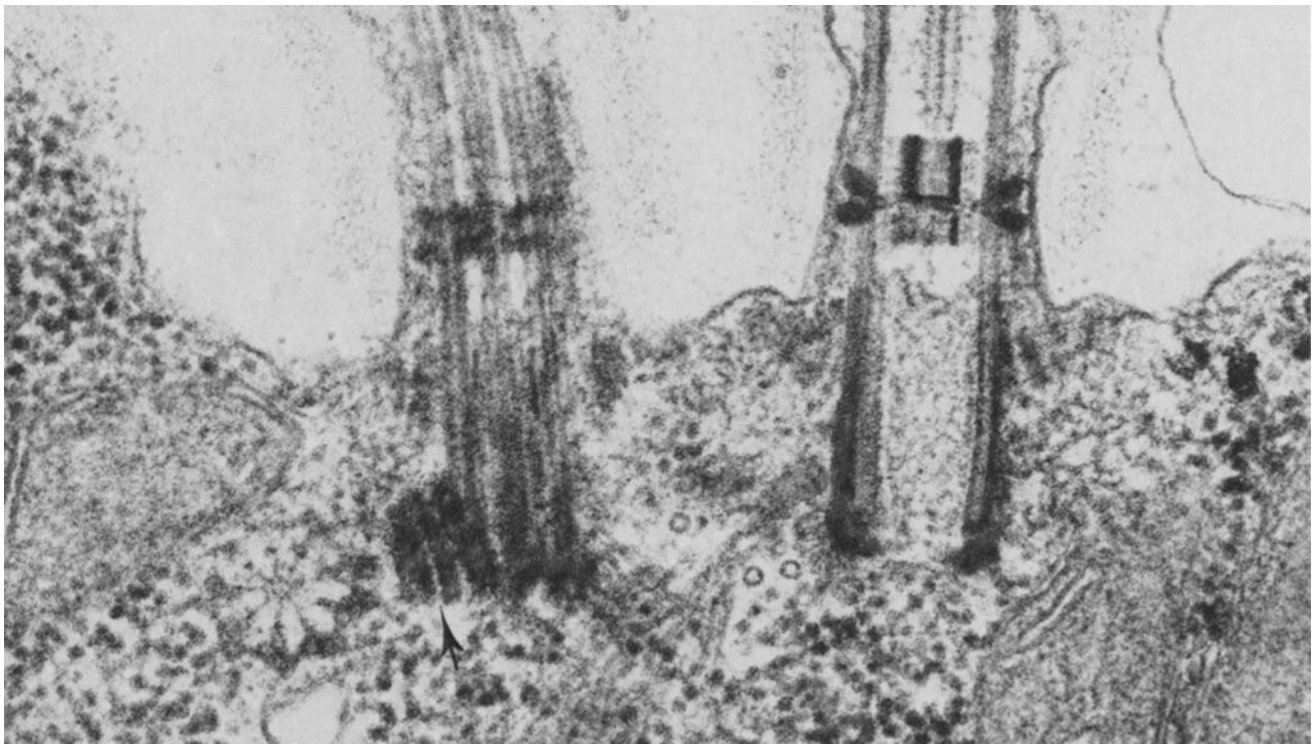

FIGURE 1 Flagellar basal region of *vfl-3*. Unlike wild-type *Chlamydomonas*, no distal or proximal striated fibers extend between the functional basal bodies. In this cell, a striated fiber (arrow) is present, but is in an abnormal position between a functional basal body and an accessory basal body. Microtubules are also present in this region but not organized into normal microtubular rootlets. × 98,500.

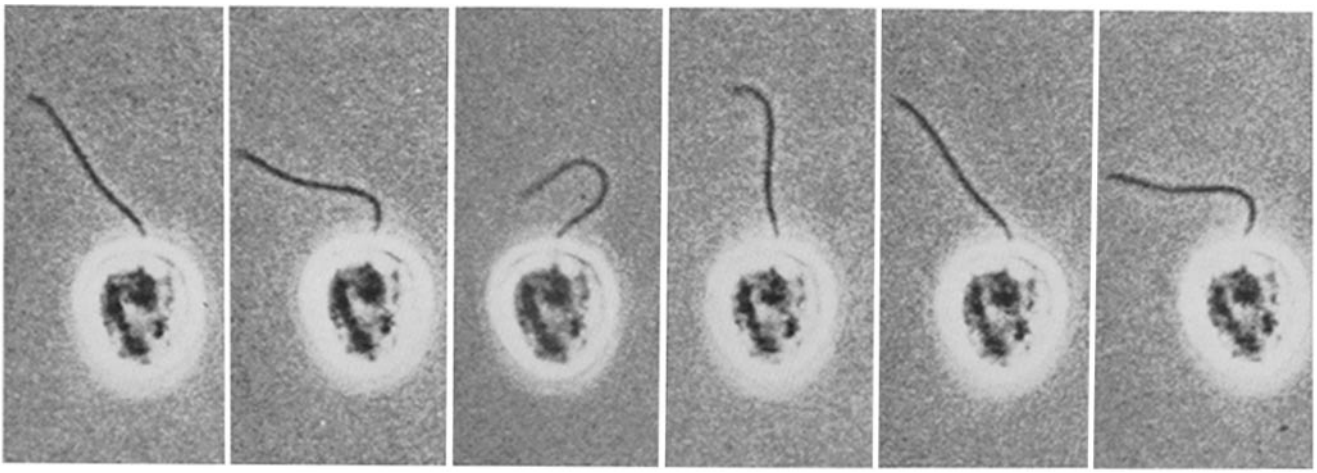

FIGURE 2 Successive movie frames showing flagellar waveform in a uniflagellated cell of *vfl-3*. The waveform is similar to that of wild type. The cell was rotating clockwise, but for ease of comparison the cell was printed in a constant orientation. 150 frames s^{-1}. × 2,100.

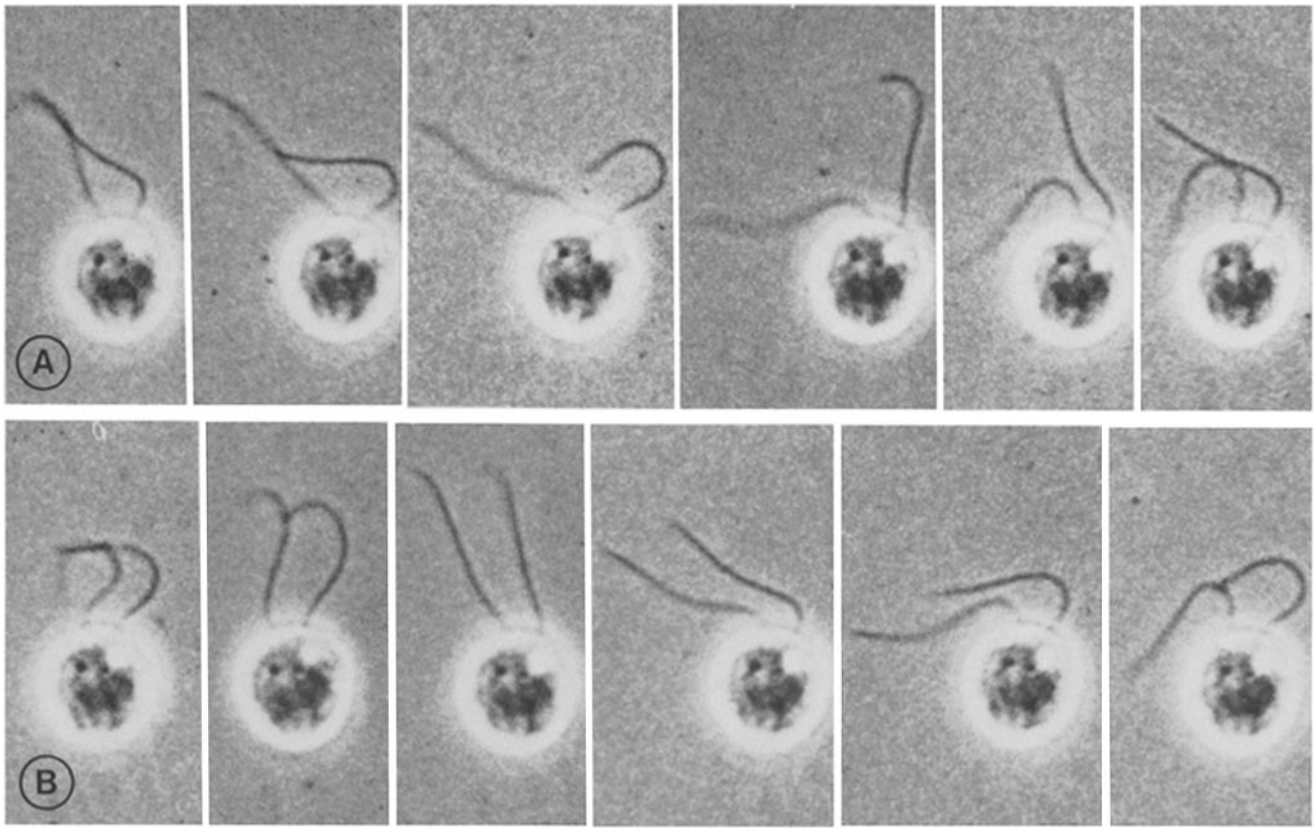

FIGURE 3 Successive frames showing flagellar beat pattern in a biflagellated cell of *vfl-3*. Although the waveform is similar to wild type, both flagella have an effective stroke in the same direction. Note that the right flagellum is beating faster than the left, leading to periods of asynchronous beating (*A*) and synchronous beating (*B*). 300 frames s^{-1}. × 2,200.

which the mutant was derived (unpublished observations). In biflagellated cells, one flagellum usually beats ~20% faster than the other, so that the cell exhibits periods of synchronous and asynchronous beating (Figs. 3 and 4). For example, in Fig. 3*A* the two flagella are beating asynchronously; in Fig. 3*B* the flagella of the same cell are beating in near synchrony. Under our conditions, one flagellum of wild type also beats slightly faster than the other. The absence of normal striated fibers therefore does not appear to have any effect on flagellar beat frequency or synchrony in *Chlamydomonas*.

Shock Response in vfl-3

After appropriate photostimulation, wild-type cells undergo a shock response in which the two flagella propagate symmetrical waves from base to tip, causing the cell to swim backwards for a short period; the cells then resume normal forward swimming (16, 28, 33). Under the conditions used here, most cells of both wild-type and *vfl-3* exhibit this response (Fig. 5). The waveform of *vfl-3* flagella during the response is virtually identical to that of wild-type flagella in

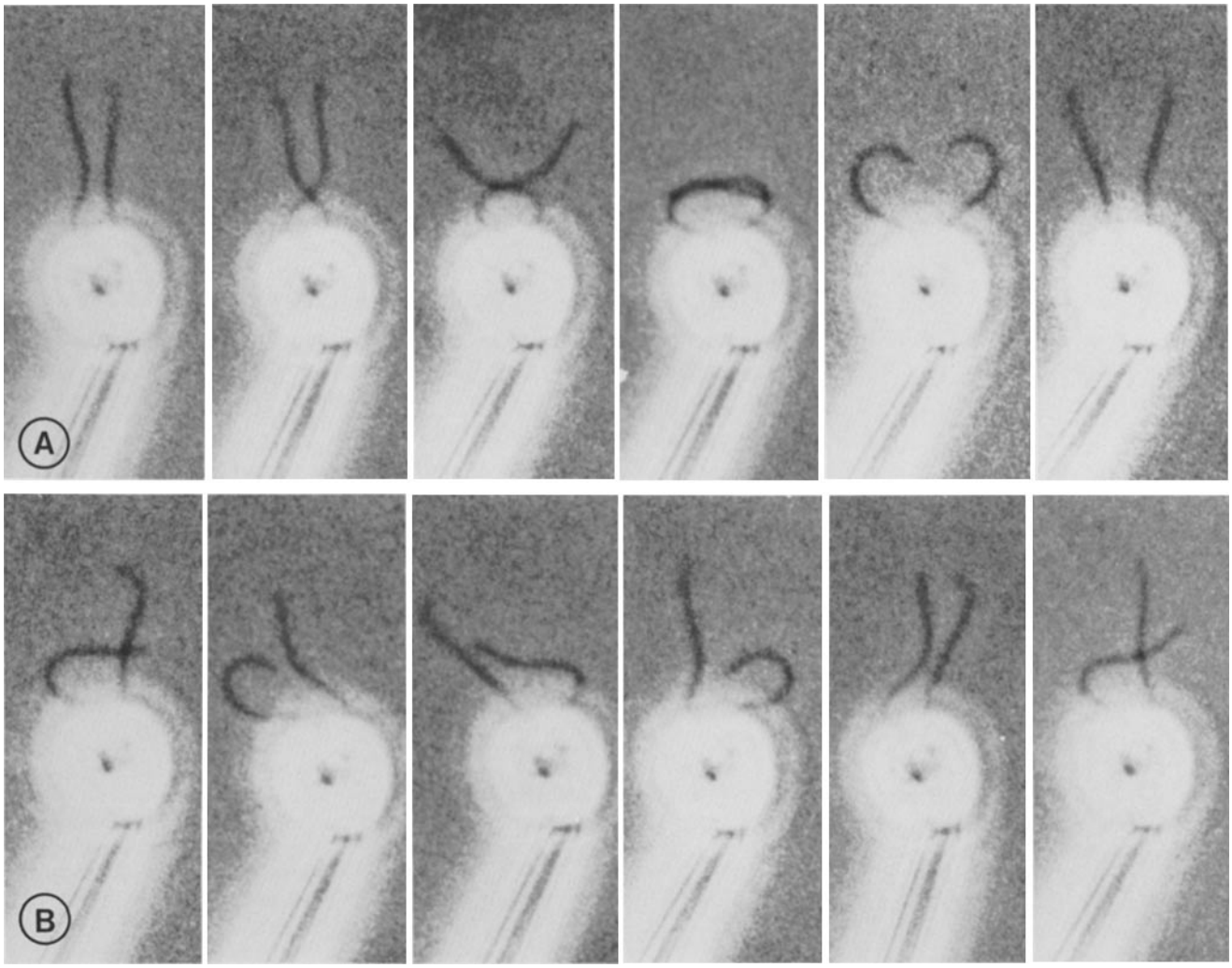

FIGURE 4 Two film sequences showing flagellar beat pattern in a biflagellated cell of *vfl-3*. The right flagellum has an effective stroke towards the left, whereas the left flagellum has an effective stroke towards the right. (*A*) Sequence showing synchronous beating; (*B*) sequence showing asynchronous beating. The micropipette that holds the cell for filming can be seen in the lower left corner. 300 frames s^{-1}. × 1,900.

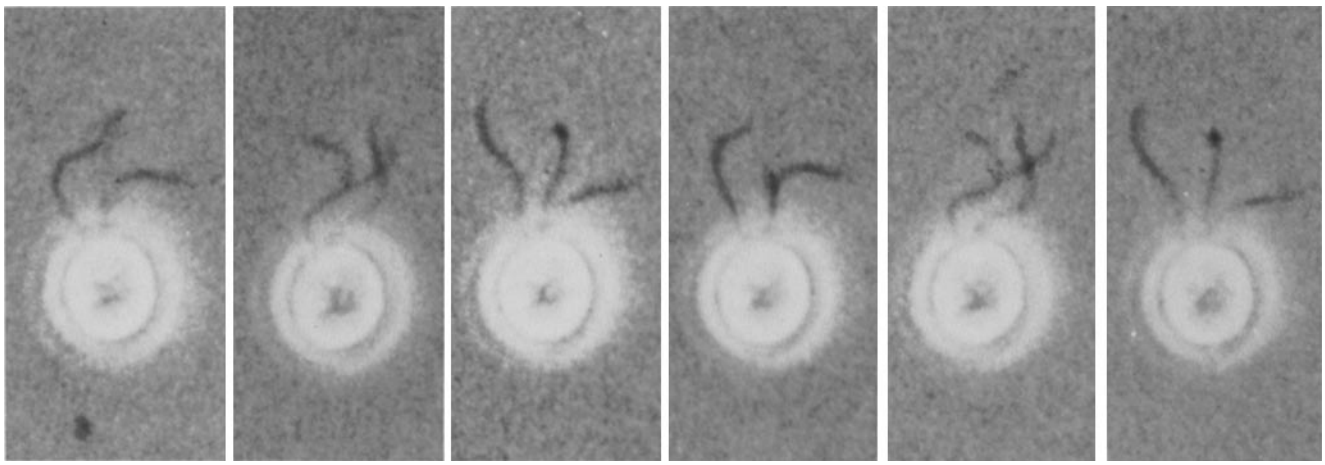

FIGURE 5 Shock response in a triflagellated cell of *vfl-3*. Like wild type, the mutant is capable of propagating symmetrical flagellar-type waves in response to appropriate stimulation. Note that the waveform in the last three frames is virtually identical to that in the first three, suggesting that all three flagella are beating at the same frequency. 150 frames s^{-1}. × 1,800.

the backwards swimming mode (5, 16, 28, 33). After about a second, the waveform spontaneously reverts to that of the normal ciliary-type beat. This response always occurs in both flagella of a cell: one flagellum never displays ciliary-type beating while the other flagellum beats with a flagellar pattern. Thus, normal striated fibers or microtubular rootlets are not required for (*a*) the ciliary-type motion characteristic of forward swimming cells, (*b*) the flagellar-type motion character-

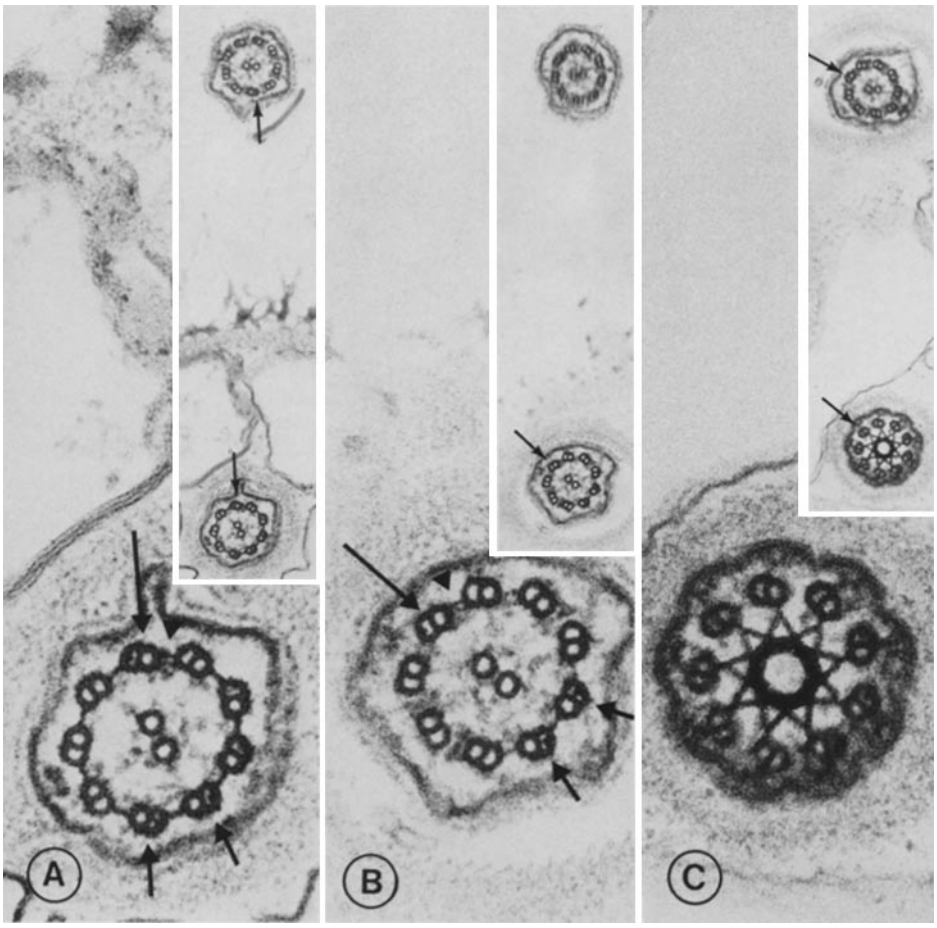

FIGURE 6 Flagellar orientation in wild type (*A*) and *vfl-3* (*B* and *C*). (*A*) In the wild-type flagellum, doublets 1, 5, and 6 have beak-like projections extending partway across the lumens of their B-tubules (arrows), and a two-part bridge (arrowhead) extends from the A-tubule of doublet number 1 to the B-tubule of doublet 2. The number 1 doublet also lacks the outer arm, although this cannot be seen here because the flagellum is sectioned proximal to the arms. (*Inset*) Low magnification micrograph showing both flagella of the cell. Note that the number 1 doublets (arrows) of the flagella face each other. × 130,000; (*Inset*) × 39,000. (*B* and *C* [sections 1 and 8 from a series]) In *vfl-3*, as in wild type, beak-like projections are present in the B-tubules of doublets 1, 5, and 6 (arrows) and a two-part bridge extends between doublets 1 and 2 (arrowhead). Also, the outer arm is missing from doublet number 1 (long arrow), although at this level arms are also absent from some of the other doublets. The transition region (*C*) and basal bodies (not shown) of the mutant are similar to wild type (13). (*Insets*) Low magnifications of the two sections showing both flagella of the cell. Note that the number 1 doublets (arrows) of the two flagella do not face each other. × 134,000; (insets) × 40,000.

istic of backward swimming cells, or (*c*) the coordinate shifts between forward and reverse beating that occur during the shock response.

Flagellar Orientation in vfl-3

Analysis of flagella of wild-type *Chlamydomonas* has revealed that the orientation of the flagella is always in the same direction relative to the direction of the effective stroke (13). The variable direction of the effective stroke in *vfl-3* suggested that its flagella may have an altered rotational orientation. To investigate this possibility, we compared the positioning of the polarity markers in the flagella of the mutant cells to that of wild type (Fig. 6). In wild-type flagella, beak-like structures are present in the lumens of the B-tubules of doublets 1, 5, and 6, a two-part bridge extends from doublet 1 to doublet 2, and the number 1 doublet lacks its outer arm. The number one doublet is invariably located on the side of the axoneme facing the other flagellum, i.e., on the side of the axoneme opposite the direction of its effective stroke (Fig. 6*A*). Individual cross-sections of *vfl-3* flagella do not always show all three markers at levels where they would be present in wild type; nevertheless, serial sections of such flagella usually reveal that all markers are present. Furthermore, the markers in *vfl-3* are always distributed in the normal pattern around the axoneme, indicating that, as in wild type, they can be used to determine the rotational orientation of the flagellum. When both flagella can be located in the same or subsequent sections, the number 1 doublet of one flagellum usually does not face the other flagellum but is facing in some other direction (Figs. 6, *B* and *C*, and 7) that varies from cell to cell. For example, the two flagella in Fig. 6, *B* and *C*, are rotated clockwise ~45° and 225° relative to their normal orientations; in Fig. 7 the flagella are rotated clockwise ~100° and 260° from normal. Assuming that the effective stroke of the mutant's flagellum is towards the number 5 and 6 doublets as in wild type (13), both flagella of the cell sectioned in Fig. 6 would have been beating in approximately the same direction, much like the cell filmed in Fig. 3. These results conclusively demonstrate that the flagella and basal bodies of *vfl-3* have abnormal rotational orientations.

DISCUSSION

In mutant *vfl-3*, which lacks normal proximal and distal striated fibers and microtubular rootlets (43), the direction of the effective stroke varies markedly between cells and between flagella of the same cell. This is an immediate consequence of abnormal rotational orientation of the flagella, as evidenced by the positioning of the structural polarity markers of one axoneme of a cell relative to its other axoneme. The striated fibers or microtubular rootlets must therefore be important in the development or long-term maintenance of proper basal body positioning and orientation. This is the first real evidence for a role for these structures, although previous investigators have proposed such a function for the striated fibers on the

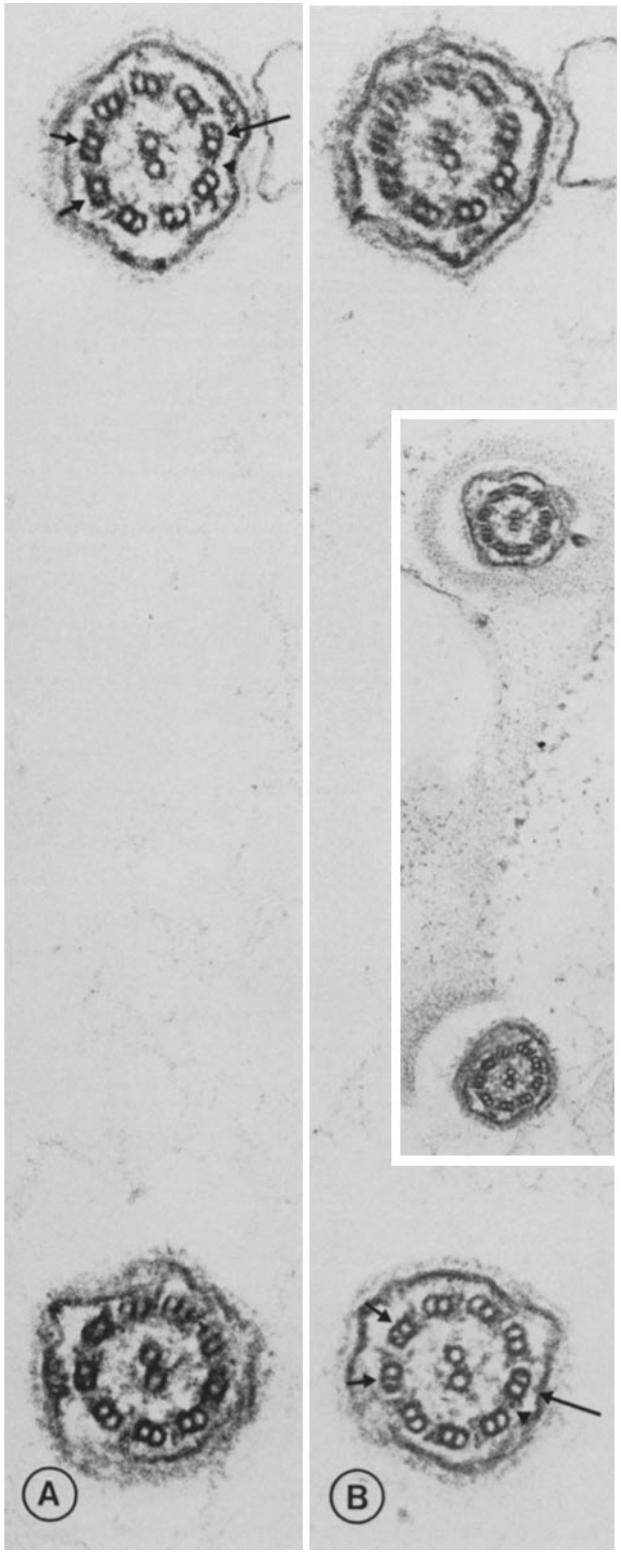

FIGURE 7 Flagellar orientation in *vfl-3*; adjacent sections of two flagella from the same cell. Each flagellum contains three doublets with beak-like projections (arrows). The number one doublet (long arrow) lacks an outer arm, and a two-part bridge (arrowhead) extends between doublets one and two. In the living cell the two flagella presumably beat perpendicular to a plane connecting the two basal bodies. (*Inset*) The two flagella at the level of their flagellar collars, confirming that both flagella come from the same cell. × 105,000; (*inset*) × 46,000.

basis of structural considerations (9, 11–13, 25, 26, 42). Because the mutant lacks both normal striated fibers and microtubular rootlets, the present study does not in itself distinguish which of these components is responsible for basal body orientation. However, the striated fibers of the *Chlamydomonas* wild-type flagellar apparatus attach to specific basal body triplets and are in appropriate positions to set up and maintain the correct rotational orientation of the flagella (13). A role for the striated fibers in flagellar apparatus morphogenesis is in good agreement with the conclusion of Wright et al. (43) that the *vfl-3* mutation is expressed early in the cell cycle.

Because a given flagellum was not observed to change the direction of its effective stroke in *vfl-3*, the flagellum is not free to rotate in the cell. Therefore, striated fibers and microtubular rootlets are not required to stabilize basal body orientation, at least over short periods of time. Structurally normal transitional fibers extend from the basal body to the cell membrane in the mutant (43); these fibers probably prevent rotation of the axoneme during its beat cycle.

Previously, we showed that flagella of wild-type *Chlamydomonas* had an inherent structural polarity related to beat direction, and proposed that the direction of the effective stroke is determined by an internal, asymmetrically distributed component of the flagellar axoneme (13). The correlation between abnormal beat orientation and abnormal positioning of the axonemal polarity markers in *vfl-3* provides further support for this hypothesis. This, plus the fact that the direction of the effective stroke of a flagellum does not vary over many thousands of beat cycles in *vfl-3*, strongly suggests that, except for setting up and/or maintaining proper basal body orientation, the striated fibers have no direct role in determining beat direction.

Striated fibers and microtubular rootlets have also been proposed to function in the dissipation of forces generated by actively moving flagella (10, 25, 26, 35, 37–40). However, *vfl-3* retains the ability to beat normally without normal striated fibers or microtubular rootlets. Moreover, recombinants between *vfl-3* and the cell wall-less mutant CW-92 (17) lack both the cell wall and normal striated fibers and microtubular rootlets, yet beat normally (H. J. Hoops, unpublished results). Therefore, even in the absence of the cell wall, neither striated fibers nor microtubular rootlets are necessary for anchoring the flagella or for prevention of large scale deformation of the cell body during flagellar movement. We cannot rule out the possibility that the base of the *vfl-3* flagellum pivots back and forth during the flagellar beat cycle. Such pivoting would decrease the propulsive action of the effective stroke (37). Uniflagellated cells of *vfl-3* rotate ~0.34 radians per beat cycle; this is ~65% of that reported for the *uni-1* mutant (5), which has normal striated fibers (14) and no identified defects in its microtubular rootlets (B. Huang, personal communication). However, because the two mutants were grown and examined under different conditions, further work will be necessary to determine if this result is significant.

A number of investigators have raised the possibility that striated fibers of various types play active roles in flagellar movement (e.g., see references 7, 15, 18, 19, 24, 28, 31, 33). Consistent with an active role are observed changes in the length and periodicity of many of these structures (22, 23, 29, 34, 41), including calcium-induced contractions (20, 30, 31). Moreover, cytochemical studies have provided evidence that

contractile proteins and ATPase activity are associated with the striated fibers of many organisms (2, 18, 21, 42).

One active role that has been proposed for the striated fibers is the initiation and control of flagellar beating (7, 24, 31, 32). However, the mutant *vfl-3* has normally active flagella, so the striated fibers do not appear to be involved in the initiation of flagellar motility in *Chlamydomonas*. This is consistent with results obtained with isolated demembranated axonemes from *Chlamydomonas*; these axonemes lack basal bodies and their associated structures, yet can be reactivated with a waveform similar to that of in situ flagella of wild-type cells (4).

Striated fibers have also been thought to play a role in flagellar coordination in many organisms, including *Chlamydomonas* (7, 15, 19, 24, 28). Although it has frequently been reported that the flagella of *Chlamydomonas* beat in synchrony (15, 16, 27, 28), in most cases asynchronous beating was also observed. High-speed movie sequences of wild-type cells indicate that, at least under the conditions of filming, one flagellum beats faster than the other, leading to periods of synchronous and asynchronous beating (8; H. J. Hoops, J. Shapiro and G. B. Witman, unpublished results). As shown here, this also occurs in *vfl-3*. Therefore, the asynchronous beating observed in this mutant cannot be taken as evidence for a role of the striated fibers in flagellar coordination. Futher work will be necessary to determine the conditions and reasons for the different beat frequencies in the two flagella of wild-type and mutant cells.

After photostimulation, the flagella of *vfl-3* change from the ciliary-type motion characteristic of forward swimming wild-type cells to the flagellar-type motion characteristic of reverse swimming cells. This response is undergone by the majority of the mutant cells under our conditions. All flagella of a cell respond in the same manner even though the basal bodies are rarely connected by striated fibers and in some cases are located several μm from one another. This indicates that (*a*) striated fibers are not required for the transition from ciliary to flagellar waveform and vice versa, (*b*) signals controlling the type of waveform operate over the entire cell and are not communicated between the flagella via the striated fibers, and (*c*), the signal that triggers the change from ciliary to flagellar waveform is not propagated from the eyespot region to the flagellar apparatus via a microtubular rootlet, a possibility discussed previously for phototactic responses (23, 24).

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