NOTES

Trailing Flagella Rotate Faster than Leading Flagella in Unipolar Cells of *Spirillum volutans*

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In unipolar cells of *Spirillum volutans*, the flagellar rotation frequency is halved, approximately, when the flagellar bundle reorientates to rotate about the cell body and reverse the swimming direction. The viscous drag resulting from a concomitant increase in flagellar wave amplitude is probably responsible for the reduced frequency of flagellar rotation.

There is convincing evidence that bacterial flagella are inert spiral structures rotated from their flagellar bases (1, 2, 9) and that they can rotate freely with respect to the cell body (3). Bacterial flagella can change their orientation and thereby reverse the direction of cell propulsion. Little is known about the mechanism by which they do this or about the movement of the flagellar bundle when it rotates about the cell body. Previous analyses of bacterial flagellar movement used cinemicrography with dark-field illumination (7, 8, 11), but reflection of light from the cell body obscured the movement of flagella when they rotated about the cell body.

To learn more about the mechanics of this movement, I filmed actively swimming unipolar cells of *Spirillum volutans* at high framing rates, thus resolving the high frequency of flagellar rotation; bright-field microscopy was used to make the flagellar bundle visible when it rotated about the cell body.

S. volutans (ATCC 19554) was grown at 30°C in ATCC medium 234. An actively growing culture with a high proportion of unipolar cells was used; unipolar cells, recently divided, have only one bundle of flagella. Older bipolar cells have longer cell bodies and two flagellar bundles, making analysis of their movement difficult; they were excluded from this study. The preparation was placed on a slide with a cover slip supported by petroleum jelly to provide a good depth of fluid and prevent fluid drift and was warmed to 28°C with a fan heater. Illumination was by xenon arc with UV filters to protect the cells and neutral density filters to adjust the film exposure. Actively swimming unipolar cells were recorded on 16-mm Ilford Pan F type 752 negative film, using a Locam camera (Redlake

Corp., Campbell, Calif.) and Zeiss WL bright-field phase microscope with a 40/0.75 neofluar or 63/1.4 planapochromat objective. The movement of most cells was recorded at 500 frames per s.

Flagellar rotation was measured for each bacterium through at least 4 and up to 12 consecutive cycles of flagellar rotation. This was done for two consecutive cycles of to-and-fro movement of the same cell. (With luck, a bacterium will swim to-and-fro in the field while the camera is running.) Only bacteria with both poles of the body clearly in focus at some time during the sequence were chosen for measurement to make sure that they were unipolar. Flagellar wave amplitude was measured by making frame-by-frame drawings of the cell body and flagellar bundle of each cell; each drawing was aligned with the previous one by keeping the axis of the cell body and the poles of the cell body in the same position throughout. The mean maximum deviation from the cell body axis of the flagellar bundle was measured to obtain flagellar amplitude. Flagellar amplitude, frequency, and cell velocity were all measured from the same film sequence. The forward velocity of the cell was measured with the cell body in the same phase of its rotation cycle at the initial and final frames; frames were aligned with an ocular graticule, and progressive movement and film speed were measured as described by Swan et al. (10). Measurements were made from cineprints, using a projector (L.W. International, Woodland Hills, Calif.) as described by Swan et al. (10).

The ultrastructure of *S. volutans* is similar to that of *S. serpens* as described by Murray and Birch-Andersen (6). *S. volutans* is a gram-negative bacterium with about 75 flagella (4) attached
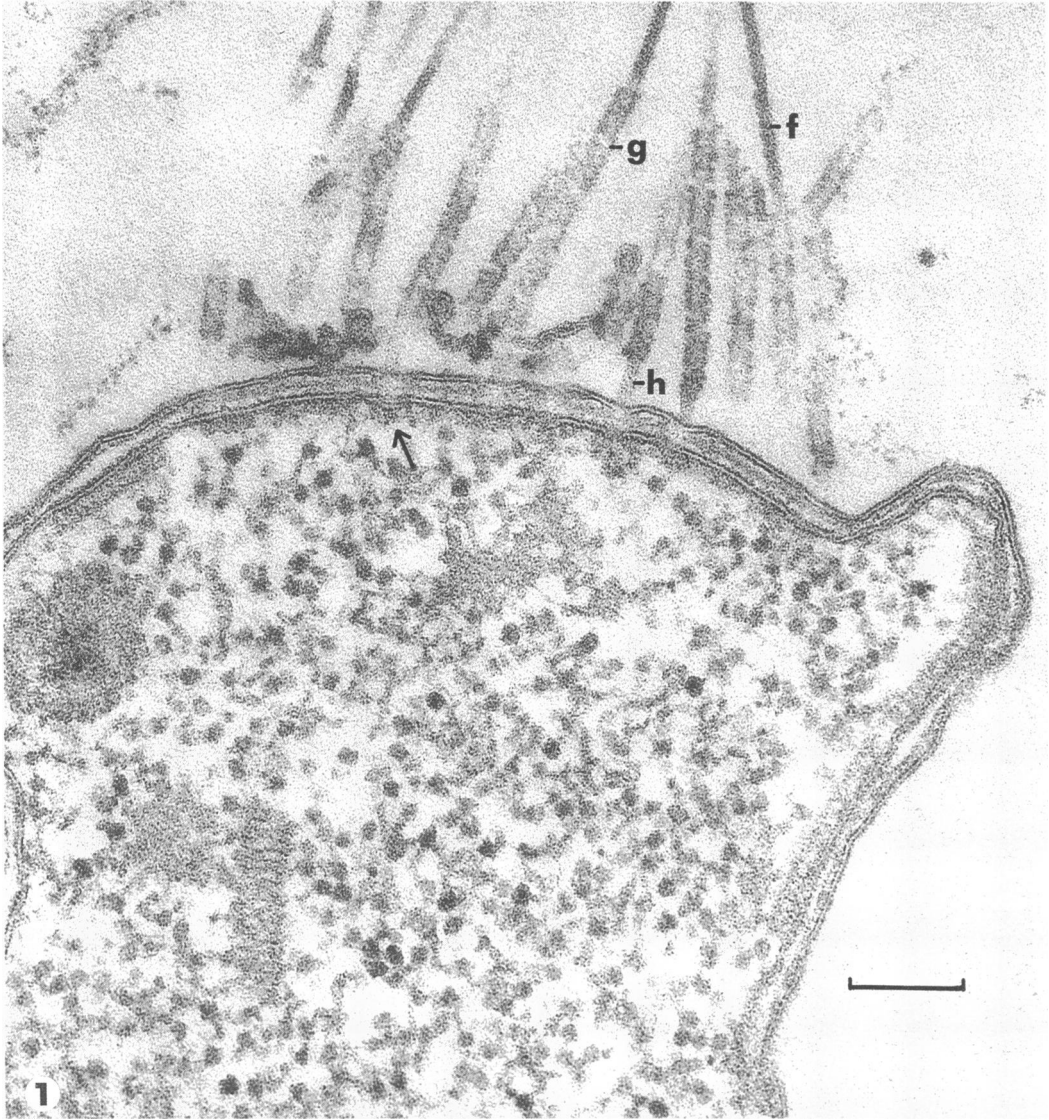
FIG. 1. Transmission electron micrograph of a cell pole in *S. volutans*. The proximal part of the flagellum (f) is surrounded by a coat of dense material (g). The flagellum is attached to a hook (h) which appears to indent the outer membrane. An electron-dense peptidoglycan layer lies between the outer and plasma membranes and appears interrupted at the insertion sites of the hooks. Some dense material lies on the cytoplasmic side of the plasma membrane below the insertion of the hook (arrow). ×149,520; Bar, 0.1 μm.

to a helical body by curved regions termed hooks (Fig. 1). The hooks terminate at flagellar bases which are thought to function in flagellar rotation by acting as a rotary motor (2).

As shown in cinemicrographs of swimming unipolar cells (Fig. 2), the flagellar bundle can change its orientation to reverse the direction of cell propulsion. In Fig. 2A, the flagellar bundle is in the trailing configuration; the bundle rotates at the rear of the cell, propelling it upward as in Fig. 3A. In Fig. 2B, the same cell has reorientated its flagellar bundle so that it rotates about the cell body in the leading configuration, propelling the cell downward as in Fig. 3B. There must be no change in direction of rotation of the flagellar rotary motor during reorientation in *S. volutans* since such a change would not result in reversal of swimming direction. The bundle remains visible during reorientation, and the flagella do not fly apart, in contrast to tumbling bacteria (5). In
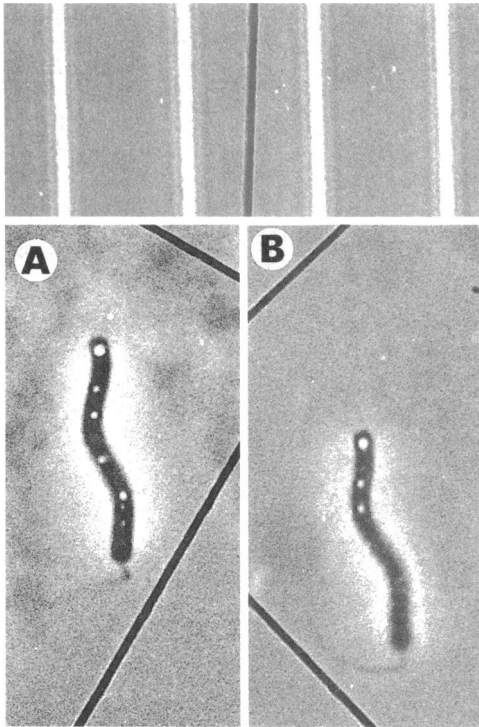
FIG. 2. Cinemicrographs of the same cell (no. 6 in Table 1). The dark crossbars near the cell are an ocular graticule which permits alignment of projected frames in movement analysis. A slide micrometer is shown above (A) and (B) as a magnification calibration (interval between lines, 10 μm). (A) The flagellar bundle is in the trailing configuration; the bundle rotates to the rear of the cell, propelling it upward (as in Fig. 3A). (B) The flagellar bundle is in the leading configuration, and the cell body is being propelled downward (as in Fig. 3B); the bundle is bent backward and extends around the proximal 5 μm of the body.
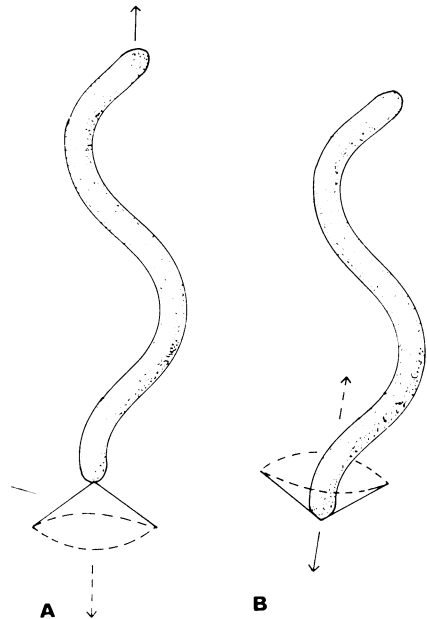

FIG. 3. Diagram of the movement of unipolar cells. The envelope of movement of the flagellar bundle is in the trailing configuration in (A) and in the leading configuration in (B). The solid arrows indicate the direction of cell movement; the dashed arrows show the direction of fluid propulsion.

S. volutans, the flagellar waves always propagate from base to tip (i.e., in a positive direction).

In this study, examination of cells undergoing reversal of swimming direction showed that active changes in motion of the flagellar bundle were involved. Winet and Keller (11) assumed that viscous drag was responsible for the flagellar bundle being bent backward. They described the movement of bipolar cells of *S. volutans* as follows: "viscous stresses would bend both bundles aftward. . . Implicit in this interpretation is the prediction that a monotrichous Spirillum has an aft bundle only." As shown in Fig. 2B, this interpretation is not valid; Winet and Keller were unable to observe this configuration of the flagellar bundle because they used dark-field illumination.

As shown in Table 1, when the flagellar bun-

dle was in the leading configuration, the frequency of flagellar rotation was significantly lower (by a factor of approximately 0.4), and the flagellar wave amplitude was significantly higher (by a factor of approximately 1.4) than when the bundle was in the trailing configuration.

Any increase in flagellar wave amplitude would increase both cell propulsion and viscous drag on the flagellum; when the flagellar bundle changes to the leading configuration and flagellar amplitude increases, the increased drag on the flagellar bundle probably results in the observed decrease in flagellar rotation frequency by placing an increased load on the flagellar rotary motor. Thus, the propulsive advantage of an increased flagellar amplitude in the leading configuration is offset by the disadvantage of a reduction in flagellar rotation frequency. The cell velocities do not differ significantly when the cell is in the leading compared with the trailing configuration (Table 1). However, cell velocity in *S. volutans* depends on additional parameters of cell movement (manuscript in preparation).

The observation that flagellar rotation frequency is reduced concomitantly with an increase in flagellar wave amplitude suggests that the flagellar rotary motor has a preset limit to its energy output.

TABLE 1. Flagellar rotation frequency, amplitude, and cell velocity of unipolar cells of *S. volutans*[a]

Cell no.	Flagellar frequency (Hz)		Flagellar amplitude (μm)		Cell velocity (μm/s)		Film speed (frames/s)
	Bundle leading (F_f)	Bundle trailing (F_r)	Bundle leading (A_f)	Bundle trailing (A_r)	Bundle leading (V_f)	Bundle trailing (V_r)	
1	75	171	5.3	3.7	95	108	500
2	28	124	5.6	3.1	47	154	350
3	15	38	4.5	3.0	3	29	500
4	29	54	4.5	3.5	27	46	500
5	40	80	5.7	3.2	95	92	500
6	19	30	6.2	5.6	51	61	530
Mean	34.3	82.8	5.3	3.7	53.0	81.7	
Ratio of means	0.4		1.4		0.6		

[a] By the paired *t* test, the following probabilities applied: $F_f = F_r$, $0.05 > P > 0.02$; $A_f = A_r$, $0.01 > P > 0.002$; $V_f = V_r$, $P > 0.1$.

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