MOTILITY OCCURRING IN ASSOCIATION WITH THE SURFACE OF THE *CHLAMYDOMONAS* FLAGELLUM

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ABSTRACT

Chlamydomonas flagella exhibit a previously undescribed form of motility. This is the rapid, bidirectional, saltatory movement of marker particles occurring in association with the extracellular surface of the flagellum.

KEY WORDS motility · cell surface · flagella · *Chlamydomonas*

With the exception of striated muscle, the most intensively studied and best understood system of cell motility is that involved in the formation and propagation of bends in eukaryotic cilia and flagella. The event basic to this motile system is the sliding of doublet microtubules relative to one another, due to the action of ATPase-containing arms on the doublet microtubules (13, 29, 34). The conversion of microtubule sliding into bend formation and propagation is less well understood and probably involves other structural components (radial links, central sheath, central pair of microtubules) of the cilium or flagellum (37, 40). The motile machinery responsible for bend propagation can function in the absence of the membrane and associated structures because isolated and demembranated flagellar or ciliary axonemes can be reactivated in vitro in a simple medium containing ATP and magnesium (4, 12).

The present report describes, for the first time, the existence of an additional type of motility associated with the flagella of *Chlamydomonas*, a rapid, saltatory movement of exogenous marker particles occurring in association with the extracellular surface of the flagellum.

MATERIALS AND METHODS

Unless otherwise stated, all observations were made on *Chlamydomonas reinhardii* strain pf 18 vegetative cells. This strain is paralyzed in terms of normal flagellar motility, and the primary morphological defect is the replacement of the central pair of microtubules by an axial core (22). Other *Chlamydomonas* strains used are listed in Table II. *Chlamydomonas* were grown in medium I of Sager and Granick (27) on a light/dark cycle (14 h/10 h), and gametes were induced as previously described (28). To obtain regenerating flagella, *Chlamydomonas* were deflagellated mechanically in a VirTis homogenizer (VirTis Co., Inc., Gardiner, N. Y.). Resorption of flagella was induced by the high-salt method of P. A. Lefebvre and J. L. Rosenbaum (manuscript in preparation).

All of the monodisperse microspheres listed in Table I were obtained from Polysciences, Inc. (Warrington, Pa.). Microspheres were coated with bovine serum albumin as described by Oreskes and Singer (20). *Escherichia coli* strain M-247 was obtained from Dr. Julius Adler (University of Wisconsin), and cultured in a medium containing 1% tryptone (Difco Laboratories, Detroit, Mich.) and 0.5% sodium chloride. This strain of *E. coli* is unable to produce bacterial flagella and hence is nonmotile. To study the association of *E. coli* with *Chlamydomonas* flagella, it was necessary to reduce the Brownian movement of the bacteria by raising the viscosity of the medium with methyl cellulose (Protoslo).

Cinematographic recordings were obtained with a Bolex H16M (L and I Photo Optical, Woodbury, N. Y.) or an Arriflex 16S movie camera (Arriflex Co. of America, Woodside, N. Y.) mounted on a Zeiss Universal microscope (Carl Zeiss, Inc., New York) equipped with phase-contrast optics. All recordings were taken at 12 frames per second on Kodak 4X-reversal 16-mm film. The films were analyzed with a model 224A Photo-Optical Data Analyzer (L-W International, Woodland Hills, Calif.).

Chlamydomonas were fixed in 1% glutaraldehyde in 15 mM sodium phosphate buffer, pH 7.0, for 1 h at 4°C, washed overnight in the same buffer, postfixed in 1% osmium tetroxide in the same buffer, dehydrated in

acetone, and embedded in Spurr's low viscosity embedding medium (33). Silver sections were cut on a Sorvall MT-2 ultramicrotome (DuPont Instruments, Sorvall Operations, Newtown, Conn.), stained in uranyl acetate and lead citrate, and photographed in a Philips EM201 electron microscope.

RESULTS

When any of the marker objects listed in Table I was mixed with any of the *Chlamydomonas* strains listed in Table II in medium I of Sager and Granick (27) or in 10 mM Tris-HCl, pH 7.0, at 25°C, the marker objects adhered to and were transported along the surface of the flagella (Figs. 1 and 2). The particle movements exhibited the following properties: (*a*) particles start and stop and remain at rest for varying periods of time; (*b*) particles change direction (180°) abruptly; (*c*) particles accelerate and decelerate very rapidly and, for most of the time they are in motion, velocity is uniform; (*d*) motion occurs in straight lines, giving the appearance of operating on "tracks"; (*e*) the movement of one particle is not influenced by the movements of others (particles can pass one another while moving in the same or opposite directions); and (*f*) particles of very different sizes show similar characteristics of movement. All of these properties are characteristic of saltatory motion as described by Rebhun (24) for intracellular particles.

Fig. 9, a plot of velocity vs. time for a single polystyrene microsphere moving along the surface of a *Chlamydomonas* flagellum, illustrates the first three of these properties. The last property is

TABLE I

Markers that Exhibit Motility in Association with the Chlamydomonas Flagellar Surface

Marker	Diameter
	μm
Polystyrene monodisperse microspheres	0.357
	0.852
Polystyrene monodisperse microspheres, bovine serum albumin coated	0.357
Carboxylate monodisperse microspheres	0.278
Carboxylate monodisperse microspheres, bovine serum albumin coated	0.278
Hydroxylate monodisperse microspheres	0.452
Primary amino monodisperse microspheres	0.52
Escherichia coli, strain M-247 (nonmotile)	

TABLE II

Cell Strains that Exhibit Flagellar Surface Motility

C. reinhardii 21 gr (wild type) vegetative cells
C. reinhardii 21 gr (wild type) gametic cells
C. reinhardii nonconditional paralyzed flagellar strains: pf 1, pf 3, pf 6, pf 14, pf 15a, pf 16, pf 17, pf 18, and pf 20 vegetative cells; pf 18 gametic cells (22, 23)
C. reinhardii JJ 89 (conditional long flagellum mutant) at both permissive (23°C) and nonpermissive (13°C) temperatures (17)
C. reinhardii CW 15 (cell wall-less mutant) vegetative cells (9)
C. moewusii wild type vegetative cells
Euglenoid flagellate (unidentified species growing in cultures of *Actinosphaerium nucleofilum*)

FIGURES 1–7 All photographs are of *Chlamydomonas reinhardii* pf 18.

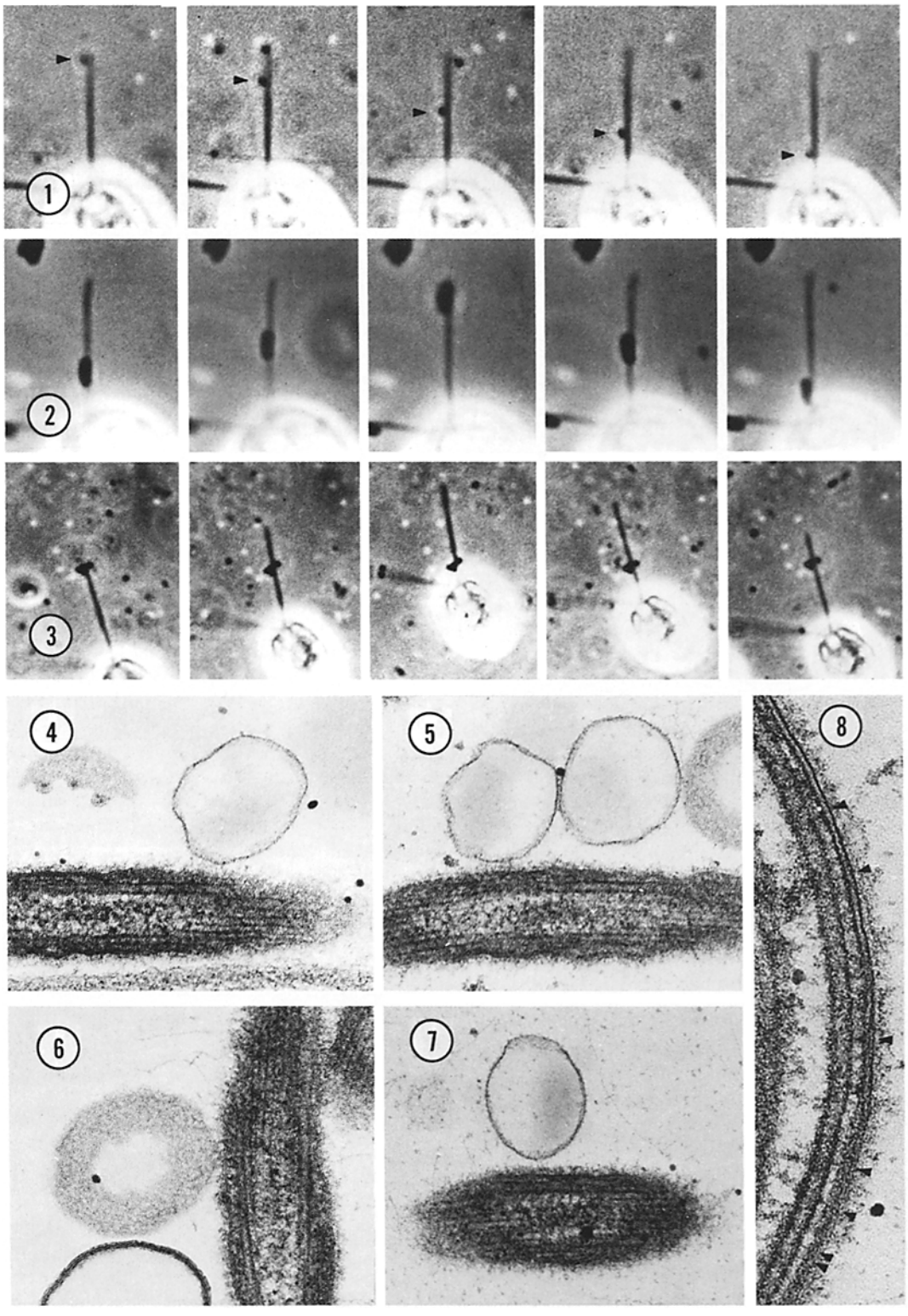
FIGURE 1 Movement of a 0.357-μm polystyrene microsphere along the flagellum. The interval between adjacent micrographs is 0.9 s. × 2,200.

FIGURE 2 Movement of an *E. coli* (nonmotile strain M-247) along the flagellum. The interval between adjacent micrographs is 2.0 s. × 2,200.

FIGURE 3 Movement of an entire *Chlamyomonas* cell back and forth along a group of polystyrene particles immobilized on a glass slide. The interval between adjacent micrographs is 2.4 s. × 1,500.

FIGURES 4–7 Electron micrographs of 0.278-μm polystyrene microspheres associated with the surface of flagella. The microspheres have been coated with bovine serum albumin so that they can be visualized in the electron microscope; this treatment does not alter their ability to be transported along the flagellum. × 64,000.

FIGURE 8 Electron micrograph illustrating the association of an outer doublet microtubule with the flagellar membrane. Material is present between the doublet microtubule and the flagellar membrane, and in certain places appears to form links between these two structures (arrowheads). A layer of fuzzy material coats the extracellular surface of the flagellar membrane. × 120,000.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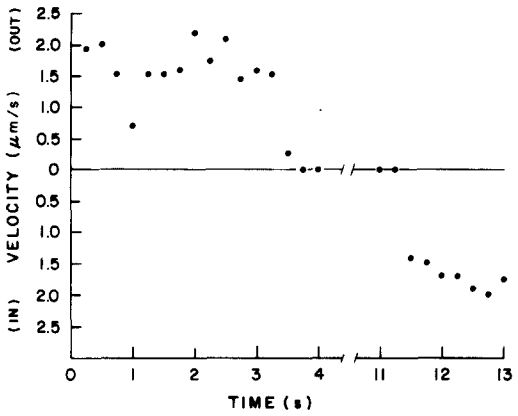

FIGURE 9 Plot of velocity vs. time for one 0.357-μm polystyrene microsphere moving along a flagellum.

shown by the following measurements: Polystyrene microspheres (0.357 μm in diam) were moved along the flagellum at an average velocity of 1.8 ± 0.4 μm/s (range of 1–3 μm/s) while the nonmotile mutant of *E. coli* (M-247) was moved at an average velocity of 1.7 ± 0.5 μm/s. *Chlamydomonas* attached by their flagella to polystyrene microspheres, which were immobilized on the surface of the glass slide, moved relative to the immobilized particles (Fig. 3) at 2.2 ± 0.6 μm/s, comparable to the velocities observed for the movement of free particles along the flagellar surface. This same phenomenon was observed to occur in association with immobilized *E. coli*.

Flagellar surface motility is dependent upon the flagellum being attached to a living cell. If cells were fixed with 1% glutaraldehyde or 1% osmium tetroxide for 5 min or if flagella were removed from a cell by gentle pressure on the cover glass, particles still attached to the surface of the flagella but no movement was observed. Flagellar surface motility continued unabated during flagellar regeneration and resorption.

To determine whether the potential to move surface particles is confined to the flagellar membrane, observations were made utilizing the cell wall-less mutant, CW 15 (9). Polystyrene microspheres attached to both the flagellar surface and the rest of the cell surface, but only those microspheres that were associated with the flagellar surface exhibited movement. Ultrastructural observations were made on CW 15 cells in connection with the present study, and no stainable material was observed to be associated with the external surface of the plasma membrane of the cell body. This is in agreement with the observations of Davies and Plaskitt (9).

When an asymmetric clump of polystyrene microspheres was observed to move along the flagellum, the axis of asymmetry did not change relative to the long axis of the flagellum, indicating that the transported object did not "roll" along the flagellar surface.

No bias in the direction of the motility (proximal vs. distal) was detected. Using 0.357-μm polystyrene microspheres, it was observed that 55% of the saltations were directed inward and that 45% were directed outward (n = 594). The average velocity of particle movement inward was 1.8 ± 0.5 μm/s (n = 8) and the average velocity outward was 1.7 ± 0.2 μm/s (n = 8).

Figs. 4–7 are electron micrographs showing the appearance of polystyrene microspheres associated with the flagellar surface of *Chlamydomonas reinhardii* strain pf 18. These cells were fixed while flagellar surface motility was occurring and then washed to remove polystyrene microspheres not mechanically associated with the flagellum. The microspheres appear to interact with the thin layer of material (previously termed the "fuzzy coat" [8] or "flagellar sheath" [25]) that coats the extracellular surface of the flagellar membrane. The close contact between the polystyrene microspheres and this material suggests that the mastigonemes, the other surface component present but poorly visualized by thin-section electron microscopy, have little importance in the association of the marker particles with the flagellar surface.

Fig. 8 is a micrograph of a longitudinally oriented section through a flagellum showing one outer doublet microtubule, the flagellar membrane, and the surface coat. Some filamentous material is present in the space between the outer doublet and the flagellar membrane (arrowheads), suggesting that the outer doublets may be linked to the flagellar surface.

DISCUSSION

Although cilia and flagella have been the subject of intensive study for many years (31), this is the first report of the existence of a rapid, bidirectional system of saltatory motility occurring in association with the flagellar surface. This motile phenomenon is independent of flagellar beating because strains of *Chlamydomonas* paralyzed with respect to the bending motility exhibit the flagellar surface motility. Although there have been a number of reports of movement of individual

marker particles associated with the extracellular surfaces of cells (1-3, 11, 15, 16, 21), these reports do not involve rapid, bidirectional movements having the characteristics defined for saltatory particle movements (24). Surface motility very similar to that reported here for *Chlamydomonas* flagella has been reported by Troyer (35) for the axopodia of the Heliozoan, *Heterophrys*. Bardele (6) suggests that both intracellular and extracellular particle movements occurring within the Heliozoan axopodium involve the lateral displacement of highly ordered membrane domains. He postulates that the motive force for these movements may result from the activity of contractile proteins attached to both the cytoskeletal array of microtubules within the axopodium and the ordered membrane domains.

The observation that whole *Chlamydomonas* move relative to immobilized polystyrene microspheres (Fig. 3) at speeds comparable to those of polystyrene microspheres moving along flagella suggests that a considerable level of force production (presumably through mechano-chemical transduction) is occurring continuously at the surface of the *Chlamydomonas* flagellum. It is reasonable to postulate the involvement of a system of contractile proteins, either forming an integral part of the flagellar membrane or localized within the flagellum and coupled in some manner to the flagellar surface.

Certain observations in the literature assume increased importance in light of this hypothesis. Morphological links have been observed between the outer doublets and the inner surface of the membrane of *Chlamydomonas* flagella (25 and Fig. 9), *Tetrahymena* cilia (5, 30), and *Colpoda* cilia (19). Cytochemical localization procedures for ATPase activity have revealed reaction product between outer doublets and the membrane in rotifer cilia (18) and *Tetrahymena* cilia (10). Magnesium-stimulated ATPase activity has been observed in purified membranes from *Tetrahymena* cilia (13) and *Chlamydomonas* flagella (36). Baugh et al. (7) reported the presence of calcium-activated ATPase activity within purified ciliary membranes of *Tetrahymena* strain BIII but not in strain W. Watanabe and Flavin (38, 39) discovered a low molecular weight calcium-activated ATPase within *Chlamydomonas* flagella; the localization and function of this ATPase are unknown. Preliminary experiments with the divalent cation ionophore A23187 suggest that the flagellar surface motility requires the presence of calcium (R. A. Bloodgood, unpublished results). Longitudinally oriented arrays of particles have been observed in freeze-fracture replicas of the membrane of *Chlamydomonas* flagella (8, 32), *Tetrahymena* cilia (30), and mollusk gill cilia (14). Actin has been searched for within *Tetrahymena* cilia by SDS-polyacrylamide gel electrophoresis but has not been detected (26).

The *Chlamydomonas* flagellar surface consists of three principal components: a single unit membrane covered by a continuous extracellular fuzzy coat and by 0.9 μm long mastigonemes. Ultrastructural observations suggested that the polystyrene particles used in the present study were interacting with the fuzzy surface coat (Figs. 4–7). Little information is available concerning the lateral mobility of these flagellar surface components. *Chlamydomonas* flagellar membranes and mastigonemes have been isolated, purified, and characterized by SDS-polyacrylamide gel electrophoresis and electron microscopy (8, 32, 41). An understanding of the mechanism of the *Chlamydomonas* flagellar surface motility will require considerable additional information concerning the structure of the flagellar membrane, the nature of the various components associated with the inner and outer surfaces of the flagellar membrane, and the localization and function of the nondynein ATPases within the flagellum.

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