

Experimental Dissection of Flagellar Surface Motility in *Chlamydomonas*

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ABSTRACT Experiments have explored the possible relationships between the flagellar surface motility of *Chlamydomonas*, visualized as translocation of polystyrene beads by paralyzed (*pf*) mutants (Bloodgood, 1977, *J. Cell Biol.* 15:983–989), and the capacity of gametic flagella to participate in the mating reaction. While vegetative and gametic flagella bind beads with equal efficiencies and are capable of transporting them along entire flagellar lengths, beads on vegetative flagella are primarily associated with the proximal half of the flagella whereas those on gametic flagella exhibit no such preference. This difference may relate to the “tipping” response of gametes during sexual flagellar agglutination (Goodenough and Jurivich, 1978, *J. Cell Biol.* 79:680–693). Colchicine, vinblastine, chymotrypsin, cytochalasins B and D, and anti- β -tubulin antiserum are all able to inhibit the binding of beads to the flagellar surface. Trypsin digestion and an antiserum directed against whole *Chlamydomonas* flagella have no effect on the ability of flagella to bind beads, but the beads remain immobile. These results suggest that at least two flagellar activities participate in surface motility: (a) bead binding, which may involve a tubulin-like component at the flagellar surface; and (b) bead translocation, which may depend on a second component (e.g. an ATPase) of the flagellar surface. Surface motility is shown to be distinct from gametic adhesiveness per se, but it may participate in concentrating dispersed agglutinins, in driving them toward the flagellar tips, and/or in generating a signal-to-fuse from the flagellar tips to the cell body. Directly supporting these concepts is the observation that bound beads remain immobilized at the flagellar tips during the “tip-locking” stage of *pf* \times *pf* matings, and the observation that bound ligands such as antibody fail to be tipped by trypsinized flagella.

Movement of marker particles on the surface of biological membranes has been widely used to study membrane fluidity and cell migration (1, 3, 4, 10, 13, 23, 24, 26). Recently, discussion has centered on possible connections between membranes and underlying cytoskeletal structures (i.e., axonemes, microtubules, microfilaments, and actomyosin complexes) (11, 12, 31). It therefore becomes of interest to learn whether surface particle movements are driven by known components of the cytoskeleton, and whether these movements can serve to transmit biological information to the cell interior.

We have addressed these questions by analyzing surface motility associated with the flagellar membrane of the unicellular eukaryote *Chlamydomonas reinhardi*. As described and discussed in detail by Bloodgood and co-workers (7, 8), the flagella will bind polystyrene microspheres, small fragments of

debris, or bacterial cells suspended in methyl cellulose, and these will be rapidly transported up and down the length of the flagellum. Each bead moves linearly along the flagellum, and if several beads are bound to the same flagellum they can pass each other as if on tracks.

We have identified two distinct phases of flagellar surface motility, bead binding and bead translocation, using as probes various enzyme, drug, and antiserum treatments. We further show that the pattern of surface motility changes at two distinct junctions in the organism's life cycle: beads are more prone to traverse the full length of gametic flagella than the full length of vegetative flagella, and beads “freeze” at the flagellar tips during the pairing stage of the gametic mating reaction. We propose that these surface motility properties reflect aspects of flagellar differentiation that serve in the mating response.

MATERIALS AND METHODS

Strains and Culture Conditions

Experiments were in most cases performed using the wild-type *C. reinhardi*, strain 137c, mating type plus (mt^+) and mating type minus (mt^-), and the paralyzed flagellar mutant, *pf-18*, mt^+ , and mt^- (35). Where indicated, strains *pf-1* and *pf-14*, mt^+ and mt^- were used (38). The *pf-18* mt^- RAB strain was obtained from Dr. R. A. Bloodgood (Department of Anatomy, University of Virginia Medical School, Charlottesville, Va.). Two of the nonagglutinating impotent mutants (*imp-2* and *imp-8*) (6, 19, 20), crossed into a *pf-18* background, were used in several experiments. The conditional nonsignaling mutant *gam-1* (16) was assayed at both the permissive temperature (25°C) and the restrictive temperature (35°C).

Vegetative cells were grown for 36 h in shaking liquid culture in Tris-acetate-phosphate (TAP) medium (22) under continuous light at 25°C, and were harvested in log-phase growth. To ensure that cells would not deplete the available nitrogen (27), an extra aliquot of TAP medium (half the volume of the original culture) was added at 12 h of growth. Single cells were freed from their mother walls by a 15–30-min treatment with autolysin prepared from the mating of wild-type gametes (21).

Gametes were prepared from 1- to 2-wk-old TAP plate cultures (27). Gametes were suspended into nitrogen-free high-salt minimal medium (NFHSM) (34), washed, and resuspended in fresh NFHSM.

Cell number was determined with a hemacytometer.

Fusion Assay and Mating Efficiency

Gametic cell fusion was monitored by allowing equal numbers of each *mt* to mate in a tube for 15 or 30 min, fixing the mating sample with two drops of 3% glutaraldehyde in 10 mM HEPES buffer, pH 7.0, and then counting the number of biflagellated cells (BFC) and quadriflagellated cells (QFC) in a sample of ~300 cells. The percent fusion was calculated according to the formula: Percent fusion = $2(QFC) \times 100 / [2(QFC) + BFC]$.

Mating efficiencies for the wild-type (*wt*) strains were generally >95%. Mating efficiencies of the *pf* mutants, as determined in *pf* × *wt* matings, averaged ~88%, possibly because *pf* cells tended to settle and were thereby somewhat less accessible to their *wt* mates.

Surface Motility Assays

Monodisperse polystyrene microspheres (0.358 μ m diameter) were obtained from Polysciences, Inc. (Warrington, Pa.). These were washed with deionized water and resuspended in NFHSM to make a concentrated stock solution which was used, at a 1:100 dilution, in all the experiments reported.

To quantitate bead binding, the diluted bead sample was presented to an equal volume of cells at 1×10^7 or 3×10^7 cells/ml. After 5 min, the cells were observed with the light microscope. Flagella were scored as either “+ bead” or “- bead”. Occasional flagella bearing more than one bead were scored only once as “+ bead”. Approximately 300 flagella were counted per assay. The percentage of flagella binding beads was calculated as: Percentage of flagella binding beads = (number “+ bead”) × 100 / (total number flagella scored).

To obtain a kinetic measure of surface motility, cells were mixed with beads as above, binding was allowed to progress for 5 min, and randomly selected cells were observed under the light microscope. The observer continuously called out

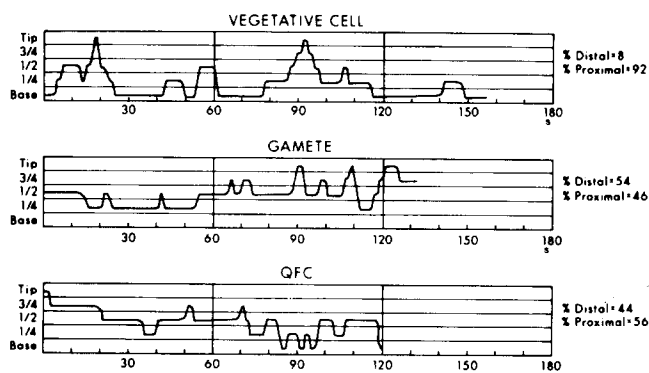


FIGURE 1 Data from assay for surface motility. Shown are representative samples of experiments done with vegetative cells, gametic cells, and quadriflagellated zygotes. (Cells in all cases have been observed for at least 2 min.)

the position of the bead on the flagellum, i.e., whether it was at the tip, the distal quarter, at the midpoint, the proximal quarter, or the base. With the aid of a stopwatch, a second individual recorded the times at which, during the translocation process, the bead was observed to pass each of the landmark positions. A given bead was monitored for ~2 min. Fig. 1 shows the type of data obtained in such experiments, giving an example from a vegetative cell, a gamete, and a quadriflagellated zygote. In diagramming this data, points were plotted and were then connected by smooth lines designed to convey the gradual progression of the bead along the length of the flagellum. In each experiment, when the data from several dozen such patterns were considered collectively, a percentage of time that the bead spent in the proximal vs. distal half of the flagellum could be calculated. Values at the halfway point were scored as “proximal” if the bead next moved to a proximal position, and “distal” if it moved on to a distal position.

Photomicroscopy

Wet mounts of both untreated and glutaraldehyde-fixed samples were photographed with a Zeiss photomicroscope equipped with phase optics under oil immersion. Exposures of ~1s duration were taken on Kodak Panatomic-X film and film was developed in Kodak Microdol-X developer.

Enzyme, Drug, and Inhibitor Treatments

Trypsin-TPCK, chymotrypsin, and soybean trypsin inhibitor (SBTI) were obtained from Worthington Biochemicals Corp. (Freehold, N. J.). Enzyme solutions were prepared daily to the specified concentrations in NFHSM. Colchicine, vinblastine sulfate, cytochalasins B and D, and phenylmethyl sulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Colchicine and vinblastine solutions were prepared directly in NFHSM to the concentrations specified. Cytochalasins B and D were each dissolved at 10 mg/ml (20 mM) in dimethyl sulfoxide (DMSO) (Sigma Chemical Co.), and these stock solutions were further diluted with NFHSM. PMSF was dissolved as a 100-mM stock solution in ethanol, and further diluted with NFHSM. In all cases, cells were spun down from their medium and resuspended in the appropriate experimental solution.

Antisera

Anti- G^+ antiserum was raised against intact, glutaraldehyde-fixed flagella from gametic mt^+ cells as described by Goodenough and Jurivich (21). Analysis of this antiserum by the methods of Adair et al. (2) reveals IgG species reacting with ~15 surface antigens of the flagellum, the pattern being indistinguishable from Fig. 3c of reference 2. Very minor reactivity is found in the tubulin region of the gel.

Anti- β -tubulin antiserum was raised against purified *Chlamydomonas* axonemal β -tubulin (30), and was the gift of Dr. G. Piperno (The Rockefeller University, New York).

RESULTS

Our experimental observations are presented in the following sequence. We first describe the bead-binding properties of the flagellar surface and reagents that inhibit this binding. We next describe reagents that obliterate bead translocation but do not affect binding, and note the effects of the various reagents employed on beads that are already bound. Finally, we analyze bead translocation patterns at various stages of the *Chlamydomonas* life cycle.

Bead Binding

In pilot experiments (25) we determined that *pf-18* mt^+ gametes bind equivalent numbers of beads over a 100-fold range of bead concentrations, the numbers dropping off only when very dilute suspensions of beads are presented. Somewhat unexpectedly, a maximum of only about one-third of the flagella bind beads at any given time. It was also noted that, even in very concentrated suspensions, flagella generally bind only one bead. About 5% of the “+ bead” flagella has two beads bound simultaneously, and only very rarely are flagella encountered bearing more than two beads. To determine whether this number is consistent throughout the various stages

of the *Chlamydomonas* life cycle, several other cell types were tested. The results, presented in Table I, show that vegetative, gametic, *mt*⁺, *mt*⁻, quadriflagellated zygotes, two of the non-agglutinating impotent mutants (*imp-2* and *imp-8*), and the conditionally nonsignaling mutant *gam-1* all bind beads with only a 25–35% efficiency.

The percentage of beads bound is also independent of the medium in which the cells and beads are suspended. Several strains were tested in NFHSM, TAP medium, and Medium I of Sager and Granick (32), and in all cases between 25 and 35% of the flagella exhibit bead binding. If cells are allowed to bind beads and are then treated with 1.5% glutaraldehyde, the beads remain attached. If, however, cells are prefixed with glutaraldehyde and are then presented with beads, the beads do not bind, indicating binding to be both an active and a specific process.

The ability of *pf* flagella to bind beads may be partially dependent on the inability of these flagella to move. Wild-type cells, if temporarily immobilized by the addition of a small amount of DMSO (3–5% vol/vol), are able to bind beads at efficiencies approaching those of their *pf* counterparts. If, however, untreated *pf* and wild-type cells are exposed to beads for 5 min (by the usual binding protocol) and are fixed as above with 1.5% glutaraldehyde before counting in the light microscope, a very different result is obtained. Such treatment has no effect on the percentage of *pf* flagella that bear bound beads, but none of the wild-type flagella exhibit bound beads under these conditions.

Effects of Reagents on Bead Binding

TRYPsin: When $1-3 \times 10^7$ gametic *pf-18* cells/ml are treated with 1 mg/ml trypsin for 15 min, no effect on the binding of beads is observed: the percentage of flagella with beads bound is identical in untreated and treated samples. The

TABLE I
Binding of Beads to Flagella at Various Stages of the Life Cycle

Strain	+ Bead	- Bead	%
Vegetative			
<i>pf-18 mt</i> ⁺	37	121	23
<i>pf-18 mt</i> ⁻	56	123	31
<i>pf-18 mt</i> ⁻ RAB	55	145	28
Gametic			
<i>pf-18 mt</i> ⁺	44	155	22
	42	143	23
<i>pf-18 mt</i> ⁻	50	156	24
	44	167	21
<i>pf-18 mt</i> ⁻ RAB	59	149	28
<i>pf-1 mt</i> ⁻	48	153	24
Quadriflagellated cells			
<i>pf-18 mt</i> ⁺ × <i>pf-18 mt</i> ⁻	41	142	29
Impotent strains; gametic			
<i>imp-2 pf-18 mt</i> ⁺	69	141	33
<i>imp-8 pf-18 mt</i> ⁺	62	162	28
<i>gam-1</i> ; Gametic			
35°C	41	113	21
	41	238	15

Cells of the strains indicated were suspended at 1×10^7 cells/ml in the medium appropriate to their stage in the *Chlamydomonas* life cycle (i.e. gametes and quadriflagellated cells in NFHSM, and vegetative cells in TAP medium). The concentrated stock solution of beads was diluted 1:100 with either NFHSM or TAP medium. Diluted beads were presented to an equal volume of cells, and were allowed to bind for 5 min. Flagella were scored in the light microscope as “+ bead” or “- bead”, and the percent of flagella binding beads was calculated.

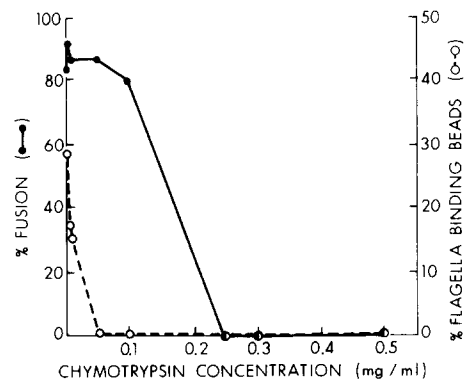


FIGURE 2 The effects of increasing concentrations of chymotrypsin on the fusion of *pf-18 mt*⁺ and *wt mt*⁻ gametes and on the binding of beads to the flagella of *pf-18 mt*⁺ gametes. The data shown are from a representative experiment. Mating types were pretreated individually for 15 min at 3×10^7 cells/ml in various concentrations of chymotrypsin, opposite mating types were mixed, and agglutination was assessed after 5 min of mating. In all but the highest concentration tested, agglutination was found to be unaffected as compared to untreated controls. At 0.5 mg/ml chymotrypsin, agglutination was severely reduced. Fusion was allowed to proceed for 15 min, and the mating reaction was stopped by the addition of 3% glutaraldehyde solution. Cells were scored in the light microscope as QFC or BFC, and the percent fusion was calculated. The dashed curve shows the concentration dependence of bead binding to the flagella of *pf-18 mt*⁺ cells. Cells were pretreated as above, and were then presented with an equal volume of beads diluted 1:100 from the concentrated stock solution. Binding was allowed to proceed for 5 min, flagella were scored by light microscopy, and the percentage of flagella binding beads was calculated.

experiment was performed with *pf-18 mt*⁺, *pf-18 mt*⁻, and *pf-18 mt*⁻ RAB cells.

The treated cells are unable to undergo sexual agglutination with untreated cells of opposite *mt* demonstrating that the trypsin treatment is indeed affecting the cells (cf., 28, 33, 37).

CHYMOTRYPSIN: The dashed curve in Fig. 2 illustrates the effects of increasing concentrations of chymotrypsin on the binding of beads. Treatment of $3 \times 10^7-10^8$ gametic cells/ml with chymotrypsin for 15 min completely inhibits bead binding at all but extremely low chymotrypsin concentrations. The solid curve in Fig. 2 displays the sensitivity of cell fusion to chymotrypsin treatment; it is seen to be considerably less sensitive than bead binding. Binding ability recovers within 45 min after removal of chymotrypsin by thorough and repeated washing, or by inhibition of the chymotrypsin by 1 mM PMSF.

COLCHICINE AND VINBLASTINE: When 3×10^7 cells/ml are presented with increasing concentrations of colchicine for 30 min, bead binding is increasingly affected (Fig. 3a, dashed line). At all concentrations tested, sexual agglutinability remains uninhibited, but the loss of bead-binding ability closely parallels the loss of the ability to fuse (Fig. 3a, solid line). When an occasional bead binds in spite of the presence of colchicine, the bead is transported normally. Removal of colchicine by washing results in the rapid (within 15 min) restoration of binding.

In contrast to the almost immediate ability of colchicine to abolish mating (29), the complete inhibition of bead binding appears to require a longer exposure to the drug. Preincubation of 3×10^7 cells/ml in 15 mg/ml (37.5 mM) colchicine for 15 min effects a 100% inhibition of fusion but only a 60–70% reduction in the percentage of flagella binding beads; a 30-min preincubation is necessary to completely abolish binding.

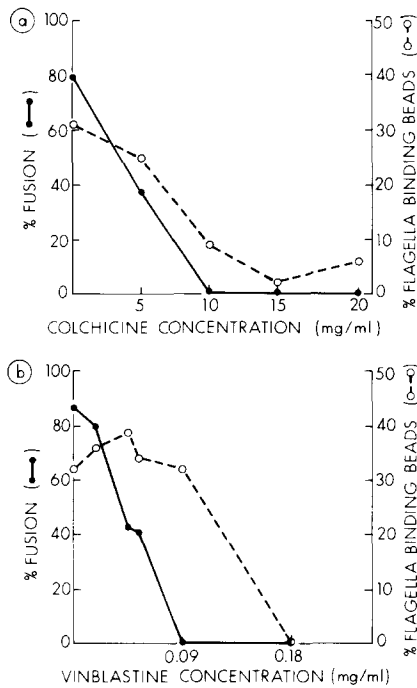


FIGURE 3 The effects of increasing concentrations of colchicine (a) and vinblastine (b) on the fusion of *pf-18 mt+* and *wt mt-* gametes and on the binding of beads to the flagella of *pf-18 mt+* gametes. The data shown are from representative experiments. Cells were preincubated at 3×10^7 cells/ml in various concentrations of colchicine or vinblastine for 30 min, opposite mating types were mixed, and fusion was allowed to proceed for 15 min. At 2-3 min after mixing, agglutination was assessed and judged in all cases of treatment to be as vigorous as in untreated controls. The mating reaction was then stopped by the addition of 3% glutaraldehyde solution. Cells were scored in the light microscope as QFC or BFC and the percent fusion was calculated. The dashed curves show the concentration dependence of bead binding to the flagella of *pf-18 mt+* cells. Cells were pretreated as above, and were then presented with an equal volume of beads diluted 1:100 from the concentrated stock solution. Binding was allowed to proceed for 5 min, flagella were scored by light microscopy, and the percentage of flagella binding beads was calculated.

Vinblastine sulfate, another antimetabolic drug, is also able to block the binding of beads to flagellar surfaces. In contrast to the gradual inhibition observed with increasing concentrations of colchicine, vinblastine causes a sharp inhibition of binding (Fig. 3b, dashed curve). This inhibition occurs between 0.09 and 0.18 mg/ml (0.1 and 0.2 mM) vinblastine, which is higher than the concentration necessary to inhibit cell fusion (Fig. 3b, solid curve). As is true for colchicine, when an occasional bead manages to bind in the presence of vinblastine it is transported normally, suggesting for both reagents that only the binding activity has been affected.

ANTISERA: Table II summarizes the effects of presenting gametes with one of several different antisera, and subsequently incubating the cells with beads. It is seen that neither an antiserum raised against *Chlamydomonas* flagella nor preimmune control serum has an effect on bead-binding ability. If cells are pretreated with an antiserum raised against *Chlamydomonas* axonemal β -tubulin, however, the beads are never observed to bind to the surface of the flagella.

CYTOCHALASINS B AND D: Fig. 4a displays the effects of cytochalasins B (closed circles) and D (open circles) on bead binding, while Fig. 4b shows the effects of parallel drug

concentrations on cell fusion. Inhibition of binding is achieved at concentrations that only partially affect zygote formation.

Effects of Reagents on Bead Translocation

TRYPSIN: Two different treatments uniquely affect the movement of the beads once they are bound to the flagella. Treatment of 3×10^7 - 10^8 gametic cells/ml with 1 mg/ml trypsin for 15 min, although without effect on the ability of cells to bind beads, disallows bead translocation; instead, the beads remain stationary in their bound positions. Subsequent treatment of the trypsinized cells with 1 mg/ml SBTI reverses the inhibition by trypsin: within 15 min after addition of SBTI, surface motility begins to reappear, and increases to its normal level by 30 min, similar in kinetics to the recovery of sexual agglutinability (18). It should be noted that the recovery of the ability to fuse requires a much longer time (18). Control experiments employing trypsin preincubated with stoichiometric amounts of SBTI demonstrate that inactivated trypsin has no effect on bead translocation.

ANTISERUM: The second means by which to inhibit bead translocation employs an antiserum (anti-G⁺) raised against intact *Chlamydomonas* gametic flagella. When presented to live *pf* gametic cells of one *mt*, the cells isoagglutinate and the resultant antigen-antibody complexes move toward the flagellar tips (21), reminiscent of the tip-locking stage of agglutination (18). As indicated in Table II, beads will bind along the length of antiserum-treated flagella, but they will not move.

Effects of Reagents on Flagella Preincubated with Beads

The experiments summarized in Table III were designed to test whether any of the treatments that inhibit bead binding or bead translocation can exert their effects on beads that are already bound to the flagellar surface. The results indicate that neither chymotrypsin nor colchicine (both of which are able to inhibit the binding of beads) is able to effect the removal of beads once they are bound. Trypsin treatment (which has no effect on bead binding, but does inhibit bead translocation) is also unable to remove bound beads, but the beads soon cease to be transported up and down the flagellar length in the presence of this enzyme.

TABLE II
Effect of Antiserum Pretreatment on Bead Binding and Surface Motility

Pretreatment (Dilution)	+ Bead	- Bead	%	SM*
Untreated control	93	204	31	Yes
	101	191	35	Yes
Preimmune control serum				
1:100	73	171	30	Yes
1:50	90	177	34	Yes
Anti-G ⁺ antiserum				
1:100	61	181	25	No
1:50	44	128	26	No
Anti- β -tubulin antiserum				
1:1	0	210	0	—

pf-18 mt+ gametes were suspended in NFHSM at 1×10^7 cells/ml. Cells were preincubated with antiserum for 5 min, an equal volume of beads was added and binding was allowed to proceed for an additional 5 min. Flagella were scored by light microscopy as "+ bead" or "- bead", and the percent of flagella binding beads was calculated.

* Surface motility (SM) was determined visually in the light microscope.

Bead Translocation Patterns in Unmated Cells

Because the flagella of *Chlamydomonas* gametes are known to differ from vegetative flagella in their ability to agglutinate and transmit sexual signals, experiments were designed to ascertain if there is any detectable difference in the surface motility of gametic vs. vegetative cells, or in *mt*⁺ vs. *mt*⁻ cells. Several different *pf* strains were used in these experiments.

Vegetative and gametic cells are found to be comparable in the number of beads bound and in the rate of bead translocation (~2 μm/s) (25). When patterns of bead translocation are

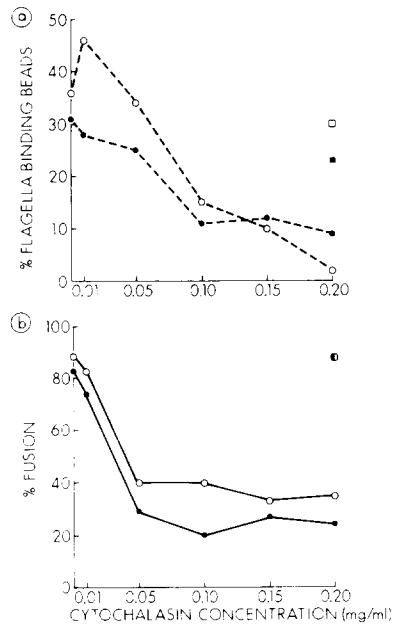


FIGURE 4 The effects of increasing concentrations of cytochalasins B and D on the binding of beads to the flagella of *pf-18 mt*⁺ gametes (a) and on the fusion of *pf-18 mt*⁺ and *wt mt*⁻ gametes (b). Cytochalasin stocks were prepared at 10 mg/ml (20 mM) in DMSO. Cells were preincubated at 1×10^7 cells/ml for 30 min before mating or assaying bead binding. All fusions were allowed to proceed for 15 min, and the mating reaction was stopped by the addition of 3% glutaraldehyde solution. Cells were scored in the light microscope as QFC or BFC and the percent fusion was calculated. Bead binding was allowed to proceed for 5 min, flagella were scored by light microscopy, and the percentage of flagella binding beads was calculated. (●, cytochalasin B, ■, 20 μl DMSO control; ○, cytochalasin D, □, 20 μl DMSO control.)

monitored as described in Materials and Methods and illustrated in Fig. 1, moreover, beads are found to engage in similar forms of motility on the two types of flagella: a given bead may traverse the entire length of the flagellum but, more commonly, it moves only a short distance, stops, and restarts in the same or opposite direction. There is, however, a distinct difference in the distribution of the beads in vegetative vs. gametic cells. As illustrated in Fig. 1 and summarized in Table IV, beads tend to move over the entire flagellar length in gametic cells, spending as much or more time in the distal half as in the proximal half. By contrast, beads bound to vegetative

TABLE III
Effects of Enzyme and Drug Treatments on Bound Beads

Treatment	% Flagella binding beads	SM*
Controls		
Untreated cells	35	Yes
Chymotrypsinized cells (0.25 mg/ml for 15 min)	0	—
Colchicine-treated cells (15 mg/ml for 30 min)	0	—
Trypsinized cells (1 mg/ml for 15 min)	33	No
Prebound beads		
NFHSM		
(5 min)	36	Yes
(15 min)	36	Yes
(30 min)	26	Yes
Chymotrypsin (0.25 mg/ml)		
(5 min)	28	Yes
(15 min)	24	Yes
Colchicine (15 mg/ml)		
(5 min)	38	Yes
(15 min)	33	Yes
(30 min)	31	Yes
Trypsin (1 mg/ml)		
(5 min)	33	No
(15 min)	32	No

pf-18 mt⁺ gametes were suspended in NFHSM at 3×10^7 cells/ml. Beads were presented to an equal volume of cells and allowed to bind for 5 min. Samples were taken, and treated with the enzyme or drug solutions as indicated. At the intervals shown, flagella were scored by light microscopy as "+ bead" or "- bead" and the percent of flagella binding beads was calculated.

* Surface motility (SM) was determined visually in the light microscope.

TABLE IV
Average Percentage of Time Beads Spend in Proximal vs. Distal Half of Flagella

	Distal	Proxi- mal	Cells ex- amined	σ_n		Distal	Proxi- mal	Cells ex- amined	σ_n
<i>mt</i>⁺ Gametic cells					<i>mt</i>⁺ Vegetative cells				
<i>pf-18</i>	71	29	(12)	30	<i>pf-18</i>	16	84	(14)	19
<i>pf-14</i>	67	33	(16)	28		4	96	(5)	8
<i>mt</i>⁻ Gametic cells					<i>mt</i>⁻ Vegetative cells				
<i>pf-18</i>	70	30	(8)	44	<i>pf-18</i>	9	91	(10)	23
	76	24	(9)	35	<i>pf-1</i>	2	98	(13)	6
<i>pf-14</i>	42	58	(10)	31	<i>pf-18 RAB</i>	9	91	(9)	11
<i>pf-1</i>	37	63	(16)	32					
	39	61	(7)	34	Quadriflagellated cells				
	25	75	(6)	32	<i>pf-18 mt</i> ⁺ × <i>pf-18 mt</i> ⁻	41	59	(11)	37
<i>pf-18 RAB</i>	47	53	(11)	35		41	59	(10)	33
	20	80	(15)	28					
	55	45	(12)	22					

flagella exhibit a strong preference for a proximal location, even though they can traverse the entire flagellar length.

In addition to moving the bead to the flagellar tip more frequently, gametic cells tend to hold beads at the tip for a longer time, the average time for gametes being 12 s and the average for vegetative cells being only 3 s.

QFCs formed as the product of a *pf-18 mt⁺* × *pf-18 mt⁻* mating have four paralyzed flagella, and as such are amenable to studies of surface motility. These cells appear to retain a gametic surface motility, that is, the beads are distributed randomly along the flagellar length (Table IV), and beads are retained longer at the flagellar tips.

By assaying bead translocation in impotent (*imp*) strains that have lost their ability to agglutinate sexually (19), and in the conditional mutant *gam-1* which fails to signal sexually at restrictive temperature (15) it was determined that the “gametic surface motility phenotype” is not dependent on the ability to mate per se. All mutant strains are found to exhibit a gametic surface motility when grown under gametogenic conditions (25).

Finally, data in Table IV reveal that patterns of surface motility are independent of mating type: *mt⁺* and *mt⁻* gametes of several *pf* strains behave similarly, as do *mt⁺* and *mt⁻* vegetative cells.

Bead Translocation Patterns during Mating

As described in detail elsewhere (18), sequential stages in the *Chlamydomonas* mating reaction are readily visualized by watching the leisurely mating behavior of two *pf* strains. Initial sites of sexual adhesion move relative to one another along the flagellar surface, a stage we term “contact migration”. The sites then move out to the flagellar tips and there remain stationary, often for minutes at a time, resulting in a stage we term “tip locking”. Finally, cell fusion signals flagellar disadhesion and a concomitant “tip unlocking.”

When two *pf* strains of opposite mating type are mixed in the presence of beads, striking changes in bead behavior occur

during the course of these phases of adhesion/disadhesion. If agglutinated flagella are in the contact-migration phase of the mating reaction and have also bound one or more bead(s) (Fig. 5), the beads move up and down the flagellum in apparently the same fashion as in unmated cells. The beads will, in fact, move past or over a contact site as if totally oblivious to its existence, with the rate of bead migration observed to be considerably faster than the rate of contact migration. As tip locking occurs, however, the beads come to rest at the extreme distal tips and surface motility stops, as if the beads have been locked into place by the tight association of the flagellar tips. Beads in this position will remain at the tips, stationary, for many minutes at a time (Fig. 6, arrows). When tip unlocking occurs, either because of “abortive pairing” (18) or as a consequence of cell fusion, beads may fall off as the tips disengage or, alternatively, they may remain with one of the flagella and resume their up-and-down migration pattern.

Trypsin Sensitivity of Antibody Tipping

Because trypsin abolishes bead translocation, and because beads lock at the flagellar tips during mating, we wished to learn whether the translocation mechanism and the tipping mechanism share a common “motor”. Sexual agglutination is trypsin-sensitive, however, so it is not possible to ask whether sexually agglutinated cells fail to tip agglutinins in the presence of trypsin. We therefore took advantage of the fact that gametic cells of one *mt* can be isoagglutinated by anti-G⁺ antiserum and that the bound antiserum is rapidly moved to the flagellar tips (21). Because anti-G⁺-mediated agglutination persists in trypsinized cells (that is, at least some surface antigens are trypsin resistant) (9), we asked whether trypsinized cells are able to tip their bound antiserum.

Wild-type *mt⁺* gametes (1×10^7 cells/ml) were treated for 15 min with 1 mg/ml trypsin, which inhibits both sexual agglutination and bead translocation. They were then washed (to prevent trypsin destruction of antibody) and immediately presented with anti-G⁺ antiserum. Initial isoagglutinating con-

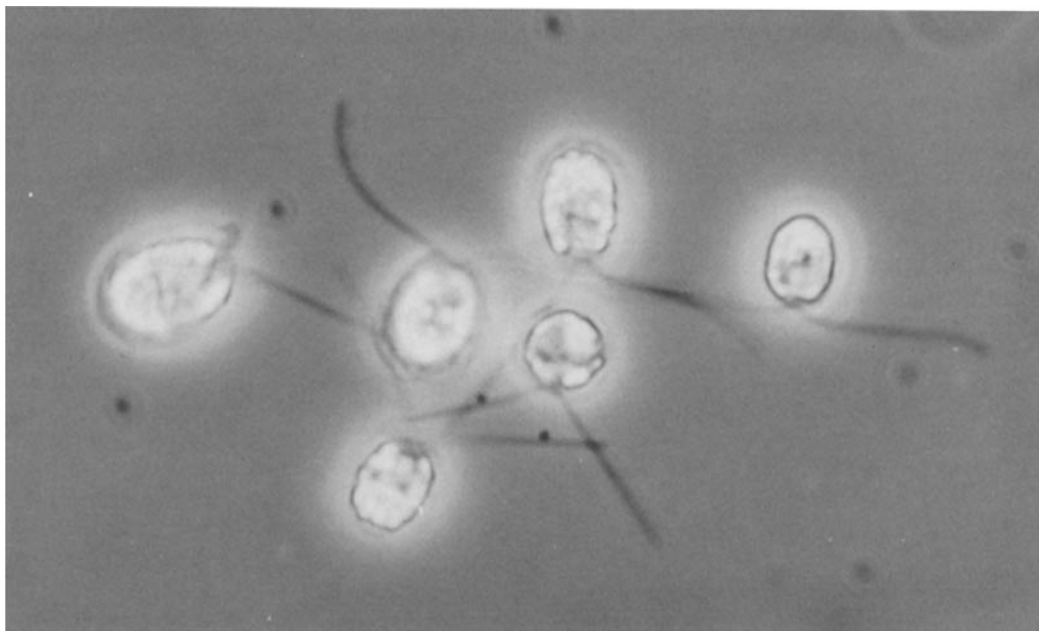


FIGURE 5 *pf-18 mt⁺* and *pf-18 mt⁻* gametes exhibiting bead binding at the contact-migration stage of agglutination. Cells were mated in the presence of beads for 5 min, then fixed with glutaraldehyde to aid in photography. The gametes depicted were all cross-linked by the fixative, an outcome that occurs only if cells are agglutinating when the glutaraldehyde is presented. ($\times 2,300$).

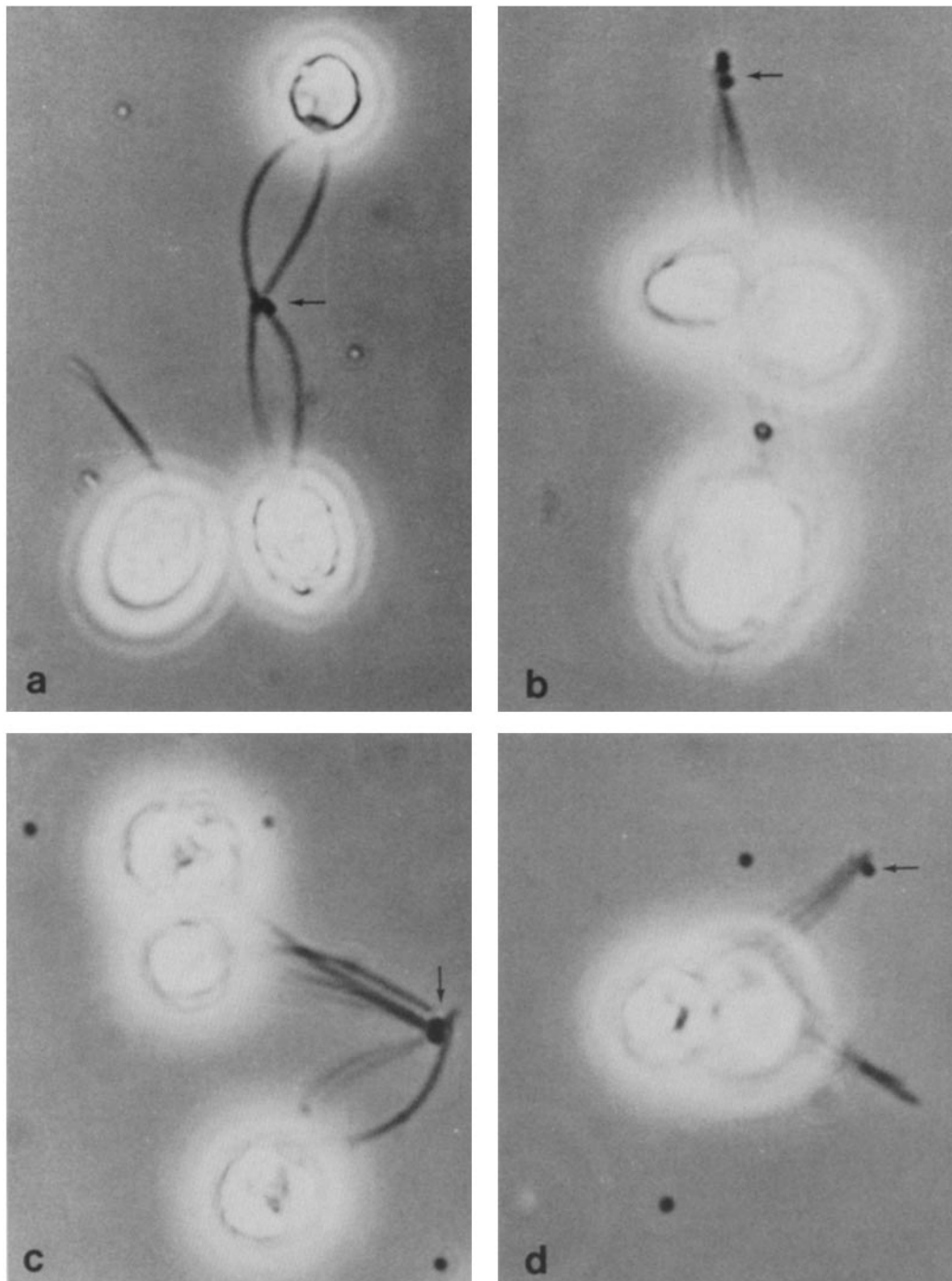


FIGURE 6 *pf-18 mt⁺* and *pf-18 mt⁻* gametes exhibiting bead binding at the tip-locking stage of agglutination. Cells were mated in the presence of beads for 5 min, then fixed with glutaraldehyde to aid in photography. Beads locked in at the agglutinating flagellar tips are indicated by the arrows. ($\times 2,700$).

tacts occur at random sites along the flagellar surfaces, as is the case for untreated gametes (21). These contacts, however, are never transported to the tips, and the cells remain tightly clumped together, much as in a colchicine-treated (29) or a *gam-1* (15) mating. Because the cells have been washed, they proceed to repair the trypsin-induced damage: within 15 min after washing, the contacts begin to move toward the flagellar tips and, by 20–25 min, the isoagglutination appears identical to that of untrypsinized control cells in the presence of antiserum.

Experiments were also performed in which washed trypsinized cells were allowed to recover in the absence of antiserum

for 0, 7, 15, or 25 min; at each interval, a sample of cells was presented with anti-G⁺, and the resultant contacts were immediately observed in the light microscope. Again, tip interactions are absent in the 7-min sample but numerous in the 15-min sample, and by 25 min virtually all contacts move immediately to the flagellar tips. Preimmune serum has no effect in parallel experiments.

Flagellar Twitching and Its Sensitivity

The *pf* strains permit the observation of an additional form of flagellar motility in *Chlamydomonas*, namely, an ability to

“twitch” (35). This movement is discernible as a frequent quivering or waving of the distal-most one-fifth of the flagellum, and is accompanied by an occasional spasmodic jerking of the entire flagellum. The two flagella of any given cell appear to twitch independently of one another, and this residual motility fails to generate an active swimming stroke. Mutant strains lacking either central pair microtubules (*pf-18*) or radial spokes (*pf-1* and *pf-14*) can twitch, and twitching seems to be more pronounced in gametic than in vegetative cells. Fig. 7 shows a series of photomicrographs of a single twitching *pf-18 mt⁺* cell. Twitching can be visualized as a “double-exposure” effect resulting from the movement of the flagella during the 1-s exposure time.

Of the reagents studied, only trypsin has any discernible effect on twitching: digestion with 1 mg/ml trypsin for 15 min totally obliterates all flagellar movement. Twitching and surface motility reappear concurrently within 15 min after washing out of trypsin or inhibition of the enzyme by 1 mg/ml SBTI. Neither chymotrypsin digestion nor any of the drug or antiserum treatments employed in this study have any obvious effects on the magnitude or pattern of the twitching response.

DISCUSSION

Bead Binding

Our assay of bead binding, which differs from that of Bloodgood et al. (7, 8), reveals that only one-quarter to one-third of the flagella in a given *Chlamydomonas* population exhibit at least one bound bead at any given time. This value is largely independent of the concentration of beads presented to the cells (25); it is also independent of the *pf* strain used and of the cells' stage in the life cycle (Table I). Finally, the value is independent of time, being equivalent for 5 or 30 min of incubation (cf., Table III). Indeed, although beads are occasionally observed to attach onto or detach from a flagellum during the course of a 30-min observation, such events are fairly uncommon. We conclude, therefore, that at the time of bead presentation, perhaps one-quarter of the flagella possesses the surface properties necessary to bind beads.

To explain why the majority of the flagella in a cell population are not engaged in bead binding at a given time, one can speculate that surface motility is, in fact, operational in all flagella but that a critical number of binding components must be adjacent to one another on the flagellar surface in order to

bind a large polystyrene bead. At the time of bead presentation, by such reasoning, roughly one-quarter of the flagella would happen to have this critical topography at some surface locale, and these would readily bind beads. For the remaining flagella, the correct binding configuration would be in the process of being attained, presumably at some modest rate. Simultaneously, and at this same rate, the correct configuration would be in the process of being lost from the flagella that had already bound beads. The result, therefore, would be a constant “steady-state” value of ~25–35%.

Inhibition of Bead Binding

We have been able to dissect surface motility into two component activities, bead binding and bead translocation, by their differential sensitivity to various experimental agents. Binding is the more sensitive of the two parameters, being hindered by the drugs colchicine, vinblastine, and cytochalasins B and D, and abolished by anti- β -antiserum and by chymotrypsin digestion.

As discussed more fully elsewhere (29), the concentrations of colchicine, vinblastine, and the cytochalasins required to inhibit bead binding are sufficiently high that one cannot conclude that their disruptive effects entail direct interaction with tubulin or actin. We present the drug studies, therefore, to indicate that bead binding can be reversibly manipulated by agents that are known to interact with cytoskeletal elements and which we find also interfere with the mating reaction (29, and Figs. 3 and 4). Further experimentation is required to establish whether or not actin and/or tubulin molecules participate in surface motility.

Bead Translocation

Once a bead has bound and engages in surface motility, two parameters can be monitored: the rate at which the bead is translocated, and its pattern of motion—that is, the distribution of the bead's position on the flagellar surface as a function of time. Although we find no difference in translocation rates when vegetative, gametic, and quadriflagellated cells are compared, two differences are evident in their surface motility patterns. First, beads localize preferentially in the proximal half of vegetative flagella but exhibit no such preference on gametic flagella nor on flagella of QFCs (Table IV). Second, vegetative cells only rarely shuttle beads to the extreme distal

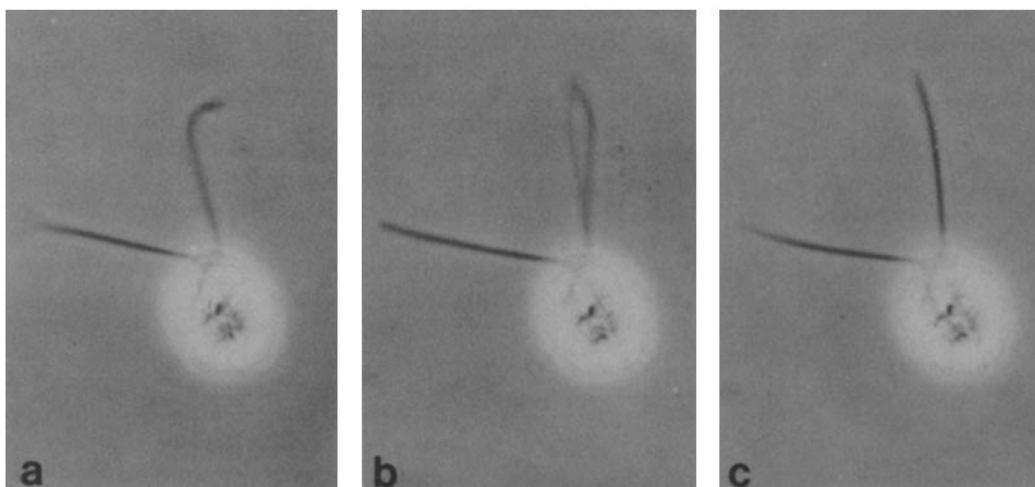


FIGURE 7 Series of photomicrographs of an untreated *pf-18 mt⁺* gamete. Sequential exposures are of 1-s duration. The twitching of the flagella during each exposure results in a “double-exposure” effect. ($\times 2,000$).

tips and, when they do, the "tipped" beads are rapidly returned to a more proximal position. By contrast, gametes and QFCs move and maintain beads at the tips for much longer periods. These differences are independent of sexual agglutinability per se: the nonagglutinating *imp* strains and the nonadhesive QFCs both retain the gametic pattern.

These observations show interesting parallels with another life-cycle-dependent property of *C. reinhardi* flagella. It has been shown that whereas vegetative and gametic flagella bind comparable amounts of the lectin concanavalin A (5), gametes (and QFCs) alone concentrate the lectin at their flagellar tips in such a fashion that isoagglutination is induced (28, 37). Similarly, only gametes (and QFCs) are able to mobilize high concentrations of anti-flagellar antibody toward their flagellar tips (21). This tipping ability has thus been identified as a specific gametic property, possibly relevant to the ability to agglutinate sexually, and the more unrestricted movement of beads on gametic flagella may reflect the acquisition of the tipping potential.

Inhibition of Bead Translocation

The process of bead translocation can be selectively interfered with by two agents: trypsin or the anti-G⁺ antiserum. The contrasting behavior of the anti- β -tubulin antiserum, that blocks binding, and the anti-G⁺ serum, that does not affect binding but blocks translocation, deserves comment. Both antisera are tipped, but whereas the anti-G⁺ antiserum tipping triggers both sexual signaling and mating-structure activation (21), the anti- β -tubulin antiserum triggers neither response and indeed inhibits normal mating (Mesland and Hoffman, unpublished observations). A possible interpretation of these findings is that (tubulin-like?) bead-binding sites associated with the flagellar surface are tied up by the anti- β -tubulin but not by anti-G⁺ immunoglobulins; that in both cases a trypsin-sensitive "motor" is used to transport all of the antiserum to the tip; and that, for anti-G⁺, beads are able to associate with additional binding sites that have been "left behind" but are unable to move because the motor mechanism has been tipped.

The third means we have found to block bead translocation is biological: as flagellar tips "lock in" during the course of sexual agglutination, bound beads move to the tips as well and stop all movement until flagellar disadhesion takes place. These observations lend support to the speculation that the tipping mechanism and the bead translocation mechanism are indeed driven by a common "motor." By this view, locking-in would somehow involve, or be accompanied by, migration of bead-binding components and their associated motors to the flagellar tips, whereupon they become reversibly fixed in place. The working model in our laboratory, moreover, is that this locked-in state, with the concomitant accumulation of a fibrous material beneath the tip membrane and an elongation of A microtubules (29), is pivotal in transmitting a signal-to-fuse to the cell bodies. In the context of this model, therefore, surface motility can be regarded as at least indirectly involved in signal generation.

Relating Surface Motility to Tipping, Agglutination, and Twitching

Three observations reported in this paper indicate that surface motility may be functionally related to tipping and to sexual agglutinability. First, beads are tipped during the locking-in phase of agglutination. Second, anti-G⁺ tipping is sen-

sitive to trypsin. Third, the kinetics of recovery from trypsin damage are comparable for sexual agglutinability, tipping, surface motility, and twitching. We propose, therefore, that the trypsin-sensitive motor identified in this study serves not only to drive surface motility but also to concentrate sexual agglutinins. If the formation of initial flagellar contacts requires that a critical number of agglutinin molecules become aggregated and that these interact with similar aggregates on the flagella of the opposite mating type, then the motor may function in this aggregation process. It may, moreover, function in contact migration and in the final tipping reaction necessary for the stable adhesions that generate sexual signals. The trypsin-sensitive twitching of *Chlamydomonas* flagella may represent yet another manifestation of the same motor mechanism.

The behavior of isolated flagella under a variety of experimental conditions is consistent with the views presented above. Isolated flagella of opposite mating types do not agglutinate sexually (17), nor do they engage in surface motility (7). If a bead is bound and the flagellum is then removed from the cell body, the bead remains attached but no longer moves (Hoffman, unpublished observation). Similarly, isolated flagella are still isoagglutinable via anti-G⁺ antiserum or concanavalin A, but tipping does not occur and the flagella remain attached to one another at points all along their lengths (5, 21). These results suggest that an active motor participates in adhesion, tipping, and surface motility, a motor that can function only if the flagellum is attached to a living cell. Whether this attachment is necessary for substrate availability, for membrane integrity, or for some other reason, is not known, nor has the motor yet been identified. An attractive candidate is, however, the membrane-associated Ca-dependent ATPase found in *Chlamydomonas* flagella (14, 36). It may be relevant to this hypothesis that Bloodgood et al. (8) have documented a Ca⁺⁺ requirement for surface motility.

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