MEMBRANE MOVEMENTS AND FLUIDITY DURING ROTATIONAL MOTILITY OF A TERMITE FLAGELLATE

A Freeze-Fracture Study

SIDNEY L. TAMM

From the Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Freeze-fracture electron microscopy was used to examine the structure of a region of plasma membrane that undergoes continual, unidirectional shear. Membrane shear arises from the continual clockwise rotation of one part (head) of a termite flagellate relative to the rest of the cell.

Freeze-fracture replicas show that the lipid bilayer is continuous across the shear zone. Thus, the relative movements of adjacent membrane regions are visible evidence of membrane fluidity. The distribution and density of intramembrane particles within the membrane of the shear zone is not different from that in other regions of the cell membrane. Also, an additional membrane shear zone arises when body membrane becomes closely applied to the rotating axostyle as cells change shape in vitro. This suggests that the entire membrane is potentially as fluid as the membrane between head and body but that this fluidity is oniy expressed at certain locations for geometrical and/or mechanical reasons.

Membrane movements may be explained solely by cell shape and proximity to rotating structures, although specific membrane-cytoskeletal connections cannot be ruled out. The membrane of this cell may thus be viewed as a fluid which adheres to the underlying cytoplasm/cytoskeleton and passively follows its movements.

KEY WORDS membrane movements · membrane fluidity · rotational motility · termite flagellate · freeze-fracture

Much attention has been focused on the fluid properties of cell membranes. Various physical probes and labelled markers have revealed that both lipids and proteins are capable of extensive lateral movements in the plane of the membrane (5, 7, 9, 18). However, many of these investigations show only that membrane lipids and proteins can be moved by certain experimental treatments, not that they really do move in vivo. A system that exhibits membrane fluidity directly would possess obvious advantages.

We recently discovered such a system. It is an unusual type of cell motility which demonstrates the fluid nature of membranes in a direct and dramatic fashion (23, 24). The movement involves continual, unidirectional rotation of one part (head) of a termite flagellate relative to the rest of the cell. Ectobiotic bacteria attached to the surface provide built-in markers for showing that the plasma membrane also turns.

Thin-section electron microscopy showed apparent continuity of the cell membrane across the surface shear zone. In addition, the membrane between head and body had the same trilaminar structure as the rest of the cell membrane. Because continual shear takes place between two apparently contiguous areas of the cell membrane, these observations were interpreted as direct, visible evidence of the fluid nature of membranes.

Two questions immediately arise from these findings. First, is the lipid bilayer really continuous across the shear zone? This basic premise must be convincingly demonstrated for the system to truly show membrane fluidity. Secondly, is the membrane in the shear zone different from the rest of the cell membrane?

Both questions are investigated here by freeze-fracture electron microscopy. This method provides extensive face views of the interior of the lipid matrix, as well as revealing the structure of specific regions in the membrane (2, 4, 19).

MATERIALS AND METHODS

Organism

The protozoan used in this study is an unnamed devescovinid flagellate from the hindgut of the termite *Cryptotermes cavifrons (21–24)*. Termites were kept in their original wood until used for obtaining flagellates.

Freeze-Fracture

Flagellates were lightly fixed *in situ* before freezing. Hindguts were teased apart in dilute fixative, consisting of 0.5% paraformaldehyde, 0.5% glutaraldehyde, and 0.1 M phosphate buffer (pH 7.0), at room temperature. Fixation time varied from 15 to 40 min. The fixed suspension was then washed three times with 0.1 M phosphate buffer by differential centrifugation. The final pellet of washed cells was resuspended in 20% glycerol, 0.08 M phosphate buffer (pH 7.0), and stored at 4°C overnight.

Cells were rapidly frozen in liquid Freon 22, cooled to its freezing point by liquid N_2. Fracturing and replication were done by standard methods in a Balzers apparatus (Balzers High Vacuum Corp., Santa Ana, Calif.) at −115°C. Replicas were examined in a Philips 300 electron microscope operated at 80 kV. The freeze-etch nomenclature proposed by Branton et al. (3) is used.

Particle Counts

The density of intramembrane particles was determined by counting the particles within rectangular areas, usually about 0.2 μm^2, on prints at × 128,000. Particles were marked as counted to avoid scoring the same particle more than once. Only those particles which cast clearly defined shadows were counted; these were typically 70–110 Å in diameter. Each area was counted three times (on triplicate micrographs), and the average number of particles was used. Differences between means were evaluated by Student's t test (Table I).

RESULTS

Structure and Rotational Motility

The morphology and rotary movements of this devescovinid have been described previously (21–24). Relevant aspects are presented here.

The anterior part (head) of the flagellate continually rotates in a clockwise direction relative to the rest of the cell (Fig. 1). Torque is generated along a microtubular-microfilamentous axostyle complex which extends through the cell body (22). Microtubules of the axostyle continue into the head and form a cup-shaped canopy under most of the head surface. The major organelles in the head (nucleus, helical Golgi apparatus, flagellar basal bodies, rootlets, and fibrous cresta) are firmly attached to the anterior extension of the axostyle, as shown by isolation of the axostyle with the entire assemblage of head organelles still intact (Tamm, unpublished results). Thus, active rotation of the axostylar rod in the cell body results in passive rotation of the entire anterior part of the cell.

In densely packed, swimming cells, such as those used in this study, the head rotates clockwise and the body rotates counterclockwise (Fig. 1*a*). Rotation velocities are difficult to measure under these conditions. However, after a short time in vitro, isolated cells stop swimming and change shape (see below); the head continues to rotate but the cell body no longer turns in the opposite direction (Fig. 1*b* and *c*). The fastest speed of head rotation observed in such cells is 0.7 rotation/s (21–23).

TABLE I

Particle Densities on P Face of Shear Zone and Body Regions of Devescovinid Plasma Membrane

Membrane region	Particles/μm^2 (mean ± SEM*)
Shear zone	820 ± 41 (4/2.8)
Body	787 ± 39 (4/2.8)

* SEM, standard error of the mean. Figures in parentheses indicate number of different cells and total area counted in square micrometers, respectively. Difference between means is not significant (see text).

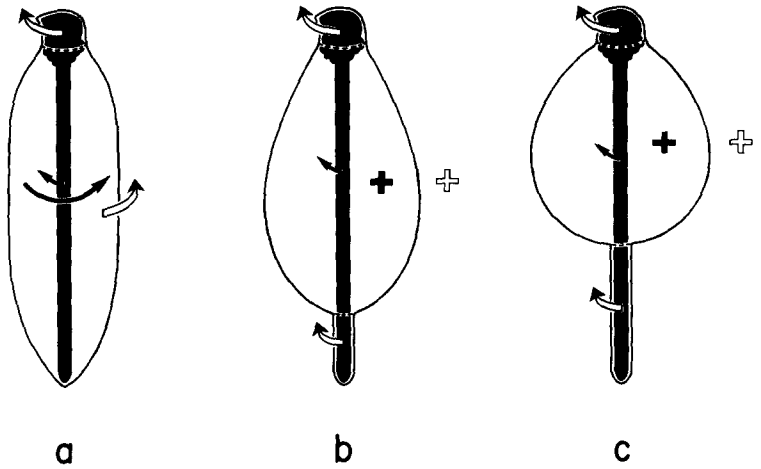

FIGURE 1 Patterns of membrane (white arrows) and cytoplasmic (black arrows) rotation in vivo (*a*) and during change in cell shape in vitro (*b*, *c*). Torque generated along the axostyle complex (black rod) in the body results in clockwise rotation (viewed from anterior) of axostyle, head organelles attached to axostyle (anterior enlargement), and axostylar projection. Membrane regions surrounding the head and axostylar projection are also turned clockwise; likewise, the membrane of the body follows the counter-clockwise rotation of body cytoplasm in vivo (*a*) or stops with the body in vitro (*b*, *c*). Dashed lines represent location of membrane shear zones. Note forward progression of an additional shear zone as the axostylar projection forms (*b*, *c*). The cell membrane is drawn slightly elevated from the axostylar projection and head cytoplasm, for illustrative purposes. Flagella and surface bacteria are omitted for clarity.

Membrane Rotation

Symbiotic rod-shaped bacteria which live in intimate association with the surface of the devescovinid (Fig. 2) provide built-in markers for following membrane movements in living cells (23). Rotation of the head includes the entire pattern of rod bacteria on its surface; likewise, rotation of the body in the opposite direction involves all of the bacteria on the body surface (23). A sharp demarcation is evident between the rotating patterns of bacteria, corresponding to a narrow belt of membrane, 1.0–1.5 μm wide, without surface bacteria. The bacterial markers therefore demonstrate continual, unidirectional rotation of one part of the cell membrane relative to an adjacent part, and localize the shear zone to a narrow belt of bacteria-free membrane between head and body. Since the circumference of the shear zone is ~30 μm, the maximal rate of membrane shear at this level is ~20 μm/s.

Devescovinids gradually change shape in vitro, leaving the posterior part of the axostyle protruding from the body as a thin spike (Fig. 1 *b* and *c*). The extension of the body membrane covering the axostylar projection is largely devoid of surface bacteria. However, remaining bacteria, as well as occasional blebs and attached debris, show that this membrane turns with the axostyle (23). An additional membrane shear zone, not present in vivo, is therefore induced at the base of the axostylar projection. Since the change in cell shape is a gradual process, the junction between body and axostylar projection—and hence the position of the secondary shear zone—gradually travels anteriorly as the axostylar projection forms. This secondary shear zone has not been studied by freeze-fracture; however, it provides insights into the dynamic state of the cell's membrane (see Discussion).

Membrane Continuity across the Shear Zone

Fractures through the shear zone membrane which include adjacent areas of body and head membrane reveal no visible boundaries of any kind separating the shear zone from other membrane regions (Figs. 3 and 4). The smooth continuum of the lipid matrix extends without interruptions or dislocations across the shear zone. There is also no change in the pattern of intramembrane particles at the border between the shear zone and body or head membranes. Freeze-fracture thus confirms previous thin-section findings (23) that the lipid bilayer is continuous across the shear zone.

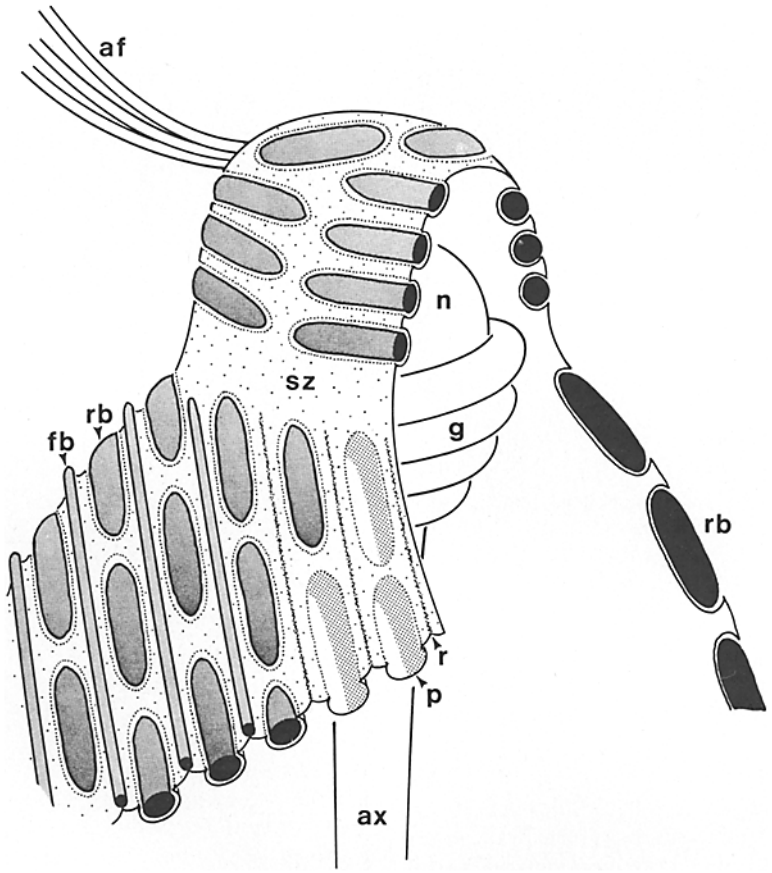

FIGURE 2 Reconstruction of surface features of the anterior end of the devescovinid. The characteristic pattern of ectobiotic bacteria attached to the surface provides landmarks for identifying different regions of the membrane in freeze-fracture replicas. Rod bacteria (*rb*) are arranged in parallel rows which run longitudinally on the body surface, and transversely on the head. The bacteria-free zone of plasma membrane between head and body (*sz*) continually shears as the head rotates. This membrane shear zone is adjacent to the anteriormost coil of the Golgi apparatus (*g*), or the midregion of the nucleus (*n*). Slender fusiform bacteria (*fb*) live only on the body surface between rows of rod bacteria. The rod bacteria reside in specialized pockets (*p*) of the devescovinid membrane; the fusiform bacteria are attached along ridgelike elevations (*r*) of the cell surface. Intramembrane particle arrays (dots) occur in the devescovinid plasma membrane (P face depicted) at the bacterial attachment sites: a double row of particles lines the edges of the pockets (shown here by a single row), and particle aggregates mark the surface ridges (cf. Figs. 3–5, and reference 1). *af*, anterior flagella (trailing flagellum not shown); *ax*, axostyle (anterior extension of axostyle microtubules into head not shown).

Particle Distribution and Density

The distribution of intramembrane particles in the shear zone membrane was compared to the particle pattern in other regions of the cell membrane. Because the bacterial attachment sites contain specialized particle arrays, only areas of head and body membrane outside these sites were used. In addition, since most replicas of shear zone membrane represented the P face, this half of the head and body membrane was selected for comparison.

Figs. 3–5 show that the distribution of particles in the shear zone membrane appears similar to that in other areas of the plasma membrane. The particle pattern is not completely dispersed, since irregular clusters of several particles are evident in most replicas.

Particle counts show similar densities on the P face of shear zone and body membrane regions

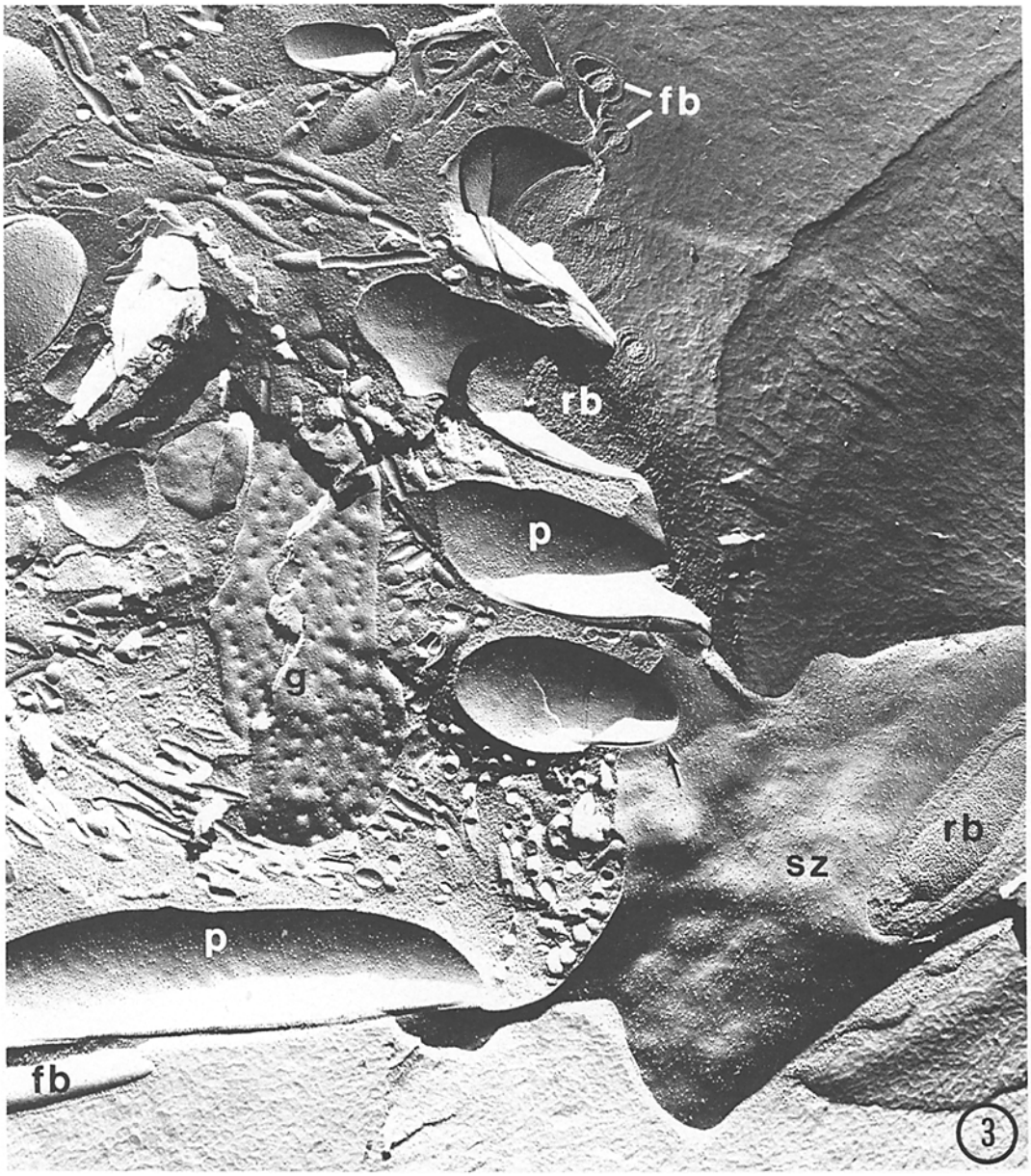

FIGURE 3 Longitudinal, slightly oblique fracture through the anterior end of the devescovinid. Note longitudinally-oriented rows of empty pockets (*p*) for rod bacteria (*rb*), alternating with fusiform bacteria (*fb*) on body. Intervening bacteria-free membrane of shear zone (*sz*; P face), and one of the transversely-oriented rod bacteria on head are visible at lower right. Besides the surface bacterial landmarks, the position of internal organelles, such as the Golgi apparatus (*g*) and nucleus (not shown here) confirm location of the shear zone. Arrow shows "seam" of particles at edges of membrane pockets holding rod bacteria. × 40,000.

(Table I); the difference between the means is not significant. Less extensive data on the head membrane give the same result.

DISCUSSION

Freeze-fracture was used to examine a region of cell membrane that undergoes continual unidirec-

FIGURE 4 Large area of shear zone membrane (*sz*; P face), including adjacent body membrane (upper left) and head membrane (lower right). Note that the smooth lipid matrix extends without interruption across the shear zone and that the particle distribution does not change between head and body. *rb*, rod bacteria in pockets; *r*, membrane ridges under fusiform bacteria; arrows indicate particle "seam" at edges of bacterial pockets. × 60,750.

tional shear. As expected, the lipid bilayer appeared continuous across the shear zone. The freeze-fracture technique provides a more convincing demonstration of membrane continuity than was possible by thin-sectioning methods (23, 24). Relative rotation of adjacent regions of the devescovinid's membrane is therefore a visible manifestation of membrane fluidity.

The same conclusion was reached 30 years ago by Kirby, who observed similar rotational movements in devescovinids from Australian and Pacific Island termites (10–12). He noted that "the surface of one part of the body turns in relation to the surface of another part, yet there is no more disruption of cytoplasmic structure, so far as microscopic appearance goes, than when different parts of any fluid body move in relation to one another" (11). Kirby realized, ahead of his time,

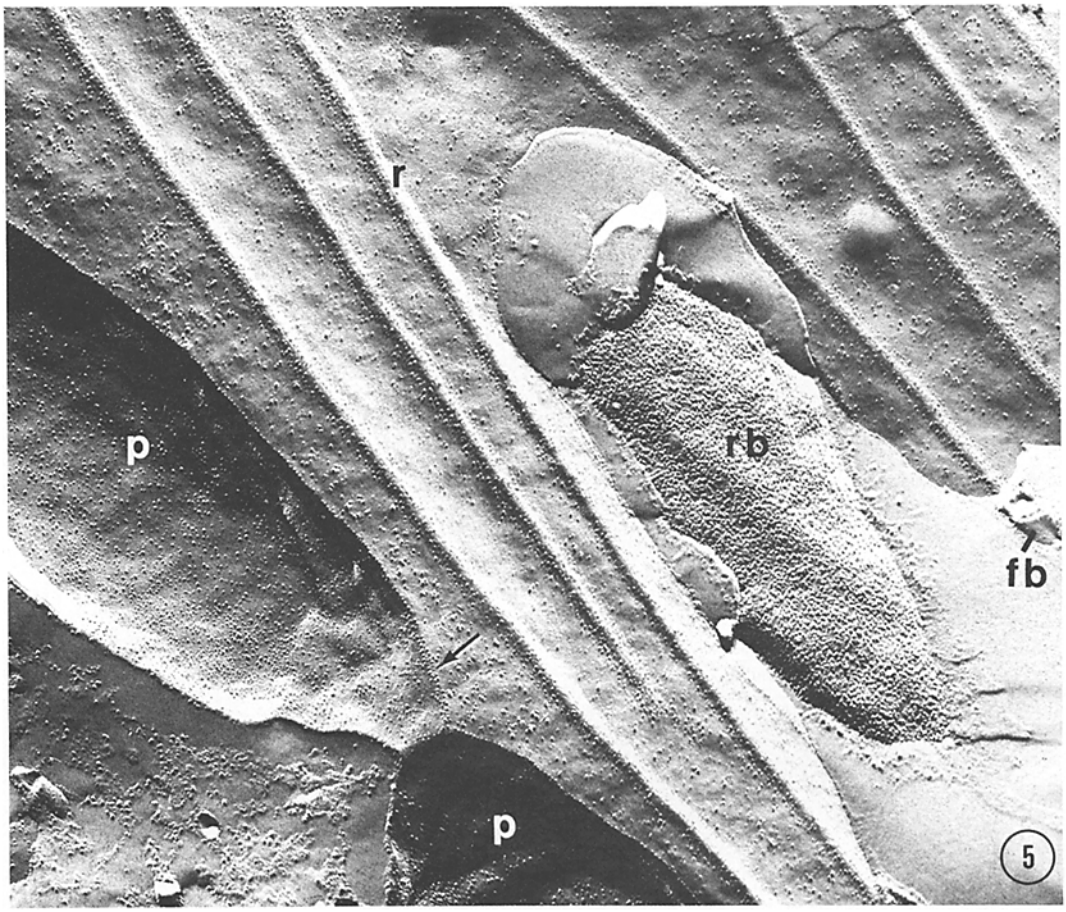

FIGURE 5 P face of body membrane. Particles are aggregated along the ridges (*r*) to which fusiform bacteria (*fb*) attach, and form "seams" (arrow) at the edges of the pockets (*p*) holding rod bacteria (*rb*). The body membrane lying between these bacterial attachment sites has a particle distribution similar to that of the shear zone membrane (cf. Table I). × 60,750.

that these movements demonstrated the "fluidity and lability of the surface layer" (11).

The obvious fluid properties shown by this membrane suggest that the membrane plays no role in maintaining the asymmetrical shape of the cell; instead, the membrane appears to passively follow the form and movements of the structured cytoplasm (see below).

Are There Regional Differences in Membrane Fluidity?

Various kinds of functional, biochemical, and structural specializations are known to occur within the otherwise continuous plasma membrane of many cells (6, 8, 9, 13–16). The dramatic exhibition of fluidity in a precisely localized region of the devescovinid plasma membrane raises the question of whether the membrane between head and body is intrinsically more fluid than that of the rest of the cell.

Although freeze-fracture images revealed no differences in the distribution of intramembrane particles between the shear zone and other membrane regions, this method does not show local differences in lipid composition (2).

However, the appearance in vitro of an additional membrane shear zone between the axostylar projection and body argues against an intrinsically more fluid membrane between head and body. The progressive movement of this secondary shear zone along the body surface shows that almost any part of the cell membrane is capable of exhibiting such fluidity. These results suggest that

the entire membrane is potentially as fluid as the membrane between head and body but that this fluidity is only expressed at certain locations for geometrical and/or mechanical reasons (i.e., cell shape and proximity to rotating structures; see below).

Does Laminar Fluid Flow Affect Particle Distribution?

It is common to infer membrane fluidity from experimentally induced redistribution of intramembrane particles (7, 9, 17, 20). Our system is novel: we start with a membrane that is demonstrably fluid, and ask, does laminar flow of the lipid bilayer affect the distribution of integral membrane proteins (particles)? Since we found a similar pattern of particles in the shear zone membrane vs. regions which are not actively shearing, the answer to this question is evidently no. The rate of membrane shear and the viscosity of the lipid bilayer apparently result in the particles being carried around without perturbing the laminar flow.

Membrane-Motility Coupling

Although specific interactions between various membrane components and cytoskeletal elements have been postulated in other systems, and may well exist in devescovinids, we believe that such connections are unnecessary to explain the bulk membrane movements described here. Considering first why the membrane of the head turns, it should be emphasized that the head is packed with organelles continuous with, or firmly attached to, the rotary motor (i.e., the axostyle). As a result, all of the anterior cytoplasm is turned by the axostyle—much like twirling a lollipop by the stick. The membrane of the head may be coupled to the underlying cytoplasm by specific connections (via axostyle microtubules, for example). Alternatively, the membrane may simply follow cytoplasmic movements by viscous coupling, arising from nonspecific molecular attractions between the membrane and cytoplasm.

Rotation of the membrane of the axostylar projection argues in favor of the latter possibility. Here, membrane, which in vivo had been turning counterclockwise with the body cytoplasm, rotates clockwise once it becomes closely applied to the rotating axostylar rod (Fig. 1). This transformation suggests that mere proximity to rotating structures is sufficient to account for membrane movements in this system.

Taken together, these findings suggest that the cell membrane of devescovinids may be viewed as a fluid which adheres nonspecifically to the underlying cytoplasm/cytoskeleton, and passively follows its movements. A similar relation between cell surface movements and cell locomotion may be true in other systems.

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