Centripetal Flow of Pseudopodial Surface Components Could Propel the Amoeboid Movement of *Caenorhabditis elegans* Spermatozoa

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ABSTRACT Latex beads and wheat germ agglutinin (WGA) were used to examine the movement of membrane components on amoeboid spermatozoa of *Caenorhabditis elegans*. The behavior of beads attached to the cell revealed continuous, directed movement from the tip of the pseudopod to its base, but no movement on the cell body. Lectin receptors are also cleared from the pseudopod (4). Blocking perexisting lectin receptors with unlabeled WGA followed by pulse-labeling with fluorescent WGA showed that new lectin receptors are continuously inserted at the tip of the pseudopod. Like latex beads, these new lectin receptors move continuously over the pseudopod surface to the cell body-pseudopod junction where they are probably internalized. Mutants altering the rate of membrane flow, and eliminating its topographical asymmetry, have been identified. Together with the observation that fluorescent phospholipids are cleared from the pseudopod of developing spermatozoa at the same rate as lectin receptors (25), these results show that there is bulk membrane flow over the pseudopod with assembly at the tip and apparent disassembly at the base. There are no vesicles visible at either the pseudopodial tip or base, so these spermatozoa must have a novel mechanism for insertion and uptake of membrane components. This membrane flow could provide the forward propulsion of spermatozoa attached to a substrate by their pseudopods.

Most motile cells exhibit a highly organized morphological polarity that is reflected in the movement of membrane components over their surface. As suggested by Bretscher (10), understanding the mechanisms underlying these directed surface movements, such as capping on lymphocytes, should provide insight into the more general problem of how cellular asymmetry is organized. Several theories have been proposed to account for the movement of the surface molecules on such cells (reviewed by Hewitt, 16). These can be subdivided into two groups. One suggests that direct or indirect linkage to cytoplasmic contractile proteins drives surface molecules through the membrane. The second predicts that movement of particular moelcules is a manifestation of continuous, polar assembly-disassembly of part or all of the cell membrane.

The amoeboid spermatozoon of the free-living nematode *Caenorhabditis elegans* is well-suited for studying membrane mobility. This 4–5 μm-long sperm is strikingly asymmetrical. A motile pseudopod extends from one end and a rigid hemispherical cell body is the other end (24, 29, 31). The acquisition of this cellular polarity and the onset of membrane mobility can be controlled by inducing differentiation of spherical spermatids into mature spermatozoa (24, 25). Many mutants altering sperm development and motility have been isolated (3, 17, 30), and these can be used for genetic analysis of the control of membrane movements.

In the previous paper (23) we described the movement of the pseudopod and the amoeboid locomotion of spermatozoa in vivo and in vitro and showed that the spermatozoa have almost no actin. Here, we used latex beads and wheat-germ agglutinin (WGA) as markers to examine the mobility of membrane components in *C. elegans* spermatozoa. Together with the evidence presented in (25) our results show that directed bulk membrane flow occurs over the surface of the pseudopod, with membrane assembly taking place at the tip

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and disassembly at the base of the pseudopod. The mechanism that drives this flow, coupled with proper substrate attachment, could provide the propulsion for amoeboid movement.

MATERIALS AND METHODS

Nematode Strains

Nematodes were grown on petri plates seeded with *E. coli* (9). Males of strain CB1490 (*him-5*) were used as source of normal sperm as described previously (4, 31). Sterile, sperm-defective mutant strains used included BA524: *fer-1(hc1ts)*, *him-5 (e1490)*; BA547: *fer-2(hc2ts)*, *him-5(e1490)*; and BA548: *fer-14*(hc*14*), *him-5(e1490)*.

Positively Charged Beads on Spermatozoa

Males grown at 25°C were picked as juveniles and maintained as virgins for 2–3 days at 25°C. These worms were transferred to a drop of sperm medium (SM) (23, 24) between two parallel vaseline strips on a glass slide and cut with a fine tungsten needle to release several hundred spermatids. The preparations were overlaid with a cover glass, creating a chamber for perfusing reagents rapidly onto the cells through the ends.

Spermatids were stimulated to differentiate into amoeboid spermatozoa by treating them with 5×10^{-7} M monensin in SM (24). After pseudopod formation, a solution of amino-containing latex beads (0.45 ± 0.20 μm Diam, 0.25% solids in SM, Polysciences, Inc., Warrington, Pa.) was perfused over the cells. Preparations were observed by light microscopy using Nomarski differential interference contrast optics. Movements of beads that bound to spermatozoa were recorded on videotape on a Panasonic Time-Lapse VTR NV8030 system and analyzed at real time or speeded up ninefold. The figures presented here are photographs of the video monitor.

Lectin Treatment and Fluorescence Microscopy

Sperm for lectin binding experiments were obtained as described above. To assess the appearance and fate of newly inserted lectin receptors on the surface of spermatozoa, we blocked WGA receptors on spermatids by treating them with unlabeled WGA (100 μg/ml, Vector Laboratories, Burlingame, Calif.) for 10 min. After washing to remove unbound lectin, the cells were treated with monensin. 15 min later, the cells were pulse-labeled with rhodamine isothiocyanate conjugated-WGA (0 μg/ml, F/P ratio = 1.0, Vector Laboratories) for 0.5, 1, or 2 min. These sperm were either fixed immediately with 1% formaldehyde plus 1.25% glutaraldehyde or chased with sperm medium for 1–5 min before fixation.

After 1–24-h fixation, cells were washed with SM and photographed with a Zeiss Universal microscope equipped with epifluorescent illumination. The light source was the 531-nm line from a krypton-argon gas laser (Control Laser, Inc., Orlando, Fla.) that was defocused and attenuated for photography. Glutaraldehyde-induced autofluorescence was bleached within a few seconds, so that only rhodamine fluorescence remained. Micrographs were taken on Kodak Tri-X-Pan film developed in Diafine to ASA 1,600.

RESULTS

Movements of Positively Charged Microspheres

Positively charged microspheres bound tightly to the surface of *C. elegans* spermatozoa. Binding was readily detected by the cessation of Brownian movement of the bead. We never observed a bead falling off a cell, nor were we able to dislodge bound beads by perfusing SM rapidly through the chambers. Beads attached as readily to the surface of the cell body as to the pseudopod. However, their behavior on these two parts of the cell was strikingly different.

Beads binding to the cell body never moved on the cell surface. It made no difference whether the cell was stationary and wiggling its pseudopod or crawling across the substrate. In contrast, beads binding to the pseudopod were always transported centripetally at a speed of 10–15 μm/min (Fig. 1). Movement of these beads began as soon as they attached to the pseudopod and continued at a steady speed over the surface until the bead reached the cell-body-pseudopod junction. The beads stopped at this junction, never moving onto the cell body or returning to the pseudopod.

We examined the movements of 32 of these beads in detail. Thirteen of them attached to the dorsal surface of the pseudopod at various distances from its base. Of these, 11 were carried over the dorsal surface parallel to the long axis of the pseudopod; two moved laterally and backward to the side of the pseudopod and then proceeded along the periphery of the pseudopod to its base. Nine beads bound at the tip of the pseudopod, six being transported along the side of the cell and three over the dorsal surface. Each of nine beads that attached to the side of the cell was transported along that side. We observed one bead that attached first to the substrate and then was picked up by the ventral surface of a pseudopod. This bead moved along the under side of the cell before stopping at the base of the pseudopod. Thus, beads attaching anywhere on the pseudopod moved directly, or nearly directly, to the base of the pseudopod.

The surface of the pseudopod of *C. elegans* sperm is covered by numerous finger-like projections (Fig. 2) that form at the tip of the pseudopod and move centripetally over the surface at a velocity of 20–45 μm/min before disappearing at the base (23). We found that, on wild-type sperm, beads moved along the pseudopod at the same speed as nearby projections, although this speed, 10–15 μm/min, was slightly lower than that previously observed. This correlation between speed of movement of beads and pseudopodial projections was also observed on *fer-2* mutant sperm. These mutant sperm produce morphologically aberrant pseudopods (29). Many *fer-2* mutant sperm lack pseudopodial projections and, therefore, exhibit no movement on the surface of their pseudopods. Others have projections but these move over the surface much more slowly than

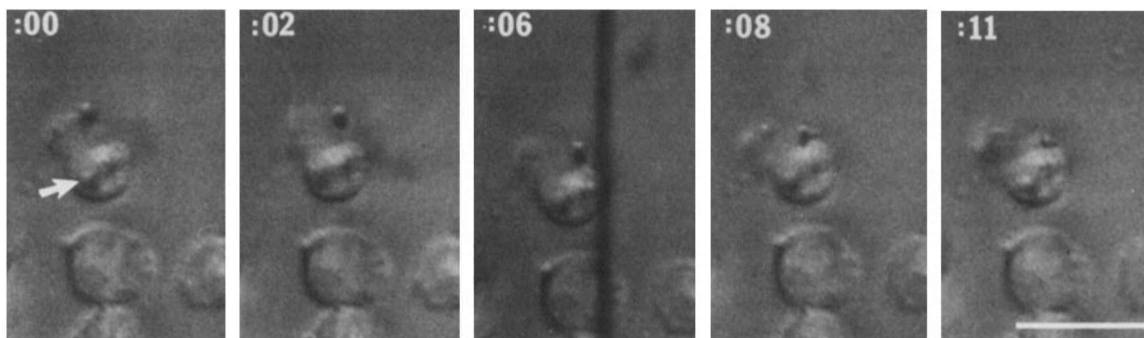

FIGURE 1 Movement of a latex bead on a wild-type spermatozoon. Numbers indicate elapsed time, in s, from attachment of bead at the tip of the pseudopod (:00) to its cessation of movement at the base of the pseudopod (:11). Arrow indicates a bead bound to the cell body that remained stationary throughout the 11-s interval. Bar, 5 μm.

on wild-type cells. Movements of beads on the pseudopods of these mutant sperm reflect this variable morphology. In some cases, beads attached to the pseudopod did not move at all (Fig. 3). On other cells, beads moved centripetally, as on wild-type sperm, but did so very slowly (Fig. 3), taking as long as 1 min to move from tip to base.

fer-14 mutant spermatozoa are morphologically indistinguishable from wild-type cells. Both their pseudopodial projections and beads that attached to their surface moved in the same direction and at the same speed as those on wild-type spermatozoa.

The behavior of beads on *fer-1* mutant spermatozoa was strikingly different from that on wild-type cells. *fer-1* mutant sperm make unusually short pseudopods. These pseudopods are, however, covered with normal-looking projections that move like those on wild-type cells (3, 29). In addition, in wild-type sperm, membranous organelles (MO's) in the cytoplasm of spermatids fuse with the surface membrane during differentiation, creating distinct pores; these fusions do not occur in *fer-1* mutant sperm (29).

Beads bound to the pseudopod of *fer-1* mutant spermatozoa either moved randomly over the surface or, more often, moved toward the cell body. However, these beads did not stop at the cell-body-pseudopod junction as on wild-type cells. Rather, they moved onto the cell body where they either stopped or continued moving, often returning to the pseudopod. Beads that landed on the cell body of these mutant sperm were not stationary. They moved over the surface, either remaining on the cell body or moving onto the pseudopod (Fig. 4). Thus, the direction and polarization of bead movement seen on wild-type spermatozoa were not observed on *fer-1* mutant sperm.

Using Lectins to Detect Newly Inserted Membrane on Spermatozoa

WGA receptors are uniformly distributed on the surface of *C. elegans* spermatids but asymmetrically distributed on spermatozoa (4). Using fluorescent lectin, we found that within a few seconds after extending a pseudopod in response to monensin the surface of these cells remains uniformly labeled. During the next 30–60 s, labeled WGA receptors are cleared from the pseudopod, resulting in the asymmetrically labeled spermatozoon (25). To determine whether this clearance is accompanied by insertion of new, unlabeled receptors, we blocked WGA receptors present on spermatids with unlabeled lectin, activated these cells with monensin, and then pulse-labeled mature spermatozoa with RITC-WGA. Fluorescence microscopy revealed unblocked and, therefore, newly inserted or uncovered WGA receptors on the surface of the pseudopod (Fig. 5 *a*) as well as dots in the cell body. Increasing the pulse time from 30 s to 2 min increased the intensity of fluorescence on the pseudopod but did not reveal insertion of new receptors on any other part of the cell. The bright dots of fluorescence within the cell body are the MO's that stained during pulse-labeling because they contain WGA receptors that are exposed on the surface by fusion (4). There is only faint fluorescence on the surface of the cell body of these spermatozoa. Similar background fluorescence was observed on cells treated with RITC-WGA in 400 mM *N*-acetyl-glucosamine, the carbohydrate specific for WGA (not illustrated). This suggests that the surface cell body fluorescence on pulse-labeled cells was nonspecific.

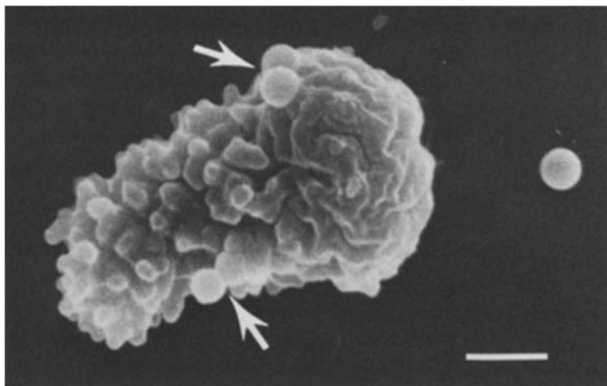

FIGURE 2 Scanning electron micrograph of a wild-type spermatozoon with attached latex beads. Arrows indicate beads attached to the cell. Bar, 1 μm.

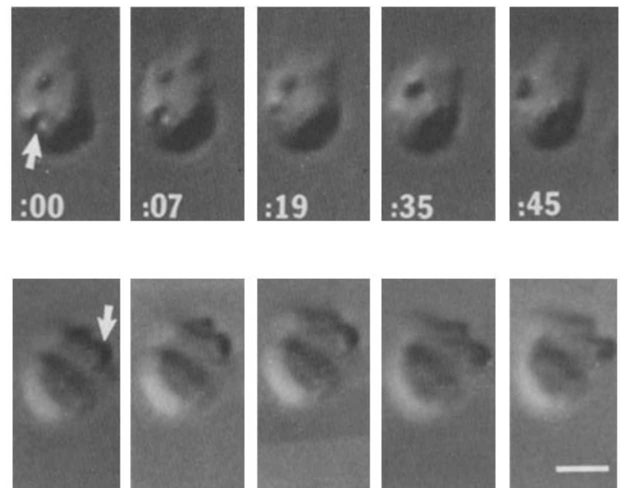

FIGURE 3 Movement of latex beads on *fer-2* mutant spermatozoa. Numbers indicate elapsed time in s. *Upper*, bead on the pseudopod requiring 45 s to move from point of attachment (*:00*) to the base of the pseudopod. A second bead (arrow) is shown that remained stationary at the cell body-pseudopod junction. *Lower*, bead attached to the tip of the pseudopod without subsequent centripetal movement. Numbers indicate elapsed time in s. Bar, 2 μm.

Locating the point of insertion of new WGA receptors more exactly required a modified pulse technique. Thus, we blocked lectin receptors on mature spermatozoa by incubating them in unlabeled WGA, then we perfused the cells in RITC-WGA followed immediately by fixative. By not washing the cells between the block and the pulse, we were able to detect only those receptors inserted during the brief exposure to fluorescent lectin. On cells pulsed for 8 s, we observed bright fluorescence predominantly at the tip of the pseudopod (Fig. 6 *a*). Increasing the pulse to 15 s labeled approximately the front half of the pseudopod; after 25–30-s pulses the entire surface of the pseudopod was labeled (Fig. 6 *b*, *c*).

Results of two controls confirmed that the fluorescent lectin detects newly inserted WGA receptors on the pseudopod of pulse-labeled cells. In the first control, we did not block WGA receptors on spermatids with unlabeled lectin. When these cells were activated with monensin and pulsed with RITC-WGA, we detected uniform fluorescence on the pseudopod and the cell body (Fig. 7 *a*). In the second control, spermatids were treated with fluorescent rather than unlabeled WGA. After activation, these cells were treated for a second time with RITC-WGA. Again, we observed evenly distributed fluores-

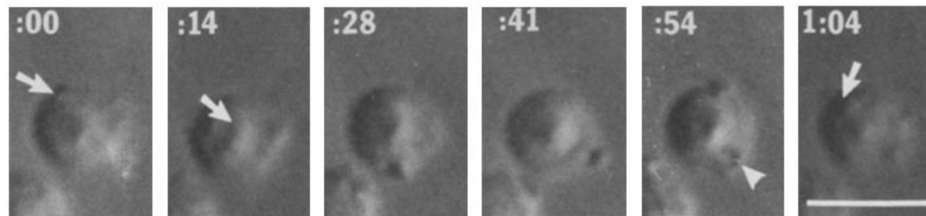

FIGURE 4 Movement of latex beads on *fer-1* mutant sperm. Numbers indicate elapsed time in s. Bead attaches to cell body (*:00*) (arrow), moves over the cell body (*:14*) to the pseudopod (*:28*), then around the leading edge of the pseudopod (*:41*, *:54*), returning to the cell body (*1:04*). At *:54*, a second bead attaches to the pseudopod (arrowhead) and moved toward the cell body in approximately the same path as the first bead. Bar, 5 μm.

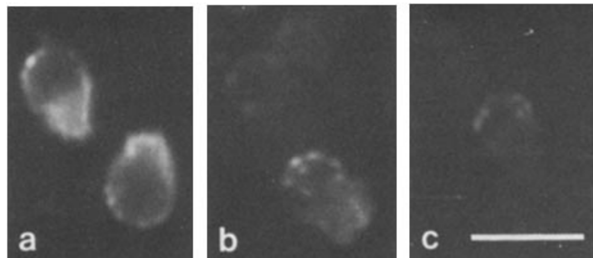

FIGURE 5 RITC-WGA pulse-chase on wild-type sperm. a. Two spermatozoa fixed immediately after a 2-min pulse in RITC-WGA. Dots around cell body are membranous organelles. b. Spermatozoon treated with a 2-min pulse followed by 1-min chase. At top are 2 spermatids. c. Spermatozoon after a 2-min pulse and a 3-min chase. Bar, 5 μm.

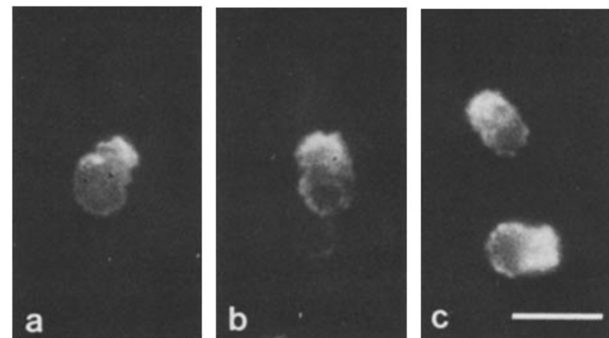

FIGURE 6 Rapid RITC-WGA pulse. See text for procedure. a. 8-s pulse; b. 15-s pulse; c. 25-s pulse. Note progression of labeling from tip to base of pseudopod as length of pulse is increased. Bar, 5 μm.

cence on the surface of these spermatozoa (Fig. 7 *b*). Both of these controls enable us to detect all WGA receptors on the surface of spermatozoa (i.e. both those initially present on spermatids and any newly inserted receptors on mature spermatozoa). The uniform distribution of fluorescence on these cells indicates that our pulse labeling of blocked cells did not detect a particularly avid subset of WGA receptors.

When pulse-labeled spermatozoa were chased with SM we observed a gradual decrease of fluorescence from the pseudopod. Thus, after a 1-min chase the fluorescence on the pseudopod was diminished compared to unchased cells and by 3 min the intensity of fluorescence on the pseudopod was about the same as that initially on the cell body and on spermatids (Fig. 5 *a–c*). We did not observe directed removal of the label from the pseudopod; rather the intensity of fluorescence diminished gradually over the entire surface. We never observed movement of labeled WGA receptors from the pseudopod to the cell body on wild-type cells. Occasionally, a faint band of fluorescence was seen at the base of the pseudopod of spermatozoa chased for 3 min, but this occurred in only 5–10% of such cells (not illustrated).

We do not know the fate of labeled WGA receptors during the chase. Four observations indicate that the label was not removed simply by dissociation of the lectin from its receptor. First, the timing of disappearance of pulse-label is the same on cells chased with unlabeled WGA (100 μg/ml) as on those chased with SM. If removal were by ligand-receptor dissociation, we would expect fluorescence to diminish more rapidly in the presence of excess unlabeled lectin. Second, Argon (4) observed that the total fluorescence on spermatids did not change after activation to spermatozoa. Third, fluorescence intensity did not decrease in pulse-labeled MO's of cells that lost their pseudopodal label during the chase (Fig. 5 *a–c*). The fourth line of evidence derives from pulse-chase experiments with mutant sperm.

The pattern of fluorescence on pulse-labeled *fer-2* mutant sperm was the same as that on wild-type cells with newly detectable WGA receptors on the pseudopod and in the fused MO's (Fig. 8 *a*). However, the time required to chase the pulse label from the pseudopods of these sperm was longer than that required for wild-type cells. Thus, after a 3-min chase, label was still detectable on the pseudopod (Fig. 8 *b*). On some cells, after a 7-min chase the intensity of fluorescence decreased to that detected on the cell body (Fig. 8 *c*). However, even after 7 min we found cells that retained fluorescence on their pseudopods (Fig. 8 *d*). If lectin were removed by dissociation, the timing of disappearance should be the same on mutant and wild-type cells. Similar results, fluorescence retained by pseudopods, was also found in *fer-3* and *fer-4* spermatozoa (4).

The pulse-labeling pattern on *fer-1* mutant sperm was also like that on wild-type cells, except that the MO's did not stain because they failed to fuse with the surface membrane. However, when these cells were chased the labeled WGA-receptors did not disappear. A typical field of pulsed, 3-min chased *fer-1* mutant sperm is shown in Fig. 9. Various labeling patterns were observed including cells with the label almost exclusively on the pseudopod or nearly entirely on the cell body. More often, the label was evident at various intensity on both parts of the cell. This indicates that the mechanism that removes lectin receptors from the surface of wild-type cells precluding their movement onto the cell body fails to function in *fer-1* mutant sperm.

DISCUSSION

The behavior of positively charged microspheres on *C. elegans* spermatozoa indicates that morphogenesis of the spermatozoan creates both a morphological polarity and an asymmetry in the behavior of membrane components on the cell. Surface movement occurs exclusively on the pseudopod, with the cell-body

membrane remaining stationary. Furthermore, movement over the entire surface of the pseudopod is directed from tip to base. Thus, our results using latex beads as markers of movement of membrane components are in agreement with results obtained by attaching inert particles to the leading lamella of vertebrate fibroblasts (2, 15, 18), free edges of cultured epithelial cells (12); neuronal growth cones (7), lamellae of Con A-treated neuroblastoma cells (19), and slime mold amoebae (26). In each case, movement of particles was centripetal (i.e., toward the cell body) and, except for slime mold amoebae where particles moved from the front to the back of the cell, movement was restricted to motile areas of the surface. However, we found that beads move over the surface of spermatozoan pseudopods 3–9 times faster than reported on the leading lamellae of fibroblasts (3.83 μm/min, 15; 1.69 μm/min, 2) and nearly 20 times faster than observed on the lamellae of neuroblastoma cells (0.8 μm/min, 19). Also, we did not observe the changes in velocity of beads during their transit that have been reported by others (15, 12). Such changes in speed may have been masked by the relatively rapid velocity at which beads moved on pseudopods and the short distance that they traveled (2–3 μm from the tip of the pseudopod to its base).

Similarity between the speed and direction of movement of attached particles and ruffles has been observed on the leading lamellae of fibroblasts (2, 18), epithelial cells (12), and motile lymphocytes (21). We observed this same correlation between the movements of beads and pseudopodial projections on sperm. Significantly, we found that the movement of beads and projections correlated on both wild-type cells and on *fer-2* mutant sperm where both beads and projections move very slowly. Thus, our results support the hypothesis that the movement of surface projections on motile cells indicates membrane movement (5, 21).

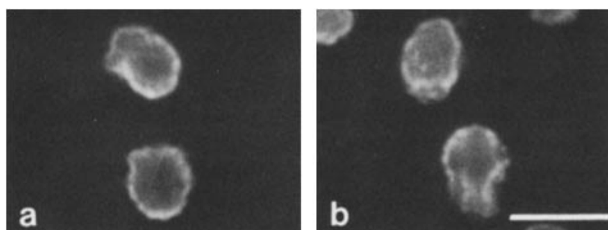

FIGURE 7 Controls for RITC-WGA pulse-chase experiment. a. Spermatozoa pulsed for 2 min with RITC-WGA without prior treatment with unlabeled WGA. b. Spermatozoa treated with RITC-WGA for min as spermatids, activated with monensin, then pulsed, as spermatozoa, with a second treatment in RITC-WGA. The entire surface along with the fused MO's is stained. Bar, 5 μm.

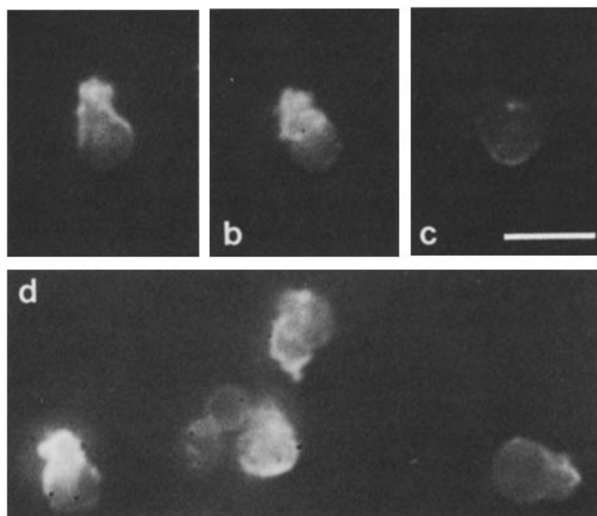

FIGURE 8 RITC-WGA pulse-chase of *fer-2* mutant sperm. a. 2-min pulse, no chase; b. 2-min pulse, 3-min chase; c. 2-min pulse, 7-min chase; d. several spermatozoa nearby the cell in c, which retained various amounts of labeled lectin on their pseudopods. Bar, 5 μm.

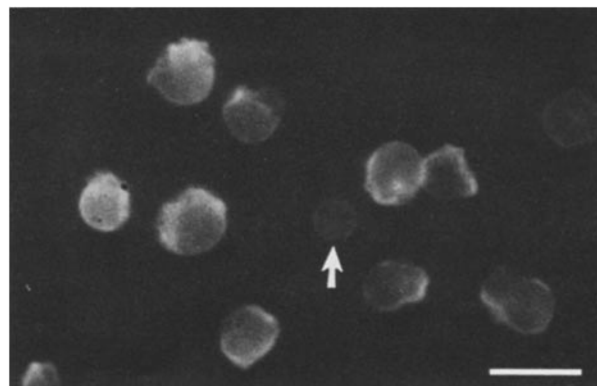

FIGURE 9 Several *fer-1* mutant sperm after 2-min RITC-WGA pulse, 3-min chase. Spermatid indicated by arrow; remaining cells are spermatozoa. Note the variety of labeling patterns. Bar, 5 μm.

Does the directed movement of beads (and projections) on the surface of sperm pseudopods reflect rearrangement of isolated membrane components or bulk membrane flow? Four theories have been proposed to account for the rearrangement of inert particles and lectin receptors on amoeboid cells and the related capping of antigens and lectin receptors on lymphocytes: (*a*) Polyvalent ligands crosslink membrane receptors that span the bilayer. This binding in some way stimulates transmembrane association of these receptors with cytoplasmic contractile elements; these cytoplasmic elements then move receptors in the plane of the membrane to one pole of the cell, forming a cap (6, 11). (*b*) Particles and macromolecules on the surface become entrained to, and thus moved by, a series of waves on the surface of motile cells. The force generating these waves derives from cytoplasmic actomyosin filaments but, unlike the first theory, this model does not require direct linkage between cytoplasmic proteins and surface components. This is the surf-riding model for cell capping (16). (*c*) There is a continuous and directed flow of lipid molecules in cell membranes, with a source at one pole and uptake at another (10). Macromolecules with diffusion coefficients slower than the lipid flow rate will be caught up in the stream and carried to the sink. To account for capping, Bretscher proposes that a molecular filter at the sink retains macromolecules on the surface while allowing internalization of lipids. (*d*) Directed bulk membrane flow occurs on the surface of motile cells, with continuous assembly of new membrane at one pole and disassembly at another. This theory (2, 7, 13, 14, 27) differs from Bretscher's by predicting that all membrane components undergo continuous flow, not just lipids.

Results of our lectin pulse-chase experiments indicate that clearance of WGA-bound lectin receptors from the surface of the pseudopod is accompanied by immediate insertion of new, unlabeled WGA receptors into the surface membrane. When we treated spermatozoa with unlabeled WGA and then briefly pulsed these cells with RITC-WGA, only the leading edge of the pseudopod was labeled. Longer pulses labeled a greater percentage of the surface of the pseudopod. This, along with the centripetal movement of microspheres, suggests that new

membrane components are inserted at the tip of the pseudopod and that membrane flows over the entire surface of the pseudopod from tip to base.

Our data agree with the bulk membrane flow model which is the only theory that predicts that clearance of a class of receptors toward one pole of the cell must be accompanied by rapid reappearance of those receptors in the cleared zone. The bulk membrane flow theory was generated to explain the centripetal movement of particles and clearance of lectin receptors from the leading lamella of fibroblasts (2, 13, 14). However, Vasiliev et al. (28), using double-label experiments, found that 2 h were required to restore initial receptor density after clearance of the original population of lectin receptors on fibroblast leading lamellae. Similar results have been obtained with capped lymphocytes (11, 22). Our observations are the first example of clearance followed by immediate insertion of new receptors in cleared areas.

Elsewhere (25), we presented further evidence suggesting that membrane flow rather than transmembrane linkage to a contractile system accounts for membrane movement on sperm pseudopods. There, we found that lectin-receptor complexes and a fluorescent phospholipid analogue integrated into the membrane bilayer and not bound to protein were cleared from the pseudopod membrane at the same rate. Lipids do not span the bilayer and cannot associate directly with cytoplasmic proteins, thereby ruling out a transmembrane control mechanism for their movement.

The ultrastructure of the pseudopod suggests that insertion of new membrane does not occur by incorporation of cytoplasmic vesicles at the tip of the pseudopod. The spermatozoan pseudopod is filled with amorphous, granular cytoplasm without vesicles, filaments, or tubules (23, 25, 29, 31). No vesicles are seen in pseudopods in any of the various fixation conditions described in the previous paper (23). Therefore, sperm must use a novel mechanism for transporting membrane components through the cytoplasm for assembly at the tip of the pseudopod.

Where is the sink for flowing membrane on the surface of *C. elegans* sperm? Latex beads stop moving at the base of the pseudopod suggesting that membrane may be disassembled at the junction between the pseudopod and the cell body. In keeping with this, we have never observed movement of newly inserted lectin receptors from the pseudopod to the cell body on wild-type cells. However, we have not been able to determine the fate of lectin receptors after they are cleared from the membrane of the pseudopod. Evidence indicating that the ligand does not simply dissociate from its receptor has been presented above. Another possibility is that the ligand-receptor complexes are internalized and diluted in the cytoplasm. *C. elegans* spermatozoa contain a series of laminar membranes in their cytoplasm that are concentrated at the junction between the cell body and the pseudopod (24, 29). Preliminary results suggest that horseradish peroxidase is taken up from outside the cell into these membranes (S. Ward, unpublished observation) but whether these structures serve as a sink for the asymmetric membrane flow on these cells remains to be determined.

We do not know how the spermatozoan controls the direction and polarity of its pseudopodial membrane movements. However, the movements of beads and lectin receptors of *fer-1* mutant sperm suggests that these spermatozoa fail to organize their surface movement. Neither the insertion of membrane at the tip of the pseudopod nor the movement of markers on the surface is impaired on *fer-1* mutant sperm, but these cells are unable to establish directed membrane movement or to restrict movement to the surface of the pseudopod. That a single mutation abolishes both the directedness and the topographic asymmetry of membrane movement suggests that these processes may be under common control. One possibility is that fusion of MO's with the surface membrane, which doesn't occur in *fer-1* mutant spermatozoa (29), may stabilize the surface of the cell body, preventing transport of surface markers off of the pseudopod. In keeping with this, we found (25) that beads attached to monensin-activated wild-type spermatids moved randomly over the surface, stopping abruptly when the cell extended a pseudopod, but that this movement did not stop after *fer-1* mutant sperm produced pseudopods. Further analysis of *fer-1* mutant sperm should provide clues about how surface movement on spermatozoa is organized.

Bulk membrane flow may be an integral component of the mechanism of amoeboid movement. Many amoeboid cells organize focal points of substrate attachment on their undersides. These attachment sites remain stationary, relative to the substrate, as the cell moves over them (20). As pointed out by Abercrombie (1), continued progression requires continual formation of new attachment sites at the leading edge of the cell. Continual membrane flow over the surface of the pseudopod of *C. elegans* sperm could provide both the membrane components needed for generating new attachment sites and the propulsion needed for amoeboid movement. Such a mechanism might not require actin filaments to provide contractile force, so it is consistent with the near absence of actin in these motile cells (23).

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