Studies on the Motility of the Foraminifera. II. The Dynamic Microtubular Cytoskeleton of the Reticulopodial Network of *Allogromia laticollaris*

JEFFREY L. TRAVIS, JAMES F. X. KENEALY, and ROBERT D. ALLEN
Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755. Dr. Travis's present address is Department of Biology, West Virginia University, Morgantown, West Virginia 26506.

ABSTRACT Lamellipodia have been induced to form within the reticulopodial networks of *Allogromia laticollaris* by being plated on positively charged substrata. Video-enhanced, polarized light, and differential interference contrast microscopy have demonstrated the presence of positively birefringent fibrils within these lamellipodia. The fibrils correspond to the microtubules and bundles of microtubules observed in whole-mount transmission electron micrographs of lamellipodia. Microtubular fibrils exhibit two types of movements within the lamellipodia: lateral and axial translocations. Lateral movements are often accompanied by reversible lateral associations between adjacent fibrils within a lamellipodium. This lateral association-dissociation of adjacent fibrils has been termed 'zipping' and 'unzipping'. Axial translocations are bidirectional. The axial movements of the microtubular fibrils can result in the extension of filopodia by pushing against the plasma membrane of the lamellipodia. Shortening, or complete withdrawal, of such filopodia is accomplished by the reversal of the direction of the axial movement.

The bidirectional streaming characteristic of the reticulopodial networks also occurs within the lamellipodia. In these flattened regions the streaming is clearly seen to occur exclusively in association with the intracellular fibrils. Transport of both organelles and bulk hyaline cytoplasm occurs bidirectionally along the fibrils.

Our previous studies (37) of the ultrastructure of the reticulopodial network of *Allogromia* demonstrated that the highly branched and anastomosed reticulopodium contains an extensive microtubule-based cytoskeleton. The microtubules were frequently bundled, forming a cytoskeletal system of far greater spatial organization than previously thought (22). By using sensitive (extinction factor of ~2,000) differential interference contrast optics, fibrillar elements were visualized within the most favorable areas of the reticulopodial networks of living organisms (37). The ultrastructural data indicated that these pseudopodial fibrils most certainly represented bundles of microtubules. The spatial distribution of microtubules within the reticulopodia reflected accurately the pattern of filopodial branching and anastomoses. This suggested that the microtubules might have a causal role in the formation and maintenance of pseudopodial form. In addition, the fibrils were oriented parallel to the direction of the cytoplasmic streaming, suggesting that they participated in some way in the intracellular transport process.

Recent advances in video-enhanced contrast microscopy (5, 6) have provided the means with which to study the cytoskeletal dynamics of living *Allogromia*. The video microscopic studies reported here were performed in part on flattened lamellipodial regions of the reticulopodial network of *Allogromia*. The results of the present studies clearly demonstrate that the microtubule-based cytoskeleton of the reticulopodial network is itself motile. The motility of these structures is consistent with the idea that they are actively involved in the formation of the filopodia. The present work also indicated that the intracellular transport of the cytoplasmic organelles and bulk cytoplasm occurs exclusively in association with the microtubules.

MATERIALS AND METHODS

Allogromia laticollaris (Arnold) were cultured as previously described (37). The organisms were rinsed at least four or five times in 'calcium-free' sea water prior to being plated on protamine sulfate-coated coverslips. Specimens were mounted between coverslips on a metal holder (22).

Video-enhanced contrast microscopy was performed according to the high

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bias-compensation video offset methods described by Allen *et al.* (5, 6). An inverted Zeiss Axiomat equipped with a planapochromatic 100 × objective (NA 1.3) and achromaticaplanatic condenser (NA 1.4) was used. This instrument was equipped with differential interference contrast, polarization, and surface reflection interference (17, 27) optics. The video system consisted of a Hamamatsu C-1000 camera (Hamamatsu Corp., Middlesex, NJ) with a chalnicon camera tube. The video signal from the camera was routed through a GYYR video timer (model G-77, FOR-A Corp. of America, Los Angeles, CA) to a Sony TVO-9000 or VO2610 time-lapse videocassette recorder. The video image was displayed on a Panasonic WV-5310 9-in monitor. We obtained video micrographs by photographing the monitor image on 35-mm Plus-X film using a Nikon macro (55 mm) lens (Nikon, Inc., Garden City, NY). Video data were recorded and stored on U-matic ¾-in videocassettes. Some of the video records were made using a Polyprocessor frame memory (Hamamatsu Corp.) to store and subtract the background pattern of the mottle due to lens defects in the microscope. In this way the signals were made clearer and easier to interpret. Electron microscopy was performed according to the methods described earlier (37).

RESULTS

General Features of the Reticulopodial Network

The reticulopodial network of *Allogromia laticollaris* is composed of numerous interconnected filopida. These filopodia are typically thin (≤5 μm diam) and cylindrical and display bidirectional streaming. Contact with the substrate may occur along the length of the filopodia. Areas of close contact between the filopodia and the substrate have been observed with surface reflection interference microscopy (Fig. 1). As the filopodia move they change their points of adhesion (36), and this process is reflected in the constantly changing pattern of close contact points revealed by surface reflection interference microscopy.

The cylindrical shape of the typical filopodium and the close packing of the microtubules and the microtubule bundles (37; see also Discussion) makes direct observation of the cytoskeletal dynamics difficult. Occasionally, parts of the reticulopodial network flatten, forming lamellipodial regions at points of adhesion to the substrate (Fig. 1) (3, 37) and at distal termini of the filopodia where they have been referred to as 'feeding fans' (23). Allen (3) has pointed out that a hyaline veil typically forms at the juncture of a bifurcating filopodium.

Induction of Lamellipodia

Lamellipodia are ephemeral. Their formation and persistence are stimulated by positively charged substrata, such as glass coated with either poly-L-lysine or protamine sulfate. Lamellipodial areas within the reticulopodial network provide an opportunity to study the cytoskeletal dynamics of the living networks. These areas are extremely thin and represent a 'two-dimensionalized' filopodium in which the arrangement of the microtubules and the pattern of cytoplasmic streaming are essentially restricted to a single plane. This flattening greatly facilitates observations of extensive areas of the network at the small focal depths inherent in high numerical aperture differential interference contrast microscopy (4) (Fig. 1). Two-dimensionalized pseudopodia are free not only from the complicating superposition of the numerous microtubule bundles that occur within the long thin filopodia, but also from the sharp phase gradients due to the enveloping plasma membrane in cylindrical filopodia.

Microtubular Cytoskeleton of the Lamellipodia

Cytoplasmic fibrils are visible within the lamellipodia (Figs. 1 and 2). The following data suggest that these fibrils corre-

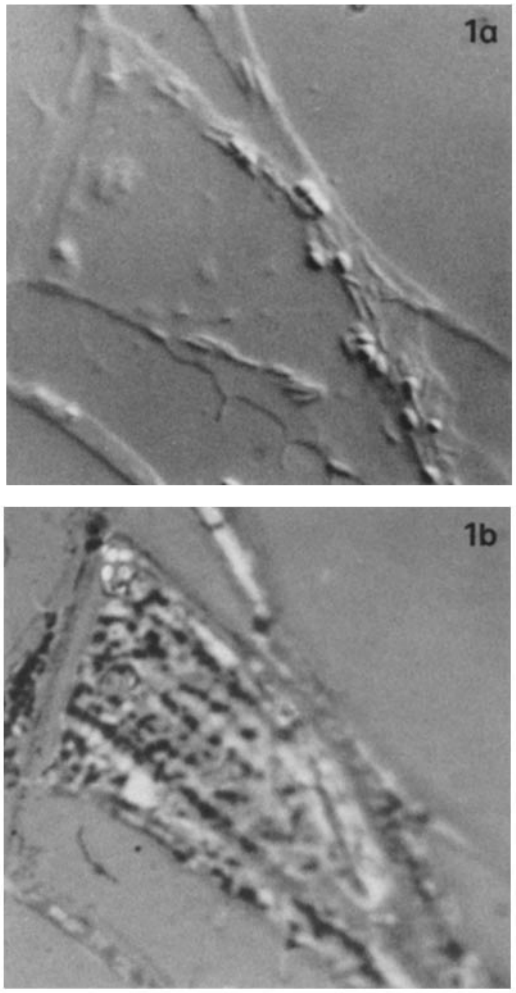

FIGURE 1 Lamellipodium formed at the site of adhesion to the substrate. (*a*) Differential interference contrast photomicrograph of a lamellipodial region of *Allogromia* reticulopodium. (*b*) Surface reflection interference photomicrograph of the same lamellipodium seen in *a*. × 2,500.

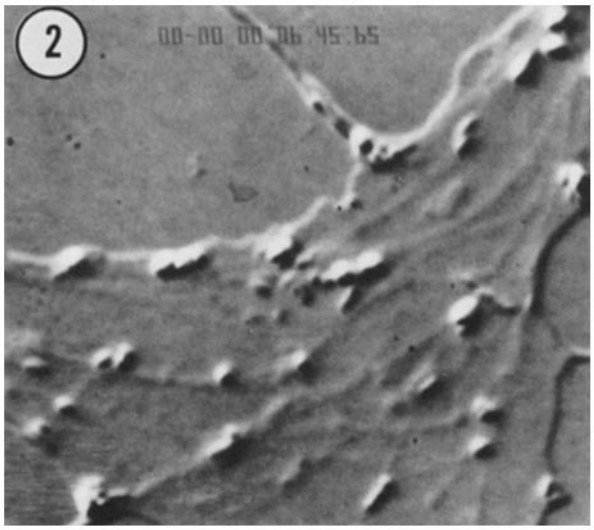

FIGURE 2 Differential interference contrast video-enhancement micrograph of an *Allogromia* lamellipodium. This image represents a single video 'field' (1/60 s) and illustrates the numerous fibrils and associated particles that may be present within such a flattened area. × 5,585.

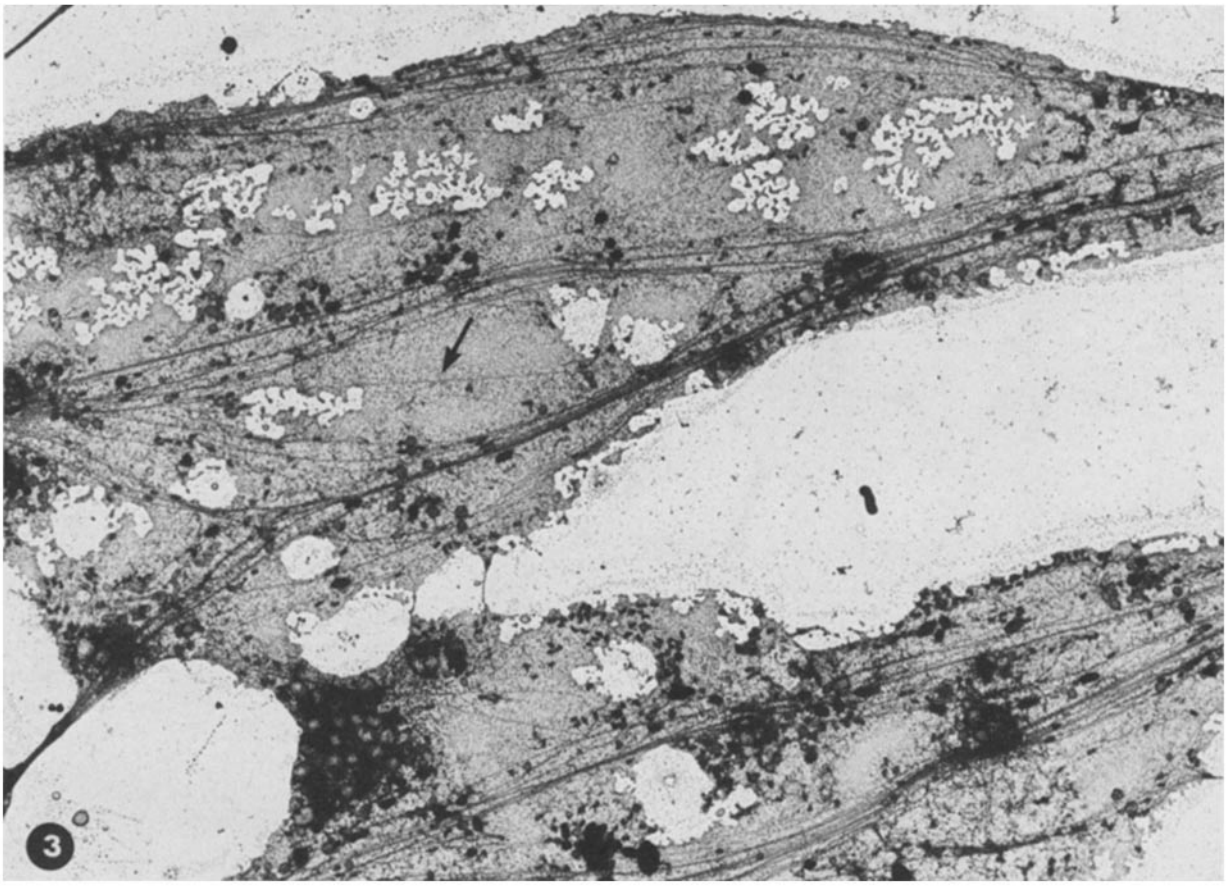

FIGURE 3 Whole-mount transmission electron micrograph of a fixed, critical-point dried lamellipodial region of the reticulopodial network of *Allogromia*. This particular region contains numerous microtubule bundles. The constituent microtubules of the bundle splay apart and rejoin the original bundles, as well as associate with microtubules derived from adjacent bundles. Numerous intracellular particles appear associated with the microtubules along their lengths. Arrow points to a single microtubule that traverses the lamellipodium. × 3,700.

spond to the microtubules or microtubule bundles observed in whole-mount transmission electron micrographs of similar flattened regions of the reticulopodial network (Fig. 3) (37). These fibrils may be straight or curving. When observed with polarization optics, these fibrils display positive axial birefringence (Fig. 4). Our whole-mount electron microscopic data reveal that in addition to bundles of microtubules, numerous single microtubules also traverse the lamellipodial areas (Fig. 3) (6, 37). On the basis of the data obtained from these parallel observations, we conclude that the fibrils detected in video microscopic observations of the lamellipodia of *Allogromia* correspond to microtubules or various-sized bundles thereof.

Lamellipodial regions are contiguous with filopodial regions. Fig. 5 is a video micrograph of a lamellipodium that contains three prominent fibrils, labeled *A*, *B*, and *C*, respectively, from top to bottom. These fibrils extend from the filopodium in the upper right corner of the micrograph, traverse the lamellipodium, and extend into smaller filopodia originating at the opposite side of the lamellipodium.

Movements of Microtubules

The fibrils corresponding to microtubules and bundles of microtubules are not stationary within the lamellipodia. They move, or are moved, both laterally and axially through the cytoplasm. Both the axial and lateral translocations of the fibrils are bidirectional; changes in direction, as well as in the velocity of the movements, appear to occur spontaneously. Occurring in concert, bidirectional axial and lateral movements of the microtubule fibrils give rise to a very complex pattern of motility and a continuously varying pattern of microtubule deployment within the lamellipodia.

Lateral Translocations: Zipping and Unzipping

Fibrils move sideways through the lamellipodial cytoplasm; often this lateral movement is accompanied by a change in the curvature of the fibrils. Routinely the fibrils bifurcate, splitting initially into two or more filaments[1] that generally branch at acute angles. The resulting branch filaments may be either straight or curved, and may in turn bifurcate further (Fig. 5). Branch filaments may recombine by an apparent 'zipping' motion to reform the original fibril. During this zipping the filaments become realigned and inosculate laterally (Fig. 6, see also Fig. 11). Alternatively, the branch filaments may continue to separate, or 'unzip', further. Branch filaments do not always reunite, but may instead zip with other adjacent filaments or fibrils. The zipping and unzipping of microtubules may occur in either direction,

[1] The terms 'fibrils' and 'filaments' are relative and their usage here is consistent with that of Allen (3). The relativity of the terms is necessarily dictated at the time of observation. Filaments are considered subunits of fibrils.

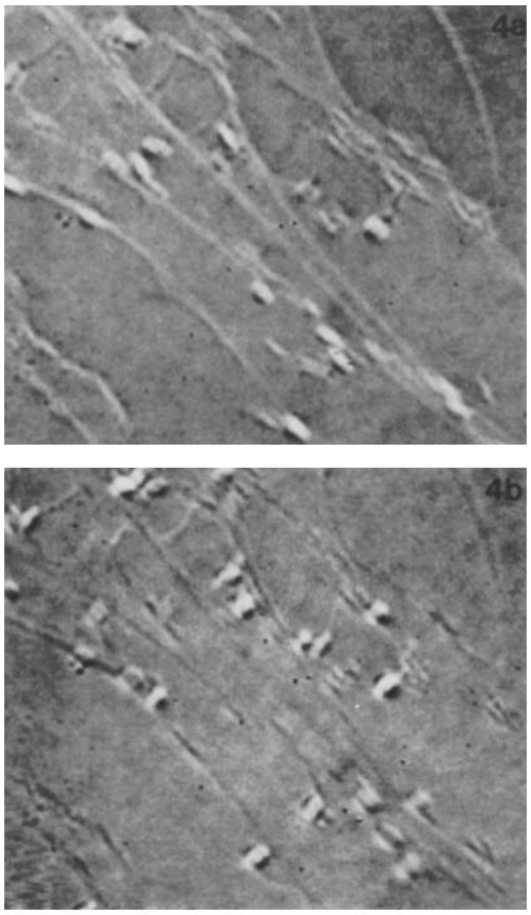

FIGURE 4 Polarized-light video-micrograph demonstrating the birefringence of the fibrils within a lamellipodium. The contrast of the fibrils reverses at opposite compensator settings (*a* and *b*). × 3,800.

towards or away from the cell body, and both are reversible (Fig. 7).

Axial Translocation of Microtubules: Filopodial Extension and Retraction

In addition to moving laterally, fibrils and filaments also translate axially. At times the leading tip of the axially translating fibril has a knobbed end, suggesting that it has a vesicle associated with it (Fig. 8). When the fibril abuts the plasma membrane at the lateral margin of the lamellipodium, it can induce the formation of a filopodium. The membrane of the newly formed filopodium advances at the same rate as did the fibril when approaching the border of the lamellipodial region, suggesting that the filopodium was pushed out by the axially moving fibril (or filament). Axial translation of the fibrils is bidirectional. If a fibril is withdrawn from a newly formed filopodium, by reversal of direction of its axial movement, the filopodium retracts (Fig. 9). The reversible processes of a filopodium forming and retracting were observed to occur repeatedly in the same area of a lamellipodial region.

Streaming Occurs Only Along the Fibrils

The bidirectional streaming characteristic of the normal reticulopodial network of *Allogromia* occurs also within the lamellipodial regions. In fact, in these regions it is clear that the translocation of cytoplasmic particles occurs only in association with the cytoplasmic fibrils. Lateral movement of the fibrils within the lamellipodia is accompanied by a change in the trajectory of the associated particle translocations. This is particularly noticeable when a fibril undergoes a change in curvature. Where fibrils bifurcate into filaments, either during zipping or unzipping, the transport occurs along all branches. There appears to be no selection of one branch over another. Filaments arising from the splitting of fibrils also serve as substrata for cytoplasmic transport. It should be stressed that cytoplasmic streaming continues to occur along the fibrils and filaments as these structures move within the lamellipodia. That the fibrils are necessary for streaming is indicated by the fact that particles not in contact with a fibril or filament do not translocate. These stationary particles commence streaming once such contact is established (Fig. 10). The movement of particles along the fibrils and filaments is always bidirectional. Many of the transported particles are asymmetrically shaped (oblate ellipsoids) and as such their orientation relative to the fibrillar elements is easily monitored. Such particles can rotate while being transported. This reorientation of particles with respect to the fibril does not affect the direction of movement. Similarly, such particles may spontaneously reverse the direction of movement along a fibril or filament without simultaneously rotating. These observations suggest that the particle maintains no fixed polarity upon which the direction of movement is dependent.

In addition to the streaming of discrete cytoplasmic particles, bulk hyaline cytoplasm is transported along the fibrillar elements of the lamellipodia (Fig. 11). A mass of cytoplasm streams through a lamellipodial region along the entire length of a fibril. The transport of the cytoplasm is accompanied by the thickening of the fibril, as if some of the cytoplasm were incorporated into the fibril concomitantly with the streaming (Fig. 11).

DISCUSSION

Cytoskeletal Morphology and the Pattern of Movement

Despite the tremendous complexity of foraminiferan motility, descriptions have become progressively more complete (3, 9, 18–21). Pseudopodia are extended and retracted; they branch, anastomose, and fuse with one another and they are capable of pulling the entire organism along the substrate, effecting its locomotion. Perhaps the most dramatic of the motile phenomena exhibited by this group of organisms is the incessant bidirectional streaming that occurs throughout the network. Cytoplasmic particles as well as bulk cytoplasm are transported at velocities that may exceed 10 μm sec^{-1}. Extracellular particles adherent to the outer surface of the pseudopodia are also transported in a saltatory manner.

A highly developed microtubular cytoskeleton exists within the reticulopodial network (37). The microtubules are generally oriented parallel to the long axis of the filopodia, and hence also parallel to the direction of the cytoplasmic streaming. Furthermore, the distribution of the microtubules mirrors quite faithfully the pattern of pseudopodial branching and anastomosis. The spatial organization of the microtubules is such that the morphology of the network could be attributed to these cytoskeletal elements (along with the sometimes attendant 5-nm filaments). It was suggested that the microtubules are also involved in the process of cytoplasmic trans-

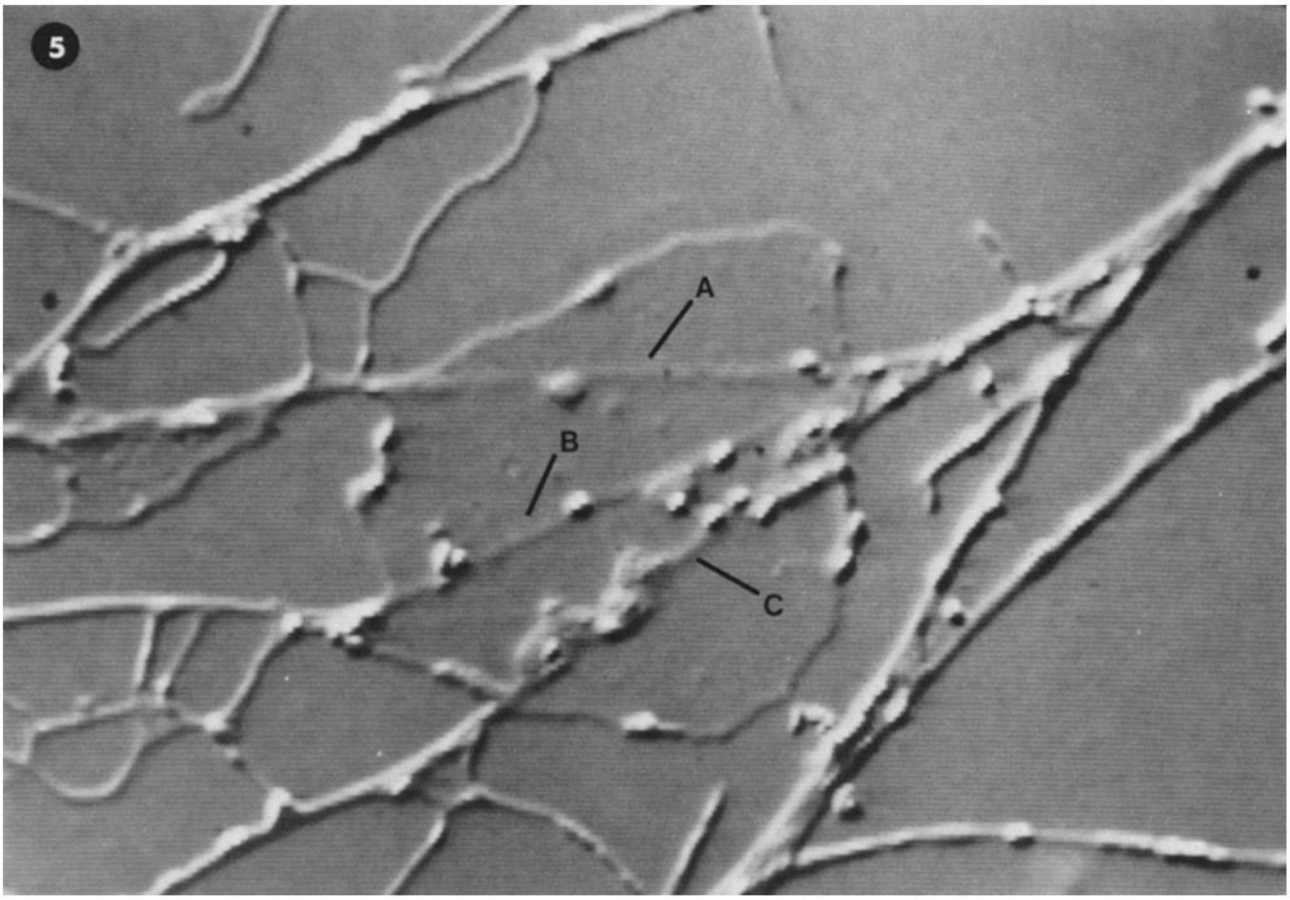

FIGURE 5 Lamellipodium with three prominent fibrils *A*, *B*, and C. All of the fibrils seem to originate from the filopodium pictured at the upper right hand corner of the image. Traversing the entire lamellipodium at divergent angles, the three fibrils appear to exit the lamellipodium and pass into small fibopodia originating from the left side of the lamellipodium. × 5,000.

port, at least insofar as they define the tracks along which the transport occurred. The video microscopic data presented in this communication provide strong confirmation.

The Visibility and Identity of Cytoskeletal Fibrils and Filaments

It has been possible to observe some fibrils in the most favorable regions of the reticulopodial network; most have been hidden from view by the sharp refractile phase gradients in filopodial regions of the network. In polycation-induced and -stabilized lamellipodial regions, the fibrils and materials moving along them are visible without interference from the membrane, which in this case forms a flat sandwich through which the fibrils and associated organelles are clearly visible and move. In effect, this preparation has 'two-dimensionalized' the reticulopodial network and offered a more penetrating look at both the fibrils and their associated organelles in motion. The fibrils that can be observed in the lamellipodial regions of the reticulopodial network are made visible by the contrast enhancement of video microscopic methods, which is sufficient to render even single microtubules visible by either polarization or differential interference contrast optics (5, 6). The range of contrasts displayed by filaments suggests that many of them are single microtubules while others are bundles.

Microtubular Displacements within the Reticulopodial Network

The most provocative of the observations on the cytoskeleton of *Allogromia* is that the microtubules display two kinds of movements: lateral and axial displacements. Such movements, occurring either separately or in combination, appear to account for many facets of the motile repertoire of the reticulopodial network. Observations of the microtubular dynamics within the lamellipodia show that the microtubule bundles branch and anastomose. Such movements of the underlying cytoskeletal elements of normal pseudopodia would account for branching and anastomosing of pseudopodial cytoplasm. Indeed, it is apparent, as predicted by ultrastructural studies (37), that the morphology of the reticulopodial network is the direct result of the microtubule interactions within the network. Clearly, membrane splitting and fusion (between filopodia) must be coordinated with these cytoskeletal movements in order to form the constantly changing pseudopodial branching and anastomosis patterns.

The Role of Microtubules in the Extension, Retraction, and Stability of Filopodia

The axial translocation of microtubules or microtubule bundles that occurs within the lamellipodia of *Allogromia*

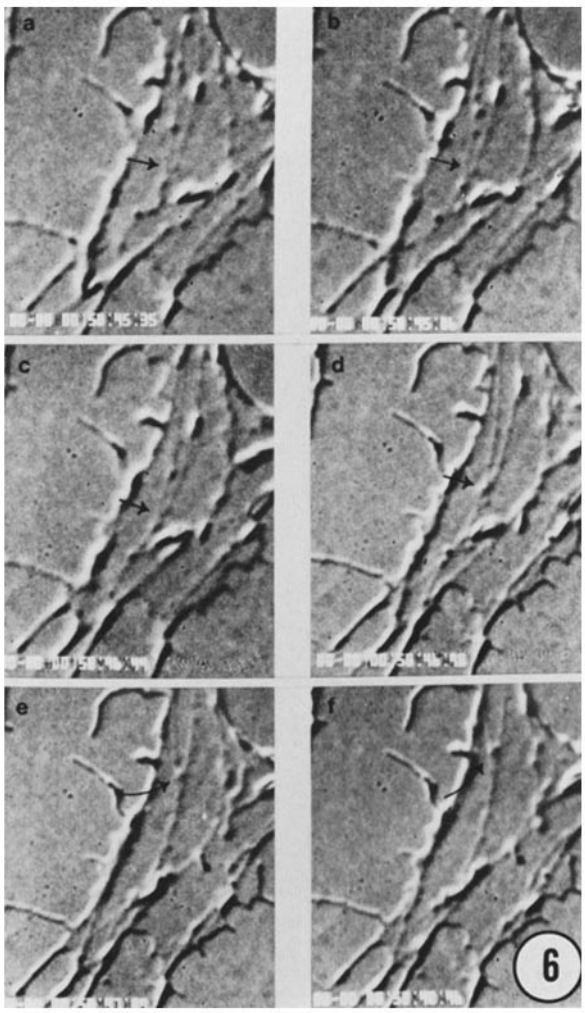

FIGURE 6 The lateral association ('zipping') of fibrils within a lamellipodium. This sequence of differential interference video micrographs illustrates the lateral association, or zipping, of two fibrils with one another. The arrow points to the junction of the two fibrils. This junction moves upwards as the fibrils zip. Times are encoded as follows: 00-00-00:minutes:seconds:hundredths of seconds. × 3,600.

may result in the extension and retraction of filopodia in this organism. Pushing of the microtubular fibril against the cell membrane has been seen to cause the protrusion of a filopodium. The fibril remains within the nascent pseudopod, serving as a rigid core, and as such is necessary for the maintenance of the filopod. When the fibril is withdrawn from the filopodium, the filopodium retracts. Filopodia typically form from the circum-oral cytoplasm at the initation of network development, but may in fact form at virtually any point within the network. It seems very likely that a similar mechanism is operative in the formation of filopodia at these other locales, but, owing to the dense packing of the microtubules and the cytoplasm (with the attendant overlapping of the phase gradients) in these areas, the process would be extremely difficult to observe.

Removal of the pseudopodial microtubules quite naturally results in the slackening and withdrawal of the pseudopod. Without such structural support one would expect that the forces of surface tension would cause a beading response similar to Allen's fibril ⇌ droplet transition (see reference 3). Such a response accompanies damage to the pseudopod through either physical (3, 30), chemical (22, 23), or pharmacological treatment (36). It is quite likely that by combining the disassembly of the microtubules with their removal or 'reeling-in' through axial transport, *Allogromia* has the ability to create a broad spectrum of pseudopodial retraction modes from the very gradual to the catastrophic.

The formation of cellular projections by the pushing of microtubules against the plasma membrane may be a wide spread occurrence in cells. Formation and withdrawal of axopodia accompanies the extension and shortening of the microtubular axonemes; assembly and disassembly of microtubules are coupled to this process (35, 39). Osborn and Weber (26) and Frankel (11) were able to provide a kinetic scheme for microtubule assembly in situ in mammalian tissue culture cells using immunofluorescent techniques. Their studies suggest that microtubules assemble from a centrally located microtubule-organizing center (centrosome?) toward the plasma membrane. Their data further suggest that the microtubules cause protuberances of the membrane where they abut it. DuPraw (10) has suggested that microtubule assembly

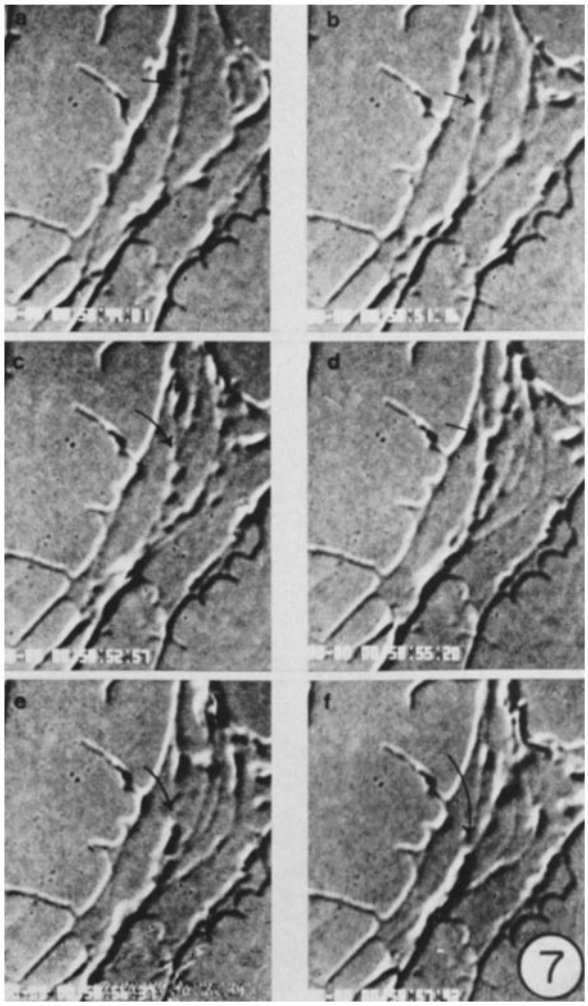

FIGURE 7 'Unzipping' of previously zipped fibrils. Differential interference contrast video micrographs depicting the continuation of the sequence shown in Fig. 6. This portion of the sequence documents the splitting, or unzipping, of the previously zipped fibrils. Arrow again points to the junction of the fibrils, which in this case moves downwards as the fibrils unzip. Timer is encoded as in previous figure. × 3,600.

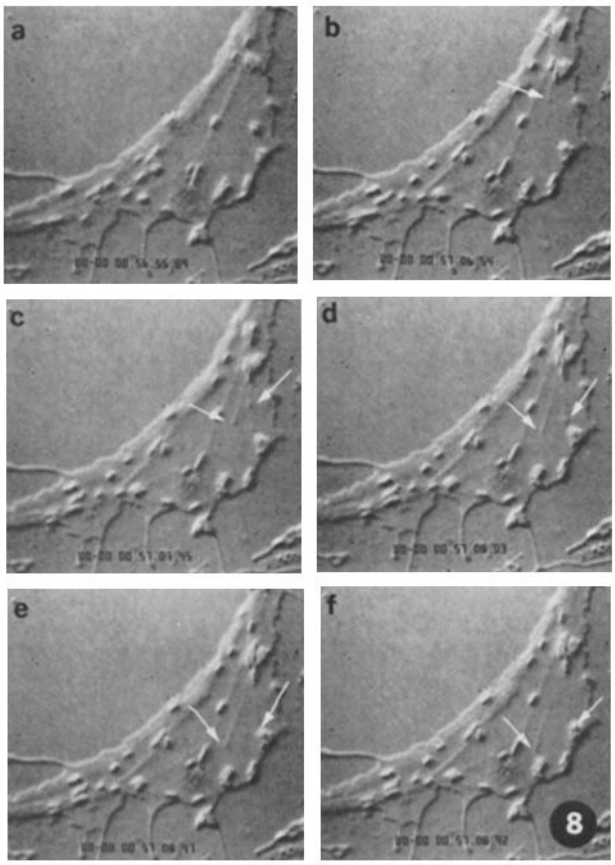

FIGURE 8 Axial translocation of fibrils through a lamellipodium. Video micrographs (differential interference optics) demonstrating the axial or lengthwise movements of two fibrils (arrows) through a lamellipodium. The fibril at the left appears to have a knobbed protuberance at its leading end. × 1,925.

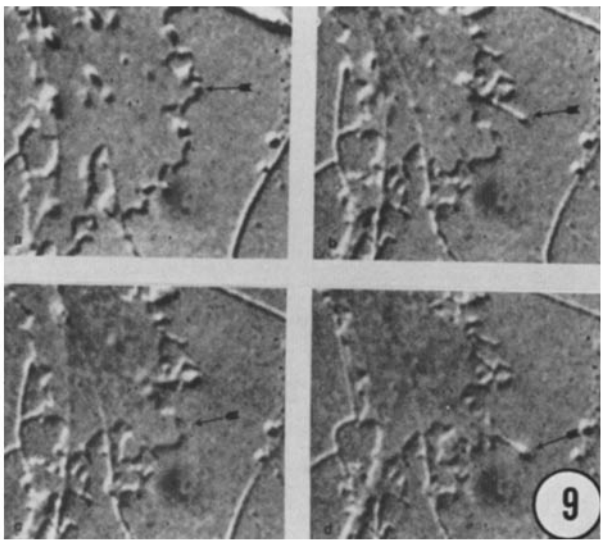

FIGURE 9 The protrusion of fibrils against the plasma membrane can form filopodia. Differential interference contrast video micrographs taken from a time-lapse sequence. This series of micrographs illustrates the extension and withdrawal of filopodia (arrows). These particular filopodia arose when an axially translocated fibril abutted the cell membrane at the margin of the lamellipodium. × 2,000.

is responsible, at least in part, for the formation of pseudopodia in honey bee embryonic cells, and microtubule assembly is thought to accompany neurite and flagellar extension (29, 34, 40). Although clearly both assembly-disassembly and axial translocation of microtubules play a role in the establishment of the reticulopodial networks, it is not yet possible to assess the relative importance of, or localize precisely, these processes.

Cytoplasmic Transport and the Microtubular Cytoskeleton

Cytoplasmic transport within the reticulopodial network of *Allogromia* is intimately coupled with the microtubular system. Cytoplasmic particles appear only to stream when they

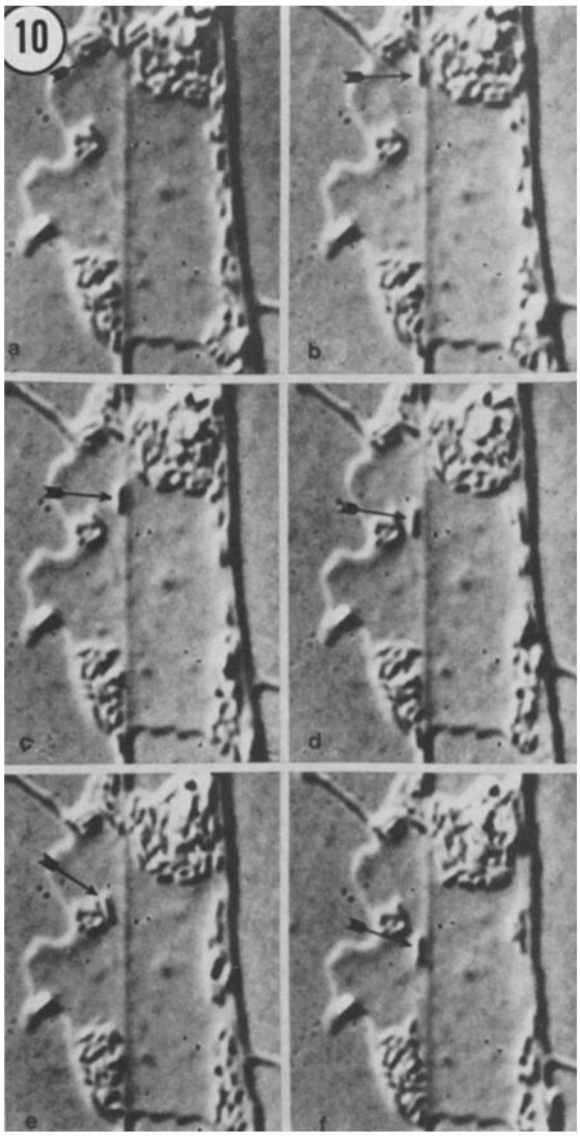

FIGURE 10 The transport of intracellular particles occurs along fibrils. The differential interference contrast sequence illustrates the association between a transported particle and a cytoplasmic fibril, and the dependence of the transport upon this association. The arrow points to a particle that moves along a fibril (*a–d*). This particle dissociates from the fibril and remains stationary for ~1 s (e) until contact is reinitiated. Movement along the fibril resumes upon the reinitiation of contact (*f*). × 2,580.

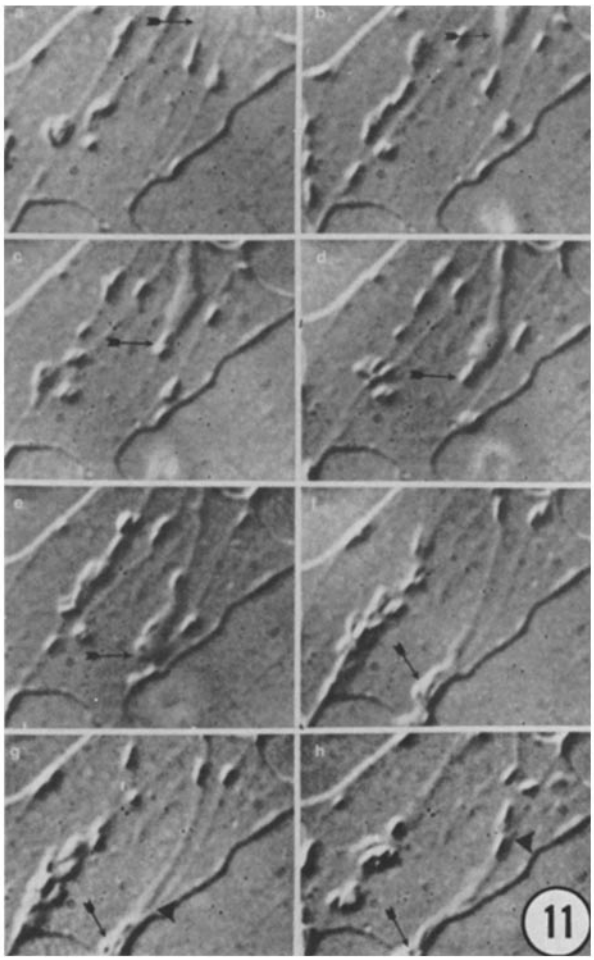

FIGURE 11 Transport of bulk hyaline cytoplasm along a fibril. Video micrographic sequence demonstrating the transport of a mass of hyaline cytoplasm (arrow) along a fibril. Oblate cytoplasmic particles are transported along with the hyaline cytoplasm. The transport of the cytoplasmic mass appears to result in the thickening of the fibril (compare *a* and *g*). The newly thickened fibril zips with the adjacent fibril zipping fibrils in *g* and *h*. × 3,100.

are in contact with a microtubular fibril or filament, as was strongly suggested by previous ultrastructural studies (37) in which particles were observed in contact with the microtubule bundles. Indeed, we frequently observed particles attached to a single microtubule in fixed and critical-point dried, whole-mount electron microscope preparations of lamellipodial areas of the reticulopodia. Video microscopic observations on similar areas show that particles often move along extremely fine filamentous elements. In no case are particles moved if not in association with a microscopically detectable filament! Since the video microscopic techniques employed in this study are sensitive enough to detect and image single in vitro assembled microtubules (6), it is reasonable to conclude that many of the particles that we observe moving are moving along a single microtubule. Microtubules translate axially through the cytoplasm of *Allogromia* reticulopodial networks. Mere attachment of a particle to a moving microtubule may be sufficient to effect its movement.

Microtubules have been implicated as providing a substratum for intracellular movements in a great variety of cells such as chromatophores, neurites, and tissue culture cells that display much less dramatic transport than *Allogromia* (16, 28). Numerous workers have concluded that such saltatory particle excursions are associated with microtubules in a nonrandom way (12, 33), and direct microtubule-organelle linkages have been observed in axoplasm in some cases (31). Recently, Allen *et al.* (7) have demonstrated continuous (non-saltatory) movements of small particles at velocities ranging from 1 to 5 μm sec^{-1} in isolated squid axoplasm. The particles move in files along linear elements (either microtubules or neurofilaments) in a manner quite reminiscent of the present observations on particle movement in *Allogromia*.

Microtubule-organelle interactions and occasionally direct linkages beween the two have been described for a number of other cells. While mitochondria are typically associated with microtubules (8, 13, 39), other organelles (2, 12, 16, 32, 38) and nuclei (15) apparently interact with cytoplasmic microtubules as well. Direct association of cytoplasmic particle transport with cytoskeletal elements has only been rarely observed in living cells or motile extracts (1, 7, 14, 24, 25). Although a vast literature has accumulated suggesting that microtubules move or direct the movement of cytoplasmic particles in cells from all four eukaryotic kingdoms, their direct involvement has yet to be proved. It would appear that *Allogromia* represents one of the very few cases in which cytoplasmic movements occur only in association with microtubules, and in which the role of the microtubules in this process can be readily observed.

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REFERENCES

1. Allen, N. S. 1974. Endoplasmic filaments generate the motive force for streaming in *Nitella*. *J. Cell Biol.* 63:270–287.
2. Allen, R. D. 1974. Evidence for firm linkages between microtubules and membrane-bound vesicles. *J. Cell Biol.* 64:497–503.
3. Allen, R. D. 1964. Cytoplasmic streaming and locomotion in marine foraminifera. *In* Primitive Motile Systems in Cell Biology. N. Kamiya and R. D. Allen, editors. Academic Press, Inc., New York. 407–432.
4. Allen, R. D., G. D. David, and G. Normarski. 1969. The Zeiss-Nomarski differential interference equipment for transmitted-light microscopy. *Z. Wiss. Mikrosk. Mikrosk. Tech.* 69:193–221.
5. Allen, R. D., J. L. Travis, N. S. Allen, and H. Yilmaz. 1981. Video-enhanced contrast polarization (AVEC-POL) microscopy: a new method applied to the detection of birefringence in the reticulopodial network of *Allogromia laticollaris*. *Cell Motility.* 1:275–289.
6. Allen, R. D., N. S. Allen, and J. L. Travis. 1981. Video-enhanced contrast differential interference contrast (AVEC-DIC) microscopy: a new method capable of analyzing microtubule-related movement in the reticulopodial network of *Allogromia laticollaris*. *Cell Motility.* 1:291–302.
7. Allen, R. D., J. Metuzals, I. Tasaki, S. T. Brady, and S. P. Gilbert. 1982. Fast axonal transport in squid giant axon. *Science (Wash. D. C.).* 218:1127–1129.
8. Aufderheide, K. 1979. Mitochondrial associations with specific microtubular components of the cortex of *Tetrahymena thermophila*. I. Cortical patterning of mitochondria. *J. Cell Sci.* 39:299–312.
9. de Saedeleer, H. 1932. Reserches sur les pseudopodes des Rhizopodes testaces. Les concepts pseudopodes lobosea, filosa et granuloreticulosa. *Arch. Zool. Exp. Gen.* 74:597–626.
10. DuPraw, E. J. 1965. The organization of honey bee embryonic cells. I. Microtubules and amoeboid activity. *Dev. Biol.* 12:53–71.
11. Frankel, F. R. 1976. Organization and energy dependent growth of microtubules in cells. *Proc. Natl. Acad. Sci. USA.* 73:2793–2802.
12. Freed, J. J., and M. M. Liebowitz. 1970. The association of a class of saltatory movements with microtubules in cultured cells. *J. Cell Biol.* 45:334–354.
13. Heggeness, M. H., M. Simson, and S. J. Singer. 1978. Association of mitochondria with microtubules in cultured cells. *Proc. Natl. Acad. Sci. USA.* 73:3863–3866.
14. Higashi-Fujime, S. 1980. Active movement in vitro of bundles of microfilaments isolated from *Nitella* cells. *J. Cell Biol.* 87:569–578.
15. Holmes, K., and P. W. Choppin. 1968. On the role of microtubules in movement and alignment of nuclei in virus-induced syncytia. *J. Cell Biol.* 51:752–762.

16. Hyams, J. S., and H. Stebbings. 1979. Microtubule associated cytoplasmic transport. *In* Microtubules. K. Roberts and J. S. Hyams, editors. Academic Press, Inc., London. 487–530.
17. Izzard, C. S., and L. R. Lochner. 1976. Cell-to-substrate contacts in living fibroblasts: an interference reflexion study with an evaluation of the technique. *J. Cell Sci.* 21:129–159.
18. Jahn, T. L., and R. A. Rinaldi. 1959. Protoplasmic movement in the foraminiferan, *Allogromia laticollaris*, a theory of its mechanism. *Biol. Bull (Woods Hole).* 117:100–118.
19. Jepps, M. W. 1942. Studies on *Polystomella* Lamarck (Foraminifera). *J. Mar. Biol. Assoc. U. K.* 25:607–666.
20. LeCalvez, J. 1938. Reserches sur les foraminifers. I. Development et reproduction. *Arch. Zool. Exp. Gen.* 80:163–333.
21. Leidy, J. 1879. Freshwater Rhizopods of America. U. S. Geological Survey Report. Government Printing Office, Wash. D. C. 12:1–324.
22. McGee-Russell, S. M., and R. D. Allen. 1971. Reversible stabilization of labile microtubules in the reticulopodial network of *Allogromia. Adv. Cell Mol. Biol.* 1:153–184.
23. McGee-Russell, S. M. 1974. Dynamic activities and labile microtubules in cytoplasmic transport in the marine foraminiferan, *Allogromia. Symp. Soc. Exp. Biol.* 28:157–189.
24. O'Brein, T. P., and K. V. Thimann. 1966. Intracellular fibers at oat coleoptile cells and their possible significance in cytoplasmic streaming. *Proc. Natl. Acad. Sci. USA.* 56:888–894.
25. O'Brein, T. P., and M. E. McCully. 1970. Cytoplasmic fibers associated with streaming and saltatory particle transport in *Heracleum mantegazzianum. Planta (Berl.).* 94:91–94.
26. Osborn, M., and K. Weber. 1976. Cytoplasmic microtubules in tissue culture cells appear to grow from an organizing structure towards the plasma membrane. *Proc. Natl. Acad. Sci. USA.* 73:867–871.
27. Ploem, J. S. 1975. Reflection-contrast microscopy as a tool for investigation of the attachment of living cells to a glass surface. *In* Mononuclear Phagocytes in Immunity, Infection and Pathology. R. von Furth, editor. Blackwell, London. 405–421.
28. Rebhun, L. I. 1972. Polarized intracellular transport: saltatory movements and cytoplasmic streaming. *Int. Rev. Cytol.* 32:92–137.
29. Rosenbaum, J. L., J. E. Moulder, and D. L. Ringo. 1969. Flagellar elongation and shortening in *Chlamydomonas*. I. The use of cyclohexamide and colchicine to study the synthesis and assembly of flagellar proteins. *J. Cell Biol.* 41:600–619.
30. Sandon, H. 1934. Pseudopodial movements of foraminifera. *Nature (Lond.).* 133:761–762.
31. Smith, D. S., J. Järlfors, and R. Beránek. 1970. The organization of synaptic axoplasm in the lamprey (*Pteromyzon marinus*) central nervous system. *J. Cell Biol.* 46:199–219.
32. Smith, D. S., U. Jarlfors, and B. F. Cameron. 1975. Morphological evidence for the participation of microtubules in axonal transport. *Ann. NY Acad. Sci.* 263:472–506.
33. Smith, D. S., J. Jarlfors, M. L. Cayer, and B. F. Cameron. 1977. Structural cross-bridges between microtubules and mitochondria in central axons of an insect (*Periplaneta americana*). *J. Cell Sci.* 27:255–272.
34. Spiegelman, B. M., M. A. Lopata, and M. W. Kirschner. 1979. Multiple sites for the initiation of microtubule assembly in mammalian cells. *Cell.* 16:239–252.
35. Tilney, L. G., and K. R. Porter. 1965. Studies on microtubules in heliozoa. I. The fine structure of *Actinosphaerium nucleofilum* (Barrett), with particular reference to the axial rod structure. *Protoplasma.* 50:317–344.
36. Travis, J. L. 1981. Motility of the reticulopodial network of *Allogromia laticollaris.* Hanover, NH: Dartmouth College; 168 p. Dissertation.
37. Travis, J. L., and R. D. Allen. 1981. Studies on the motility of the foraminifera. I. Ultrastructure of the reticulopodial network of *Allogromia laticollaris* (Arnold). *J. Cell Biol.* 90:211–221.
38. Wagner, R. C., and M. D. Rosenberg. 1972. Endocytosis in Chang liver cells: the role of microtubules in vacuole orientation and movement. *Cytobiologie.* 7:20–27.
39. Watters, C. D. 1966. Studies on the motility of the helizoa. Princeton, NJ: Princeton University; 132 p. Dissertation.
40. Witman, G. D. 1975. The site of *in vivo* assembly of flagellar microtubules. *Annu. NY Acad. Sci.* 253:178–191.