

# Studies on the Motility of the Foraminifera

## I. Ultrastructure of the Reticulopodial Network of *Allogromia laticollaris* (Arnold)

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**ABSTRACT** *Allogromia laticollaris*, a benthic marine foraminifer, extends numerous trunk filopodia that repeatedly branch, anastomose, and fuse again to form the reticulopodial network (RPN), within which an incessant streaming of cytoplasmic particles occurs. The motion of the particles is saltatory and bidirectional, even in the thinnest filopodia detected by optical microscopy. Fibrils are visible by differential interference microscopy, and the RPN displays positive birefringence in polarized light. These fibrils remain intact after lysis and extraction of the RPN in solutions that stabilize microtubules (MTs). Electron micrographs of thin sections through these lysed and stabilized cytoskeletal models reveal bundles of MTs. The RPNs of living *Allogromia* may be preserved by standard EM fixatives only after acclimatization to calcium-free seawater, in which the streaming is normal. The MTs in the RPN are typically arranged in bundles that generally lie parallel to the long axis of the trunk and branch filopodia. Stereo electron micrographs of whole-mount, fixed, and critical-point-dried organisms show that the complex pattern of MT deployment reflects the pattern of particle motion in both flattened and highly branched portions of the RPN. Cytoplasmic particles, some of which have a fuzzy coat, are closely associated with, and preferentially oriented along, either single MTs or MT bundles. Thin filaments (~5 nm) are also observed within the network, lying parallel to and interdigitating with the MTs, and in flattened terminal areas of the filopodia. These filaments do not bind skeletal muscle myosin S1 under conditions that heavily decorate actin filaments in controls (human blood platelets), and are ~20% too thin to be identified ultrastructurally as F-actin.

For most of the motile phenomena exhibited by the various metazoan cell types, one can find counterparts within the Protista. Indeed, several protists have lent themselves to elegant biophysical (2, 3, 18) and, more recently, biochemical (16, 33) investigations into all aspects of their motility. Bearing this in mind, we have undertaken a study of the motility of *Allogromia laticollaris* (6), a marine benthic foraminifer, as a possible model for the types of bidirectional particle transport observed in metazoan neurons. *Allogromia* extends slender filopodia that may reach a length of several millimeters. These filopodia repeatedly branch and fuse with one another, giving rise to a continuous anastomosing reticulopodial network (RPN). Within this network there is a constant bidirectional streaming of cytoplasm and particles (30). In the thinnest filopodia and in flattened areas of the RPN, particle motion is most easily observed, and here it is readily apparent that the motion is

saltatory in character (1, 46–48). The most slender of the filopodia detectable in the light microscope are <100 nm in diameter.

The presence of structurally distinct anisotropic regions (stereoplasm), while well established in the heliozoa (18, 57) and radiolaria (5, 13, 14), remains uncertain in foraminiferan reticulopodia. Schmidt (52) showed that the filopodia of a foraminifer displayed positive birefringence. More recent investigations have failed to detect the presence of a stereoplasmic rod within RPNs leading earlier descriptions to be dismissed as diffraction artifacts (1, 61). Jahn and Rinaldi (30) and Allen (1) both described the presence of intracellular fibers that they were not able to capture with the somewhat more primitive photographic methods then available. Sandon (50) pointed out that a pseudopodial core can appear if the surrounding seawater is made hypertonic, although ordinarily it is undetecta-

ble. Watters (61) has suggested that the ensuing osmotic water loss may cause lateral associations of real fibrillar elements.

Early ultrastructural studies on the RPN were obstructed by the failure of standard fixatives to preserve the network. Upon contact with most fixatives, the RPN displays a characteristic "beading-reaction," in which the constituent filopodia suddenly become thinner, producing as a result many small, spherical droplets giving the overall appearance of a beaded necklace. Typically, the string of droplets was disrupted and lost during the subsequent steps in sample preparation. All but the thickest pseudopodia were completely destroyed, and the larger ones showed little internal structure (63). McGee-Russell and Allen (41) managed to preserve the RPN intact at the light microscope level through a prefixation step of "stabilizing" the RPN in seawater fortified with the addition of  $MgCl_2$  to levels  $\geq 30\%$  (wt/vol). With this technique, the presence of microtubules (MTs) oriented parallel to the long axis of the pseudopodia was demonstrated (41). Because the orientation of the MTs coincides with the direction of the intracellular movements, we sought to investigate more fully the distribution of the MTs in the reticulopodia and their relationship to other cytoskeletal elements and to the transported particles. This communication presents the results of our ultrastructural studies; in it we document the occurrence of intracellular fibrils composed of bundles of MTs, the association of intracellular particles with the MT bundles, and the occurrence within the RPN of  $\sim 50\text{-}\text{\AA}$ -thick filaments that do not bind the S1 fragment of chicken skeletal muscle myosin under the same conditions in which the thin filaments of human blood platelets (controls) are decorated.

## MATERIALS AND METHODS

### Culture

Cultures of the foraminifer, *Allogromia laticollaris*, were maintained in glass bottles in a medium consisting of 26% Foy's Erdschreiber in Instant Ocean (Aquarium Systems, Inc., Eastlake, Ohio) as described by Lee (35), except that the medium was made 10 mM in Tris-HCl (pH 8.1). Diatoms, algae, and bacteria growing in cultures served as prey.

### Light Microscopy

Individual *Allogromia* were placed between two glass coverslips separated by plasticene feet; the preparations were then sealed with Valap (Vaseline-lanolin-paraffin, 1:1:1) and examined with a Zeiss Photomicroscope equipped with either sensitive differential interference contrast (DIC) optics or phase contrast. Polarized light microscopy was performed using Nikon rectified polarizing optics mounted to the same stand with a 10-mm spacer ring above the objective. High extinction DIC micrographs and videomicrographs were obtained with a Zeiss Axiomat. For photomicrography, single flash illumination from a high pressure xenon arc strobe (Chadwick-Helmuth Co., Inc., Monrovia, Calif.) was used.

### Perfusion and Lysis

Microperfusion chambers originally designed by Molé-Bajer and Bajer (43) and subsequently modified by McGee-Russell and Allen (41) were used in all perfusion experiments. These chambers allow rapid perfusion rates with minimal mixing of solutions within the observation chamber. The foraminifers were lysed in a solution of the following constitution: 0.05 M PIPES,  $5 \times 10^{-4}$  M  $MgSO_4$ ,  $1 \times 10^{-3}$  M EGTA, 0.2–1.0% Triton X-100, and 10–15% dimethyl sulfoxide (DMSO) (58). The cells for S1 experiments were lysed and stabilized in 0.005 M PIPES, 0.05 M KCl, 0.0075 M  $MgCl_2$ , 0.001 M EGTA, in the same concentrations of Triton X-100 and DMSO.

### Electron Microscopy

**FIXATION:** *Allogromia* were acclimated to "calcium-free" artificial seawater (CaFSW), a solution of 0.390 M NaCl, 0.049  $MgCl_2$ , 0.026 M  $Na_2SO_4$ , 0.008 M KCl and 0.002 M  $NaHCO_3$  buffered to pH 7.9–8.1 with 0.01 M Tris-HCl. No

calcium ion chelators were added to this solution, and  $[Ca^{2+}]$  was estimated to be  $\sim 5 \times 10^{-5}$  M by means of atomic absorption spectroscopy (kindly performed by Dr. Christopher Cronan). Fixation was carried out with 2–3% glutaraldehyde and 0.04% tannic acid in 0.1 M Na-cacodylate buffer at pH 7.4. After two or three rinses in buffer the specimens were postfixed in 0.5%  $OsO_4$  in 0.1 M Na-cacodylate buffer pH 6.0 (39) for 2–5 min at 0°C. After the specimens were rinsed in distilled  $H_2O$  at 0°C, they were carried through 30 and 50% ethanol for 10 min each, and stained *en bloc* for 5–10 min in 0.5% (wt/vol) uranyl acetate in 70% ethanol. After destaining overnight in 70% ethanol, dehydration was carried to completion with either ethanol or acetone.

**THIN SECTIONS:** *Allogromia* were allowed to extend an RPN for at least 30 min on glass coverslips that had been sprayed with Teflon, polished, and then coated with 1.5  $mg\ ml^{-1}$  poly-L-lysine or protamine sulfate. After fixation and dehydration, specimens were embedded in Epon (36) or Epon-Araldite. The cured resin wafers were separated from the coverglass by immersion in liquid  $N_2$ . Individual foraminifers were selected, cut out, and mounted on blank Epon blocks. Thin sections were cut with a Porter-Blum MT-1 ultramicrotome (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.). Sections were contrasted with uranyl acetate and lead citrate.

**WHOLE MOUNT TEM:** Cells were placed on protamine-sulfate-coated, carbon-over-Formvar gold or stainless-steel 200-mesh grids. After fixation and staining as described above, grids were dehydrated in acetone and critical-point-dried from liquid  $CO_2$  (64) in a Bowmar CPX-990 apparatus (Bowmar Instrument Corp., Fort Wayne, Ind.). Electron micrographs and stereo pairs were obtained with a JEOL-100 CX electron microscope equipped with a goniometer stage, at an accelerating potential of 100 kV.

**SCANNING ELECTRON MICROSCOPY:** The foraminifers spread on 9-mm diameter glass coverslips were fixed, dehydrated, and critical-point-dried as described above, then coated with 10 nm Au-Pd in a Hummer II sputter coater, and examined in either a CWIK Scan 105 A or a JEOL JSM-35 scanning electron microscope. Alternatively, cells were prepared with the osmium-thiocarbonylhydrazide-osmium (OTO) procedure (29, 32); in the latter case, coverslips were sputtered with Au-Pd ( $\sim 40$  nm), to increase conductivity, before protamine sulfate coating and plating of the foraminifers.

## RESULTS

Pure cultures of *Allogromia laticollaris* display striking polymorphism (7). Several authors have suggested that the various morphotypes are specific to the many different stages of the complex life cycle of this monothalamous foraminifer (40). The typical specimens selected for this study have a more or less spherical cell body (Fig. 1) covered by a flexible unmineralized organic test (8). The diameter of the cell body increases with the age of the animal and ranges from 20 to several hundred micrometers. There is generally a single aperture in the test, around which the test is thickened. An isthmus of cytoplasm, the peduncle, extends through this channel in the aperatural thickening. The peduncle separates the intrathalamous cytoplasm from that of the extrathalamous or circum-oral cytoplasm (8). Filopodia extend from the circumoral region. These are formed as slender (generally  $>5\ \mu m$  in diameter), rigid structures that are capable of extending great distances through the medium, unsupported by the substratum (31, 49). The filopodia branch repeatedly, and the branch pseudopodia subsequently fuse with one another, giving rise to a continuous structure, the RPN.

The RPN may become quite expansive, with the main trunks extending several millimeters. The RPN is a feeding apparatus that captures food particles and transports them toward the cell body. Prey, such as diatoms and bacteria, are captured and collected into large masses that are accumulated within basketlike enclosures of the RPN close to the cell body (Fig. 2). Transport of the food particles occurs along the external surface of the reticulopodia. Prey organisms are frequently seen adherent to pseudopodia that are completely suspended above the substratum (Fig. 2).

The reticulopodial network is a highly dynamic structure. The individual pseudopodial elements continuously extend, retract, branch, and fuse with one another. The most striking

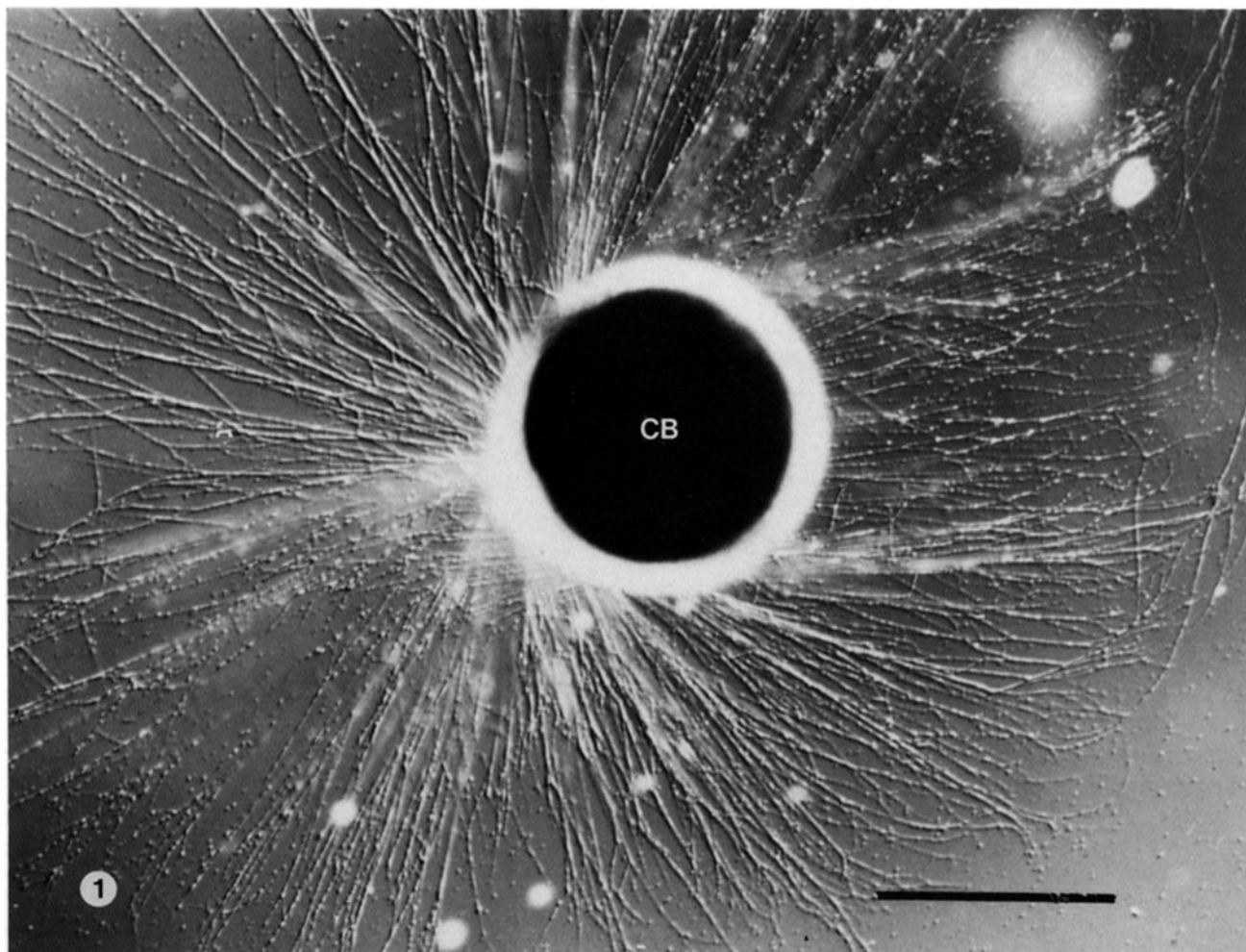


FIGURE 1 Survey light micrograph of living *Allogromia* (DIC optics). This extensive reticulopodial network illustrates the radially extended trunk filopodia, and the complex interconnection of these with the branch filopodia. CB, cell body, Bar, 100  $\mu\text{m}$ .  $\times 335$ .

aspect of the motility of the RPN, however, is the movement of cytoplasmic particles. Within the network there is an incessant streaming of ground cytoplasm and particles. The particle translocations are bidirectional in all parts of the RPN (1, 30), even in the thinnest filopodia detectable with the optical microscope ( $\sim 50$  nm). While in the larger filopodia, particles appear to move in well-defined streams of cytoplasm, the movement of single particles is saltatory in nature, occurring at velocities often  $>10 \mu\text{/s}$  (48).

To characterize the cytoskeletal elements of the reticulopodia ultrastructurally, it was necessary to overcome the beading damage in response to fixatives. The ability of high concentrations of  $\text{Mg}^{2+}$  to "stabilize" the *Allogromia* networks to electron microscope fixatives (41) suggested that these solutions might be acting antagonistically to  $\text{Ca}^{2+}$  and that calcium ions might be responsible for the beading reaction. When *Allogromia* specimens have been washed in CaFSW several times they still retain the ability to extend normal networks. This lowering of external  $\text{Ca}^{2+}$  concentration makes it possible to preserve the RPN with more conventional fixation techniques.<sup>1</sup> Apparently high external concentrations of  $\text{Ca}^{2+}$  prevent the preservation

<sup>1</sup> After these present studies were initiated, S. M. McGee-Russell and R. Trautwein (Department of Biological Sciences, State University of New York, Albany) demonstrated that lowering the external  $[\text{Ca}^{2+}]$  allowed fixation of *Allogromia strain NF* (Lee), without the rigors of

of the microtubule-based cytoskeleton.

Thin sections of fixed and embedded *Allogromia* RPN show that large numbers of microtubules are present within the filopodia. As reported for another *Allogromia* species (41), the MTs are oriented parallel to the long axis of the pseudopodia (Fig. 3). Contrary to the case reported for *A. strain NF* (Lee), MTs in *A. laticollaris* pseudopodia are predominately arranged in bundles (Figs. 4 and 5). Frequently, larger filopodia contain multiple bundles of MTs (Fig. 4). Smaller filopodia branching from the main trunks contain bundled MTs that appear to diverge from larger bundles that run along the main trunk. This finding suggests that the formation of branch pseudopodia involves the redistribution of the microtubules bundles of the trunk pseudopodia.

The RPN is positively birefringent (Fig. 6a) when examined in a polarizing microscope. This birefringence remains after lysis of the plasma membrane in solutions that are known to stabilize microtubules (Fig. 6b and c) (See Materials and Methods). Examination of thin sections of these lysed cytoskeletal models reveals bundles of microtubules often packed in a loose, hexagonal array (Fig. 5c). High extinction DIC microscopy reveals the presence of numerous linear elements

prefixation "stabilization." Their work was presented in a platform session of the 5th International Congress of Protozoology, held in New York City, 26 June–2 July 1977.

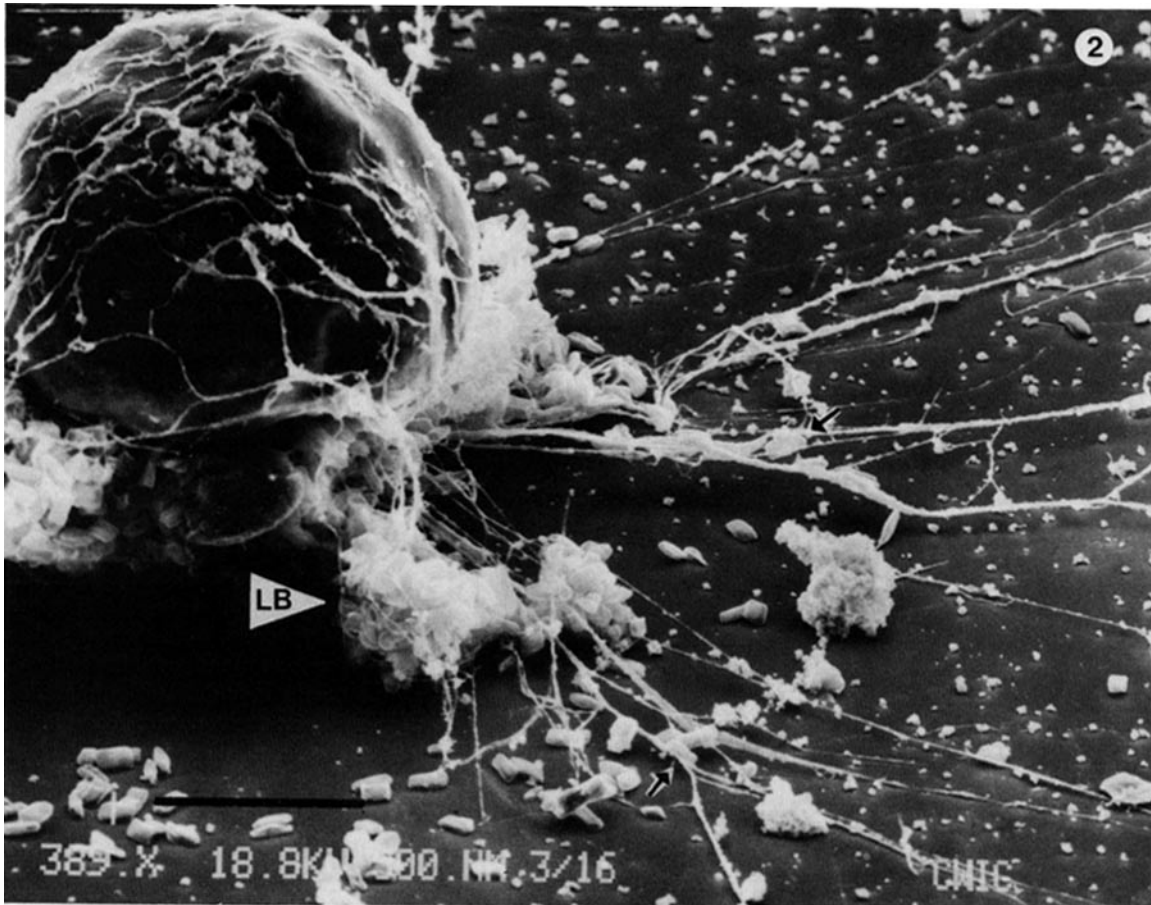


FIGURE 2 Scanning micrograph of an *Allogromia* that has captured diatoms and accumulated them in netlike enclosures of the RPN, called "lunch boxes" (LB). Many diatoms are adherent along filopodia (arrows). The RPN has extended over the exterior surface of the test which covers the cell body. Bar, 50  $\mu\text{m}$ .  $\times 535$ .

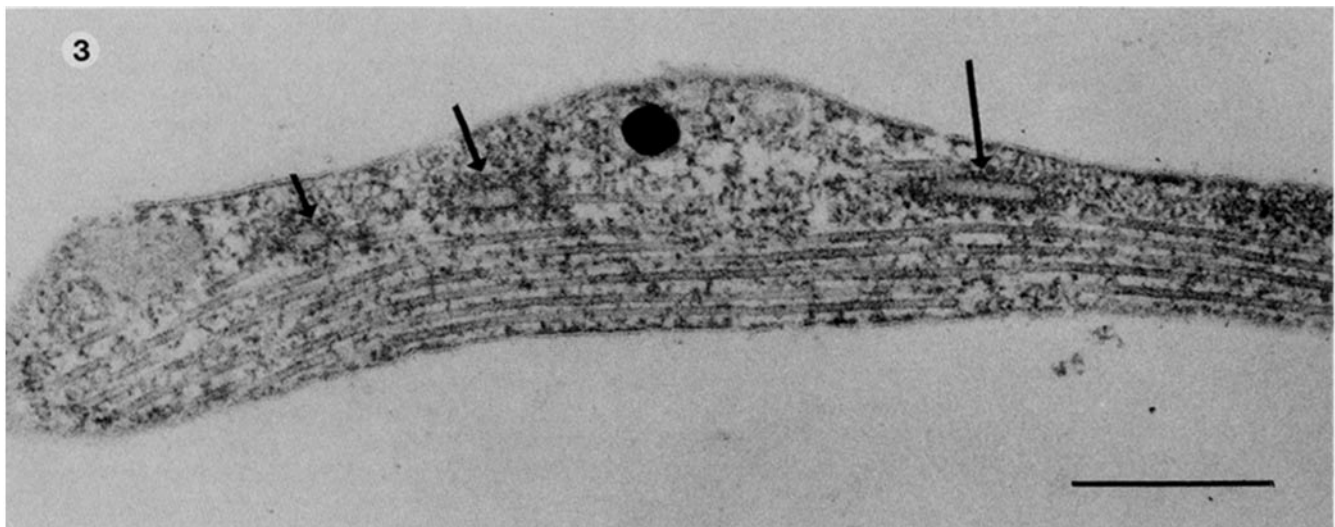


FIGURE 3 Thin-section electron micrograph of foraminiferan that was acclimatized to CaFSW before fixation. The MTs are aligned parallel to the long axis of the filopod. These MTs are not smooth-walled but rather appear to possess side projections along their length. One class of small elliptical vesicles appears to have a fuzzy investing layer around its limiting membrane (arrows). Bar, 500 nm.  $\times 53,250$ .

within the pseudopodia of living *Allogromia* (Fig. 6d). These fibrils lie parallel to the long axis of the pseudopodia and appear to delineate the paths along which the streaming particles move. In tannic acid-stained thin sections the microtu-

bules appear "fuzzy" as though coated with surface projections. These projections often possess a regular 25-nm periodicity along the length of the tubules (Figs. 3 and 5). The periodicity is particularly noticeable between adjacent MTs, where on

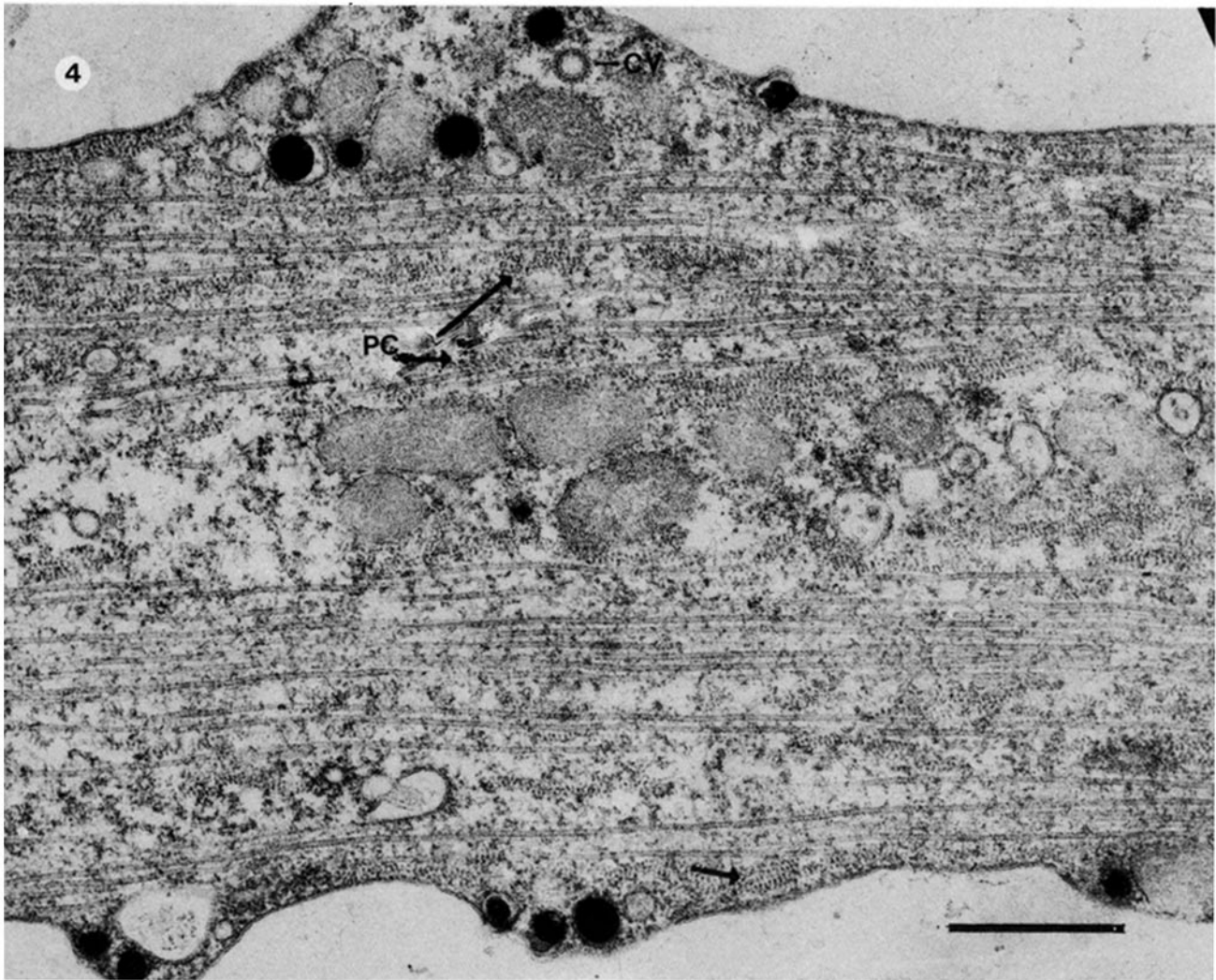


FIGURE 4 Thin-section EM of a portion of a more complex pseudopod. The MT appear to be organized in distinct tracts or bundles. Cytoplasmic vesicles of varying shape and electron density appear limited to the exterior of bundles. One class of these vesicles appears to be coated (CV). Paracrystalline material (PC) may be seen throughout this filopodium. Bar, 1  $\mu\text{m}$ .  $\times 24,000$ .

occasion bridges are observed between the MTs such as those observed in Fig. 5b.

Many different types of vesicles are present throughout the RPN. One class in particular appears to be bristle coated and presumably arises through endocytosis (Fig. 4, CV). Another very common variety is small, oblate ellipsoidal vesicles that have electron-lucent cisternae and appear to have a fuzzy coating around the cytoplasmic surface of their limiting membrane (Figs. 3, 4, 9, and 10). All the different types of granules appear to be in contact with the adjacent MTs. This association seems to occur preferentially at the periphery of MT bundles. Often the granules and vesicles are located between two MT bundles. Cytoplasmic particles also appear closely associated with the plasma membrane; indeed images of fusion between vesicle membrane and plasmalemma have been encountered.

One especially prominent feature observed within the reticulopodia is densely staining paracrystalline structures (Figs. 4, 7a, and 11). At higher magnifications these have a helical symmetry (Figs. 7b and 11). These structures are most probably identical to those reported to occur in the peripheral intrathalamous cytoplasm of *Allogromia* by Hauser and Schwab (25), who have proposed that these helical paracrystalline bodies

probably represent a different supramolecular arrangement of tubulin monomers. Indeed, we often observe these helical structures to be continuous with the MTs, as if they were an intermediate stage in microtubule assembly or disassembly (Fig. 7b).

### *Three-dimensional Organization of the Reticulopodial Network*

We wished to determine how the microtubules are organized within the extensive reticulopodial network. As an alternative to three-dimensional reconstruction from serial thin sections, we prepared stereo pairs of transmission electron micrographs of whole-mounted, fixed, and critical-point-dried *Allogromia*. Although 100 kV is insufficient accelerating potential to provide high resolution, achromatic images of the thickest pseudopodia, the thinner ones, and especially the spread regions that form at attachment points yield quite satisfactory micrographs (Figs. 8–10). The bundles of MTs and the granules associated with them are clearly visible in these preparations. Stereo micrographs (Figs. 8–10) of these whole mounts show how the bundles of MTs are arranged in the RPN. Fig. 8 is a

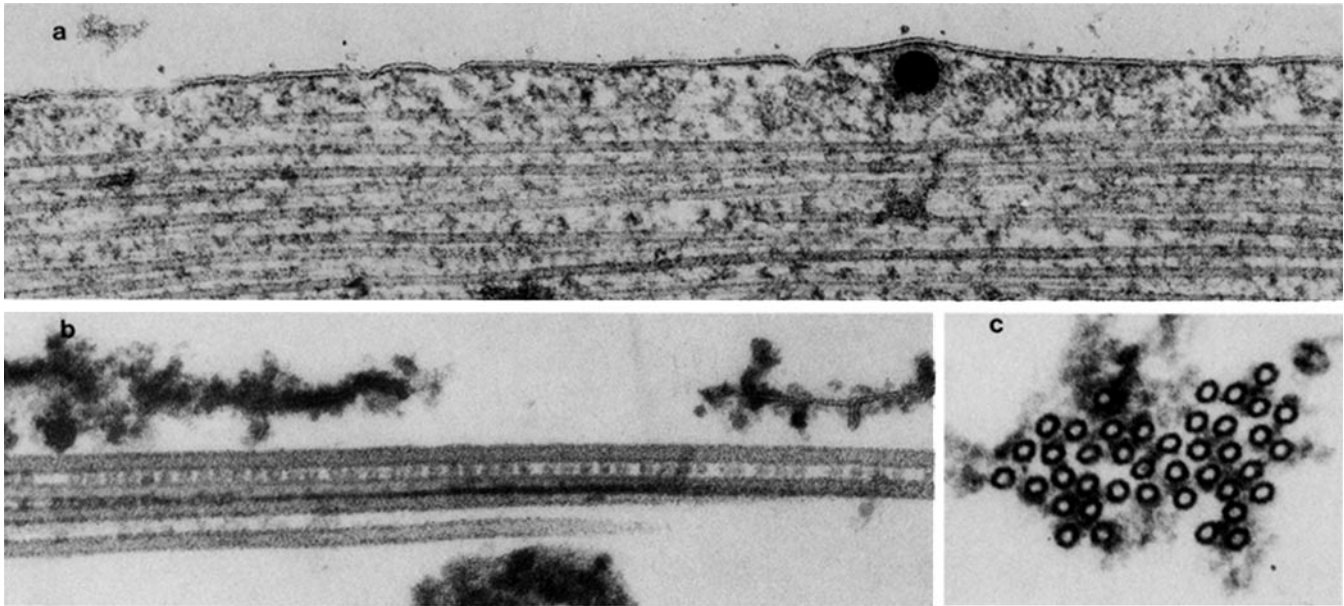


FIGURE 5 (a) Thin section through an MT bundle in a filopodium showing side projections along MTs. 66,670 $\times$ . (b) Microtubule pair from a lysed cytoskeletal model of a foraminiferan, demonstrating periodic cross-bridging between the tubules.  $\times$  117,000. (c) TEM of thin section through lysed and stabilized filopod. a,  $\times$  40,000; b,  $\times$  80,000; c,  $\times$  100,000.

stereo image of a flattened, branched portion of an RPN. The area to the left side of this image is more flattened than those to the right. Within the pseudopodia the MTs are arranged in bundles or cables. Note that these MT cables frequently branch along their lengths. The resulting branches may rejoin the original bundle or, alternatively, diverge and join another bundle. Small numbers of MTs or even a single MT can split off from one bundle and join another (Fig. 8, arrow). Frequently, the MTs of a cable splay apart into groups of single MTs, which run parallel to one another for a distance before associating again to reform a bundle (Figs. 8 and 10).

Cytoplasmic particles lie in close contact with single MTs or bundles of MTs and, when asymmetrical, tend to be oriented parallel to the MTs (Figs. 9 and 10). This is particularly evident in the case of the small, ellipsoidal, fuzzy coated vesicles seen in thin-sectioned material (Fig. 3) and in stereo whole mounts (Figs. 9, and 10 [arrow]).

Fig. 9 shows a typical cylindrical trunk pseudopodium that contains several thick MT bundles, and a few thinner bundles extending into an attachment region shown in the lower right corner of the image. The cytoplasmic particles that appear much larger in the light microscope can be seen in their correct size and perspective to lie adjacent to or between bundles of MTs. Several very small filopodia of  $\sim$ 50 nm in diameter and from 0.3 to 0.7  $\mu$ m long extend outward from the lateral margins of this spread attachment region.

Flattened portions of the RPN show the tendency of the MT bundles to splay out and branch into smaller and smaller groups. Fig. 8 demonstrates this particularly well. Furthermore, it can be seen that even a single microtubule that traverses a broad lamellipodial region may have a particle attached to it (Fig. 8). Similarly, Fig. 10 also shows the association of vesicles with a single MT.

### 5-nm Filaments Are Present within the Filopodia

Fine (5-nm-thick) filaments are observed in regions of the reticulopodial network that are not in intimate contact with the

substratum. Electron micrographs of these areas show that these filaments extend parallel to, and often interdigitate with, the MT (Fig. 11). In spread regions of the RPN, where MT cables splay out, these filaments run parallel to the individual MTs.

The hypothesis that the thin filaments might be actin, despite being some 20–25% thinner than would be expected, was tested by several attempts to label cytoskeletal models of *Allogromia* with chicken skeletal muscle myosin S1. While S1 in concentrations from 1 to 10 mg/ml consistently labeled the 6- to 7-nm filaments of human blood platelet controls, the same concentrations failed to label the 5-nm filaments of lysed *Allogromia*. The lysis medium failed to preserve the 5-nm filaments as well as it preserved the 6- to 7-nm filaments of platelets. Searching through 50 grids failed to reveal a single labeled 5-nm filament among those that remained intact in the lysis medium. By way of contrast, S1-labeled microfilaments in the platelet controls could be found in minutes. A rare exception was the unlabeled microfilament adjacent to an MT in Fig. 12*b*. In the *Allogromia* preparations, many unlabeled filaments were found that were not adjacent to MTs.

## DISCUSSION

Our results demonstrate that MTs play a pivotal role in the cytoskeletal apparatus of *Allogromia laticollaris* reticulopodial networks. The positive birefringence of the pseudopodia would appear to be in large measure caused by these MTs. More importantly, however, the branching patterns of individual pseudopodia in a network are faithfully mirrored by the branching and rapprochement of the underlying MT cables. This finding suggests that these cytoskeletal elements are necessary for the maintenance, if not the formation, of the reticulopodial patterns. Clearly, maintenance of form in the RPN depends on the integrity of the MT system. Urea (42), colchicine (58), or griseofulvin<sup>2</sup> treatment results in the “beading” of

<sup>2</sup> J. L. Travis and J. F. X. Kenealy. Unpublished data.

the filopodia accompanied by the loss of rigidity and eventual collapse of the network. Failure to preserve the MTs by chemical fixatives causes similar destruction of the network, al-

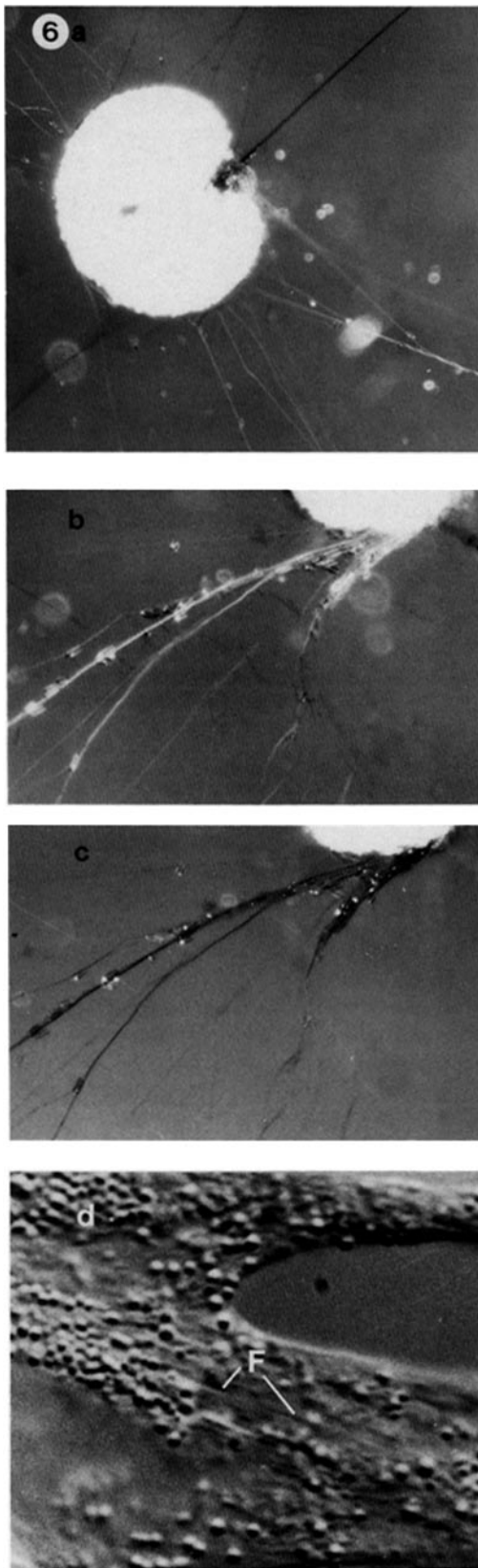


FIGURE 6 (a) Polarized light micrograph demonstrating birefringence of RPN. +4° Brace-Köhler compensation. (b and c) *Allo-*

though in this latter case the influx of  $Ca^{2+}$  seems responsible for MT disruption. Schliwa (51) showed that ionophore-induced  $Ca^{2+}$  influx caused beading of *Echinospaerium axopodia*. It may well be that standard EM fixatives preserve internal cell components only after changing the permeability of the cell membranes. Huang and Mazia (27) had to pretreat *Stentor* with 10 mM EDTA to avoid fixation-induced contraction of the myonemes. In addition, Luftig et al. (37) have reported that fixing with glutaraldehyde in MT polymerization buffer at physiological temperatures increased both the total number and average length of MTs preserved in tissue culture cells.

Besides fulfilling a structural role, it seems likely that MTs are involved in the transport of the intracellular particles in *Allogromia* RPN, at least inasmuch as they define paths along which the particles move. This contention is supported by a number of observations. First, the granules within the pseudopodia are associated with the MTs and the MT cables; in fact, these particles often appear in contact along the length of the MTs. These MTs may be either single or in bundles. Second, just as the distribution of MTs seems to mirror the pseudopodial patterns of the reticulopodia, so too does it reflect the pattern of motility observed. Granules are frequently observed to change streams in the middle of a pseudopod, reverse direction, accelerate, decelerate, or stop, remaining altogether stationary for varying periods of time (1, 30, 48). The behavior of the streaming particles is saltatory (1, 46-48). Typically, these changes in direction and velocity occur at points of attachment to the substratum (1). It is in these same regions of particle movement change that one sees the connection of MT cables with one another. Here either adjacent cable may join together or individual MTs, splay out, and connect with adjacent bundles. Often the MTs crisscross one another. The complex pattern formed by this weaving of MT fibers would allow a particle moving along one fiber to: (a) continue along the same path, (b) cross over to another, intersecting path, or (c) reverse directions along the same path or change to another path and reverse directions along it. Thus, if we can understand how particles are moved in their association with MT bundles, we can explain the complexities of the particle behavior in terms of the MT pattern. All of the types of particle movements in the reticulopodial network may be characterized as occurring along MTs or MT cables. The spatial distribution of these cytoskeletal elements reflects accurately the myriad pathways possible.

The fuzzy lateral projections occurring along the MTs may be of particular relevance to the mechanism of the transport of intracellular particles and ground cytoplasm within the RPN. In favorable cases, these projections can be seen organized into regularly spaced cross-bridges between adjacent MTs (Fig. 5). If these projections represented mechanochemical transducers, rather than static structural cross-links, a sliding MT mechanism might be the basis of much of the motility of the RPN. Such a mechanism could explain the occurrence of multiple velocities of particle translocation within the same filopodium (1); for example, a particle attached to or moving along an MT would move at successively higher velocities as this MT slid

*gromia* lysed in MT-stabilizing solutions. Two micrographs at opposite compensator settings demonstrate the retention of birefringence in cytoskeletal models. (d) High extinction DIC flash micrograph of a portion of RPN of living foraminiferan. Intracellular particles appear associated with numerous cytoplasmic fibrils (F).  $\times 6,000$ .

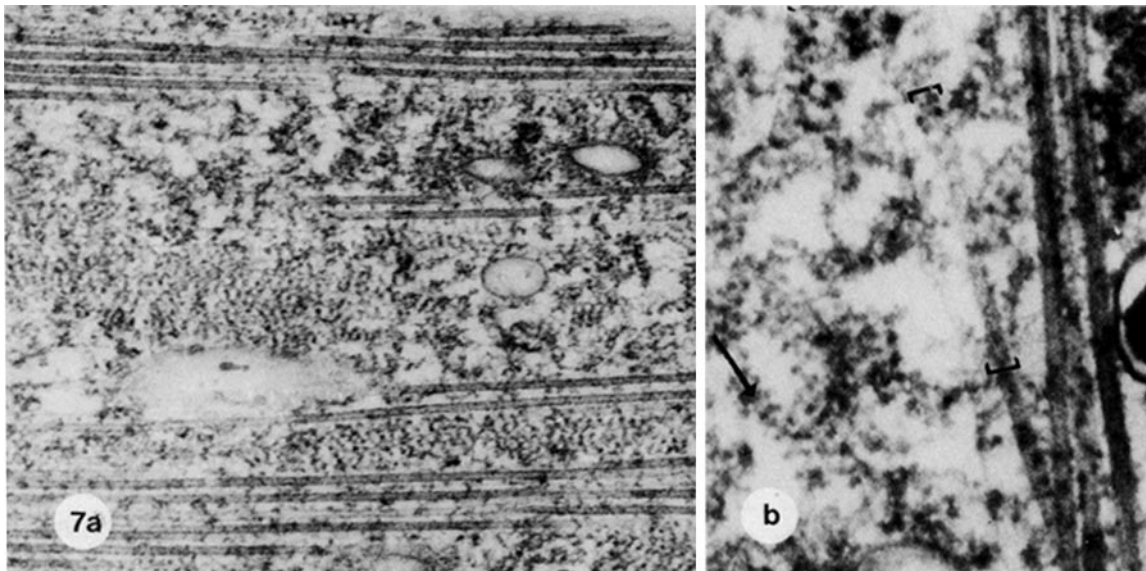


FIGURE 7 (a) Thin section through filopodium showing paracrystalline material striated between MT bundles.  $\times 58,000$ . (b) Electron micrograph demonstrating helical nature of paracrystalline material (arrow) and region of continuity between the end of an MT and a paracrystal (brackets).

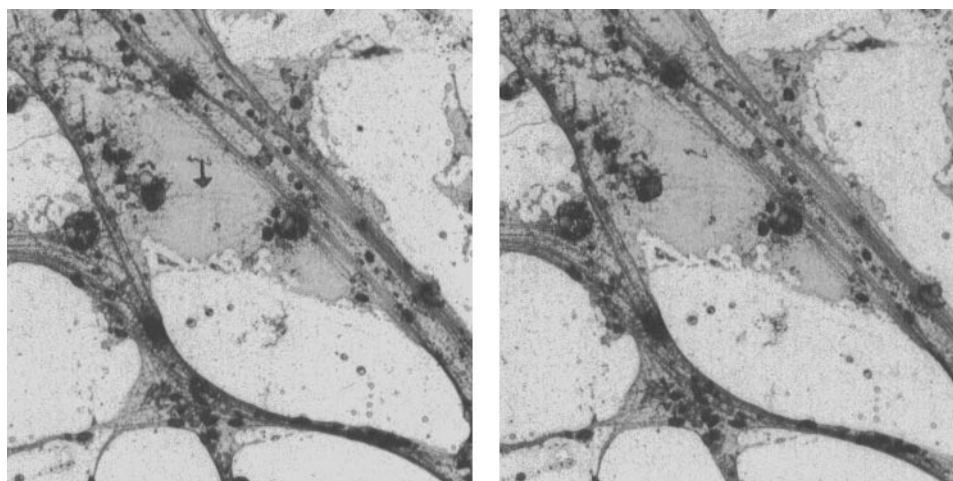


FIGURE 8 Stereo image demonstrating the splaying apart and interconnection of MT bundles. Arrow points to a single MT. Note pattern of MTs and associated intracellular particles in region of filopod criss-crossing in lower left corner of image.  $\times 7,500$ .

along the next, and each successive, MT, in piggyback fashion. Similarly, the fuzzy coat that surrounds the cytoplasmic surface of many of the vesicles (Figs. 3 and 10) could serve either to attach the vesicles to MTs, or as mechanochemical transducer molecules that propel them along stationary MT "tracks."

The present work may lay to rest the question of whether foraminiferan reticulopodia contain "stereoplasmic" elements or not. It is clear that *Allogromia* lacks an axonemal structure such as those observed in heliozoans (57) and radiolarians (5, 13, 14). However, the filopodial MTs are arranged preferentially in bundles, as are those of *Iridia* (38), and individual filopodia may contain numerous bundles (Fig. 9). It is likely that these MT cables are the "fibrils" described by previous authors (1, 30). The presence of these many MT bundles, and their propensity for alternately splaying apart and anastomosing, lends credence to Watters' (61) suggestion that linear elements may at times associate laterally, giving the impression of a stereoplasmic core.

The literature of cell motility abounds with examples of MT-directed movements of organelles and cytoplasm (28, 56, for review). These include axoplasmic transport (9-11, 21, 55), saltations of lysosomes in mammalian tissue culture cells (22, 59, 60), particle transport in heliozoan axopods (18, 57), pigment granule migration in vertebrate chromatophores (12, 23), and mitosis (4, 46, 47). The data in all cases show that movement occurs along paths that are parallel to intracellular microtubules. To date, however, no causal role for microtubules has been clearly demonstrated in any translocation system. Indeed, the elegant experiments of Edds (18, 19) and the observations of Fitzharris et al. (20) demonstrate that the axonemal MTs of *Echinospaerium* need not be present in order that particles saltate inside axopodia.

Numerous morphological studies have shown that microfilaments (MFs) are implicated in intracellular movements in a wide variety of metazoan cells (44, 47). Several types of these intracellular particle movements, particularly those of certain



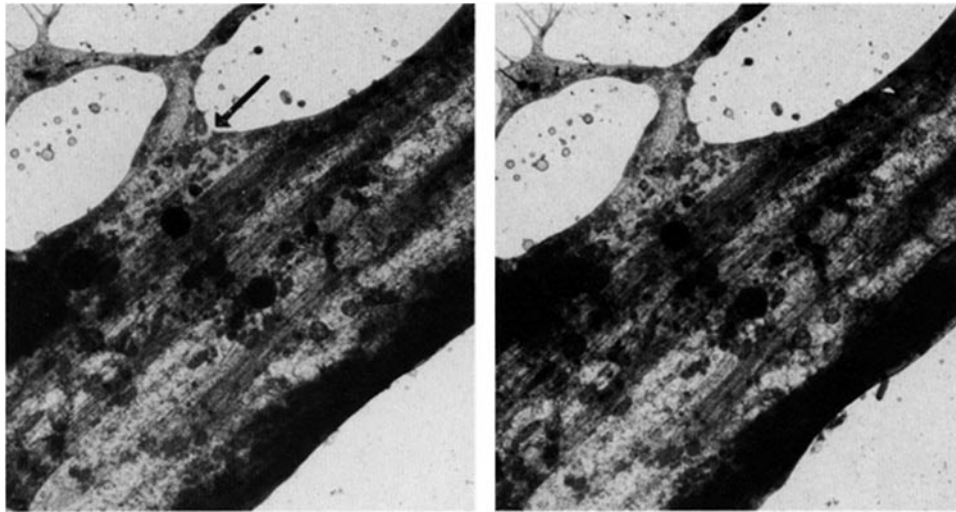


FIGURE 9 Multiple MT cables, each with its own associated intracellular particles, can be observed within a single filopodium. The region in the upper left corner of image is attached to the support film. A spread area containing a filamentous network has formed at this contact site. Three small filopodia project vertically from the center of this spread region. Arrow points to a region that contains accumulations of small elliptical, fuzzy coated vesicles. Similar vesicles are seen oriented along the MT cables.  $\times 8,855$ .

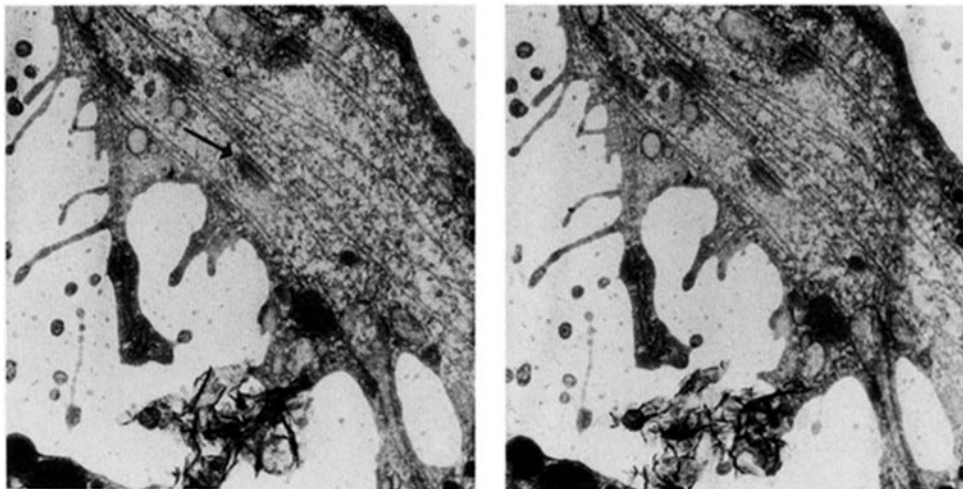


FIGURE 10 Stereo transmission micrograph of a portion of whole-mount, fixed, and critical-point-dried animal. The association between small vesicles and MT (arrow), as well as the splaying behavior of individual MTs of a bundle can be seen in this flattened region. Fine filaments are seen in the cytoplasm, some of which extend parallel to the MTs.  $\times 16,900$ .

pigment cells, are blocked by colchicine and cytochalasin B, suggesting that either an MT or MF system, or perhaps a combination of the two, can be responsible for the movements.

In the case of axoplasmic transport, fast and slow components are traditionally distinguished by their relative velocities. The slow component exhibits a rate of  $\sim 1$  mm/d (26, 62), while the fast component may attain rates of 400–500 mm/d (11, 45). Organelle translocations are not readily observed in living vertebrate nerves, but in one study (21) they were shown to consist of saltatory transport with velocities of  $\sim 1$   $\mu\text{m/s}$ . Particle motions in neurites extending from explanted embryonic ganglia correspond well with this velocity range (9–11). Electron micrographs of vertebrate nerves frequently demonstrate a close association between axoplasmic organelles and the neuronal MTs (9, 10, 55).

The motion of particles within the RPN of *Allogromia* (1, 30, 48) is similar in pattern to that seen in nerve preparations. The main difference is that the velocities of particles in the foraminifer are much greater. Neurons possess three intracel-

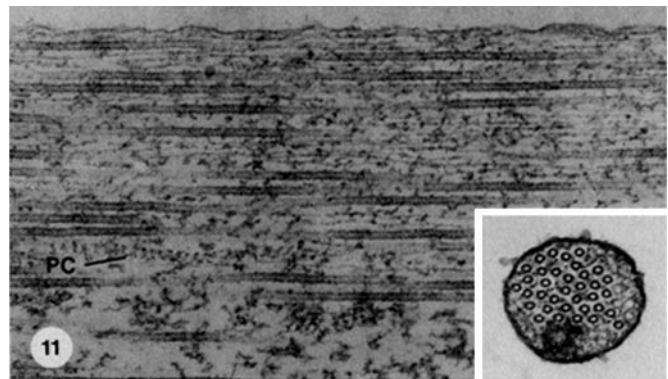


FIGURE 11 Thin-section transmission electron micrograph of a portion of a filopod not in contact with the substratum. Numerous fine (5-nm) filaments lie parallel to the MTs. Helical paracrystalline structures (PC) are also seen.  $\times 49,900$ . Inset: Cross section of filopodium showing relative diameters of the MTs and thin filaments that appear as electron-dense "dots" from this aspect.  $\times 47,900$ .

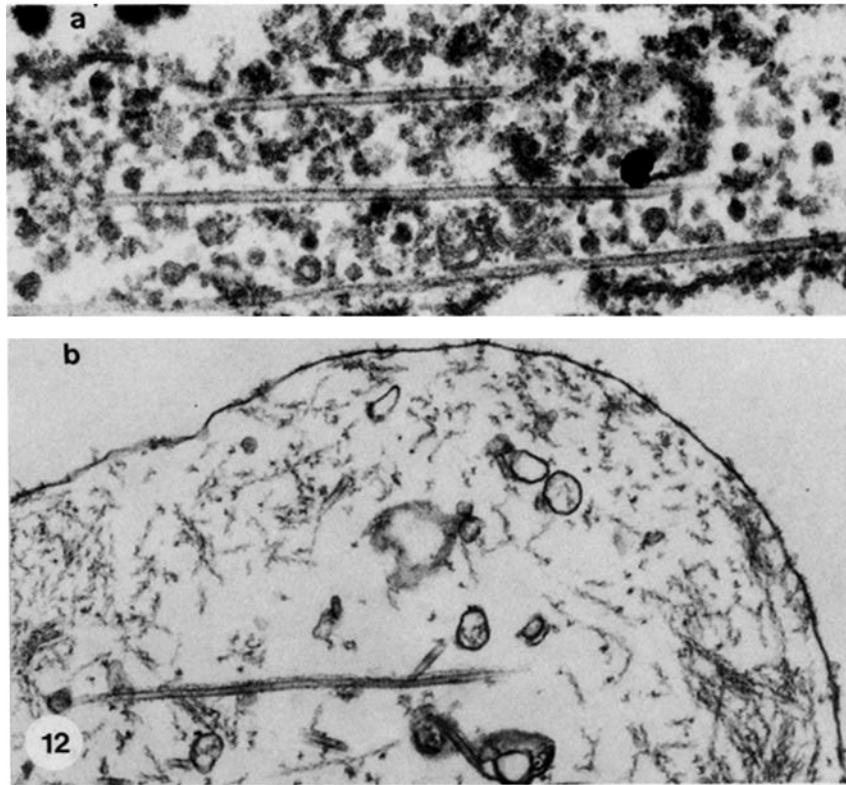


FIGURE 12 (a) Thin-section electron micrograph of lysed and stabilized filopodium demonstrating the maintenance of the close association between the thin filaments and the MTs. This particular filopodium was incubated with skeletal myosin, and the filaments remain undecorated.  $\times 83,300$ . (b) Human blood platelet lysed, stabilized, and incubated with S1. Note the plethora of decorated actin filaments.  $\times 45,100$ .

lular fiber systems. MFs (actin) occur at the growth cones as well as within the neurites (15, 34, 65), and neurofilaments (26, 53) are present in neurites in addition to the MTs. All these elements, or combinations of them, have been postulated to be the force transducers powering axoplasmic transport. *A. laticollaris* apparently possesses only two such fiber systems within its RPN: MTs and the 5-nm non-actin filaments. The similarity between the type of intracellular transport in *Allogromia* and that displayed by vertebrate neurons suggests that foraminiferans represent an excellent model system in which to study cytoplasmic transport. The lack of 10-nm filaments would appear to offer the significant advantage of simplicity in the analysis of this form of movement.

There is, however, a third component to the cytoskeleton of *Allogromia*, the helical-paracrystalline forms described originally by Hauser and Schwab (25). The interchangeability of these helical elements and MTs has been described previously (25), and McGee-Russell (42) noted the formation of paracrystalline material in the RPN of *Allogromia* strain NF (Lee) upon the administration of 0.75 M urea. This treatment causes the reversible destruction of the MT-based cytoskeleton (42) in a manner analogous to that of colchicine (59). Because of the extremely dynamic nature of the RPN, it is possible that these helical structures represent an intermediate assembly form of tubulin, or perhaps a storage form that may be easily transported to sites of rapid MT assembly.

Cytoplasmic granules appear closely associated with MTs in *Allogromia*, but 5-nm filaments are present as well. In *Allogromia*, fine filaments can be observed in the medial portions of the pseudopodia, where they lie parallel to and interdigitate with the MTs. At present, the possibility that these 5-nm

filaments are actin appears unlikely, because they are significantly thinner than actin (54) and fail to bind S1 under conditions that label platelet MFs. Thus, the close association between the 5-nm filaments and MTs appears not to represent an *in vivo* analogue of the MT-MF interactions described as occurring *in vitro* (24). Nevertheless, the occurrence of these unidentified fine filaments in such close proximity to the MTs, and their interdigitation, suggests that their interaction might play an important role in the motility of the RPN.

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