MOTILITY IN ECHINOSPHAERIUM NUCLEOFILUM

I. An Analysis of Particle Motions in the Axopodia and a Direct Test of the Involvement of the Axoneme

KENNETH T. EDDS

From the Department of Biological Sciences, State University of New York at Albany, Albany, New York 12222. Dr. Edds' present address is the Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT

The motion of particles in the axopodia of Echinosphaerium nucleofilum is saltatory. In the present study, photokymograph records of 123 motions from six axopodia have been analyzed. Particles followed rectilinear paths of from 1 to 15 μm while in continuous motion at an average velocity of 0.66 ± 0.32 μm/s. The velocity of the particles was variable in 36% of the cases measured. Some motions were punctuated by pauses either before continuing in the same direction or reversing. Frequently, several particles were moving at the same velocity, but neighboring particles showed no motion or moved in the opposite direction. Two particles occasionally contacted one another and traveled as a unit for varying lengths of time but subsequently moved independently. These motions reflect the underlying mechanism of motive force production. Furthermore, a glass microneedle can be substituted for the microtubular axoneme in the axopodia. In these artificial axopodia, bidirectional particle motions occurred which were similar to those in normal axopodia. Colchicine, at the threshold dose for axonemal dissolution, had no affect on these particle motions. It is concluded that the microtubular axoneme is not responsible for particle motions and also that individual microtubules are unlikely candidates for motive force production in this system.

The axopodia of Echinosphaerium nucleofilum are needle-like projections up to 300 μm in length that radiate from the cytosome and are supported longitudinally by two coiled sheets of cross-linked microtubules, the axoneme (5, 9, 12). In Echinosphaerium the prevalence of cytoplasmic microtubules has suggested a possible role for microtubules in intracellular motility. The majority of ultrastructural studies have shown microtubules to be the only linear element present in the cytoplasm. However, Hovasse (4) has reported "skins of microfibrils," as well as microtubules, to be present in the axopodia, an observation that more recent works have not confirmed. Thus, since alternatives are lacking, cytoplasmic motility has been attributed to the microtubules.

The axopodia constitute a convenient system for studying particle motions because their geometry confines the motions to the long axis of the axopodia. The one-dimensional motions are readily analyzed with a photokymograph.
ize these motions in the hope that the analysis would reveal aspects of saltatory motion not attainable from ultrastructural studies. Furthermore, the data provide a base-line for subsequent work in which the hypothesis that the axoneme is responsible for cytoplasmic motility is tested directly. Experiments were carried out to determine whether a glass needle could be used to form an artificial axopodium and, if so, whether particle motions, comparable to those in a normal axopodium, would occur.

MATERIALS AND METHODS

Cultures of *E. nucleofilum* were obtained from Carolina Biological Supply Co. (Burlington, N. C.) and cultured in Marshall's medium containing mixed ciliates as a food source.

Specimens were observed with a Zeiss Photomicroscope II equipped with Nomarski differential interference optics. A planachromat 100/1.25 oil immersion objective was used at a working aperture of ca. 1.0, producing an optical section thickness considerably below 1 μm.

Motions of particles were recorded on 16-mm cine film with a camera driven by a synchronous motor at 8 frames/s.

The cine films were projected with an L-W Photo Optical data analyzer (L-W Photo Inc., Van Nuys, Calif.) so that the particle motions of interest were displayed on the slit of a Waters photokymograph (waters Instruments, Inc., Rochester, Minn.). In this device, the image of a particle is focused on a slit, and a sheet of photographic film is drawn past the slit at a known and uniform velocity. Thus, a continuous record is produced of the distance a particle travels as a function of time. The velocity of a particle in an axopodium can be determined from the slope on the photokymograph record. Zero slope indicated that the particle was stationary; positive slopes, in the following photokymograph records, represent velocities of particles moving toward the axopodial tip, while negative slopes indicate motions toward the cytosome. Velocities were judged variable if the slope on the photokymograph record deviated from a straight line by more than the particle width while in continuous motion i.e., between direction changes or stationary periods.

Lengths of particle motions were measured as the distances moved between direction reversals or periods of no motion.

The photokymograph records followed particles for 30-60 s. Normal axopodia that were in contact with the substratum were chosen for analysis because they remained in a single plane of focus for a sufficient time to allow filming.

The glass needles were mounted in a small piece of modeling clay and so positioned on the stationary microscope stage that the needle could be slipped between the slide and coverslip. The needle was prepositioned and held stationary, while the slide and organism were moved relative to the needle with the mechanical stage.

Colchicine was obtained from Calbiochem (San Diego, Calif.). All experiments utilizing colchicine were recorded with green light to minimize photolysis to lumicolchicine.

RESULTS

Movements in Axopodia

The moving particles in the axopodia of *E. nucleofilum* are dense granules and mitochondria (12) and were seen with Nomarski differential interference optics as spheres (ca. 0.5 μm in diameter) or ellipsoids (ca. 1.5 μm long and 0.5 μm wide). As might be expected from the gradual taper of the axopodia, there were more particles per unit length near the axopodial base than at its tip (Fig. 1).

The motion of particles is both rectilinear and bidirectional. In a particular region, several particles appeared to be moving at the same velocity, giving the impression of bulk flow; however, neighboring particles, during the same period, showed no movement or proceeded in the opposite direction (e.g., Fig. 2, between 5 and 10 s).

The motion of any particle resulting in a net displacement included either: a continuous rectilinear motion at constant velocity (Fig. 2, particle D from 1 to 10 s) or varying velocity (Fig. 2, particle E), a series of successive motions in the same direction separated by periods of no motion (Fig. 3, particle B), or a series of motions punctuated by direction reversals, sometimes pausing before reversing (Fig. 2, particle B).

Particles frequently approached one another, made contact and travelled as a unit, i.e., in tandem, for a variable period of time, whereupon each acted independently. These "strings of particles" underwent changes in velocity and direction together (Fig. 3).

Analysis of Motions in Axopodia

Particle motions occurring throughout the most distal half of different axopodia were analyzed. This region was selected because it remained in a single plane of focus for a sufficient period of time to allow filming.

Analysis of the velocities of 123 motions of 85 particles revealed two categories: those with con-
FIGURE 1  A photomicrograph illustrating a normal axopodium. The membranes and cytoplasm of the axopodium and cortical surface layer are continuous. Note the small knobs of cytoplasm (arrows). Particle motions were apparent in these knobs which contained no microtubules. × 1,800.

stant velocity (64%) and those with variable velocity (36%). A histogram of velocities of all movements showed that the range was from 0.2 to 2.0 μm/s, the average velocity of all motions was 0.66 ± 0.32 μm/s (± 1 SD). The average velocity of all inward motions was 0.75 ± 0.30 μm/s, while that for outwardly moving particles was 0.57 ± 0.29 μm/s (Fig. 4).

The duration of the movement of inwardly directed particles averaged 3.9 ± 2.6 s and that of the outwardly directed motions averaged 4.3 ± 3.1 s.

The average distance travelled in one continuous motion was 3.1 ± 2.5 μm for inward motions and 2.4 ± 1.7 μm for outwardly moving particles (Fig. 5). Most of the distances (80%) were between 1–5 μm in length; however, continuous motions up to 15 μm were observed and motions up to 50 μm have been reported (3, 13).

The relationship between distance travelled and the velocity of motions was analyzed. When the average velocity of each motion was plotted as a function of the distance travelled and a linear regression analysis performed on those data, a line was generated whose slope was significantly ($P < 0.001$) different from zero. This indicates that there was a tendency for faster moving particles to travel farther (Fig. 6).

**Movements in Other Structures**

The cytoplasm of an axopodium is continuous with that of the cortical surface layer (Fig. 1), and particles move back and forth between these two regions. The velocities of particles in the cortex are in the same range as the velocities reported here for axopodial particles; however, the average velocity is generally slower in the cortex (3, 13). Furthermore, an exception to the rectilinear nature of particle motions was the finding that the particles moving from the axopodia into the cortex rarely followed the projection of the axoneme into the cortex, but rather moved, while remaining in a single plane of focus, into the cortical cytoplasm along a curvilinear path parallel to the membrane.

The cytoplasm of the cortical layer or the axopodia formed sheetlike pseudopodia (pharopodia). In this structure, some particles exhibited saltatory motions oriented radially to its geometric center, and others underwent saltation seemingly at random. Several particles partially circumscribed the periphery of the pharopodium apparently while in contact with the membrane (Fig. 7). This was the second situation of curvilinear motion parallel to a membrane.

Small knobs of cytoplasm arose apparently at random from the cortex and the axopodia (Fig. 1). Particles were seen to move in and out of these structures; no microtubules are seen in thin sections of the knobs (10).

**Artificial Axopodia**

It is possible to push a glass needle through the cytosome and into the cortical region of the opposite side, thus raising the membrane and cortical cytoplasm from underneath into the form of an artificial axopodium (Figs. 8 and 9). Since artificial axopodia can be formed from any point...
FIGURE 2  A photokymograph record of motions of particles in the axopodia. See text.

FIGURE 3  A photokymograph record showing three particles which approach one another, contact, and then move as a unit. See text.
on the surface of the cytosome, all portions of the cortical surface are equally extensible.

As the glass needle was withdrawn, the artificial axopodium simultaneously decreased in length, indicating that no other structural support remained. In contrast, normal axopodia could be extended beyond their existing length with a glass needle, but when the needle was withdrawn the axopodia shortened to essentially the same length as before insertion of the glass needle (Fig. 10). Therefore, the axoneme appears to support these axopodia up to the original termination.

Particle motions were apparent in the artificial axopodia. The motions along the entire length were analyzed with a photokymograph (Fig. 11) and were found to be similar to the motions occurring in normal axopodia. The velocity of motions averaged $0.65 \pm 0.21 \mu m/s$, regardless of direction. The average length of one continuous motion was 2.4 $\mu m$ for inward motions and 2.6 $\mu m$ for outward motions. There were no continuous motions observed for distances greater than 5 $\mu m$.

The cytoplasm extended by a glass needle did not always retain the shape imposed by the needle. Occasionally, the cytoplasm that was extended into an artificial axopodium would show increased local motility which resulted in a small pseudopodium that was perpendicular to the long axis of the glass needle. Further extension of the cytoplasm with the needle stretched this pseudopodium into a thinner layer of cytoplasm.

It was also possible to pull a sheet of cytoplasm away from the surface of the cytosome with a glass needle.

![Figure 4](image1.png)  
**Figure 4** A histogram of average velocities of particles in the axopodia.

![Figure 5](image2.png)  
**Figure 5** A histogram of the distances travelled by the particles in the axopodia while in continuous motion.

KENNETH T. EDDS  *Motility in Echinospaerium. I* 149
FIGURE 6 A plot of the average velocity and one standard deviation of particles as a function of distance travelled. A line was generated from the data points by linear regression analysis. The slope is significantly \( P < 0.001 \) different from zero, indicating a trend for faster moving particles to travel farther. \( N \), number of particles.

FIGURE 7 A photograph made from two frames of a 16-mm cine film of a pharopodium formed approximately halfway along an axopodium. Note that the particle (arrow) has moved several microns in 1 s. Marker, 10 \( \mu \text{m} \). \( \times 1,000 \).

DISCUSSION

Particle Motion Analysis

The majority of particle motions in the axopodia of *Echinosphaerium* fit all previously described criteria for saltatory motion (7); however, in some instances there was one variation from those criteria.

This difference was the variability of velocity during some motions of the particles. This variability reflects the true nature of the particle motions and appears to have been overlooked in previous studies. Although Fitzharris et al. (3), using frame by frame analysis, reported that the velocity of one particle in an axopodium was more or less uniform during a 50-\( \mu \text{m} \) excursion, careful examination of their data reveals a variability in the velocity that is similar to that observed in this study. Berlinrood et al. (1), using the same method as described here, have shown that in nerve fibers there was a variation in the velocity of some saltations (see Fig. 6 of reference 1).

The variation in velocity may be explained by postulating either that the motive force applied to or by the particle is changing, or that the viscous drag exerted on the particle is locally variable within the axopodia and the motive force is small enough to be affected by the variations in viscosity.
It is interesting to speculate that saltatory motion is the result of a localized translation in a set of linear elements that produces motion in all particles associated with those linear elements that move. If the linear elements are free to slide past one another, a particle associated with the elements would proceed at a uniform velocity. If, on the other hand, there is some degree of ephemeral association between neighboring linear elements, then the particles would show a variable velocity because of the variations in viscous drag thus produced.

A comparison of average velocities for both directions (IN vs. OUT) in the data pooled from six axopodia indicated a slightly higher average velocity for particles moving toward the cytosome (Fig. 4). In the analysis of the motions in individual axopodia, the average velocity of inwardly directed particles was not consistently higher than that of outwardly directed particles, i.e., in some axopodia the average velocity of particles was the same in both directions. Indeed, if there were a continuous preferential inward transport the distal portions of the axopodia would ultimately lose all particles. This was never observed.

When the average velocity of each saltation was compared to the distance travelled in a linear regression analysis, the resulting equation of the line had a slope that indicated a significantly ($P < 0.001$) higher velocity for motions of longer dis-
An artificial axopodium formed by inserting a glass needle through the cytosome and underneath the cortex of the opposite side. \( \times 1,900 \).

It is evident from these experiments that the membrane and cortical cytoplasm in *Echinospaerium* are easily deformable with a glass needle (Figs. 9 and 12). Particle motions similar to those in a normal axopodium were apparent within these mechanically formed axopodia. When the glass needle was withdrawn, the axopodia simultaneously decreased in length because there were no structures present to support the cytoplasm. Furthermore, extension of an ax-

**Artificial Axopodia**

It is evident from these experiments that the membrane and cortical cytoplasm in *Echinospaerium* are easily deformable with a glass needle (Figs. 9 and 12). Particle motions similar to those in a normal axopodium were apparent within these mechanically formed axopodia. When the glass needle was withdrawn, the axopodia simultaneously decreased in length because there were no structures present to support the cytoplasm. Furthermore, extension of an ax-

**Artificial Axopodia**

It is evident from these experiments that the membrane and cortical cytoplasm in *Echinospaerium* are easily deformable with a glass needle (Figs. 9 and 12). Particle motions similar to those in a normal axopodium were apparent within these mechanically formed axopodia. When the glass needle was withdrawn, the axopodia simultaneously decreased in length because there were no structures present to support the cytoplasm. Furthermore, extension of an axopodium was facilitated by the deformability of the membrane and cortical cytoplasm.
FIGURE 10 A glass needle can be inserted into a normal axopodium. When extended beyond its existing length with a needle followed by withdrawal of the needle (Fig. 10) yielded the normal axopodium at essentially its original length. This work confirms the numerous studies of Tilney and coworkers (see reference 11) which demonstrated that an axoneme was present within every axopodium and was necessary for the maintenance and growth of this structure.

The particles in the artificial axopodia exhibited bidirectional saltatory motions within the cytoplasm (Fig. 11). The average particle velocity was $0.65 \pm 0.21 \mu m/s$, regardless of direction, and was indistinguishable from the value obtained for normal axopodia ($0.66 \pm 0.32 \mu m/s$).

The length of motion was, on the average, similar to those in normal axopodia ($2.5 \mu m$ vs. $2.7 \mu m$ in normal axopodia). The fact that motions for distances longer than ca. $5 \mu m$ were not observed in artificial axopodia may be characteristic of the relatively short artificial structures ($50 \mu m$ vs. $300 \mu m$ in normal axopodia) or it may be a manifestation of the absence of tracks possibly formed by microtubules that are present in normal axopodia. This interpretation is supported by the fact that the disappearance of microtubules in tissue cells after treatment with colchicine results in a substantial reduction of the average path length of saltations to $2-3 \mu m$, but does not abolish these motions (reference 8, discussed in reference 7).

Several workers (5, 9, 12) have seen microtubules that are peripheral to the main axonemal bundle in the axopodia, and thus it might be argued that some single microtubules might have been forced up into the artificial axopodia or the cortical layer during the formation of an artificial axopodium, and that these microtubules may be involved in producing the motion of particles. Therefore, the obvious investigation of thin sectioning an artificial axopodium was attempted, but it has not been possible yet to preserve the structure for thin sectioning during the fixation procedures of either Tilney and Porter (12) or Roth et al. (9).

Nonetheless, two other lines of evidence support the view that individual microtubules are not directly involved in producing the motive force for particle motions in *Echinosphaerium*. First, particle motions continued unaffected when the artificial axopodia were bathed in $2.5 \times 10^{-3} M$ colchicine. This is the threshold dose used to cause dissolution of the axoneme (10). A concentration of $2.5 \times 10^{-2} M$ is more effective; however, this
FIGURE 11 A photokymograph record of particle motions in an artificial axopodium showing that normal bidirectional particle motions occur.

ARTIFICIAL AXOPODIUM

FIGURE 12 A pharopodial sheet can also be drawn away from the cortical surface with a glass needle. Normal particle motions were apparent in this structure. × 900. Marker, 10 μm.

higher concentration had an adverse affect on the membrane and caused blebbing in normal axopodia and membrane rupture in the artificial axopodia. Thus, in concentrations of colchicine higher than $2.5 \times 10^{-3}$ M it was not possible to form or maintain an artificial axopodium. The effect on the membrane, while noteworthy, is not understood. Tilney (10) has reported that treatment with ca. $10^{-2}$ M colchicine, while affecting the dissolution of the axoneme, had no effect on particle activity, i.e., particles continued to undergo saltation in both directions while the axopodia were shortening; however, the net movement of particles was inward as a result of axopodial shortening.

Secondly, particles move in and out of the small
knobs of cytoplasm that arise from the cortical surface layer and axopodia, but these contain no microtubules when examined in the electron microscope (10). Tilney thus reported two types of cytoplasmic motion in *Echinosphaerium*, one of which appears to be independent of microtubules.

On the basis of the experiments reported here and the above arguments, it is proposed that the microtubules in normal axopodia orient a system of contractile proteins in the cytoplasm, while acting only as a structural support for the axopodia.

In conclusion, it has been shown that the microtubular axoneme is not responsible for producing particle motions. It also appears that individual microtubules are unlikely candidates for motive force production in this system. Regardless of what produces the motion of particles, the analysis of motions also provided here may offer some insight for the formulation of a mechanism that will explain how these motions occur.

This work was supported by grant GM 18854 from the National Institute of General Medical Sciences to Dr. R. D. Allen.

Portions of this work were presented at the 12th Annual Meeting of the American Society for Cell Biology, St. Louis, Mo., 1972 and at the 13th Annual Meeting of The American Society for Cell Biology, Miami, Fla., 1973.

Received for publication 18 December 1974, and in revised form 12 March 1975.

REFERENCES

1. BERLINROOD, M., S. M. McGEE-RUSSELL, and R. D. ALLEN. 1972. Patterns of particle movement in nerve fibers in vitro; an analysis by photokymography and microscopy. *J. Cell. Sci.* 77 11:875–886.

2. COMLY, L. 1973. Microfilaments in *Chaos carolinensis*. Membrane association, distribution and heavy meromyosin binding in the glycerinated cell. *J. Cell Biol.* 58:230–237.

3. FITZHARRIS, T., R. A. BLOODGOOD, and J. R. McINTOSH. 1972. Particle movement in the axopodia of *Echinosphaerium*: evidence concerning the role of the axoneme. *J. Mechanochem. Cell Motility.* 1:117–124.

4. HOVASSE, R. 1965. Ultrastructure comparée des axopodes chez deux Heliozoaires des genres *Actinosphaerium* et *Raphidiophys*. *Protistologica.* 1:81–88.

5. MACDONALD, A. C., and J. A. KITCHING. 1967. Axopodial filaments of heliozoa. *Nature (Lond.).* 215:99–100.

6. POLLARD, T. D., and E. D. KORN. 1973. Electron microscopic identification of actin associated with isolated amoeba plasma membranes. *J. Biol. Chem.* 248:448–450.

7. REBHUN, L. 1972. Polarized intracellular particle transport: saltatory movements and cytoplasmic streaming. *Int. Rev. Cytol.* 32:93–137.

8. ROISEN, R. 1969. Movement of cells and intracellular particles in tissue culture: the effects of colchicine. Ph.D. Thesis. Princeton University, Princeton, N. J.

9. ROTH, L. E., D. J. PhILAJA, and Y. SHIGENAKA. 1970. Microtubules in the heliozoan axopodium. I. The gradion hypothesis of allosterism in structural proteins. *J. Ultrastruct. Res.* 30:7–37.

10. TILNEY, L. G. 1968. Studies on the microtubules in Heliozoa. IV. The effect of colchicine on the formation and maintenance of the axopodia and the redevelopment of pattern in *Actinosphaerium nucleofilum* (Barrett). *J. Cell Sci.* 3:549–562.

11. TILNEY, L. G. 1971. Origin and continuity of microtubules. In *The Origin and Continuity of Cell Organelles.* J. Reinert and H. Ursprung, editors. Springer-Verlag Inc., New York.

12. 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. *Protozoa.* 50:317–344.

13. WATTERS, C. 1968. Studies on the motility of the heliozoa. I. The locomotion of *Actinosphaerium* and *Actinophrys*. *J. Cell Sci.* 3:231–244.