

Bidirectional Organelle Transport Can Occur in Cell Processes That Contain Single Microtubules

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ABSTRACT Intracellular organelle transport was studied in a new model system, the giant freshwater amoeba *Reticulomyxa*. The amoeba extends a large reticulate network of cytoplasmic strands in which various phase-dense organelles can be seen to move at a rate of up to 25 $\mu\text{m/s}$. This combined light and high voltage electron microscopic study shows that organelles move bidirectionally in even the finest network strands that contain only a single microtubule. In terms of microtubule-associated intracellular transport, this observation defines a minimum set of conditions necessary for such movement. The implications of this finding for possible models of force generation are discussed.

In addition to their role in mitosis and ciliary movement, microtubules are regarded as elements along which intracellular organelles are transported (reviewed in references 1, 2). Ultrastructural studies have correlated cytoplasmic organelle movement with the presence of microtubules in neurons (3, 4), pigment cells (5, 6), protozoan systems (7, 8), and several other cell types (9, 10). The dependence of some forms of intracellular movement upon an intact system of microtubules (2, 5, 11) further demonstrates that microtubule-organelle associations are more than just coincidental. Furthermore, the increasing likelihood that cytoplasmic dynein-like molecules exist (12, 13) suggests that microtubules might even be directly involved in providing the motive force for organelle translocation. Previous studies have shown that bidirectional movement of organelles can take place in the presence of microtubule arrays in which the vast majority of microtubules are of uniform polarity (14–16). Using a unique new model system in a combined light and electron microscopic study, we show here that bidirectional movement of intracellular organelles can occur in processes containing single microtubules, thus representing the least complex “minimum model” able to support bidirectional movement. The nature of the microtubule-organelle interactions and the force producing motor are currently under investigation.

MATERIALS AND METHODS

The Amoeba: A giant multinucleated freshwater amoeba closely resembling the myxamoeba *Reticulomyxa filosa* (17) was used in this study. It was discovered growing on the sides and floor of a freshwater aquarium. The naked cell body of this organism consists of an interconnected system of thick tubes (100 μm or more in diameter) that under optimal conditions can cover an area of several square centimeters. The organism can easily be kept in petri dishes filled with tap water and supplied three times a week with flaked tropical fish food. Better defined living conditions, life cycles, food uptake, etc., are currently being investigated.

Light Microscopy: Pieces of this amoeba were allowed to settle and spread on Formvar-covered electron microscope grids mounted on coverslips. The amoeba was viewed through the coverslip with a 100X Zeiss Plan objective on a Zeiss Standard microscope. The phase-contrast image was projected onto a video monitor with a Dage MTI 65 videocamera (Dage-MTI Inc., Michigan City, IN) and simultaneously recorded at real time speed with a NEC videorecorder. Photographs were taken from the video monitor, using a Ronchi Ruling slide (Edmund Scientific Co., Barrington, NJ) to reduce the video lines of the monitor, with Ilford Pan F film and developed in Microphen (Ilford).

Electron Microscopy: For high voltage preparations, the amoebae were fixed directly on the light microscope with 1% glutaraldehyde in a dilute microtubule stabilization buffer of 6 mM PIPES, 2.5 mM HEPES, 2 mM EGTA, and 0.2 mM MgCl_2 , pH 7.0 (10% PHEM [18]), then transferred to petri dishes containing fixative for at least 10 min. The amoebae were postfixated with 0.4% OsO_4 (4 min), treated with 0.2% tannic acid (1 min), dehydrated in a graded series of ethanol, critical-point-dried with CO_2 , and coated with a thin layer of carbon. The preparations were viewed in a Kratos high voltage electron microscope (National Center for Electron Microscopy, Lawrence Berkeley Laboratories) operated at 1,500 kV accelerating voltage. Specimens prepared for transmission electron microscopy were processed in petri dishes as above except that they were embedded in Epon following dehydration. Thin sections were stained with uranyl acetate and lead citrate and then examined in a Siemens 102 microscope operated at 80 kV.

RESULTS

The organism used in this study, a giant amoeba closely resembling *Reticulomyxa*, has a large central protoplasmic area surrounded by an extensive (a few centimeters in length) reticulopodial network similar to that of the marine foraminifer *Allogromia* (7, 8). This network is composed of a highly dynamic, extensively branched, system of membrane-bound filamentous strands (Fig. 1). The entire organism can translocate several centimeters per day, often leaving behind a soft “shell” probably consisting of, or containing, waste material. Preliminary antibody staining suggests the presence of both actin- and tubulin-containing structures within the extended networks. A more complete account of this new model system will be given later.

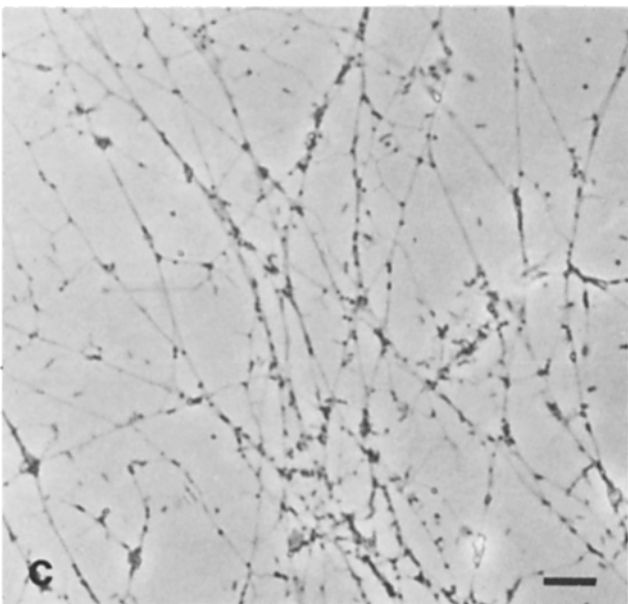
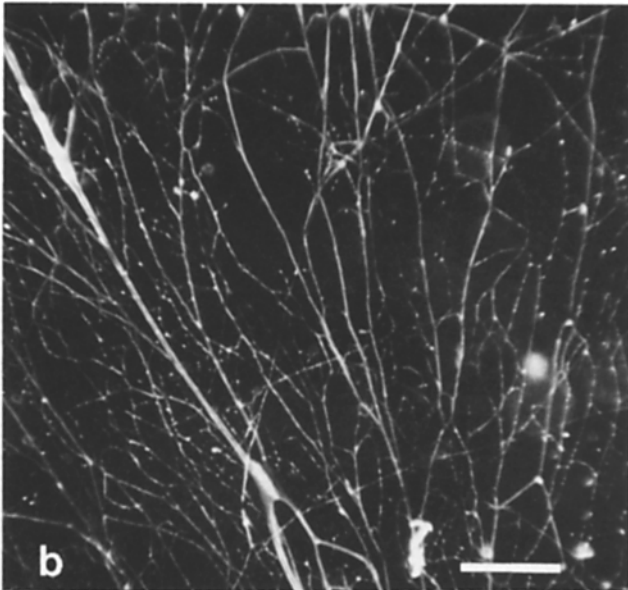
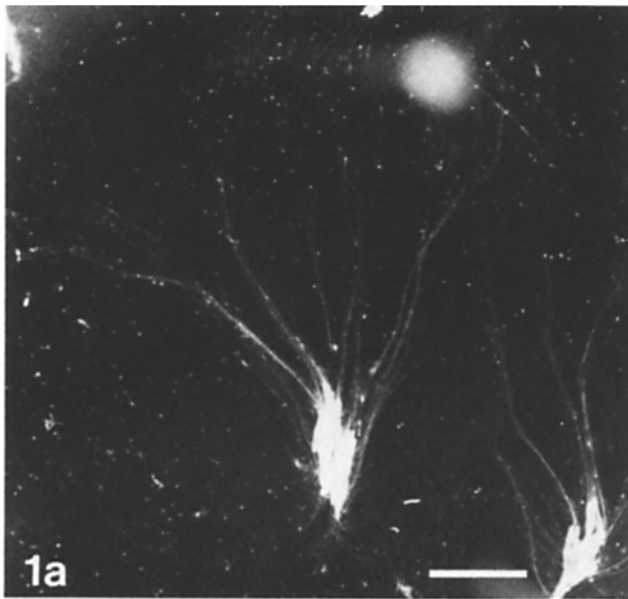


FIGURE 2 Electron micrograph of a sectioned strand demonstrating the complete membrane enclosure of transported organelles within a strand containing a few microtubules. No organelles have yet been observed attached to the outside of a strand. Bar, 100 nm. $\times 51,000$.

Very rapid (15–25 $\mu\text{m/s}$) bidirectional streaming of many small phase-dense organelles or organelle aggregates in a saltatory fashion is the most conspicuous feature of this ameba. Although at least three distinct classes of organelles can be readily seen, this study is concerned with the movement of just the predominant class of organelles with a diameter of 0.25–0.40 μm . Because of their affinity for the laser dye rhodamine 123, a mitochondrial specific stain (19), and the presence of double membranes and cristae-like structures visible in some thin sections, these organelles are tentatively identified as mitochondria. In wide, flattened portions of the strands, longitudinal streaming occurs as if these organelles follow one another along discrete tracks, with many apparent tracks per strand. Lateral organelle movement perpendicular to the main flow is never seen. Even in the very finest strands (diameter ~ 100 nm), organelle movement also occurs in both directions. Frequently, two organelles in the same fine strand can be seen to move simultaneously in opposite directions. Organelles often are larger than the strand diameter and strands locally “expand” to accommodate their passage. These organelles are transported completely within the strands (Fig. 2). Immunofluorescence microscopy, thin section and whole-mount electron microscopy show microtubules to extend parallel to the long axes of the strands. There appears to be a positive correlation between the diameter of a strand and the number of microtubules it contains. Very fine strands contain only one to three microtubules. We have yet to find a strand without microtubules.

Organelle movement along the finest strands of the ameba’s network was recorded on videotape at high magnification in

FIGURE 1 Overview of the *Reticulomyxa*-like ameba. (a) Photograph of an entire organism (with part of another) attached to an aquarium wall. Note the large white protoplasmic central mass and the extensive thin network. Bar, 5 mm. $\times 3.5$. (b) A dark-field image of the network from a different organism at a slightly higher magnification. Bar, 1.0 mm. $\times 13$. A detached ameba (or a piece of it) could be seen to extend such a network in <1 h. (c) Phase-contrast image showing the intricacy of the finer branches of the network. Note the abundance of “particles” within the strands. Bar, 10 μm . $\times 675$.

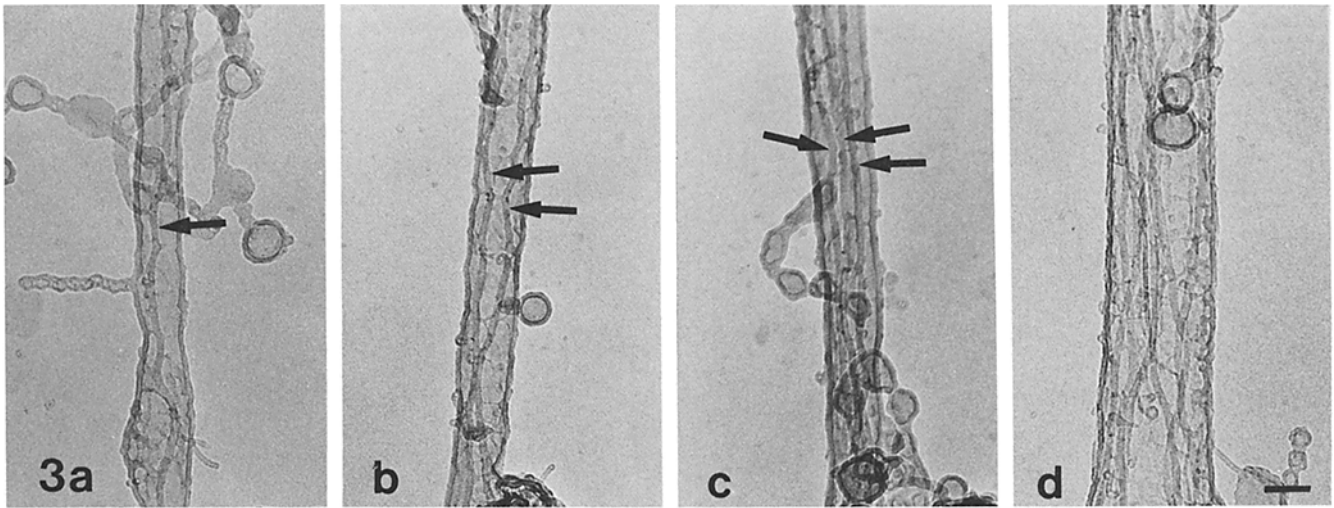


FIGURE 3 High voltage electron micrographs of whole mount preparations showing strands containing one microtubule (a), two microtubules (b), three microtubules (c), and at least six microtubules (d). This series demonstrates the relative ease with which strands containing one microtubule are distinguished from those containing two or more. Stereo micrographs of all strands were taken and examined. Stereo pairs were not included here because (at least in the very thin strands) they did not yield significant new information. Bar, 100 nm. $\times 62,000$.

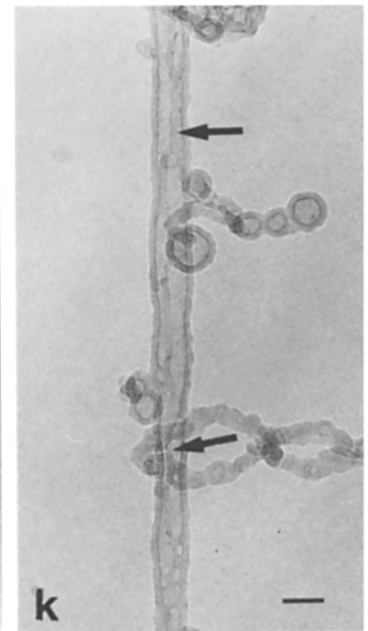
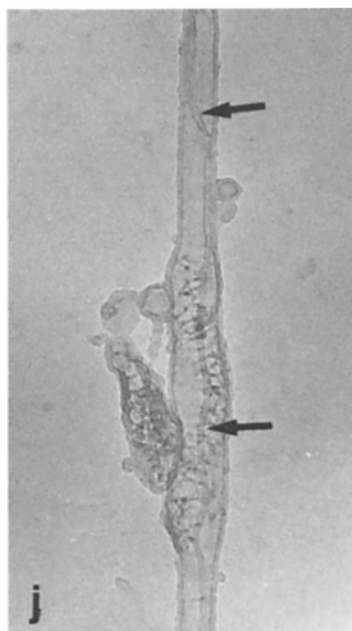
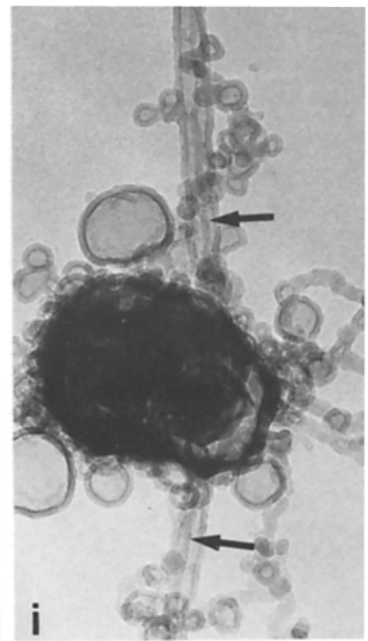
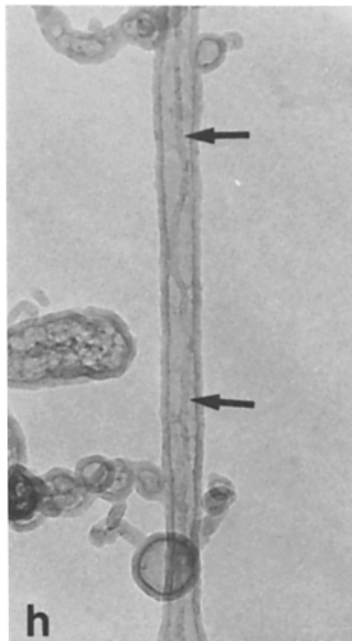
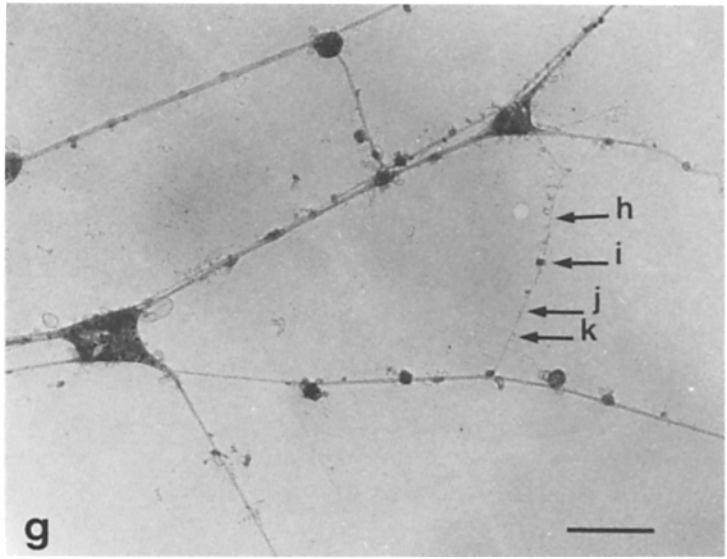
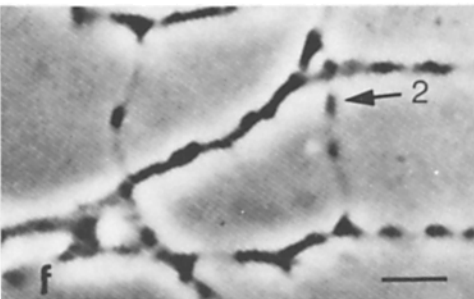
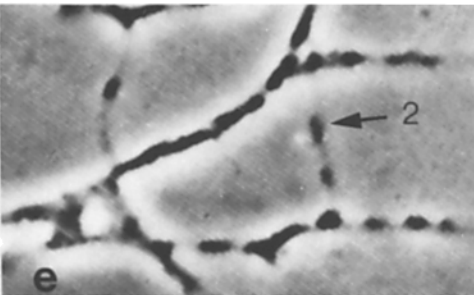
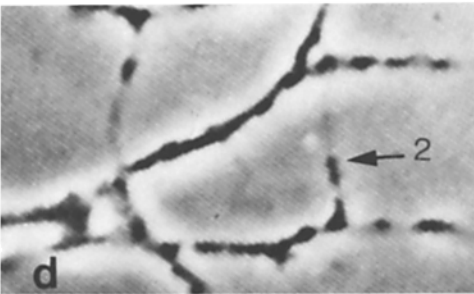
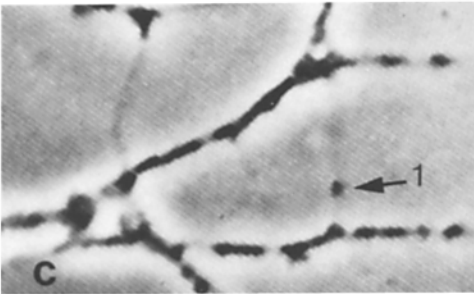
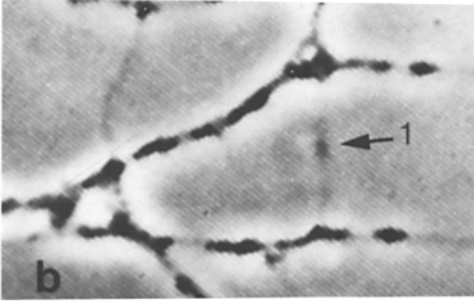
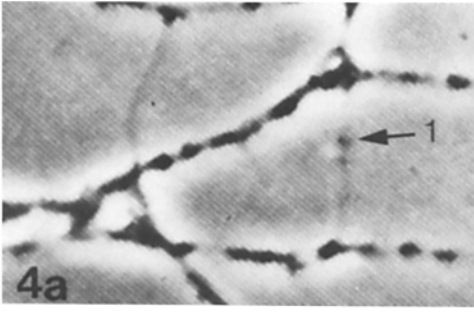
a phase-contrast microscope. After establishing that bidirectional motility did occur along one of these fine strands, preparations were fixed immediately and processed for whole mount electron microscopy. Granular elements and microtubules could easily be identified within the networks upon examination in a high voltage electron microscope. Processes containing single microtubules, which are quite abundant, were easily distinguished from those containing two or more (Fig. 3). In at least eight separate preparations, bidirectional organelle movement was observed along strands which, by electron microscopy, could be shown to contain single microtubules (Fig. 4). The entire length of a strand was inspected to assure only a single microtubule was present. In a few instances, we found part of a strand exhibiting bidirectional movement to contain two microtubules, while the remainder of the strand contained only one.

DISCUSSION

Using a new model system, the giant amoeba *Reticulomyxa*, to study organelle movements along microtubules, we show conclusively that bidirectional organelle transport can occur in close association with a single microtubule. This observation is in agreement with previous findings which suggested that the intrinsic polarity of microtubules is not relevant for bidirectional movement in cilia, heliozoan axopodia, fish melanophores (14), and neurons (15, 16); it extends the findings of Hayden and Allen (20) and others (21, 22), who observed unidirectional organelle movement along linear elements containing one microtubule and bidirectional move-

ment along thin networks of "filaments." Therefore, the factor(s) that provide directionality for movement are extrinsic to the microtubule proper. Though our observations do not solve the question of how transport along microtubules is brought about, their significance lies in the fact that they provide a conceptual framework for considerations of the mechanism and define a minimum set of conditions that have to be fulfilled by future models. In terms of a model that involves cytoplasmic dynein-like ATPases attached to either the microtubule or the surface of the transported organelle, at least two possibilities must be considered: (a) unlike ciliary dyneins, the cytoplasmic dynein-like ATPase is "promiscuous" and can exert a driving force in each direction; and (b) alternatively, two different dynein ATPases coexist on the same organelle, one specific for either direction. On the other hand, models proposing a cooperative interaction between a contractile (possibly actomyosin) component and microtubules in the development of the motive force for organelle movement are intrinsically independent of the polarity of microtubules and depend solely on the correct orientation of the polar actin filaments (see, for example, reference 23). The finding of congruence between tubulin and actin staining in the filopodial networks of *Reticulomyxa* and the observation of thin filaments oriented parallel to microtubules (unpublished observations) are in agreement with the latter hypothesis and indirectly support it. While as yet no experimental evidence is available to distinguish between the various possibilities, we feel that this cell model is amenable to various experimental approaches that may further our understanding of the underlying mechanisms.

FIGURE 4 An example of bidirectional particle movement in a single strand containing a single microtubule. (a-f) Video-recorded sequence demonstrating particle movement in one of the very small strands. Particle one (a-c) joins a smaller particle and both proceed down the strand. Particle two (d-f) is followed by several others in an upward movement across the strand. This entire sequence (a-f) encompassed six seconds. Bar, 5 μm . $\times 1,800$. (g) Low magnification whole mount electron micrograph of the same area videotaped in (a-f) Bar, 5 μm . $\times 2,400$. The letters h, i, j, and k refer to corresponding areas of the following four high magnification micrographs of the strand exhibiting bidirectional particle movement, and demonstrate the presence of only a single microtubule (arrows). Bar, 100 nm. $\times 62,000$.



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Note Added in Proof: After submission of this manuscript, bidirectional organelle transport along single microtubules was reported by two other laboratories (25, 26).

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