

Research Campaign. We thank Drs J. Creanor, P. A. Fantes and R. A. Gray and Mr A. B. Sanderson for assistance in some experiments and for helpful discussions.

Received 21 April; accepted 4 June 1986.

1. Mitchison, J. M. & Nurse, P. *J. Cell Sci.* **75**, 357-376 (1985).
2. Yoneda, M., Ikeda, M. & Washitani, S. *Dev. Growth Differentiation* **20**, 329-336 (1978).
3. Newport, J. W. & Kirschner, M. W. *Cell* **37**, 731-742 (1984).
4. Horisberger, M., Vonlanthen, M. & Rosset, J. *Archs Mikrobiol.* **119**, 107-111 (1978).
5. Nurse, P., Thuriaux, P. & Nasmyth, K. *Molec. gen. Genet.* **146**, 167-178 (1976).
6. Benitez, T., Nurse, P. & Mitchison, J. M. *J. Cell Sci.* **46**, 399-431 (1980).
7. Creanor, J. & Mitchison, J. M. *J. Cell Sci.* **69**, 199-210 (1984).
8. Elliott, S. G. *Molec. gen. Genet.* **192**, 212-217 (1983).
9. Creanor, J. & Mitchison, J. M. *J. Cell Sci.* **58**, 263-285 (1982).

Propulsion of organelles isolated from *Acanthamoeba* along actin filaments by myosin-I

Richard J. Adams & Thomas D. Pollard

Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205, USA

Eukaryotic cells are dependent on their ability to translocate membraneous elements about the cytoplasm. In many cells long translocations of organelles are associated with microtubules¹⁻³. In other cases, such as the rapid cytoplasmic streaming in some algae, organelles appear to be propelled along actin filaments⁴. It has been assumed, but not proven, that myosin produces these movements. We have tested vesicles from another eukaryotic cell for their ability to move on the exposed actin bundles of *Nitella*⁵ as an indication that actin-based organelle movements may be a general property of cells. We found that organelles from *Acanthamoeba castellanii* can move along *Nitella* actin filaments. Here, we report two different experiments indicating that the single-headed non-polymerizable myosin isozyme myosin-I (ref. 6) is responsible for this organelle motility. First, monoclonal antibodies to myosin-I inhibit movement, but antibodies that inhibit double-headed myosin-II do not. Second, ~20% of the myosin-I in homogenates co-migrates with motile vesicles during Percoll density-gradient ultracentrifugation. This is the first indication of a role for myosin-I within the cell and supports the suggestion of Albanesi *et al.*⁷ that myosin-I moves vesicles in this way.

We saw directed motion of vesicles and aggregates of vesicles from *Acanthamoeba* on the substrate of *Nitella* actin bundles (Fig. 1). Movements were in the same direction as those of endogeneous algal organelles but substantially slower (Fig. 2b),

the average rates of movement being $0.24 \pm 0.11 \mu\text{m s}^{-1}$ (mean \pm s.d., $n = 50$) compared with $\geq 40 \mu\text{m s}^{-1}$ for endogeneous movements⁸. The distribution of rates of movement for *Acanthamoeba* vesicles was wide (Fig. 2b) and varied from batch to batch. The vesicles in some preparations were never seen to move; the cause of this variation is unknown. Further, in any one extract, not all of the vesicles that settle onto the substrate were seen to move. We could follow moving vesicles for tens or hundreds of micrometres; the data in Fig. 2 represent the average rate measured over a distance of 20-140 μm . Rates of motion were relatively constant over these distances without the saltations or interruptions characteristic of organelle movements within live cells. Most of the movements that ceased in the field of view appeared to result either from damage in the substrate or from steric interference. We are confident that the movements of *Acanthamoeba* vesicles is independent of those of *Nitella* organelles for several reasons: no endogeneous organelles are seen to move with this speed and all that show any signs of movement are lost from the preparation soon after dissection. (Experiments were not performed on dissected *Nitella* preparations while endogeneous organelles were still moving.) To check this, two preparations of dissected *Nitella* were incubated in 5 mM *N*-ethylmaleimide for 5 min to inhibit the activity of the endogeneous myosin species⁹. After treatment with 7 mM dithiothreitol to neutralize the *N*-ethylmaleimide, no endogeneous organelles moved but organelles in *Acanthamoeba* extracts moved normally. The enzyme responsible for their motion would, therefore, appear to be associated with the vesicles themselves.

Acanthamoeba contains two distinct species of myosin^{6,10,11} which are candidates for the motor driving of these vesicles along the actin bundles of the *Nitella* cortex. The two species differ, both physically and enzymatically: myosin-I is enzymatically highly active⁶ but, unlike *Acanthamoeba* myosin-II and other known myosins, it consists of only one heavy chain which lacks the long α -helical tail required for polymerization into thick filaments^{6,7}. Like most other myosins, myosin-II has two heads and a tail and can form bipolar filaments.

We used monoclonal antibodies to show that myosin-I but not myosin-II is required for organelle movements along actin filaments. Antibody M1.5 inhibits stoichiometrically the actin-activated ATPase of myosin-I (ref. 12) and antibodies M2.3, M2.10 and M2.26 all stoichiometrically inhibit the actin-activated ATPase of purified myosin-II as well as the contraction of actin gels in crude cytoplasmic extracts¹³. These four antibodies react with the heavy chains of either myosin-I (M1.5) or myosin-II (M2.3, M2.10 and M2.26) among the various proteins in cellular extracts^{12,14}. M1.5 and other monoclonal antibodies to myosin-I also bind to nuclear proteins¹². In blind experiments antibody M1.5 completely stopped all organelle movement on

Table 1 Antibody inhibition of movements

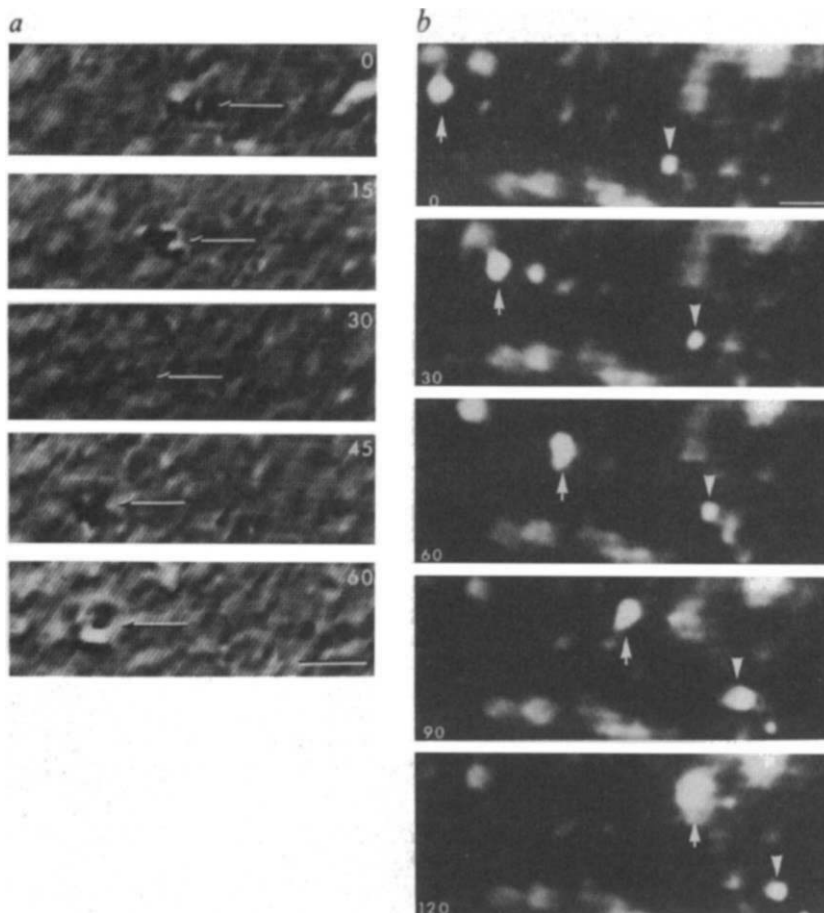
Experimental preparation	Antigen	Antibody: myosin ratio	Presence of movement
Untreated extract			+
Untreated extract on substrate pretreated with 5 mM <i>N</i> -ethylmaleimide then dithiothreitol			+
Extract treated with antibodies*:			-
M2.3	Myosin-II	10:1	+
M2.10	Myosin-II	20:1	+
M2.26	Myosin-II	20:1	+
M1.5	Myosin-I	20:1	-
		2:1†	+/-
		0.2:1	+
		0.02:1	+
Alice‡	Chicken muscle myosin	-	+

* Results of blind experiments in which the experimenter did not know the treatment of the organelle preparations.

† Results of two sets of blind experiments carried out at this ratio of antibody to antigen showed inhibition in one but not the other experiment.

‡ Monoclonal antibody raised against chicken muscle myosin; does not cross-react with *Acanthamoeba* myosins.

Fig. 1 a, Organelles (arrows) moving on a substrate of *Nitella* actin bundles, photographed at 15-s intervals. The image-processing procedure (see below) causes the image of the organelles to change from frame to frame. Photographs prepared from moving organelles recorded on videotape. Scale bar, 10 μm . Actin bundles oriented horizontally. **b**, Phagocytic vesicles rendered visible by feeding the cells fluorescent beads before homogenization. Fluorescent organelles (arrows) are seen against the autofluorescence of the *Nitella* chloroplasts. Photographs taken at 30-s intervals from an unprocessed video-recording. Scale bar, 10 μm . Actin bundles oriented horizontally.



Methods. *Acanthamoeba castellanii* were grown in liquid culture as described previously⁶. Organelles were prepared freshly each day starting from approximately 0.2 g wet weight of compacted cells, washed three times in 50 mM NaCl. The cells were resuspended in 1 ml of homogenization buffer (60 mM potassium glutamate, 2 mM EGTA, 2 mM MgCl₂, 10 mM imidazole pH 6.4, 0.1 mM benzamide, 1 mM phenylmethylsulphonyl fluoride and 4 mM ATP) and disrupted by eight strokes with a tight-fitting pestle in a 7-ml Dounce homogenizer. The homogenate was cleared of unbroken cells and large particles with a 5-s spin at 10,000 g in a bench-top Eppendorf centrifuge. The resulting supernatant was used for all the experiments. In some experiments cells were prepared with fluorescent phagocytic vesicles. Washed cells were incubated, with occasional mixing, for 15 min at room temperature in a dense suspension of rhodamine-labelled polyacrolein beads in 50 mM NaCl. (Beads of ~0.5 μm diameter were prepared by the method of Margel *et al.*²³). Labelled cells were further washed three times in cold saline and processed as before. *Nitella* was dissected as described by Sheetz and Spudich⁵ in a buffer of 20 mM KCl, 4 mM EGTA, 4 mM MgCl₂, 10 mM sucrose, 5 mM imidazole pH 7.0 and 2 mM ATP. The suspension of organelles was mixed 1:1 with homogenization buffer containing 0.2 M sucrose and added to a region of exposed *Nitella* ectoplasm with a microcapillary. Motion of organelles on *Nitella* was observed on an upright Zeiss microscope with a $\times 40$ water-immersion, phase-contrast objective. At this magnification movements were too slow to be discerned by eye but became obvious when speeded up by time-lapse video microscopy. The microscope was fitted with Dage-MIT Newvicon video camera, the output of which was passed through a Quantex digital image processor (DS 30) and Colorado Video analogue video processor (604) before being recorded at 1/18 real-time on a National Panasonic (NV 8030) time-lapse video recorder. Detection of movement of small vesicles on the complex substrate was difficult, so the following image-processing regime was used to selectively enhance moving particles. The image processor continuously subtracted an image of the non-moving *Nitella* background from the incoming picture. Thus, only differences between the two images are seen, that is, objects that moved. This technique is complicated because the *Nitella* substrate distorts to some extent during the experiment (~20 min). To overcome this, the background image was itself slowly averaged using a rolling average, with the incoming image, that replaces the stored image every 128 frames. It is necessary to ensure that the rate of averaging is slower than the rate of detection of the differences (the rate of recording on the video recorder). The contrast of the difference image from the digital processor was further enhanced with the Colorado Video analog processor.

Nitella actin bundles, whereas antibody inhibitors of myosin-II (M2.3, M2.10 and M2.26) had no obvious effect on organelle movements (Fig. 2a). The average rate of movement was $0.23 \pm 0.09 \mu\text{m s}^{-1}$ (mean \pm s.d., $n = 12$) in the presence of myosin-II antibodies. A ratio of M1.5 antigen binding sites (this antibody is an immunoglobulin M) to estimated myosin-I content of the extract (1.3 μmol per kg of wet cells⁶) of 20:1 reproducibly inhibited motion whereas a ratio of 2:1 inhibited motion in one but not a second of two sets of experiments. Ratios of 0.2:1 or 0.02:1 did not inhibit movement (Table 1). These experiments provide evidence that the movement of the organelles is driven by the smaller myosin species, myosin-I. The rate of motion of these organelles is ~10 times higher than that recently reported for plastic beads coated with myosin-I on a *Nitella* preparation⁷.

The composition of the homogenate used for these experiments is highly heterogeneous so we do not yet know which organelles are moving. But by pre-labelling phagocytic vesicles of the cells with fluorescent beads we could show that these were among the organelles that move in our experiments (Fig. 1b). This could be a subset of the total moving population.

We used cell fractionation to test whether myosin-I is physically associated with the motile vesicles (Fig. 3). When the crude extracts used in the motility experiments were centrifuged on a Percoll gradient, organelles sedimented into the density gradient and were separated from the soluble components at the top of the tube. Organelles that came to equilibrium at a density of 1.08–1.13 g ml^{-1} were motile on *Nitella* actin bundles. This fraction was also greatly enriched in myosin-I relative to myosin-II, which remains essentially in the supernatant. Approximately 20% of the myosin-I in the extract sedimented with these motile organelles. Tests with specific monoclonal antibodies¹⁰ showed that both myosin-IA and -IB are associated with organelles. Therefore, there is a stable interaction of myosin-IA and -IB with organelles that persists through centrifugation, is insensitive to ATP and is competent to move organelles along actin bundles.

These experiments show that an actomyosin system can provide the force for some membrane movements within the cell, as has been speculated based on experiments with myosin-coated beads⁵⁻⁷. The heterogeneity of the organelles in our preparation and the fact that we used a cell-free system do not

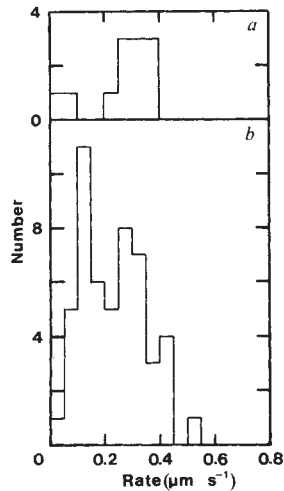


Fig. 2 Distribution of velocities of *Acanthamoeba* vesicles on a *Nitella* actin bundle substrate. *a*, Movement of 12 organelles in the presence of antibodies to myosin-II; *b*, accumulated results from 50 organelles measured in control preparations.

Methods. The net speed of movement for a vesicle was measured from the replay of a videotape recording of an experiment. The positions at the beginning and end of a timed motion were marked on the video monitor. The screen coordinates of these two points were read using a Colorado Video analyser (321) and the corresponding displacement calculated with account being made of lateral distortions introduced by the video camera. Speeds were recorded for displacements of 20–140 μm .

yet permit us to identify directly the nature or role of these movements in the cell. Immunofluorescent localization of myosin-I in the cell^{12,15} reveals a cortical concentration which suggests an involvement of myosin-I-powered movements in endocytosis or surface movements in addition to organelle movements. Such processes may be important in other cells since myosin-I has now been identified in *Dictyostelium*¹⁶ and a

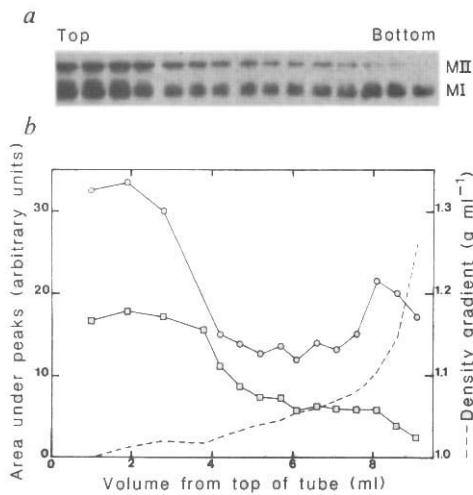


Fig. 3 *Acanthamoeba* cytoplasmic extract (0.5 ml) was layered onto 9.0 ml of 1.08 g ml^{-1} Percoll in homogenization buffer and centrifuged at 27,000 r.p.m. in a Beckman Ti 50 rotor for 15 min. The gradient was unloaded from the bottom of the tube; the densities of Percoll in the collected fractions were determined from refractive indices. Aliquots of these fractions were electrophoresed on 7.5% gels²⁴, transferred to nitrocellulose²⁵ and radioimmunologically stained with antibodies to myosin-I and -II (*a*). The autoradiogram shown in *a* was scanned using a soft-scanning laser densitometer to show the distribution of the antigens in the fractions collected from the gradient. MI, myosin-I; MII, myosin-II. *b*, Relative areas under the peaks of myosin-I and -II were plotted for each fraction through the gradient. Circles, myosin-I; squares, myosin-II.

similar ATPase has been identified as the link between the actin filaments and the membrane in microvilli¹⁷. Previous results which suggested a possible role for actin filaments in organelle movements in other cell types^{1,18–22} may also be mediated by proteins like myosin-I.

This work was supported by NIH research grants GM 26338 and GM 26132 (to T.D.P.) and a Postdoctoral Fellowship from the Muscular Dystrophy Association (to R.J.A.).

Received 10 March; accepted 2 June 1986.

- Schliwa, M. *Cell Muscle Motil.* **5**, 1–82 (1984).
- Schnapp, B. J., Vale, R. D., Sheetz, M. P. & Reese, T. S. *Cell* **40**, 455–462 (1985).
- Allen, R. D. *et al. J. Cell Biol.* **100**, 1736–1752 (1985).
- Kachar, B. *Science* **227**, 1355–1357 (1985).
- Sheetz, M. P. & Spudich, J. A. *Nature* **303**, 31–35 (1983).
- Pollard, T. D. & Korn, E. D. *J. Biol. Chem.* **248**, 4682–4690 (1973).
- Albanesi, J. P. *et al. J. Biol. Chem.* **260**, 8649–8652 (1985).
- Kamiya, N. & Kuroda, K. *Bot. Mag.* **69**, 544–554 (1956).
- Chen, J. C. W. & Kamiya, N. *Cell Struct. Funct.* **1**, 1–9 (1975).
- Maruta, H. & Korn, E. D. *J. Biol. Chem.* **252**, 6501–6509 (1977).
- Pollard, T. D., Stafford, W. F. & Porter, M. E. *J. Biol. Chem.* **253**, 4798–4808 (1978).
- Hagen, S. C., Kiehart, D. P., Kaiser, D. A. & Pollard, T. D. *J. Cell Biol.* (in the press).
- Kiehart, D. P. & Pollard, T. D. *J. Cell Biol.* **99**, 1024–1033 (1984).
- Kiehart, D. P., Kaiser, D. & Pollard, T. P. *J. Cell Biol.* **99**, 1002–1014 (1984).
- Gadasi, H. & Korn, E. D. *Nature* **286**, 452–456 (1980).
- Cote, G. P., Albanesi, J. P., Ueno, T., Hammer, J. A. & Korn, E. D. *J. Biol. Chem.* **260**, 4543–4546 (1985).
- Collins, J. H. & Borysenko, C. W. *J. Biol. Chem.* **259**, 14128–14135 (1984).
- Edds, K. T. *J. Cell Biol.* **66**, 145–155 (1975).
- Bradley, T. J. & Satir, P. *J. Supramolec. Struct.* **12**, 165–175 (1979).
- Isenberg, G., Schubert, P. & Kreuzberg, G. W. *Brain Res.* **194**, 588–593 (1980).
- Goldberg, D. J., Harris, D. A., Lubit, B. W. & Schwartz, J. H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7448–7452 (1980).
- Brady, S. T., Lasek, R. J., Allen, R. D., Yin, H. L. & Stossel, T. P. *Nature* **310**, 56–58 (1984).
- Margel, S., Beitel, U. & Ofarim, M. *J. Cell Sci.* **56**, 157–175 (1982).
- Laemmli, U. K. *Nature* **229**, 680–685 (1970).
- Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354 (1979).

Lateral proton conduction at lipid–water interfaces and its implications for the chemiosmotic-coupling hypothesis

Michel Prats, Justin Teissière
& Jean-François Tocanne*

Centre de Recherche de Biochimie et de Génétique Cellulaires du CNRS, 118 Route de Narbonne, 31062 Toulouse Cedex, France

The driving force in energy-transducing membranes is now recognized to be linked to a flux of protons between membrane-bound donors and acceptors. The question of whether the proton pathway is delocalized within the two bulk aqueous phases on each side of the membrane (delocalized chemiosmotic theory¹), or is localized at its surface (semi-localized hypothesis²) still remains open to discussion^{3,4}. Using an original fluorescence monolayer technique, we have recently been able to provide direct experimental evidence that a phospholipid–water interface can act as an efficient proton conductor^{5,6}. This observation strongly supports the semi-localized hypothesis². In the present study, comparisons between surface potential and fluorescence measurements in monolayers provide additional evidence of a facilitated proton conduction along phospholipid–water interfaces. They suggest the existence of an induced steep surface pH gradient from a more acidic surface towards the bulk. They also show that lateral proton transfer along the surface of a biological membrane would alter the surface potential of that membrane.

The surface potential ΔV which is measured for lipids in monolayers is known to originate primarily from the polar headgroups and to depend on their ionization state⁷. Therefore, since the lipid is the probe itself, this technique affords an elegant way of observing the lateral movements of protons and of

* To whom correspondence should be addressed.