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Organelle Movement in *Actinophrys sol* and Its Inhibition by Cytochalasin B

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Summary. Movement of extrusomes in the heliozoon *Actinophrys sol* was characterized at surfaces of the cell body and giant food vacuoles where microtubules are absent. Extrusomes moved in a saltatory manner at an average velocity of 0.5 μms^{-1}. The highest velocity observed was 2.1 μms^{-1}. Cytochalasin B (50 µg/ml) strongly inhibited extrusome movement at the surfaces of newly-formed food vacuoles, suggesting that the actomyosin system is involved in the organelle transport in *Actinophrys*.

Key words: actinophryid, actomyosin, extrusome, heliozoa, organelle transport.

INTRODUCTION

Transport of intracellular organelles is a ubiquitous feature of eukaryotic cells (e.g. Rebhun 1972, Hyams and Stebbings 1979, Schliwa 1984). In many instances, microtubules have been postulated as important elements along which bidirectional particle transport takes place (Koonce and Schliwa 1985, Hayden and Allen 1984, and references therein). In addition to the microtubule-associated movement, there are other examples in which organelles are transported in the absence of microtubules (reviewed in Hyams and Stebbings 1979), which include movement of organelles (extrusomes and mitochondria) in the heliozoon cell surface.

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Edds (1975a) showed that organelle movement of the heliozoon *Echinosphaerium* still occurred in artificial axopodia where a glass microneedle substituted for the microtubular axoneme, and colchicine did not inhibit the motion in either the normal or the artificial axopodia (Tilney 1968, Edds 1975a). Organelle movement is also known to take place in the cortex of the heliozoon cell body where no microtubules are present (Fitzharris *et al.* 1972, Suzaki and Shigenaka 1982). The actomyosin system has therefore been suggested as a possible force-generating mechanism for this movement, although there is no direct evidence available so far.

Actinophrys cells form giant food vacuoles when they are fed with large ciliates such as *Colpidium* and *Paramecium* (Patterson and Hausmann 1981, Hausmann and Patterson 1982). Extrusomes move in the cytoplasm at the surfaces of such food vacuoles. Motions of individual extrusome particles can be observed easily on

food vacuoles, because this region of the cell is most distant from the cell body cytoplasm that usually hinders precise observation of extrusomes due to optical disturbances. In the present study, we characterized particle movement at the food vacuole surface and demonstrated that cytochalasin B inhibited particle movement there, which further strengthens the evidence for the involvement of the actomyosin system in organelle transport in heliozoa.

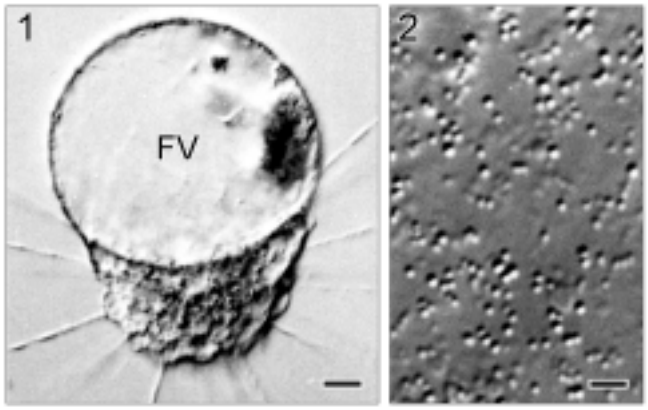
MATERIALS AND METHODS

The source of *Actinophrys sol* and the culture method have been described by Patterson (1979). Prior to every experiment, cells were kept in a conditioning medium (0.01% Knop solution containing 5 mM Hepes-KOH buffer at pH 7.0) for more than 1 h to make them adapted to the solution. Formation of food vacuoles was induced by adding *Colpidium* spp. (ciliate) as prey. For light microscopy, a Zeiss inverted microscope (IM 35) was used with a 63x plan apochromatic oil immersion objective and Nomarski differential interference contrast optics. Movement of extrusomes was recorded at real time speed with a video recorder (JVC BR6400 TR), and analyzed afterwards frame by frame. Cytochalasin B (Sigma) was dissolved at 5 mg/ml in dimethylsulfoxide (DMSO) before it was diluted with the above medium.

RESULTS AND DISCUSSION

Actinophrys sol formed giant food vacuoles when it was fed with large ciliates. The size of such food vacuoles sometimes exceeded the initial size of the cell body (Fig. 1). The food vacuole was surrounded by a thin layer of cytoplasm, in which numerous particles moved in a saltatory manner (Fig. 2). These particles are extrusomes (Patterson 1979), which can be seen as refractile spheres of about 0.5 µm in diameter under the light microscope. Since these organelles are known to discharge their contents when the cells capture food organisms, they are implicated in the process of prey adhesion and membrane supply for making food vacuoles (Suzaki *et al*. 1980; Patterson and Hausmann 1981; Hausmann and Patterson 1982; Sakaguchi *et al*. 1998, 2001).

Movement of these cytoplasmic particles was examined in detail at the food vacuole surface. Particles moved at variable velocities, with a range from ~ 0 to 2.1 μms^{-1} (Fig. 3). The average velocity was 0.55 μms^{-1}, which was almost the same as the one recorded at the surface of the cell body (0.52 μms^{-1}, Table 1). Particles moved independently from each other



Figs 1, 2. Light micrographs of *Actinophrys sol*; **1** - low magnification picture showing a giant food vacuole (FV); **2** - highly magnified picture showing many extrusomes at the surface of a food vacuole. Scale bars: 10 µm (1); 2 µm (2).

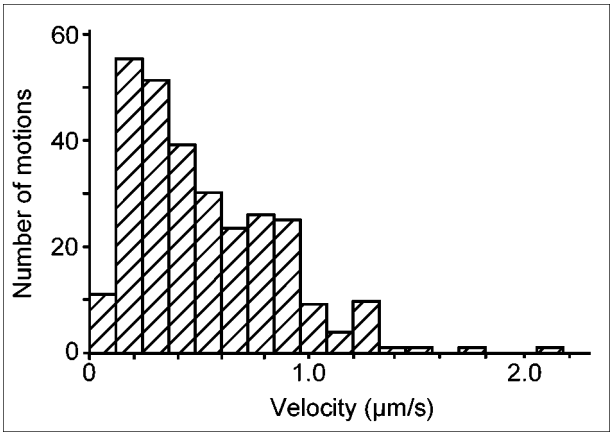


Fig. 3. Histogram of velocities (distance moved in 1 s) of particles at the surface of a newly-formed food vacuole. 10 motions were examined for each of 30 particles (total 300 motions examined).

with no evident track of movement, and frequently showed changes in direction and velocity (Figs 4, 5). The degree of regularity of the particle movement was examined by calculating Jarosch's regularity quotient (Qr, the ratio of mean square displacement of double intervals to the mean square displacement of single intervals) according to Kamiya (1959). Qr for particle movement of the food vacuole surface was 2.13 (30 particles were traced in both 5 and 10 s intervals), which suggests that the motion can be regarded as an irregular one with some energy-dependent driving process for it.

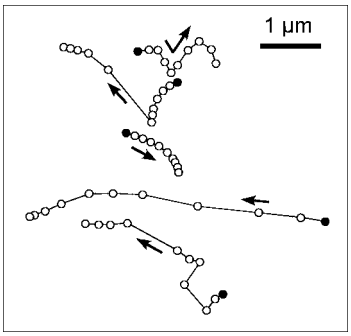
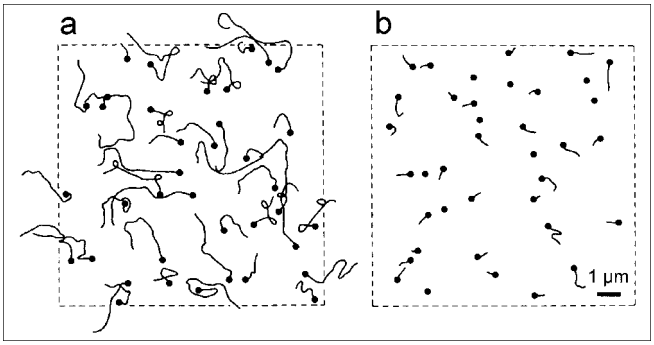


Fig. 4. Tracing of particles moving at the surface of a food vacuole. Each open circle represents the position of the particle at 1 s intervals (started from the position shown by the closed circles). Arrows indicate directions of movement.



Figs 5a, b. Effect of cytochalasin B on particle movement at the surface of newly-formed food vacuoles (about 30 min after formation); **a** - control movement in 1% DMSO; **b** - 30 min after treatment with 50 µg/ml cytochalasin B in 1% DMSO. Particles were traced for 10 s from the position shown by the closed circles.

Table 1. Effect of cytochalasin B on particle movement at the surfaces of cell body and newly-formed food vacuole*.

		Average velocity ($\mu ms^{-1} \pm SD$)	Sample number**
Control***	Cell body	0.52 ± 0.34	30
	Food vacuole	0.55 ± 0.34	30
Cytochalasin B treated (50 µg/ml, 30 min)	Cell body	0.37 ± 0.15	38
	Food vacuole	0.05 ± 0.04****	38

* Examined about 30 min after formation; ** 9-10 particles were traced for each of 4 experiments; *** containing 1% DMSO; **** significantly different from control food vacuole ($p<0.001$).

Movement of particles at the surface of newly-formed food vacuoles (within 3 hours after formation) was inhibited by cytochalasin B at 50 µg/ml (Fig. 5 and Table 1). Particle movement resumed when the cells were washed in a control medium that did not contain cytochalasin B (the control medium contained 1% DMSO, which did not show any effect either on cell shape or on particle motility). Average velocity of the particles slightly decreased at the surface of the cell body, but it was not significant as compared with the inhibition observed at the food vacuole surface (Table 1).

The plasma membrane of the food vacuole is considered to be derived from the investing membrane of the extrusomes, which becomes available after the discharge of their contents to the prey organisms (Hausmann and Patterson 1982). This newly-formed membrane has different characteristics from other cell body surfaces, i.e., it is very adhesive to the prey organisms (Suzaki *et al*. 1980, Hausmann and Patterson 1982), and is easily fused with food vacuole membranes of neighboring heliozoon cells to form a large common food vacuole (Bovee and Cordell 1971, Patterson and Hausmann 1981). These properties of the food vacuole membrane may suggest that this new membrane surface possesses a reduced or a different type of surface coat. A large increase in the frequency of cell fusion after experimental removal of the surface coat has been shown in *Echinosphaerium* (Vollet and Roth 1974). Cytochalasin B inhibited particle movement most effectively at the food vacuole surface but had little or no effect in other parts of the cell (Table 1). This difference might be due to different permeability characteristics of the food vacuole membrane.

The inhibitory effect of cytochalasin B suggests that the actomyosin system might be involved in force generation for particle movement, although its exact mecha-

nism is still an open question. Microfilaments have been observed in the cytoplasm of *Echinosphaerium* (Tilney *et al.* 1966, Edds 1975b, Suzaki *et al.* 1980) and *Actinophrys* (Hausmann and Patterson 1982). They are, however, located either deep in the cytoplasm (Tilney *et al.* 1966) or in the food vacuole-forming pseudopodia (Suzaki *et al.* 1980, Hausmann and Patterson 1982), without any direct interaction with moving extrusomes. Movement of extrusomes is known to take place always along the inner surface of the plasma membrane and was inhibited when the extrusomes were removed from contact with the plasma membrane by treatment with chloral hydrate (Suzaki and Shigenaka 1982). Therefore, it seems to be plausible that the driving force for the movement of extrusomes may be generated through interaction with meshwork of single actin filaments located in the close vicinity of the inner surface of the plasma membrane, not with large bundles of microfilaments located deeper in the cytoplasm. The next and crucial step to understand the molecular mechanism of movement of the extrusomes is to demonstrate the presence of F-actin between the cell membrane and the food vacuole.

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