# MOTILITY IN A "CELL SAP" OF THE SLIME MOLD *PHYSARUM POLYCEPHALUM*

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## 1. Introduction

Cytoplasmic streaming in plasmodia of the myxomycete *Physarum polycephalum* is exceedingly vigorous compared to that of plant cells or amoeba. The fluid part of the cytoplasm streams at a rate up to about 1.3 mm/sec, changing direction every 2–3 min. Considerable effort has been devoted to elucidating the physico-chemical basis of cytoplasmic movement in this system [1]. It has been shown [2, 3] that both an actin and a myosin can be prepared from *Physarum*. Since i) it is an act of faith that a basic mechanism common to all cellular movement will be found and ii) there is an analogy between the response of cytoplasmic streaming and of muscle actomyosin *in vitro* to the action of various factors (e.g. the addition of ATP to the plasmodia may augment the motive force [1]), it is believed that ATP splitting by an actomyosin system creates the driving force for cytoplasmic streaming.

"Cell sap" squeezed out from plasmodia by high-speed centrifugation was found to contain a 20 S peak considered to be primarily actin-like material as well as appreciable $Ca^{2+}$-ATPase activity [4]. The mechanochemical force in striated muscle is in all probability generated as the result of an interaction between ATP and the active sites of the actin and myosin filaments. The very generation of force does not therefore seem to require the presence of the Z and M lines which hold together the thin and the thick filaments, respectively, or the chemically inactive shaft of the myosin filaments. We therefore considered the possibility that in the above described "sap", interaction between the presumably free actin filaments and the *Physarum* myosin (which is known to be very soluble under physiological conditions) in the presence of ATP might lead to the movement of the filaments relative to the surrounding fluid, i.e. the fluid will flow past the filaments.

## 2. Experimental

In order to obtain massive streaming which could be visualized under the microscope, it is obvious that the region observed should be anisotropic and contain many filaments which are parallel to each other. It has been claimed by Kamiya [5] that (presumably) F-actin filaments in a glycerol model of plasmodia of *Physarum* gather closer to form fibrils on addition of ATP and that there exists a correlation between fibril formation and motive force production in plasmodia. "Liquid crystal" formation by rod-like macromolecules is known to be affected by the concentration as well as by environmental factors such as ionic strength etc. [6]. In addition, parallel orientation might be facilitated by confining the filaments in a microcapillary whose internal diameter (i.d.) is not too large in comparison with the length of the filaments. We therefore introduced the sap into microcapillaries of 20–100 $\mu$m i.d. and 0.5–3.0 cm long. At both ends, the capillaries were wider (about 1 mm i.d.). Liquid occupied 0.5–2.0 cm in each of the wide ends.



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Microplasmodia of *Physarum polycephalum* were cultured at 23°C in shaken flasks containing 25 ml of semi-defined medium [7]. Thirty hours after inoculation plasmodia from 16 flasks were allowed to sediment in a 250 ml measuring tube at 4°C. All subsequent operations were carried out in the cold. After decanting the supernatant, the plasmodia were washed 3 times by a solution containing 0.3 M KCl and 1 mm EDTA pH 7.0. The plasmodia were centrifuged at 35 000 *g* for 10 min and the pellet obtained homogenized in a motor-driven Teflon homogenizer. In some of the preparations, ATP was added to give a final concn. of 5 mM. The homogenate was centrifuged at 10 000 *g* for 10 min. Three layers could be observed in the test tube: the lowest comprised of nuclei while the top, compact, layer contained membranes and other particles. Cytoplasmic solution containing small granular particles separated between these two layers. This solution was sucked by a syringe and transferred into a test tube. We made sure that the whole procedure did not exceed 2 hr. A drop was sucked into a microcapillary and observed under the microscope (magnification 125–250 X).

## 3. Results

A rapid movement of up to about 1 mm/sec of the suspended particles could be observed. Movement of the "cell sap" started within 1 min after its introduction into the capillary. It was significantly faster than the viscoelastic streaming which was observed immediately after sucking. The meniscus of the liquid in the wider ends moved in the same direction as the particles indicating that the particles were passively dragged by the streaming fluid.

When the microcapillary was relatively narrow (about 50 μm), the direction was reversed after 2 min, the period of oscillation increasing and the maximal velocity decreasing with time. After about 20 min of this oscillatory movements, streaming continued slowly in one direction only for about 1 hr and then stopped. During this period streaming was maintained even against an opposing hydrostatic pressure difference of up to 4 mm water created by tilting the capillary. The initial values of the velocity of streaming and of the period of the oscillations were quite comparable to those observed in the microcapillary strands of the *intact Physarum*.

When wider capillaries (about 100 μm i.d.) were used, the movement of the particles was jerky during the first 1–2 min, even though they all moved together. Later on, streaming took place in one direction for about 4–5 min at the much higher velocity of 1 mm/sec. It then stopped for a while, changed direction – now at a much smaller velocity – for about half a minute. This cycle repeated itself 3–4 times.

In all experiments, new particles appeared towards the end of the streaming.

## 4. Discussion

The presence of actin and myosin in *Physarum* does not prove that these proteins cause the cytoplasmic streaming despite the role they are known to play in the mechanochemical machinery of muscle. If, however, we assume that some kind of interaction between these two proteins is responsible for the streaming then our experiments seem to indicate that cytoplasmic streaming in the intact cell may at least be partially attributed to free actin and myosin, i.e. actin filaments and myosin which are *not* bound to the plasmalemma or organized into "brushes" like the sarcomere arrays of striated muscle. Cell-free extracts, obtained from *Amoeba proteus* by centrifugation, exhibited characteristic patterns of streaming in the presence of ATP [8] and contained 50–70 Å and 160 Å filaments [9, 10]. The thick filaments bear some superficial resemblance to the myosin filaments of striated muscle and on their removal, the preparations exhibited little or no ATP-stimulated movement. The thin filaments formed specific ATP-dissociable complexes with muscle heavy meromyosin (HMM) that were indistinguishable from those of HMM with F-actin from muscle; these indirect methods were claimed to provide *tentative*, though reasonable, identification of the thin filaments as F-actin and of the thick filaments as myosin. The evidence for the presence of (soluble) actin and myosin in the *Physarum* sap was, however, direct and beyond doubt.

If indeed free actin and myosin *are* responsible for streaming both in the sap and in the intact *Physarum* then one may reverse the argument and apply a *synthetic* rather than an *analytical* approach to the mechanochemical basis of cytoplasmic streaming. Parallel to proving the presence of these proteins in the vari-

ous non-muscular motile cells or trying to correlate the effects of different physical and chemical factors on cell motility and on the actomyosin system *in vitro*, one may look for the possibility that an aqueous solution in which we dissolved actin, myosin and ATP might exhibit active streaming. Such a system, compared to an intact cell, or even an "extract" will have the following advantages: i) it is not contaminated by other, especially macromolecular, species the role of which is unclear; ii) we are free to vary the concentrations of all the components (e.g. the ratio of actin to myosin); and iii) we may have better control on phase transitions such as sol–gel transformations so that we can see how and if such transitions may be correlated with streaming.

Such an attempt has been made (A. Oplatka and R. Tirosh, submitted to Biochim. Biophys. Acta) and proved to be successful: natural actomyosin (myosin B) in the presence of ATP was introduced into microcapillaries similar to those utilized in the present work. The actomyosin was extracted from *Physarum polycephalum* and from rabbit's striated muscle and the protein concentration was similar to that found in *Physarum* [2,3]. These systems, under the proper conditions, exhibited vigorous oscillatory streaming which was very reminiscent of cytoplasmic streaming in the intact *Physarum*. The new particles which appeared towards the end of streaming in the *Physarum* sap seem to be superprecipitated actomyosin, in analogy to the same phenomenon which was observed in the experiments with myosin B.

Active streaming of ATP-containing actomyosin solutions in microcapillaries may thus serve as some kind of a model system for *cytoplasmic streaming*, in analogy to the use of actomyosin *threads* as models for muscular *contraction*. Such streaming, as well as the sliding of filaments in active muscle, may also be considered as special cases of "active transport" in which hydrodynamic streaming is *coupled* to a chemical (enzymatic) process.

If, indeed, mechanical impulses [11] which lead to muscular contraction are generated in the region where interaction between myosin and actin takes place, then filament formation by myosin does not seem to be obligatory for force *generation*. We are therefore searching now for active streaming in solutions containing actin, HMM (or HMM subfragment-1) and ATP.

In view of the above, one may wonder whether streaming in the microcapillary strands of *Physarum* originates from a pressure difference established between the adjoining bulky regions. Such a pressure difference has been claimed to result from either a "contraction" of an actomyosin matrix associated with a "gel–sol" transformation or a contraction of the plasma membrane due to a force generated in attached filaments of actin interacting with myosin [5]. The region where the native force is produced may as well be in the microcapillary strands themselves in which actin, and maybe also myosin, filaments are oriented parallel to the axis of the strands.

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