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Rapid Report

A soluble motor from the alga *Nitella* supports fast movement of actin filaments in vitro

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Abstract

In the streaming cytoplasm of the Characean algae cell, the movement of organelles along actin bundles occurs at a striking rate of up to 60 $\mu m\ s^{-1}$. To further characterize the molecular mechanisms responsible for this phenomenon, we have reconstituted the movement of actin filaments in vitro using defined biochemical components. We report that only a soluble cytoplasmic fraction devoid of organelles and filamentous material supports the movement of fluorescent-labeled actin filaments on glass at a rate of up to 60 $\mu m\ s^{-1}$. This fraction also contains the K^+-EDTA ATPase and the actin-activated Mg^{2+} ATPase activities characteristic of myosin proteins. Therefore, on the basis of these observations, we conclude that *Nitella* cells have a soluble pool of non-filamentous myosin molecules with the mechanochemical properties expected for a motor responsible for cytoplasmic streaming in vivo. The preparation and conditions described here should be useful for the purification of this translocator.

Keywords: Actin; Myosin; Motility; Video microscopy

The cells of the Characean algae have been extensively used as a model for studying cytoplasmic streaming. We have previously reported that, in the extruded cytoplasm of these cells, the fast movement of organelles along actin cables can be visualized for an extended period of time by video microscopy [1]. In addition, based on structural analysis of the *Nitella* cytoplasm, we have proposed that the streaming is produced by the translocation of the endoplasmic reticulum cisternae along the cortical actin bundles [2]. Several attempts have been made to identify the actin-based translocator responsible for this type of movement. Kato and Tonomura [3], for example, isolated a filamentous form of myosin with all the physical and enzymatic properties that characterize myosin II; however, its mechanochemical properties were not studied. In addition, Grolig et al. [4] have detected and localized two proteins in Chara cells that reacts with a monoclonal antibody against the heavy chain of the mouse fibroblast myosin II, although their final identity was not determined. Therefore, the nature of the translocator responsible for the movement of organelles along actin cables in these cells remains to be established.

In the present work, we further characterize the molecular mechanisms responsible for actin-based motility in *Nitella* cells by reconstituting the movement of actin filaments in vitro using defined biochemical components. First, we dissociated the *Nitella* cytoplasm on the surface of a coverslip and visualized the movement of isolated actin bundles using video-enhanced interference microscopy. Second, in order to better characterize the motor proteins responsible for these movements, we prepared a cellular extract in which the motile activity was preserved and could be easily analyzed.

The visualization of the actin filaments moving on glass was performed using a Zeiss Axiomat microscope in the differential interference contrast mode equipped with an internally corrected 100X, 1.3 numerical aperture (NA) planapochromatic objective. The aperture of a 1.4 NA condenser was fully illuminated with a 100 W mercury lamp aligned for critical illumination [1]. The optical image was projected out of the camera port of the microscope onto a Newvicon Dage-MTI 70 video camera (Dage-MTI,

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Michigan City IN). Using these conditions, both the movements of organelles along actin bundles and the gliding of actin bundles on the glass surface could be visualized in the same field (Fig. 1). Immediately after extruding the cytoplasm, a large number of organelles could be seen moving unidirectionally along the actin bundles at a speed of 60 μm s^{-1} (Fig. 1a,b). However, after 5 to 10 min, almost all of the organelles had fallen off the surface of the actin bundles and stuck to the glass coverslip. Interestingly, the movement of actin bundles on the surface of the coverslip could be observed continuously for several hours in the presence of ATP (Fig. 1c,d). This movement of actin bundles which occurs in the absence of an organized cytoplasm indicates that the motor molecules can dissociate from the organelle wall and remain soluble in the cytoplasm.

In order to better characterize the *Nitella* cytoplasmic factors responsible for the actin-based motility, we performed biochemical fractionation of extruded cytoplasm under conditions in which the motile activity was preserved. For this purpose, individual giant internodal cells of the algae *Nitella* (Carolina Biological Supply Co.) were isolated from neighboring cells and rinsed with distilled water. These cells were blotted on tissue paper, transected at one end to drain the fluid from the giant central vacuole, and then the cytoplasm was squeezed out using a pair of forceps which tips were covered with Teflon tubing. For the preparation of a 'soluble' and an 'organelle' fractions, the cytoplasm was collected in one volume of a buffer containing 200 mM sucrose, 25 mM KCl, 25 mM NaF, 2.5 mM DTT, 2 mM EDTA, 0.5 mM PMSF, 10 μg/mL of pepstatin, leupeptin and aprotinin, and 20 mM imidazole (pH 7.2). The cytoplasm extruded from 50–100 cells was pooled and centrifuged at 15 000 $\times$ g for 20 s using an Eppendorf microfuge. The pellet, containing mainly chloroplasts and nuclei (organelle fraction 1), was discarded and the supernatant centrifuged at 150 000 $\times$ g for 30 min at 4°C using a Beckman TL-100 tabletop ultracentrifuge to obtain the 'soluble' fraction of the cytoplasm and a pellet consisting of cytoskeletal filaments and microsomal membranes (organelle fraction II). The presence of organelles and filamentous material in all of these fractions was assayed by video-enhanced interference microscopy.

To determine the ability of the various subcellular fractions to support motility, aliquots of each fraction were incubated with fluorescent-labeled actin filaments as described by Kron and Spudich [5] in a buffer containing 15 mM imidazole, 2 mM $MgCl_2$, 1 mM EGTA, and 2 mM

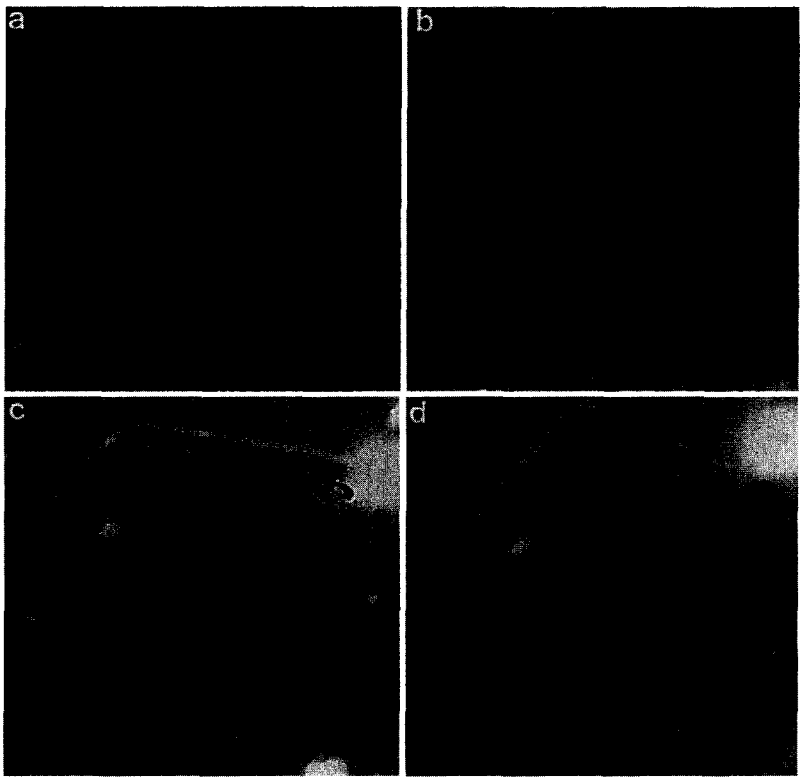

Fig. 1. In vitro movements of organelles and actin bundles from extruded *Nitella* cytoplasm. (a and b) Video micrographs showing a large number of organelles moving along native actin bundles with a maximal speed of up to 60 μm s^{-1}. Organelles could be seen actively moving in cytoplasmic preparation within 5 to 10 min after extraction. (c and d) Video micrographs of actin bundles gliding on the surface of the glass coverslip. This movement becomes more evident 5 to 10 min after extrusion of the *Nitella* cytoplasm when most of the organelles have fallen off the surface of the actin bundles and attached to the glass. Note that in this particular experiment, the ends of the actin cables have associated themselves and the whole bundle is rotating counterclockwise. Organelles that remain attached to the actin bundle move clockwise (arrows).

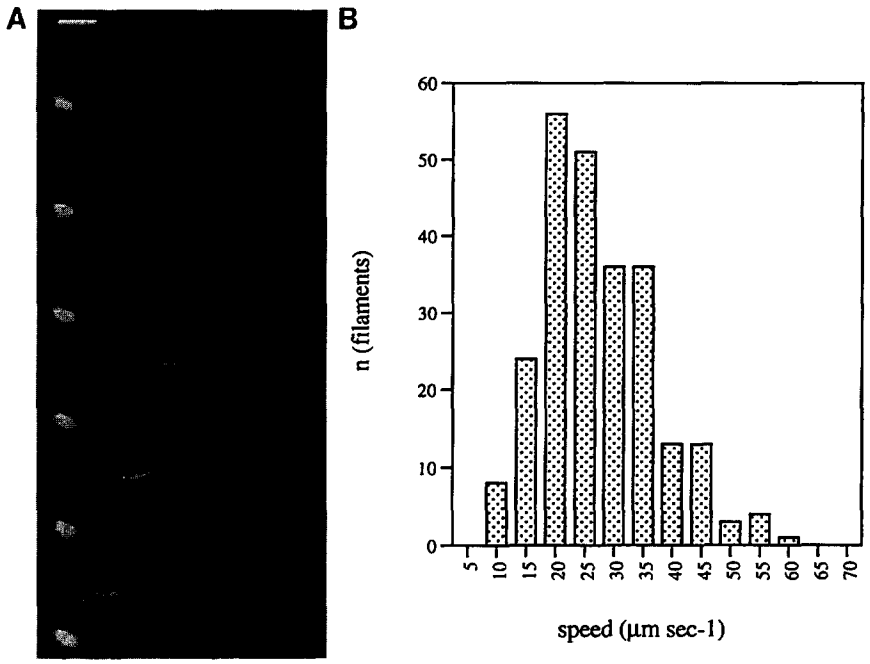

Fig. 2. In vitro movement of fluorescently labeled actin filaments on a glass surface supported by the soluble fraction of the *Nitella* cytoplasm. (a) Micrograph of consecutive video frames showing the movement of an individual fluorescently labeled rabbit actin filament on a glass coverslip at a speed of 60 μm s^{-1}. Note that the movement of the filaments becomes more evident when compared to the position of a still actin aggregate present in the upper right corner of each frame. The bar in the lower right corner is equivalent to 5 μm. Time between frames is 67 ms. (b) Histogram representing the rate of movement for individual actin filaments on a glass surface supported by the soluble *Nitella* cytoplasmic fraction. Actin filaments move at speeds ranging from approximately 10 μm s^{-1} to 60 μm s^{-1}. Note that the faster moving filaments display a speed which is similar to the rate of the *Nitella* cytoplasmic streaming in vivo.

ATP (pH 7.5). The visualization of the fluorescent-labeled actin filaments moving on the glass surface was done using a Zeiss Axiomat microscope equipped with a 100 ×, 1.3 NA objective. The image was projected onto a Newvicon or SIT video camera (Dage-MTI) and recorded on a video recorder or in an optical memory disc recorder (Panasonic). Still photographs were taken directly from the video monitor. After biochemical fractionation, the *Nitella* soluble cytoplasmic supernatant was the only fraction that supported the movement of fluorescent-labeled actin filaments on the glass surface, at average speeds ranging from 10 μm s^{-1} to 60 μm s^{-1} (Fig. 2A). This faster movement occurs at the speed which is characteristic of the cytoplasmic streaming in vivo. Almost 85% of the actin filaments moved smoothly on the glass surface. Another type of movement was produced when one-third of the actin filaments were stuck to the glass and the remaining part either waved or underwent vigorous oscillatory motion at a speed of around 3 cycles s^{-1} ($n = 50$) (Fig. 3).

The length of the filaments varied dramatically with the time of incubation. At early times, the field of observation had an average population of 20 actin filaments approximately 5 μm in length. However, during the movements the filaments became fragmented into hundreds of 1 μm filaments, perhaps due to the shearing force developed by the interaction with the motor and the glass surface. 15 min after adding the soluble cytoplasmic fraction, almost all the long filaments present in the original field had fragmented (data not shown). The breakdown of individual filaments may be produced when myosin heads in a rigor

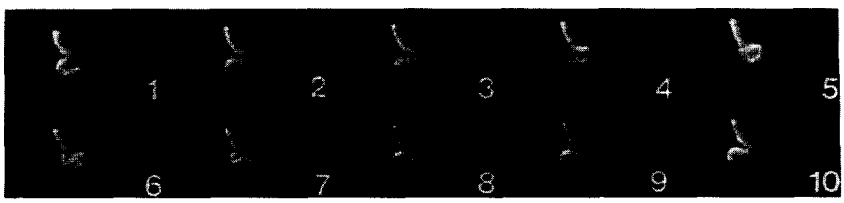

Fig. 3. Oscillatory motion of actin filaments on glass supported by the soluble *Nitella* cytoplasmic fraction. Micrograph of consecutive video frames taken every 33 ms showing the oscillatory motion of a single fluorescent actin filament on glass. This movement is produced when the upper one-third of the actin filament is stuck to the glass surface, maybe due to the action of some actin binding proteins, while successive waves of bending are propagated toward the free end. This oscillatory motion takes place at a speed of 3 cycles s^{-1}. Note that in this experiment the lower part of the filament is 'waving' to complete a cycle every eight frames.

Table 1
ATPase activities present in the soluble *Nitella* cytoplasmic fraction

Conditions	nmol/min per mg
K^+-EDTA	21.76 ± 3.28
Mg^{2+}	7.96 ± 1.13
Mg^{2+} + F-actin	20.46 ± 1.52

The different ATPase activities were measured from a soluble *Nitella* cytoplasmic fraction which had been concentrated 5-fold by ultrafiltration. ATP hydrolysis was measured using the radioactive method of Pollard and Korn [6]. Note that the soluble cytoplasmic fraction contains a high ATPase activity in K^+ EDTA buffer and displays a low Mg^{2+}-ATPase activity which is significantly activated by F-actin. Data represent mean ± S.E. for five determinations.

state of other actin binding proteins present in the soluble cytoplasmic fraction bind a portion of the filament on the glass surface and the rest of the active myosin heads generate movements. This mechanism would result in a motile part of the filament pulling away from the still part with a force higher than the filament resistance and therefore cause fragmentation. Careful studies have still to be made to determine the force developed by the *Nitella* myosin. Transient re-orientation of the myosin heads before the fragmentation may produce forces vectorially different, resulting in the waving and the oscillatory movement of the motile part of the filaments.

The presence of myosin proteins in the soluble fraction was also determined by measuring the K^+-EDTA and actin-activated Mg^{2+} ATPase activities which are characteristics to this proteins. For this purpose, the soluble fraction of the *Nitella* cytoplasm was concentrated 5-fold with a Centricom™ 30 unit (Amicon®). ATP hydrolysis was assayed by measuring the release of ^{32}P from [γ-^{32}P]ATP during a 10 min incubation period at 30°C [6]. The protein concentration was determined according to the method of Bradford [7], using bovine serum albumin as a standard. The Mg^{2+} ATPase activity was measured in the presence or absence of F-actin (12.5 μM), in a buffer containing 2 mM ATP, 2 mM $MgCl_2$, 10 mM imidazole (pH 7). The buffer for the determination of the K^+-EDTA ATPase activity contained 500 mM KCl, 2 mM EDTA, 2 mM ATP, 20 mM imidazole (pH 7.5). The soluble fraction had a K^+-EDTA activity of 21.76 nmol min^{-1} mg^{-1}. The Mg^{2+}-ATPase activity was stimulated by actin (12.5 mM) from a value of 7.96 to 20.46 nmol min^{-1} mg^{-1} (Table 1). These activities are mechanochemical properties characteristic of proteins of the myosin family [8].

Myosin molecules can be classified into several different families, according to their molecular structures and physicochemical properties [9]. The speed of movement supported by these molecules is of 1–8 μm/s for myosin II [10], and of 0.03–0.1 μm/s for myosin I [8]. Because the speed of the in vitro movement described here for the *Nitella* myosin is at least one order of magnitude faster than the rate reported for any other type of myosin, we currently do not know what type of myosin is responsible for this fast movement. However, through the analysis of the data presented here, we can speculate on the identity of the *Nitella* myosin responsible for the fast actin-based movement. It is highly unlikely, for instance, that the myosin II protein previously described by Kato and Tonomura [3] would be responsible of the fast movement of actin filaments described here, since the soluble cytoplasmic fraction which supports this movement has been prepared under conditions designed to avoid the presence of myosin filaments. In addition, the *Nitella* myosin is capable of moving organelles along actin filaments, while myosin II has not been previously associated to organelle movement. In order to support organelle movement, the fast *Nitella* cytoplasmic myosin would have to bear an organelle-binding domain which is known to be absent in the myosin II molecule. Thus, among the members of the myosin protein superfamily, myosin I and V are good candidates to support the type of movements described in this report. Interestingly, the association of myosin I and myosin V types of molecule with the translocation of organelles in other cell types [11,12] suggests that this type of protein may be powering organelles in the *Nitella* cell. However, no previous evidence has been reported which suggests the presence of protein members of the non-filamentous myosins I or V families in these cells.

In summary, although the evidence for the identity of the motor with myosin is indirect, our results suggest that a non-filamentous type of myosin is responsible for the fast movement of organelles and actin filaments supported by a soluble cytoplasmic fraction in vitro. In addition, the preparation and conditions described here should be useful to follow the purification of this protein. Further studies on the structure of these proteins using biochemical and molecular techniques may then provide critical information on the mechanisms by which the high-speed movement is generated by this translocator.

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