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Invited article

Characean Actin Bundles as a Tool for Studying Actomyosin-Based Motility

TERUO SHIMMEN*

Department of Botany, Faculty of Science, University of Tokyo, Hongo, Tokyo 113

At the inner surface of the stagnant chloroplasts of Characeae cells, bundles of actin filaments having uniform polarity are anchored. These bundles are responsible for generating the motive force of cytoplasmic streaming. It is now possible to induce movement of either beads coated with foreign myosin or organelles associated with myosin along the characean actin bundles. The Ca^{2+} sensitivities of the reconstituted movements are consistent with those of the actin-activated myosin ATPases. The use of reconstituted systems is finding wide application in the detection of various myosins in materials from which myosin is not significantly purified. Furthermore, sliding velocities and the Ca^{2+} regulation of myosins bound to organelles are now being determined.

Key words: Actin — Calcium — Characeae — Cytoplasmic streaming — Myosin.

The actomyosin system is one of the most widespread motile machineries in the plant and animal kingdoms. The elucidation of the molecular mechanism of the motive force generation by the interaction of actin with myosin was carried out in skeletal muscle. The sliding hypothesis for muscle contraction was proposed in 1954 by Huxley and Niedergerke (1954) and by Huxley and Hanson (1954). Two years later the sliding mechanism was proposed by Kamiya and Kuroda (1956) to explain the generation of the motive force for cytoplasmic streaming in Characeae. Based on the velocity profile, they concluded that the interaction of an organized gel surface and a sol phase produces the force which causes the interfacial sliding of the endoplasm. Stimulated by the novel findings, attention is now focused on the molecular mechanism of the sliding. At present, it has been almost established that the sliding of endoplasm at the sol-gel interface is the direct manifestation of myosin sliding along actin filaments. This is reviewed thoroughly by Kamiya (1986).

At the inner surface of chloroplasts, bundles of actin filaments are anchored (Kamitsubo, 1966; Nagai and Rebhun, 1966; Palevitz *et al.*, 1974; Williamson, 1974; Palevitz and Hepler, 1975; Kersey and Wessells, 1976; Nothnagel *et al.*, 1981). Kato and Tonomura (1977) isolated a myosin-like protein from *Nitella flexilis*.

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Abbreviations: ATP, adenosine 5′-triphosphoric acid; EDTA, ethylene diamine tetraacetic acid; EGTA, ethyleneglycol-bis-(β-aminoethylether) N, N, N′, N′-tetraacetic acid; HMM, heavy meromyosin; NEM, N-ethylmaleimide.

Physiological studies revealed that myosin is localized in the flowing endoplasm (Chen and Kamiya, 1975, 1981; Kamitsubo, 1981). Nagai and Hayama (1979) observed protuberances on endoplasmic vesicular organelles. These protuberances are associated with actin bundles through small globular bodies, which may be composed at least of the functional head of myosin or myosin aggregates. Williamson (1979) found filaments associated with endoplasmic reticulum and suggested that the filaments contain myosin. At present, direct evidence to show the involvement of myosin in characean cytoplasmic streaming is lacking. However it is reasonable to call the endoplasmic factor myosin, based on its ability to slide along actin bundles in a Mg-ATP-dependent manner (Williamson, 1975; Tazawa *et al.*, 1976; Shimmen, 1978). HMM from skeletal muscle myosin forms arrowhead complexes on characean actin filaments. The arrowheads point in the direction opposite to that of the cytoplasmic streaming, indicating that the putative characean myosin moves in the same direction relative to the polarized actin filaments as myosin moves in the muscle sarcomere (Kersey *et al.*, 1976).

Recently a novel method has been developed to study actin-myosin sliding using characean actin bundles. Sliding of beads coated with foreign myosin can occur along characean actin bundles. Using this reconstitution method, we can directly measure the velocity of myosin sliding along actin filaments. The movement of foreign myosin along characean actin bundles has been briefly reviewed by Williamson (1986).

Sliding of Skeletal Muscle Myosin along Characean Actin Bundles

Sliding between characean actin bundles and skeletal muscle myosin was first realized by Kuroda and Kamiya (1975). Endoplasmic droplets isolated from characean cells sometimes contain chloroplasts detached from ectoplasm. When actin bundles are attached to the chloroplasts, the chloroplasts rotate actively presumably due to the same mechanism as that of native cytoplasmic streaming. After the endoplasmic drop is mechanically demembranated, chloroplast rotation is inhibited by NEM treatment. The chloroplast rotation can be reactivated upon the addition of skeletal muscle HMM.

Since the quantification of the actin-myosin interaction using the chloroplast rotation system is difficult, two other methods have been designed to induce myosin movement along characean actin bundles *in situ*. In the first method, an internodal cell is cut open parallel to its long axis and the endoplasmic layer is exposed directly to the external medium (Kuroda, 1983; Sheetz and Spudich, 1983a). After the flowing endoplasm is washed away, beads coated with skeletal muscle myosin are applied onto the inner surface of chloroplasts where the actin bundles are anchored (Sheetz and Spudich, 1983a). The second method, called the intracellular perfusion method, was developed by Shimmen and Yano (1984). The procedures are schematically shown in Fig. 1. After both cell ends are cut off, the vacuole is perfused by a medium containing EGTA, a Ca^{2+} chelator. A drastic decrease in the Ca^{2+} concentration in the vacuole results in a disintegration of the tonoplast (tonoplast-free cell,

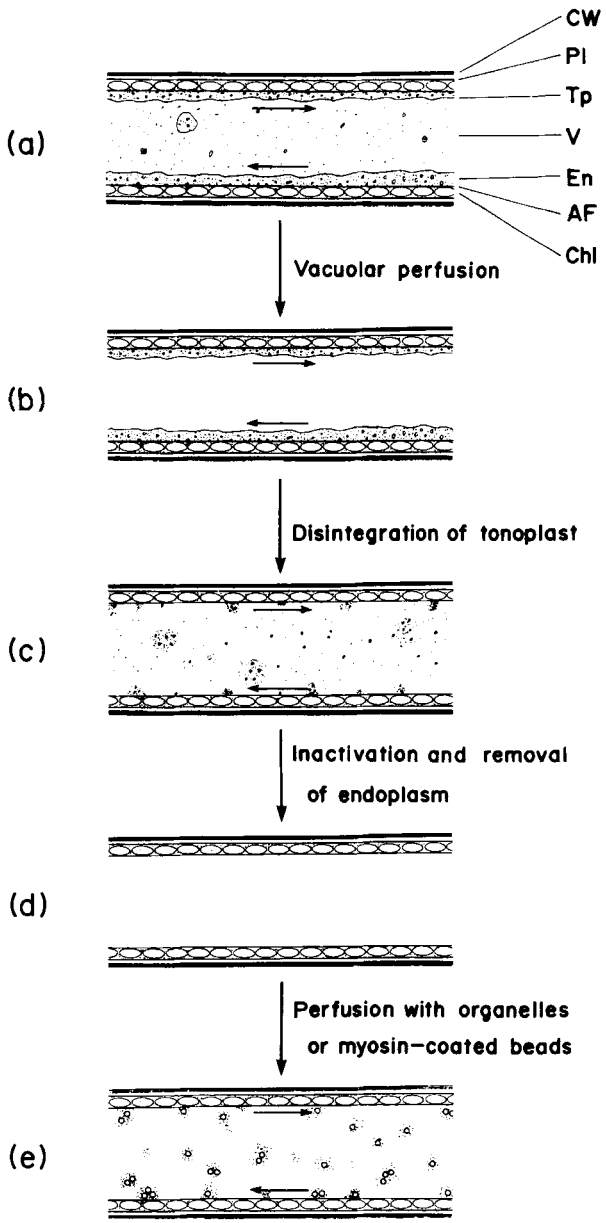

Fig. 1. Procedures for the induction of movement of myosin-coated beads or organelles along characean actin bundles. a: Intact cell. b: Vacuole-perfused cell. c: Tonoplast-free cell. d: Endoplasm-free cell. e: Reconstituted movement. CW, cell wall; Pl, plasmalemma; Tp, tonoplast; V, vacuole; En, endoplasm; AF, actin filaments; Chl, chloroplast. For further explanation, see the text.

Tazawa *et al.*, 1976). After the disintegration of the tonoplast, most of the endoplasm disperses, but some remains attached to actin bundles and continues to stream. By intracellular perfusion with a medium containing EDTA (Shimmen, 1978) or by treating the tonoplast-free cell with NEM (Chen and Kamiya, 1975), the endoplasm, which contains the putative characean myosin is inactivated. After the inactivated endoplasm is effused out by perfusion, beads coated with skeletal muscle myosin are introduced and both cell ends are closed by ligation to avoid the passive movement of intracellular fluid. Beads coated with rabbit skeletal muscle myosin move at a rate of 1–10 μm/sec (Sheetz and Spudich, 1983a, b ; Shimmen and Yano, 1984, 1986). The direction of bead movement is the same as that of cytoplasmic streaming (Sheetz and Spudich, 1983a). The success in moving purified skeletal muscle myosin along characean actin bundles strongly supports the hypothesis that the actomyosin generates the motive force of cytoplasmic streaming.

Ca^{2+} Regulation of myosin movement

Ca^{2+} regulation of the actin-myosin interaction is linked to either actin or myosin. Myosin-linked regulation is subdivided in two groups (Table 1). In skeletal muscle, myosin lacks Ca^{2+} sensitivity and Ca^{2+} regulation is mediated by troponin-tropomyosin which is closely associated with the actin filaments (Ebashi, 1980). The movement of beads coated with skeletal muscle myosin along characean actin bundles is independent of the Ca^{2+} concentration (Vale *et al.*, 1984 ; Shimmen and Yano, 1985 ; 1986). When the troponin-tropomyosin complex purified from skeletal muscle is incorporated into characean actin bundles, the reconstituted movement becomes sensitive to Ca^{2+}. Specifically, movement does not occur in the absence of Ca^{2+} but is activated at Ca^{2+} concentrations higher than 10^{-7} M (Shimmen and Yano, 1985, 1986). These results clearly demonstrate that Ca^{2+}-sensitizing mechanism is absent in characean actin bundles.

When a characean cell generates an actin potential at the plasmalemma, cytoplasmic streaming quickly stops and recovers slowly (Tazawa and Kishimoto, 1968). A transient increase in the concentration of cytoplasmic free Ca^{2+} causes the cessation of cytoplasmic streaming (Williamson and Ashley, 1982 ; Tominaga *et al.*, 1983). The

Table 1. Various mechanisms of regulation of actomyosin by Ca^{2+}

Mode of regulation	Example
Actin-linked regulation	Skeletal muscle(troponin-tropomyosin)[1]
Myosin-linked regulation	
Direct regulation	Scallop muscle(direct binding of Ca^{2+} to myosin)[2]
Indirect regulation	Vertebrate smooth muscle(myosin phosphorylation)[3]

[1] Ebashi (1980).
[2] Kendrick-Jones *et al.* (1970).
[3] Adelstein *et al.* (1982).

lack of Ca^{2+} sensitivity of the actin bundles strongly suggests that the Ca^{2+} sensitizing mechanism of characean cytoplasmic streaming is associated with the myosin present in the endoplasm.

The absence of Ca^{2+} sensitivity in characean actin filaments makes it possible to examined myosin-linked Ca^{2+} regulation by the reconstitution method.

Direct Ca^{2+} regulation

In scallop muscle, Ca^{2+} binds to the myosin light chain and activates the actin-activated myosin ATPase activity (Szent-Györgyi *et al.*, 1973). Latex beads coated with scallop myosin also slide along characean actin bundles. The movement does not occur in the absence of Ca^{2+} but is activated by calcium (Vale *et al.*, 1984; Kohama and Shimmen, 1985). When the myosin is desensitized by removing the regulatory light chains, the movement becomes insensitive to Ca^{2+}. Readdition of the regulatory light chains to the desensitized myosin reestablishes the Ca^{2+} sensitivity (Vale *et al.*, 1984).

The giant myosin thick filament which is 20 μm in length can be isolated from the anterior byssus retractor muscle of *Mytilus*. The myosin ATPase is activated upon the addtion of purified skeletal muscle actin (i.e., lacking troponin-tropomyosin). The actin-activated ATPase is dependent on Ca^{2+}, indicating that the myosin thick filamests have Ca^{2+} sensitivity. This is supported by the observation that the movement of the thick filaments of myosin along characean actin bundles is activated by Ca^{2+} (Yamada *et al.*, 1987).

Physarum myosin also exhibits Ca^{2+} sensitivity, however, the actin-activated ATPase is inhibited by Ca^{2+} (Kohama and Kendrick-Jones, 1986). Likewise, the movement of *Physarum* myosin along the characean actin bundle is also inhibited by Ca^{2+} (Kohama and Shimmen, 1985).

Indirect Ca^{2+} regulation

In vertebrate smooth muscle, contraction is also regulated by Ca^{2+}. When intracellular free Ca^{2+} is increased, Ca^{2+} puls calmodulin activates a myosin light chain kinase, resulting in the phosphorylation of the light chains of myosin which is the active form. The dephosphorylation of the light chains by myosin light chain phosphatase results in the inactivation of the myosin ATPase (Adelstein *et al.*, 1982). Movement of turkey gizzard muscle myosin along characean actin bundles occurs when the light chain is phosphorylated, but no movement occurs when the light chain is in the dephosphorylated form (Sellers *et al.*, 1985).

In *Dictyostelium*, actin-activated myosin ATPase is regulated by the phosphorylation of either the light chain or the heavy chain of myosin. The ATPase activity is enhanced by the dephosphorylation of the heavy chain and is inhibited by its phosphorylation (Kuczmarski and Spudich, 1980). The phosphorylation of the heavy chain is regulated by the Ca^{2+}-calmodulin complex (Maruta *et al.*, 1983). In addition, the actin-activated myosin ATPase of *Dictyostelium* is also regulated by the phosphorylation of the myosin light chain, although involvement of Ca^{2+} is not known. The actin-activated ATPase is high in the light chain-phosphorylated form but low in the

dephosphorylated form. *Dictyostelium* myosin with the phosphorylated light chains moves actively along characean actin bundles, however, myosin containing only dephosphorylated light chains moves slowly (Griffith *et al.*, 1987).

Acanthamoeba cells contain two types of myosins; single headed nonpolymerizable myosin, IA, IB, and double headed myosin II (Maruta and Korn, 1977; Maruta *et al.*, 1979). The actin-activated ATPase of myosin IA and IB is regulated by heavy chain phosphorylation. The activity is high in the phosphorylated form but low in the dephosphorylated form. The activity of the heavy chain kinase is insensitive to Ca^{2+} or the Ca^{2+}/calmodulin complex (Hammer *et al.*, 1983). In accordance with the actin-activated ATPase, beads coated with dephosphorylated myosin IA or IB can not move on characean actin bundles but those coated with phosphorylated myosin IA or IB can move (Albanesi *et al.*, 1985).

The above observation shows that the regulation of the actin-activated myosin ATPase by the direct binding of Ca^{2+} to the myosin molecule or by myosin phosphorylation is exactly reflected in the sliding movement of myosin along characean actin bundles.

Movement of exogenous organelles

Transfer of exogenous organelles onto characean actin bundles was first succeeded by Shimmen and Tazawa (1982). Organelles in the flowing endoplasm are isolated from *Chara* cells and are introduced into a *Nitella* cell whose endoplasm has been inactivated and effused out. Since the *Chara* organelles can move on *Nitella* actin bundles after the soluble fraction has been removed, it is concluded that myosin is bound to endoplasmic organelles in Characeae.

Adams and Pollard (1986) observed the movement of organelles of *Acanthamoeba* along *Nitella* actin bundles. The movement was inhibited by an antibody raised against myosin I but not by an antibody raised against myosin II. Some of the motile organelles were identified as phagocytic vesicles (Adams and Pollard, 1986). Thus, the reconstitution experiment clearly demonstrated the function of myosin I in the cell.

Active cytoplasmic streaming is observed in pollen tubes. The involvement of actomyosin in the generation of motive force is suggested by the fact that actin filaments are present in pollen tubes (Condeelis, 1973; Perdue and Parthasarathy, 1985) and that cytoplasmic streaming is inhibited by cytochalasin B (Franke *et al.*, 1972; Mascarenhas and Lafountain, 1972). However, information about the contribution of myosin is completely lacking as a consequence of the difficulty in isolating myosin. Organelles isolated from lily pollen tubes move actively along characean actin bundles in an ATP-dependent manner. The movement is inhibited by pretreating the pollen tube organelles with either NEM or heat. It was strongly suggested that 'myosin' is bound to pollen tube organelles (Kohno and Shimmen, 1988). The movement of pollen tube organelles along characean actin bundles is inhibited at Ca^{2+} concentration higher than 10^{-6} M (Kohno and Shimmen, 1988). Since characean

actin bundles lack Ca^{2+} sensitivity (Shimmen and Yano, 1986), it is concluded that the pollen tube myosin has an inhibitory type of myosin-linked Ca^{2+} regulation. Native cytoplasmic streaming in pollen tube is also inhibited by increasing the cytoplasmic Ca^{2+} concentration using a Ca^{2+} selective ionophore, A23187. In this study, it was found that the inhibition of cytoplasmic streaming by Ca^{2+} *in situ* is linked not only to myosin but also to actin (Kohno and Shimmen, 1987).

Velocity of Myosin and Organelle Movement

The velocities of myosin and organelle movement along characean actin bundles are summarized in Fig. 2. The velocity of movement of purified myosin along characean actin bundles is almost consistent with its actin-activated ATPase activity (Sheetz *et al.*, 1984; Vale *et al.*, 1984). An exception is the *Acanthamoeba* myosin I.

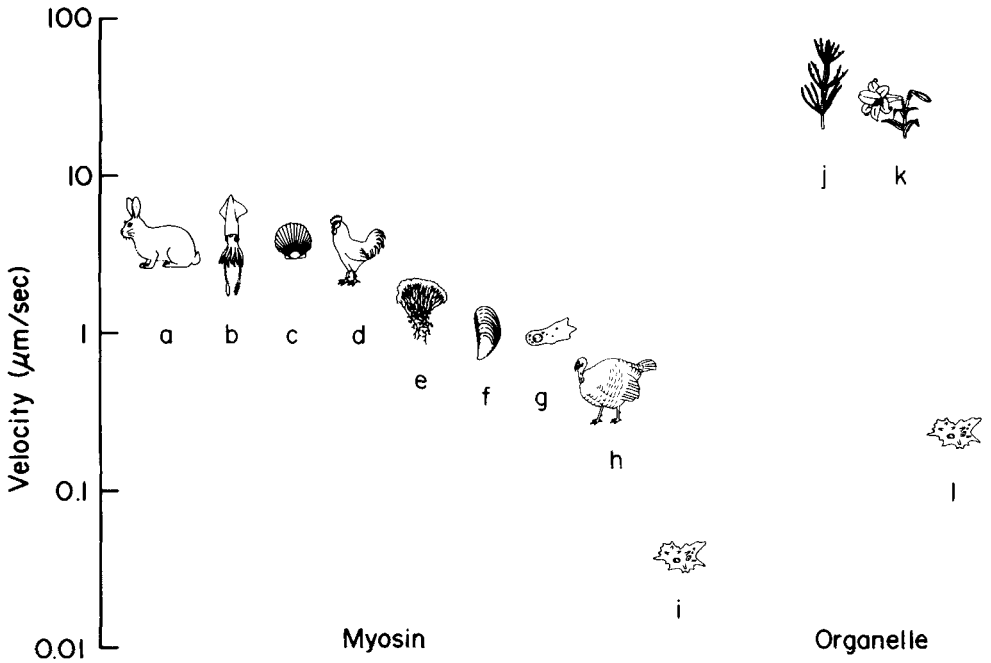

Fig. 2. Competition on characean actin bundles by various myosins and organelles. a: Skeletal muscle myosin of rabbit, *Oryctolagus* (Shimmen and Yano 1986). b: Siphon muscle myosin of squid, *Loligo* (Vale *et al.*, 1984). c: Adductor muscle myosin of scallop, *Placopecten* (Vale *et al.*, 1984). d: Breast muscle myosin of chicken, *Gallus* (Vale *et al.*, 1984). e: Plasmodium myosin of true slime mold, *Physarum* (Kohama and Shimmen 1985). f: Anterior byssus retractor muscle myosin of blue mussel, *Mytilus* (Yamada *et al.*, 1987). g: Amoeba myosin of cellular slime mold, *Dictyostelium* (Sheetz *et al.*, 1984). h: Gizzard smooth muscle myosin of turkey, *Meleagris* (Sellers *et al.*, 1985). i: Myosin I of *Acanthamoeba* (Albanesi *et al.*, 1985). j: Endoplasmic organelles of Characeae (Shimmen and Tazawa, 1982). k: Pollen tube oraganelles of lily, *Lilium* (Kohno and Shimmen, 1988). l: Organelles of *Acanthamoeba* (Adams and Pollard, 1986).

The unusual single head myosin I has a high ATPase activity but the velocity on characean actin bundles is very low (Albanesi *et al.*, 1985). Chicken myosin moved much faster than turkey myosin. However, it must be stressed that chicken myosin was isolated from skeletal muscle (Vale *et al.*, 1984) but the turkey myosin was supplied from gizzard smooth muscle (Sellers *et al.*, 1985).

The most striking feature in Fig. 2 is that plant 'myosin' moves much faster than animal myosin. The maximal sliding velocity between myosin and actin filaments in skeletal muscle contraction is about 6 μm/sec (Crowder and Cooke, 1984), which is almost the same as the velocity of skeletal muscle myosin along characean actin bundles. The velocity of native cytoplasmic streaming in pollen tube is about 5 μm/sec (Kohno and Shimmen, 1988). However, pollen tube organelles move at a rate of about 30 μm/sec on characean actin bundles. Three possibilities are suggested to account for the accelerated movement of pollen tube organelles on characean actin bundles. 1) Characean actin bundles are so thick that the pollen myosin can make good contact with actin filaments. 2) In pollen tubes, the cytoplasmic streaming in one direction is close to that in the opposite direction so that the rate of flow is diminished by the drag from the counter streaming. 3) The viscosity of the cytosol in pollen tubes may be higher than that of intracellular medium of the tonoplast-free cells. At any rate, it is clear that plant (Characeae and pollen tube) myosins have a capacity to move much faster than the animal myosins. Plant myosins may have intrinsically higher ATPase activity or perhaps their molecular organization on the organelles may be responsible for higher velocity. The importance of the molecular organization is suggested by the observation that *Acanthamoeba* organelles move 10 times faster than purified myosin I on characean actin bundles (Albanesi *et al*, 1985; Adams and Pollard, 1986).

Concluding Remarks

The biological role of the actomyosin system in plant and animal cells is the conversion of the chemical energy of ATP to a mechanical sliding force. Quantitative studies of actomyosin are usually carried out by measuring actin-activated myosin ATPase activity. The movement of myosin-coated beads or organelles along the characean actin bundle directly reflects the velocity of sliding between actin and myosin molecules. In the present method, it is possible to directly measure the actin-myosin sliding velocity *per se*, which is biologically important. Since characean actin bundles lack Ca^{2+}-sensitivity, it is possible to analyze myosin-linked Ca^{2+} regulation. By incorporating troponin-tropomyosin into characean actin filaments, actin-linked regulation can also be realized (Shimmen and Yano, 1985, 1986). The molecular mechanism of actin-myosin sliding can also be studied with the present method using subfragments of myosin (Hynes *et al.*, 1987), or monoclonal antibodies raised against different sites of the myosin molecule (Flicker *et al.*, 1985).

In addition to the present method, two kinds of reconstitution systems have been developed using purified actin. In the first method, actin filaments are polarized on

a carbon-coated electron microscope grid using severin which binds to the barbed end of the actin filaments. Myosin-coated beads can move along these actin filaments (Spudich *et al.*, 1985). In this method, severin must be purified from *Dictyostelium* to orientate actin filaments. In the second method, single actin filaments labeled with a fluorescent dye can move along myosin that has been immobilized on a glass surface (Kron and Spudich, 1986). Although the characean actin system lacks complete biochemical characterization, it has the following advantages. Since the polarity of the actin bundles is known in relation to the direction of cytoplasmic streaming, the direction of movement of foreign myosins and organelles in relation to the polarity of the actin filament is easily determined. Furthermore, the movement can be observed with an ordinary light microscope. Using the present method, we can analyze the sliding velocity of myosin bound to organelles without purification. This is the most obvious advantage of this reconstitution method. We did not purify myosin, but concluded that pollen tube organelles are associated with myosin. Critically speaking, it is functionally myosin, since it slides along actin bundles using the hydrolysis energy of ATP. In intestinal microvilli, the function of the 110 kD protein-calmodulin complex was not known. Since the protein complex can move on characean actin bundles, it was suggested that it is a translocator along actin filament (Conzelman and Mooseker, 1987). Using the sliding activity along microtubules, a new translocator protein along microtubule, kinesin, was discovered in addition to dynein (Vale *et al.*, 1985). A similar approach in studying actin-based motility may be possible using the reconstituted movement on characean actin bundles.

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