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Interaction of pollen F-actin with rabbit muscle myosin and its subfragments (HMM, S_1)

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Summary. Actin purified from maize pollen grains can be polymerized into F-actin which increased the ATPase activities of proteolytic fragments (HMM, S_1) of rabbit muscle myosin. The values of K_{app} is 232 μM for HMM and 290 μM for S_1, which are six- and seven-fold higher than those of rabbit muscle F-actin under the same conditions. Pollen actin and rabbit muscle myosin form hybrid actomyosin showing increase in viscosity and turbidity of solution. Viscosity and turbidity of the actomyosin dropped and then increased again with addition of ATP. Polymerized pollen actin can be decorated in vitro with both rabbit muscle HMM and S_1 to form an arrowhead-shaped structure like that observed in living plant cells. The results show that pollen actin is similar to muscle actin at a qualitative level. But there are differences between them at a quantitative level.

Keywords: Pollen actin; Rabbit muscle myosin; Heavy meromyosin; Myosin subfragment 1.

Abbreviations: HMM heavy meromyosin; S_1 myosin subfragment 1; ATP adenosine-5'-triphosphate.

Introduction

Interaction of actin filaments with myosin coupled with ATP hydrolysis is the fundamental process for generation of force to drive actin-dependent intracellular motility such as muscle contraction, cytoplasmic streaming and organelle movement in nonmuscle cells. Kinetic analysis of this process has been performed in animal and fungal cells (Fraser et al. 1975, Gordon et al. 1976, Margossian and Lowey 1978, Hatano et al. 1980, Stein et al. 1981, Greene et al. 1983, Katoh et al. 1984). Actin is widely identified in many plant tissues (Staiger and Schliwa 1987) and has been purified from some plant cells (Andersland and Parthasarathy 1992, Villanueva et al. 1990, Liu and Yen 1992). But the biochemical characterization of plant actin is inadequate. It is now believed that some intracellular movements in plant cells are driven through interaction between actin and myosin (Heslop-Harrison and Heslop-Harrison 1989). However, interaction between plant actin and myosin is poorly studied to date because of the difficulty in obtaining sufficient quantities of purified plant actin and myosin for experimental analysis. We have developed a biochemical procedure of purifying biologically active actin from maize pollen grains, the yield of which is enough for its biochemical characterization (Liu and Yen 1992, Yen et al. 1992). Here we report interaction of purified pollen actin with rabbit muscle myosin and its subfragments, which is an extension to our previous study on pollen actin.

Materials and methods

Preparation of proteins

Rabbit skeletal muscle actin was purified by the method of Spudich and Watt (1971). It was further purified by chromatography through a 2 × 90 cm column of Sephacryl S-200 (Pharmacia). Pollen actin was prepared by the method as described previously (Liu and Yen 1992). Both pollen and muscle actin were electrophoretically pure and were dissolved in Tris buffer (pH 8.0) which contained 2 mM Tris, 0.1 mM $CaCl_2$, 0.05% NaN_3, 0.75 mM mercaptoethanol, and 0.2 mM ATP. Rabbit muscle myosin was prepared by the method of Kielly and Harrington (see Pollard 1982). Heavy meromyosin (HMM) was prepared by digestion of rabbit muscle myosin with trypsin using the method of Margossian and Lowey (1982). Rabbit muscle myosin subfragment 1 (S_1) was prepared by cleaving myosin with papain according to the method of Margossian and Lowey

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(1982). HMM and S_1 were further purified through DEAE-cellulose chromatography.

Determination of concentration of proteins

Concentrations of proteins were determined by spectrophotometry, using extinction coefficients as follows:
$E^{1\%}_{290}$ = 6.2/cm for actin, $E^{1\%}_{280}$ = 5.3/cm for myosin,
$E^{1\%}_{280}$ = 8.1/cm for S_1, and $E^{1\%}_{280}$ = 6.0/cm for HMM.

Assay of ATPase

ATPase activities of both HMM and S_1 were determined according to Margossian and Lowey (1978). The concentration of P_i liberated from ATP by the enzymes was determined according to the method of Le Bal et al. (1978).

Viscometry

Viscosity was measured in a water bath (20 °C) with an Ostwald viscometer with capacity of 4.0 ml. The outflow time was 29.80 s for buffer. Relative viscosity was determined as the ratio of the outflow time of sample to that of buffer.

Measurement of turbidity

Change in turbidity was determined by measuring O. D. at wavelength of 420 nm in a Shimadzu UV-240 spectrophotometer.

Electron microscopy

Samples were negatively stained by the improved method of U. Aebi (see Cooper and Pollard 1982). Pollen actin was polymerized at room temperature for 2 h by addition of KCl and $MgCl_2$ to concentrations of 100 mM and 2 mM, respectively. It was diluted to final concentrations of 0.05 mg/ml and was applied onto carbonized formvar-covered grids. About a minute later a drop of HMM or S_1 was added onto the grid. Then it was stained with 1% uranyl acetate for 30 s. Grids were observed in a JEM 100 electron microscope at 80 kV.

Results

Activation of HMM ATPase

One of the very important properties of actin is its activation of myosin ATPase activity. Figure 1 shows that the activity of HMM ATPase was evidently activated by pollen F-actin. The relationship between activation of HMM ATPase and concentration of F-actin was almost linear. Figure 2 shows double reciprocal plots of HMM ATPase activities vs. concentrations of actin from which kinetic parameters of activation of HMM ATPase by pollen actin could be deduced. K_{app} is the concentration of actin half of that required for maximal activation. The K_{app} value deduced from Fig. 2 is 232 μM which is six fold higher than that of rabbit muscle actin (Table 1). It implies that pollen actin is less effective in activating rabbit muscle HMM ATPase than rabbit muscle actin under the same conditions. Other nonmuscle actin such as

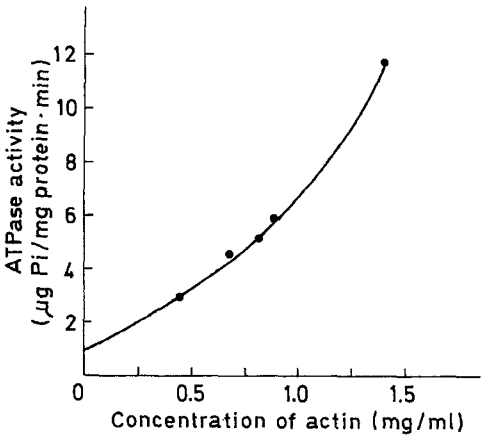

Fig. 1. Activation of Mg ATPase of rabbit muscle heavy meromyosin by pollen F-actin at various concentrations. The ATPase activity was analysed in buffer containing 10 mM Tris (pH 7.9), 2.5 mM ATP, 2.5 mM $MgCl_2$, and 25 mM KCl at 23 °C. The concentration of HMM was 0.15 mg/ml

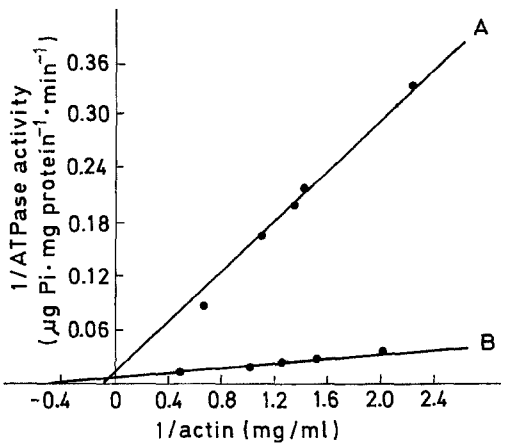

Fig. 2. Activation of Mg ATPase of rabbit muscle HMM by pollen and muscle F-actin. Double reciprocal plots are shown for activation as a function of actin concentration at room temperature (23 °C) in buffer containing 10 mM Tris (pH 7.9), 2.5 mM ATP, 2.5 mM $MgCl_2$, and 25 mM KCl at 23 °C. The concentration of HMM was 0.15 mg/ml. *A* Pollen F-actin, *B* rabbit muscle F-actin

Table 1. Activation of Mg ATPase of HMM and S_1 by pollen F-actin in comparison with rabbit muscle F-actin

	HMM		S_1	
	K_{app}[a]	V_{max}[b]	K_{app}[a]	V_{max}[b]
Pollen actin	232.6	112.1	290.0	166.7
Muscle actin	38.7	112.1	38.7	166.7

[a] Values are μM
[b] Values are μM

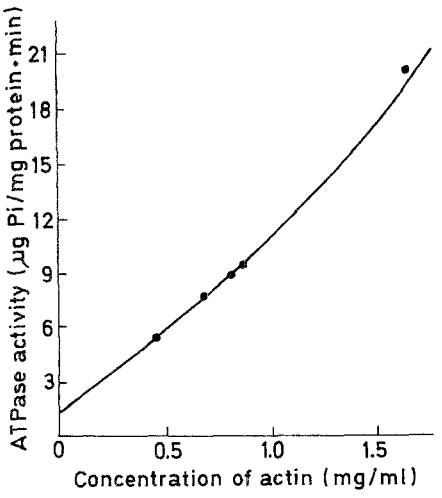

Fig. 3. Activation of MgATPase of rabbit muscle S_1 by polymerized pollen actin in various concentrations. The ATPase activity was measured in solution containing 10 mM Tris (pH 7.9), 2.5 mM ATP, 2.5 mM $MgCl_2$, and 25 mM KCl at temperature of 23 °C. The concentration of S_1 was 0.1 mg/ml

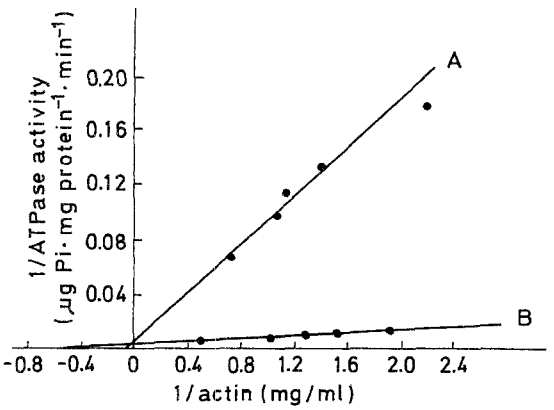

Fig. 4. Activation of Mg ATPase of rabbit muscle S_1 by polymerized pollen and rabbit muscle actins. Double reciprocal plots are shown for activation as a function of actin concentration at room temperature (23 °C) in buffer containing 10 mM Tris (pH 7.9), 2.5 mM ATP, 2.5 mM $MgCl_2$, and 25 mM KCl. The concentration of HMM was 0.15 mg/ml. *A* Pollen F-actin, *B* rabbit muscle F-actin

Acanthamoeba actin is also less effective in its activation of muscle HMM than muscle actin (Gordon et al. 1976). But pollen actin was the least effective in activation of HMM ATPase among all nonmuscle actins which have been reported so far.

Activation of myosin subfragment 1 (S_1) ATPase activity

The activity of S_1 ATPase was markedly activated by polymerized pollen actin just like the case of HMM.

Table 2. Mg ATPase activity of rabbit muscle HMM and S_1 in the presence of pollen F-actin in comparison with rabbit muscle F-actin

	HMM	S_1
Pollen actin (1.25 mg/ml)	8.33	13.15
Muscle actin (1.25 mg/ml)	56	125

Values are μg P_i/mg protein × min

Figure 3 indicated that the relationship between S_1 ATPase activity and concentration of pollen F-actin was linear. Figure 4 shows double reciprocal plots of S_1 ATPase activities vs. concentrations of pollen actin. The K_{app} value of pollen F-actin was 290 μM, which is about seven fold of that of rabbit muscle F-actin (Table 1). The ATPase activities of HMM and S_1 in the presence of pollen or muscle F-actin are shown in Table 2. It indicates that HMM ATPase activity was lower than that of S_1 in the presence of pollen F-actin. This was similar to the case of the other nonmuscle F-actins. But both HMM and S_1 ATPase activities were less activated by pollen F-actin than by muscle or other nonmuscle F-actins (Gordon et al. 1976).

Viscosity change

When pollen F-actin was mixed with rabbit muscle myosin the viscosity of the solution increased markedly (Fig. 5 A). It implies that they formed a hybrid actomyosin. If ATP is added to the muscle actomyosin solution the viscosity decreased rapidly to the level of initial clearing phase and again increased rapidly. When ATP was again added to the hybrid actomyosin viscosity dropped rapidly to the value which was lower than that of the initial clearing phase and then increased to the value which was less than that at the initial stage. After three cycles of binding and dissociation the viscosity of the hybrid actomyosin solution containing pollen F-actin and rabbit muscle myosin was much less than that at the initial stage. The differential viscosity after addition of ATP reduced with each cycle, however, the viscosity of muscle actomyosin solution containing rabbit muscle F-actin and myosin was almost the same as that at the initial stage (Fig. 5 B). It shows that pollen F-actin lost ability to bind to rabbit myosin with each cycle of binding and dissociation. In other words, with each cycle less and less pollen F-actin combined with myosin. But rabbit muscle F-actin retained the same ability to combine with myosin after a cycle of binding

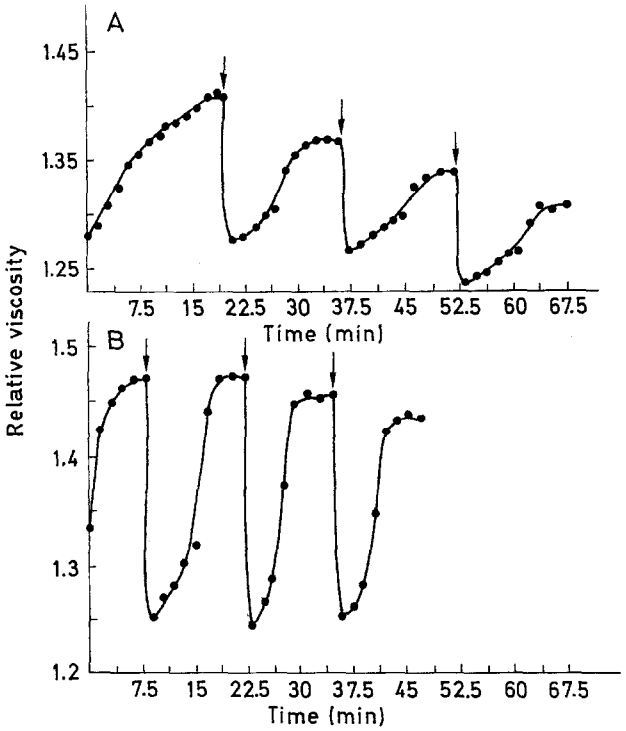

Fig. 5. The viscosity change of actomyosin at 20 °C which showed reversible binding of F-actin with myosin. 1 ml of F-actin (1.0 mg/ml) was mixed with 3 ml of myosin (0.6 mg/ml). The mixed solution contained 0.25 mg/ml F-actin, 0.45 mg/ml myosin, 0.4 M KCl, 8 mM Tris, and 0.5 mM $MgCl_2$. Arrows indicate positions at which ATP was added. The concentration of ATP was 20 mM. The volume of ATP added was 10 µl. **A** Pollen F-actin and rabbit muscle myosin. **B** Rabbit muscle actin and myosin

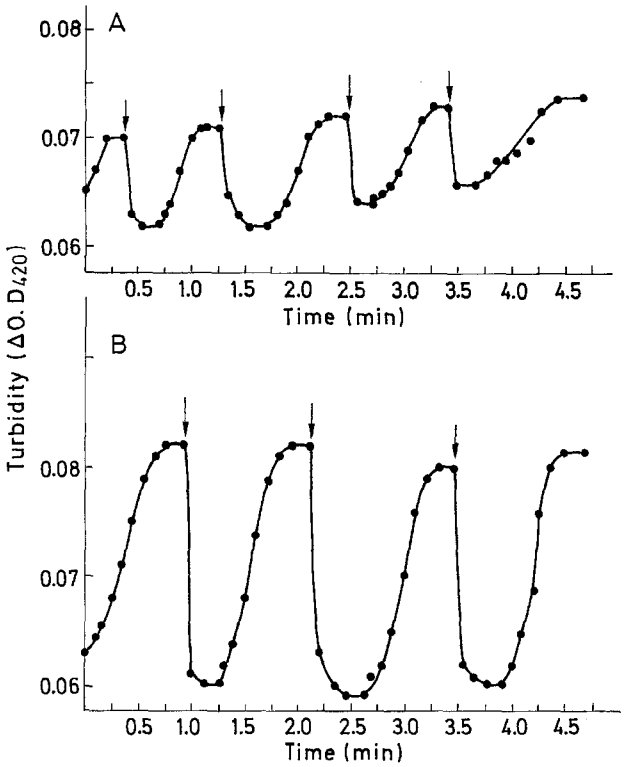

Fig. 6. The turbidity change of actomyosin at 20 °C which showed reversible binding of F-actin with myosin. 1 ml of F-actin (1.0 mg/ml) was mixed with 3 ml of myosin (0.6 mg/ml). The mixed solution contained 0.25 mg/ml F-actin, 0.45 mg/ml myosin, 0.4 M KCl, 8 mM Tris, and 0.5 mM $MgCl_2$. The concentration of ATP was 20 mM. The volume of ATP added was 10 µl. **A** Pollen F-actin and rabbit muscle myosin. **B** Rabbit muscle actin and myosin

and dissociation. Figure 5 also shows that less rabbit myosin was bound to pollen F-actin than to rabbit muscle F-actin under the same conditions.

Turbidity change

Measurement of turbidity change is another method to analyze combination of myosin with F-actin. When pollen F-actin was mixed with myosin the turbidity increased rapidly. It dropped and increased again as ATP was added to the solution just like the case of viscosity change. Figure 6 shows the turbidity change of actomyosin in the presence or absence of ATP. Turbidity of actomyosin which consisted of pollen actin and rabbit muscle myosin did not decrease to the level of the initial clearing phase but levels above that of the initial clearing phase with repeated addition of ATP. It again increased rapidly to the value which was higher than that at the initial stage. The differential turbidity after addition of ATP was less and less with each cycle. This also implied that pollen F-actin lost its ability to bind with rabbit muscle myosin which was in accordance with the result obtained by viscometry. On the other hand, rabbit muscle F-actin was still unaffected in combining with myosin after three times of addition of ATP.

Electron microscopy of decoration of pollen F-actin with HMM and S_1

When polymerized pollen actin was treated with rabbit muscle HMM typical arrowhead-shaped complexes could be observed, which was similar to other non-muscle actin (Fig. 7 A). Pollen F-actin was also decorated by S_1 showing arrowheaded structure on actin filaments which was similar to that decorated by HMM (Fig. 7 B). It can be seen from Fig. 7 that polymerized pollen actin was less cleanly decorated by S_1 than HMM under the same conditions. Decoration of pollen F-actin by S_1 (Fig. 7 B) is similar to that of the other plant F-actin (Andersland and Parthasarathy 1992). Furthermore, the in vitro polymerized pollen

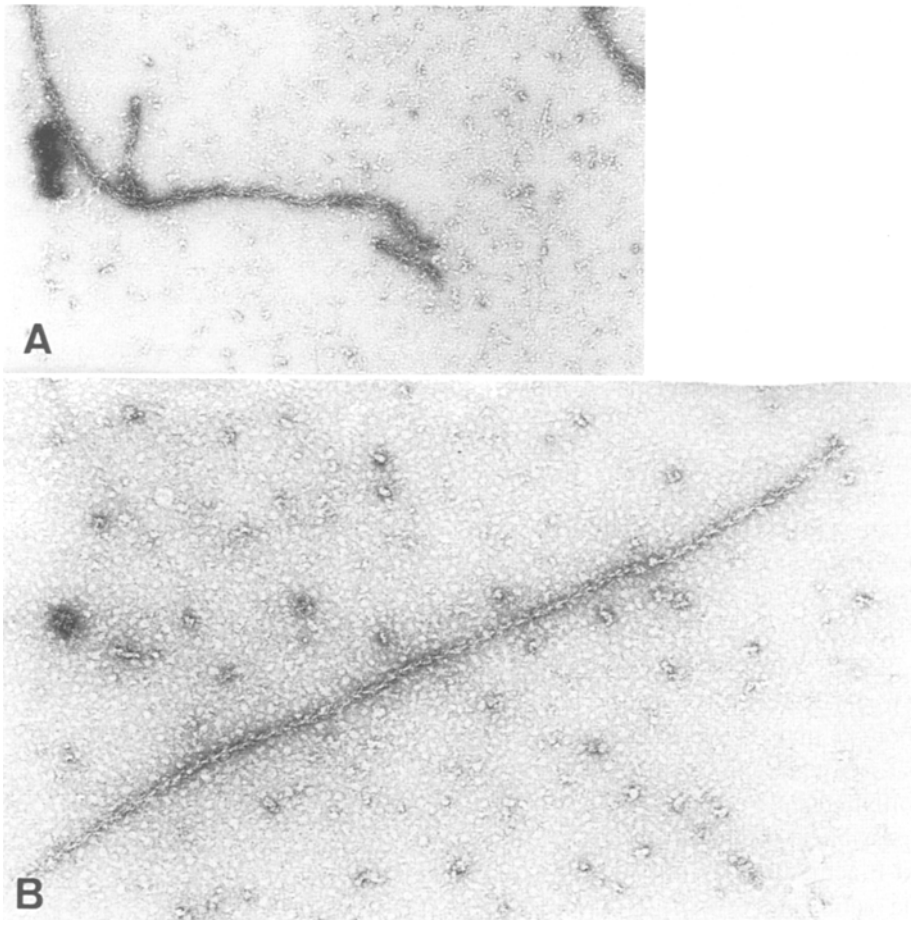

Fig. 7. Electron micrographs of decoration of polymerized pollen actin with HMM (**A**) and S_1 (**B**). Samples were negatively stained with 1% uranyl acetate. The arrowheaded structures on F-actin formed after it was mixed with HMM or S_1. A, × 50,000; B, × 75,000

actin was decorated by HMM as they are in living cells (Condeelis 1974).

Discussion

Increasing importance has been placed on the functions of actin in plant cells in recent years (Staiger and Schliwa 1987). Although actin has been studied in many plant tissues, the progress in its biochemical and biophysical characterization is relatively slow due to the difficulty in obtaining enough amount of plant actin for this purpose. Actin has been highly conserved during evolution. But Hennessey et al. (1993) concluded that animal and plant actins form distinct groups according to their structures and properties. Animal and fungal actins have been characterized in detail, but plant actin remains poorly understood. Pollen actin, a representative of plant actin, certainly has properties which are different from those of actins from other sources. Hepler et al. (1993) demonstrated that animal actin, though it can work in fungal cells, cannot copolymerize with plant actin to assemble actin filaments when it is microinjected into living plant cells. This means that there does exist an appreciable difference between plant and animal actins. In this sense, it is worth investigating how plant actin is different from animal actin.

This study compared the binding of rabbit muscle myosin to pollen and muscle F-actins and assayed kinetics of activation of rabbit muscle HMM and S_1 by pollen F-actin. It aimed at further characterization of plant actin. Results in this paper indicate that pollen actin has similar physicochemical properties to those of rabbit muscle actin. For example, it can interact with muscle myosin like muscle actin. Other properties which were described in our previous papers (Liu and Yen 1992, Yen et al. 1992) are also similar to those of muscle actin. But on the other hand, there are differences at a quantitative level between plant actin and muscle actin. We once assayed the activation of rabbit muscle myosin ATPase by polymerized pollen actin and found it to be the least effective among non-muscle actin reported so far. The kinetics of its activa-

tion of HMM and S_1 ATPases indicates that pollen F-actin was less effective than muscle and *Acanthamoeba* actin (Gordon et al. 1976). The ability of pollen F-actin to combine with muscle myosin was also lower than muscle actin under the same conditions. In addition to this, we noticed in our laboratory that pollen F-actin was less cleanly decorated by HMM and S_1 than muscle or Physarum F-actin under the same conditions. Therefore, we believe that the difference between plant actin and muscle actin is bigger than that between fungal and muscle actin.

The results presented here seem to suggest that pollen F-actin is not quite fit to cooperate with rabbit muscle myosin, i.e., the efficiency is low when they work together. In living plant cells the counterpart of actin is plant myosin. Plant actin and myosin may match well to fulfil their physiological functions in cell. Plant myosin which was identified in several plants, unlike actin, is not highly conserved during evolution (Ma and Yen 1989, Tang et al. 1990, Vahey et al. 1982). It will be very interesting to purify plant myosin and investigate the interaction of plant myosin with plant actin. The biochemical procedures of pollen myosin purification was reported recently (Yokota and Shimmen 1994). So it is possible at present to study the in vitro sliding of plant F-actin along plant myosin like the case of muscle actin and myosin (Yanagida et al. 1986, Toyoshima et al. 1989) and then to get a fundamental understanding at the molecular level of the mechanism of interaction between plant actin and myosin in living cells. It will also provide information on the difference between the mechanism of actin-based cell motility in plant cells and that in animal cells. We think that present work on plant actin and myosin has opened a way to systematic study on the mechanism of actin-based cell motility in plant cells.

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