CMLS, Cell. Mol. Life Sci. 56 (1999) 227–232
1420-682X/99/040227-06 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Myosins from plants

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Abstract. Molecular cloning and sequence analysis of myosin genes from *Arabidopsis thaliana* and electron microscopic observation of a myosin from characean alga have revealed that overall structure of plant unconventional myosins is similar to that of the class V myosins. These plant unconventional myosins have two heads, a coiled-coil tail of varied length and a globular tail piece at the end. The tail piece is probably a site for membrane interaction. Characean myosin is of special interest because it can translocate actin filaments at a velocity several times faster than muscle myosin, which must have evolved to support the quick movement of animals in the struggle for their lives.

Key words. Plant; myosin; myosin family; unconventional myosin; motor protein; cytoplasmic streaming; sliding velocity.

Kuhne originally described his finding that he could extract protein from frog muscle with a concentrated cooking salt solution in his book published in 1864 [1]. He named the protein myosin, which means a protein from muscle (*myos* in Greek). Seventy-five years later, it was found that myosin has adenosine triphosphate (ATP) hydrolyzing activity [2]. It is now generally accepted that myosin is a mechanochemical enzyme that has a catalytic domain (head) which produces force by cyclic interaction with actin and hydrolysis of ATP. Recent studies have identified a number of proteins which have regions homologous to the catalytic domain of muscle myosin (conventional myosin or myosin II) [3–5]. Some of these new proteins were able to translocate actin filaments, just like muscle myosin. It is considered, therefore, that myosins are a family of mechanochemical enzymes that hydrolyze ATP and support many important motile activities in both animal and plant cells.

Myosins from plants seem to be involved in many motile activities in plant cells, including cytoplasmic streaming. This streaming transports essential molecules and organelles to distribute them homogeneously in large plant cells. As the ordered structure of striated muscle facilitated the understanding of the fundamental mechanism of muscle contraction (the sliding filament theory) [6, 7], simple circulatory cytoplasmic streaming in characean alga has provided useful information about the fundamental mechanism of cytoplasmic streaming. Shown in figure 1 is a drawing of *Chara corallina* grown in our laboratory. Between the branched nodes are internodal cells. The internodal cells are giant cylindrical cells with lengths up to 20 cm and diameters up to 1 mm. The cytoplasm of internodal cells flows in a direction nearly parallel to its long axis. It goes up along one hemicylinder to the node and comes down along the other. There exist areas between the two hemicylinders where the direction of flow reverses (neutral zones). Kamiya and Kuroda deduced that the motive force of streaming is generated near chloroplasts lining the cytoplasmic face of the plasma membrane [8]. It was found later that there are arrays of cables on the surface of chloroplasts that run parallel to the direction of streaming [9]. These cables turned out to be bundles of actin filaments [10] having the same polarity [11]. Figure 2 shows fluorescence-labeled actin bundles on chloroplasts in *C. corallina*. It is not so hard to imagine that cytoplasmic streaming is generated by myosin-like motor proteins which move on the actin bundles. Hydrodynamic considerations suggested that the myosin should move either with an attached membrane vesicle or endoplasmic reticulum to produce bulk

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streaming [12]. Figure 3 illustrates schematically the geometrical relationship between the chloroplasts, the actin bundles, plant myosins and membrane vesicles in characean cells.

In this review, we will briefly mention conventional myosin (myosin II) from plants and describe the recently identified unconventional myosins.

Plant conventional myosin

In the days when only conventional myosin was known, researchers applied the same methods used for the purification of muscle myosin to plant tissue. Plant tissue was homogenized in a high ionic strength buffer solution, and the extract was dialyzed against a low ionic strength buffer solution. The precipitate that formed was collected by centrifugation and dissolved in a high ionic strength buffer solution. This method exploits the filament-forming property of muscle myosin. It forms thick filaments at low ionic strength, and these filaments dissolve into myosin monomers at high ionic strength. Myosin monomers again form thick filaments when diluted with water or dialyzed against a low ionic strength buffer solution. This property, together with its abundance in muscle, provided a very simple purification procedure. Myosins were prepared in this way from *Nitella flexilis* [13], *Egria densa* [14] and tomato [15].

Peptides with a wide variety of molecular weights were detected immunochemically in various plants using antibodies against animal conventional myosin [16–19]. It should be noted, however, that myosin antibody does not always cross-react with myosin from other species. Thus it requires quite fortuitous conditions for an

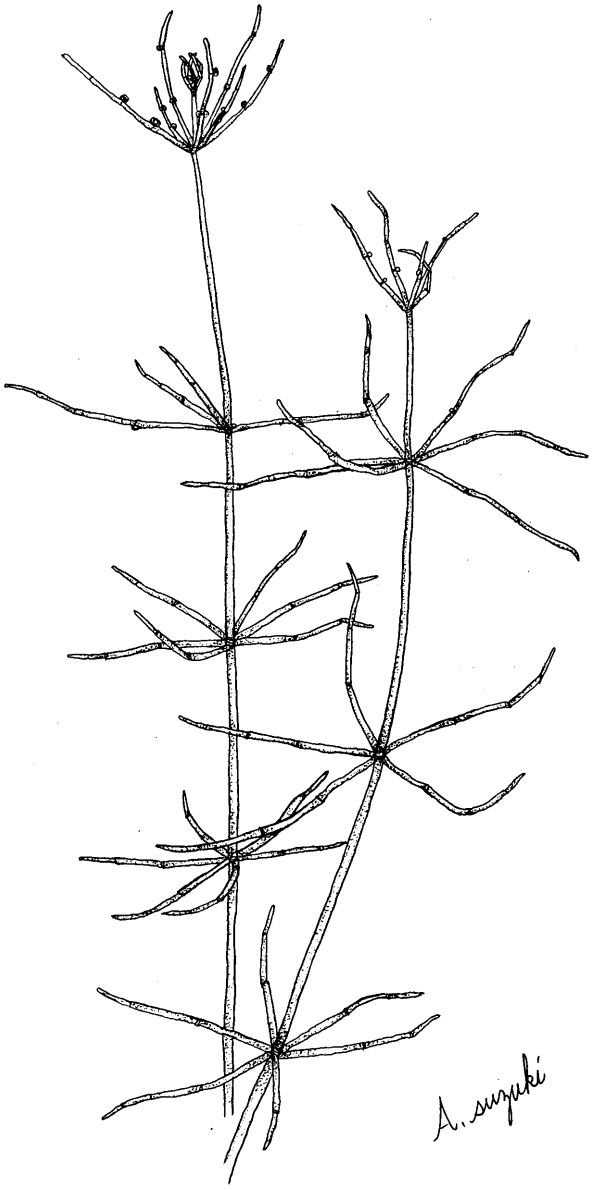

Figure 1. *C. corallina*. Between branched nodes are large internodal cells.

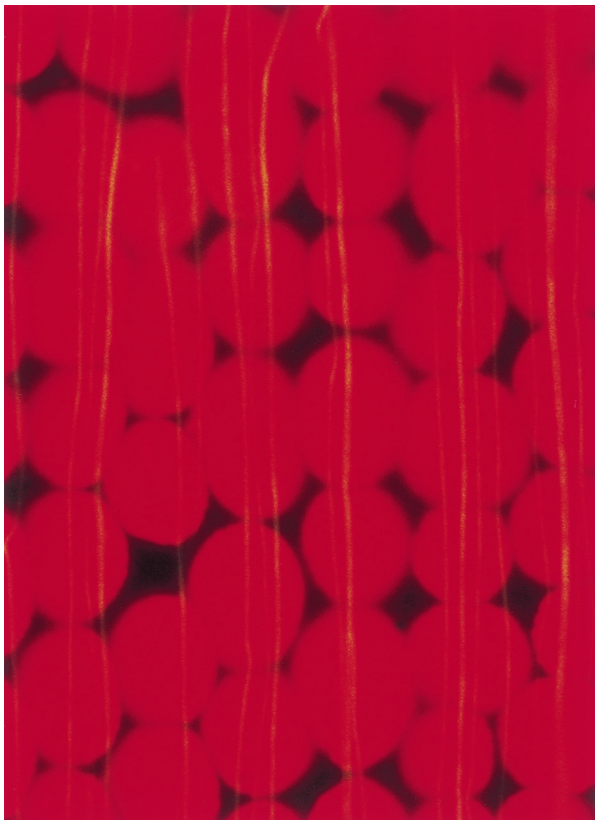

Figure 2. Actin cables on chloroplasts. Actin cables were labeled with fluorescein isothiocyanate-conjugated rabbit heavy mero-myosin. Their parallel alignment is somewhat distorted during this labeling process. Red fluorescence of chloroplasts is due to chlorophyll.

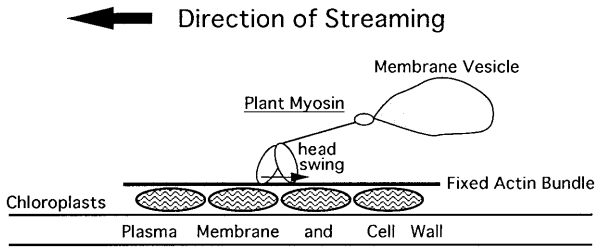

Figure 3. Mechanism of cytoplasmic streaming in characean cells.

antibody against animal myosin to cross-react with myosin from another kingdom. An anti-pan myosin antibody raised against conventional myosin heavy chain from mouse 3T3 cells is known to recognize almost all muscle and nonmuscle conventional myosin. However, Yokota and Shimmen reported that no component in crude extract of lily pollen tube was recognized by the antibody [20]. The same antibody also did not recognize any components in crude extract of *C. corallina* [K. Yamamoto, unpublished result].

Plant unconventional myosins

The discovery of the unconventional myosin, myosin I (single-headed myosin), having a special globular tail structure capable of interacting with membranes [21], reminded researchers of the importance of the interaction between plant myosin and membrane. Such a myosin does not form filaments at low ionic strength due to the globular structure at its tail and cannot, therefore, be purified by the same procedure used for muscle myosin. Chromatographic separation must be employed instead [20, 22]. Coprecipitation with F-actin is effective if myosin is properly protected from denaturation in the absence of ATP [23]. Plant unconventional myosin seems to have a tendency to stick to glassware and dialysis membranes. It is advisable to use plastic ware throughout the purification procedure.

Active plant unconventional myosin fractions have been detected using the in vitro motility assay [24]. This assay utilized fluorescencently labeled actin filaments to identify myosin activity. When myosin was attached to a cover slip, the fluorescent actin filaments were translocated by these myosin molecules on the glass surface if ATP was supplied. Movement of actin filaments can be observed under a fluorescence microscope. Images were taken by a charge connected diode (CCD) camera with an image intensifier, and the velocity of each actin filament was recorded and analyzed. There are many ATPases in crude plant cell extract. This motility assay detects only the motile activity of myosin and is not disturbed by the presence of other ATPases.

Proteolytic degradation is another obstacle that must be overcome to purify plant unconventional myosins. Proteolytic enzymes are quite enriched in the plant vacuole. The large cylindrical characean internodal cells are advantageous in this respect. Their vacuole can be perfused [25] with a solution containing protease inhibitors [22]. The large size of characean internodal cells also allows one to obtain an adequate amount of cytoplasm, which facilitated the biochemical purification of unconventional myosin [22]. Kohno et al. used lily pollen tube just after germination as another way to avoid proteolytic degradation of plant unconventional myosin since the vacuole is not yet fully developed [26]. However, polyacrylamide gel electrophoresis revealed that their preparation contained three polypeptides with molecular weights of 170, 145 and 120 kDa. All three peptides were recognized by antibodies raised against the 170- and 145-kDa peptides, suggesting that the 145- and 120-kDa polypeptides are degradation products of 170 kDa [20].

Biochemical characterization of plant unconventional myosins

The molecular weight of plant unconventional myosin heavy chains obtained biochemically so far differ considerably. The myosin heavy chain from lily pollen tube has a molecular weight of 170 kDa, as mentioned above [20], whereas that from characean alga is 230 kDa [22, 23]. The characean myosin is a unique two-headed unconventional myosin (fig. 4 and [36]), but the shape of lily pollen tube myosin has not yet been observed. Myosin heavy chains with a molecular weight of about 170 kDa were detected in other higher plants by using antibody against lily pollen tube myosin [27, 28], but this antibody did not recognize characean myosin [T. Shimmen, personal communication]. Similarly, an antibody directed against the characean myosin did not recognize any components in crude extracts of several higher plants, including lily pollen tube [K. Yamamoto, unpublished observation].

Myosins I and V in plants

Antibodies against myosins I, II and V detected polypeptides having molecular weights of 125, 205 and 190 kDa, respectively, in crude extracts of *Lilium* and *Nicotiana* pollen tubes [29]. However, as noted before, the antibodies used in this study were those raised against animal myosins. It was not shown that these polypeptides recognized by the antibodies had any of the characteristics of myosin, i.e. coprecipitation with F-actin in the absence of ATP or being labeled with ATP by ultraviolet (UV) irradiation. Sequence analysis

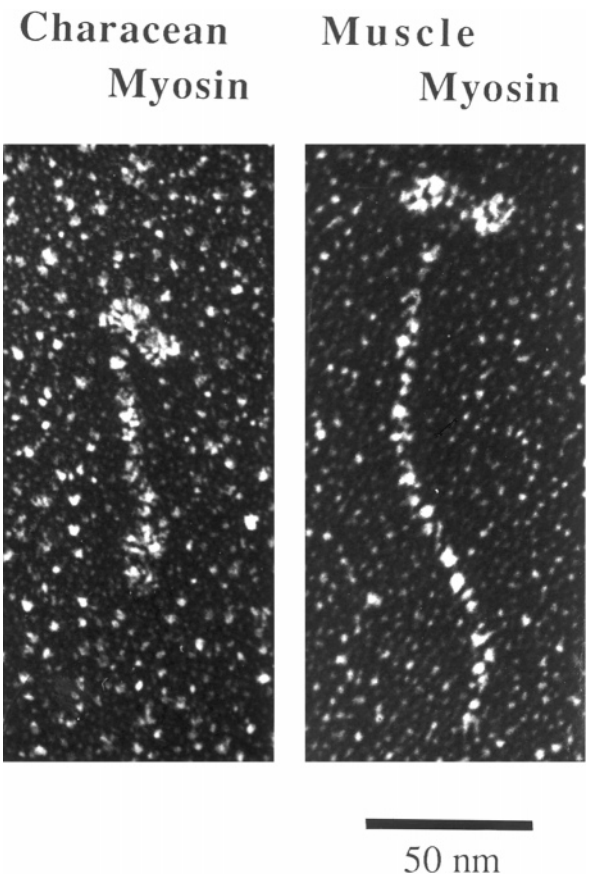

Figure 4. Electron micrograph of characean myosin. Characean myosin (left) has two heads with similar size and shape to those of muscle myosin (right, courtesy of N. Kanzawa), but it has a short tail and a globular tail piece at its end.

of *Arabidopsis* myosin genes indicated that their overall structures are related to that of myosin V [30], but there is no evidence for the presence of genes closely related to those of myosins I and II.

ATPase activity of plant unconventional myosin and its regulation by Ca^{2+}

The plant unconventional myosins obtained so far had very low ATPase activity compared with that of muscle myosin. The activity in the presence of Mg (Mg-ATPase activity) was 1 to 2 nmol Pi/min mg [20, 22] compared with 40–60 nmol Pi/min mg for rabbit skeletal muscle myosin. The Mg-ATPase activity was activated about 100-fold by the addition of saturating amounts of rabbit F-actin [20, 22]. The extent of the activation was similar to that of muscle myosin. Plant unconventional myosins are so labile that it is difficult to estimate the actual fraction of intact myosin in the final preparations. Thus true ATPase activity may not be so different from that of muscle myosin.

Since cytoplasmic streaming in plants is regulated by Ca^{2+} concentration [31, 32], the effects of Ca^{2+} on the activity of plant myosin were investigated. Yokota and Shimmen reported that the activity of lily pollen tube myosin is sensitive to Ca^{2+} [33]. They also detected immunochemically that their myosin fraction contained calmodulin (CaM) and suggested the possibility that CaM is a light-chain component of plant unconventional myosin [E. Yokota and T. Shimmen, personal communication]. It is already known that some animal unconventional myosins contain several CaM molecules as light chains [34]. However, no effect of Ca^{2+} on the activity of characean myosin was observed [22, 23]. Tominaga et al. suggested that characean cytoplasmic streaming is regulated by a Ca^{2+}-CaM-dependent phosphatase because ATPγS and phosphatase inhibitors suppressed the recovery of streaming once it was stopped by Ca^{2+}, and protein phosphatase I addition recovered streaming even in the presence of Ca^{2+} [35]. It is possible that the mechanism of calcium regulation in higher plants is different from that in lower plants.

Structural features of plant unconventional myosins

Characean myosin is the only plant myosin whose structure has been revealed by electron microscopic observation [36]. It has two heads like muscle myosin (fig. 4), but its tail is short and there is a globular structure at its end. This structure agrees well with what is expected for a plant myosin responsible for cytoplasmic streaming. The globular tail piece probably contains a site(s) for membrane interaction. This characean myosin was coprecipitated with membrane vesicles composed of phosphatidyl serine [36], which is abundant in the cytoplasmic face of plasma membrane [37], but not with phosphatidyl choline, which is abundant in the outer face of plasma membrane. This tail structure is similar to that of myosin V observed in electron microscopic studies [38].

Molecular cloning and sequence analysis of *Arabidopsis thaliana* genes revealed that there are two classes of unconventional myosins, ATM and MYA [30, 39, 40]. These myosins have a coiled-coil region in the tail of varied length and a globular tail piece. The presence of coiled-coil sequences suggests that these unconventional myosins also have a two-headed structure. Figure 5 shows the comparison of the tail region of characean myosin, MYA1 and ATM1. If the size of the motor domain of these myosins is almost the same, the difference in the length of the coiled-coil portion (fig. 5) may explain the difference in the molecular weight of heavy chains between myosin from higher plant (170 kDa) and that from characean alga (230 kDa). The difference

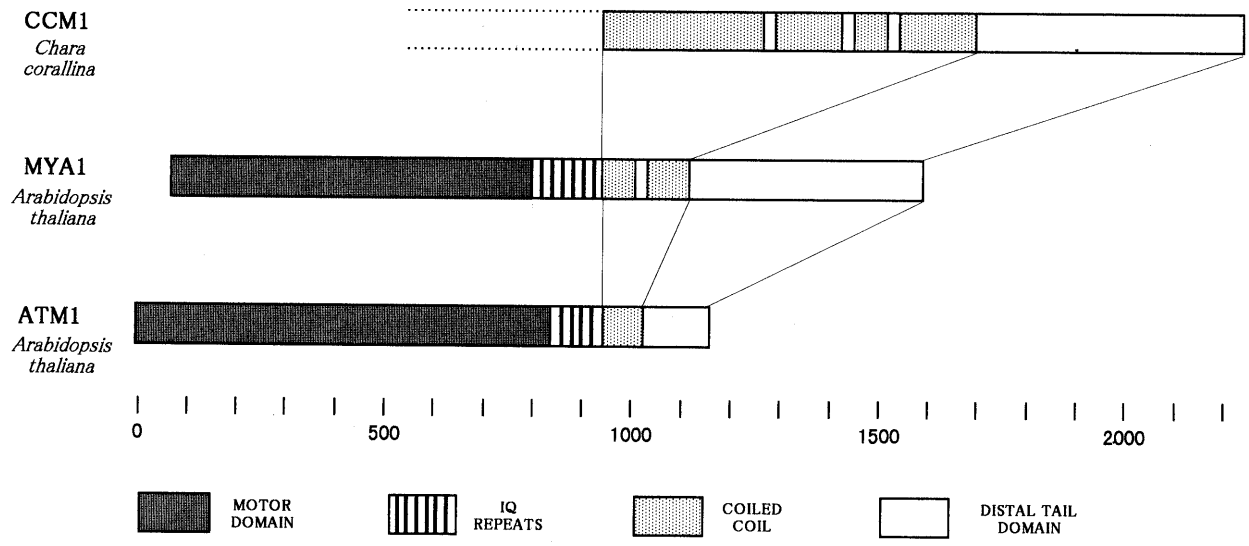

Figure 5. Comparison of tail region of characean myosin, MYA1 and ATM1. Cloning of the complementary DNA (cDNA) encoding characean myosin from a *C. corallina* cDNA library was done by immunoscreening using a polyclonal antibody directed against characean myosin [T. Kashiyama and K. Yamamoto, unpublished result]. Scale indicates amino acid number.

in the molecular weight of the coiled-coil portion between MYA1 and characean myosin is about 60 kDa (fig. 5).

Motile activity

Characean unconventional myosin is a very interesting mechanochemical enzyme because it can translocate actin filaments at a velocity several times faster than muscle myosin, which must have evolved to support quick movement of obligate heterotrophs, or animals, in the struggle for their lives. The sliding velocity of actin filaments in freely shortening muscle is about 10 μm/s at best [41], whereas the velocity of cytoplasmic streaming (equal to the sliding velocity of myosin on actin cables, fig. 3) in characean cells is 50 to 70 μm/s ([42] and table 1). Myofibrils, the contractile apparatus in muscle cells, are constructed from a series of contractile units called sarcomeres (about 2 μm in length). The contraction speed of the free moving end of a muscle cell is, therefore, far greater (50,000–100,000 times depending on the length of the muscle cell) than that of the individual sarcomere. Since both ends of the muscle are connected to bones near the joint, a small contraction of the muscle can cause a large movement of the skeleton. Thus, the requirement for muscle myosin to translocate actin filaments fast is not so tight, or animals gained the required speed not by fast myosin but by the arrangement of muscles in their body structure.

From electron microscopic observations, it is clear that the high velocity of characean myosin does not result from long power stroke in one cycle of ATP hydrolysis, because head size is almost the same as that of muscle myosin. Since the ATPase activity of characean myosin is not so high, we think that its motor domain changes conformation much faster than muscle myosin when it interacts with actin.

It should be noted that not all plant myosins are fast. The velocity of cytoplasmic streaming differs among plant species (table 1), and so might the actin translocation activity of plant myosins. Lily pollen tube myosin translocates actin filaments at velocity of 6–10 μm/s [20], which is comparable to the sliding velocity in muscle.

Although only a few plant unconventional myosins have been characterized so far, as in animal cells, there must be several other types of myosin participating in various motile activities in plant cells. Molecular cloning and genetic engineering experiments as well as biochemical characterization of these myosins will expand our understanding about their diverse roles in plant cells.

Table 1. Rates of cytoplasmic streaming in various plants*.

Plant	Organ	Rate (μm/s)	Temp.
Elodea canadensis	leaf	10	20
Oryza sativa	root hair	6.7	20
Lilium longiflorum†	pollen tube	6.6	25
Nitella mucronata	internode	52	20
Chara braunii	internode	75	27

* Taken from [42].
† These data alone were taken from [20].

Acknowledgements. The authors wish to thank Aya Suzuki for allowing us to use her drawing of *C. corallina* in this review.

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