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# Cytoplasmic streaming in plants

Teruo Shimmen[1] and Etsuo Yokota

Plant cells are surrounded by a cell wall composed of polysaccharides and hence can change neither their form nor their position. However, active movement of organelles (cytoplasmic streaming or protoplasmic streaming) is observed in plant cells, and involvement of the actin/myosin system in these processes has been suggested. Successful biochemical and biophysical approaches to studying myosins have extensively promoted the understanding of the molecular mechanism underlying these phenomena.

**Addresses**
Department of Life Science, Graduate School of Science, Himeji Institute of Technology, Harima Science Park City, Hyogo 678-1297, Japan
[1]e-mail: shimmen@sci.himeji-tech.ac.jp

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## Introduction

In 1956, Kamiya and Kuroda [1] published a paper that came to represent an important milestone in elucidating the molecular mechanisms underlying cytoplasmic streaming in plants. Using giant cells of the alga *Nitella*, they found that the motive force of streaming is generated by active sliding of sol endoplasm along gel ectoplasm (this is known as the Sliding theory. Morphology of a characean cell is shown in Figure 1 of [2]). Further extensive studies revealed that cytoplasmic streaming is caused by the motor protein myosins associated with streaming organelles actively sliding along actin filaments (reviewed in [2]). Myosin is a molecular motor which slides along actin filaments using the hydrolysis energy of ATP and is involved in various phenomena including muscle contraction. The sliding velocity on the actin filament varies considerably among species of mysoin. The velocity of cytoplasmic steaming supported by the actin–myosin system also varies to a great extent. Several micrometers per second is the most common velocity. A maximum velocity amounting to 100 μm/sec is observed in Characeae.

In order to identify the underlying molecular mechanism of cytoplasmic streaming, the myosin molecules involved had to be identified; however, until the *in vitro* motility assay — where we succeeded in inducing sliding movement of fluorescently labeled actin filaments on a glass surface coated with crude extract from pollen tubes of lily (*Lilium longiflorum*) [3] — it had been almost impossible because there was no method available to do this in crude extract from plants. Using this strategy, myosin was first isolated from lily pollen tubes [3] and later from internodal cells of *Chara corallina* [4,5]. The velocities of the sliding movement of myosins observed in the *in vitro* motility assay were consistent with those of cytoplasmic streaming in cells of plant species from which myosins had been isolated. In addition, immunostaining studies have shown co-localization of this myosin with streaming organelles [6,7], indicating that these myosins are indeed involved in cytoplasmic streaming.

The present article aims to introduce recent progress in studies on the molecular mechanism of cytoplasmic streaming in plants, especially the identification and characterization of myosin, and the molecular mechanisms underlying the organization of actin filaments in the cell.

## Characterization of plant myosins

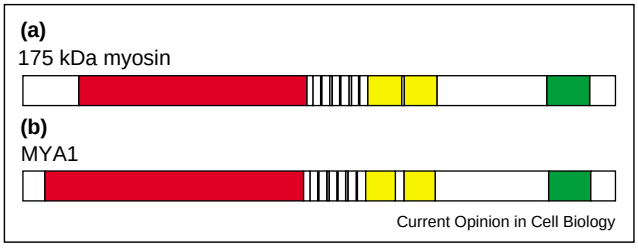
Myosins can be classified into 18 subfamilies, myosins I–XVIII, and three subfamilies of myosin have been reported in plants, myosins VIII, XI and XIII [8]. Myosins isolated from *C. corallina* [7,9] and from higher plants [10,11••,12] belong to the myosin XI subfamily.

In tobacco, two myosin XIs have been isolated — 170 kDa myosin (the molecular mass of one heavy chain of which is 170 kDa) and 175 kDa myosin (with a 175 kDa heavy chain) [10].

Figure 1 illustrates the domain structure of the heavy chain of higher plant myosin XIs — the 175 kDa myosin of tobacco (Figure 1a) and MYA1 of *Arabidopsis thaliana* (Figure 1b). The heavy chains of both myosins comprise an amino-terminal motor head region, a neck region containing six IQ motifs, an α-helical coiled-coil region and a carboxy-terminal DIL domain. *Chara* myosin XI has fundamentally the same structure, with a longer α-helical coiled-coil region [9].

The presence of this α-helical region suggests that the two heavy chains can associate by twisting this region together into a coiled-coil to form a stable dimer that has two heads and a tail, as seen with other myosin subfamily members. In fact electron microscopy has revealed that *Chara* myosin has two heads and a long tail with a globular structure [13], and the 175 kDa myosin of tobacco has also been shown to have a similar structure, a tail with two globular structures (Figure 2) [11••]. It has been

**Figure 1**




Domain structure of higher plant myosin XI. **(a)** 175 kDa myosin of tobacco. **(b)** MYA1 of *Arabidopsis thaliana*. Red: head domain; blue: neck region comprising six IQ motifs; yellow: α-helical coiled-coil domains; green: DIL domain. Reproduced from [11••] by copyright permission of Oxford University Press.

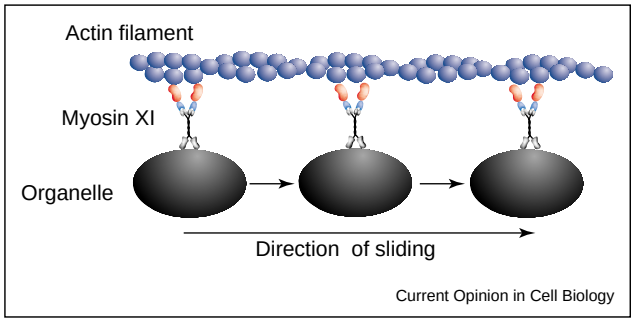
suggested that the amino-terminal head region of the myosin XI heavy chains have ATPase and motor activity and hence is involved in sliding along actin filaments using the hydrolysis energy of ATP and that its tail region is concerned with binding to organelles (Figure 2) [8].

Isolation of plant myosins has made it possible to analyze their biochemical characteristics. The sliding activity and actin-activated ATPase activity of both the 170 kDa and 175 kDa myosins of tobacco are inhibited by $Ca^{2}$ at physiological concentrations [10,14]. Moreover, biochemical and immunological analysis indicated that the light chains of both myosins are calmodulin, and analysis of the amino acid sequence showed the presence of six IQ motifs in the light-chain-binding domains of each heavy chain [11••].

Cytoplasmic $Ca^{2+}$ levels transiently increase and cytoplasmic streaming transiently ceases upon generation of an action potential at the plasma membrane in Characeae such as *Chara* and *Nitella* [2]. *Chara* myosin, however, is insensitive to $Ca^{2+}$, even though this myosin also has IQ motifs and an association of calmodulin with its heavy chain has been suggested [15]. Moreover, studies using a model in which plant cells are stripped of their membranes unequivocally demonstrated that cytoplasmic streaming in Characeae is reversibly inhibited by $Ca^{2+}$ [2]. On the other hand, pharmacological studies suggested that protein phosphorylation is involved in this (reviewed in [2]). This was recently confirmed using motility assays of cell extracts from *Chara* cells and the phosphatase and kinase inhibitors, okadaic acid and staurosporine, respectively [16•]. However, a target of the protein kinase is not elucidated yet. It seems that regulation of myosin activity is mediated by direct binding of $Ca^{2+}$ to light chain (calmodulin) in higher plants but $Ca^{2+}$-dependent phosphorylation in *Chara*. However, the possibility of regulation via dual mechanisms also remains. Further biochemical studies are needed in order to gain a comprehensive view of $Ca^{2+}$ regulation of plant myosin.

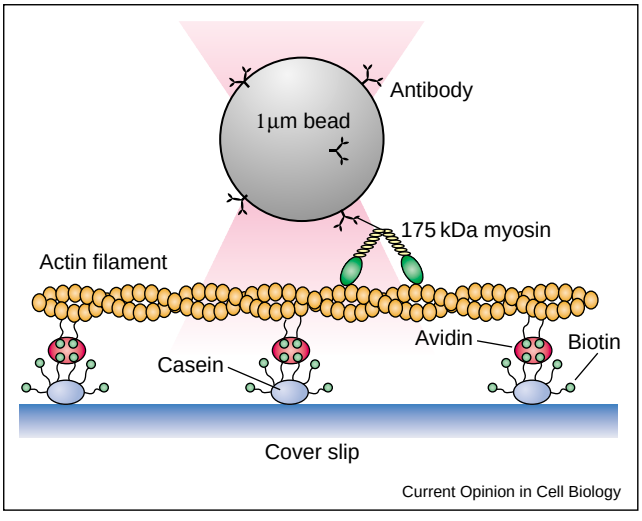
Motility analysis using a single myosin molecule (optical trap nanometry) can reveal precise mechanochemical characteristics of motor proteins. This approach was first carried out for plant myosins on the tobacco 175 kDa myosin [11••] (Figure 3). Analysis of motility of polystylen beads attached to a single molecule of 175 kDa myosin revealed that it can move along the glass-bound actin filament for a long distance without detachment from it (this is known as processivity), with steps of 35 nm

**Figure 2**




Structure and function of myosin XI, which comprises two head domains (red), two neck regions (blue) bound by calmodulin and a tail. In organelle transport, it is thought that myosin binds to organelles via its carboxy-terminal tail and organelles are transported by myosin sliding along actin filaments. (Original figure courtesy of K Hashimoto and H Tahara.)

**Figure 3**




Optical trap nanometry of the higher plant myosin XI, 175 kDa myosin from tobacco. A partially biotinylated actin filament is attached to a biotinylated-casein-coated glass surface via avidin (red). Polystylen beads are coated with antibody against the carboxy-terminal sequence of the myosin XI, and then with myosin XI to be analyzed. The bead is captured within the optical trap and brought into contact with the glass-bound actin filament to induce sliding movement. Biotin, green; casein, light blue. Reproduced from [11••], by copyright permission of Oxford University Press.

(i.e. the size of the displacement generated by myosin per ATP hydrolysis cycle at 7 μm/s), indicating that this myosin XI is the fastest known processive myosin motor [11••]. This processivity enables a small number of higher plant myosin XIs to transport organelles along actin filaments over long distance.

In contradiction, myosin XI of *C. corallina* might be a non-processive motor. This suggestion is based on an *in vitro* assay in which the sliding velocity of actin filaments on a glass surface coated with *Chara* myosin was shown to be sensitive to a change in myosin density [17•]. It was further supported by Kimura *et al.* [18•], who used single-molecule analysis to show that the small step size (19 nm) and the dwell time (i.e. the time the myosin is bound to actin per ATP hydrolysis cycle) observed for myosin could not explain the high-velocity sliding movement seen in characean cells (up to 100 μm/s, depending on the measuring temperature). This velocity is almost ten times higher that that of cytoplasmic streaming in cells of higher plants and that of actin–myosin sliding in skeletal muscle. They postulated that single *Chara* myosin proteins cannot generate this high-velocity motion; however, multiple *Chara* myosin molecules sliding on the same actin filament, resulting in an accelerated release of ADP, can. Kashiyama *et al.* [19] also tried to establish a reason for such high-velocity streaming for *Chara* myosins, expressing a chimaeric myosin protein comprising the motor domain of *Chara* myosin XI and the neck and tail domains of *Dictyostelium* myosin II in *Dictyostelium*. However, the velocity of this chimaeric myosin did not reach that of *Chara* myosin XI.

## Cargoes carried by myosin

Under the microscope, one can observe movement of various organelles in plant cells, and immunohistochemistry experiments have revealed that myosin XIs are associated with these organelles [6,7,20]. Visualisation of the Golgi apparatus [21] and peroxisomes [22•] in plant cells using green fluorescent protein (GFP) indicated that translocation of these organelles are also carried out via the actin–myosin apparatus.

The 170 kDa and 175 kDa myosins of tobacco are associated with different organelles, indicating that these myosins can recognize their target organelle(s) [12]. It is suggested that the cargo-binding domain of myosin XIs is located in their carboxy-terminal region, as described above. Identification of the cargo-binding domain of these myosins and the myosin–receptor on the surface of streaming organelles will be an important next step.

## Actin filaments as a track for myosin motors

In plant cells, actin filaments form bundles. As actin filaments *per se* have no capacity to form a bundle, involvement of crosslinking protein is suggested. Using pollen tubes from the lily, two actin-bundling proteins had been isolated [23,24] and identified to be plant villin [25,26]. In *A. thaliana*, villins were also identified by a molecular biological approach [27]. In root hair cells of *Hydrocharis dubia*, plant villins are co-localized with actin bundles and microinjection of antibodies disorganizes the transvacuolar strand and the actin bundles [26,28]. These plant villins bundle actin filaments in a uniform polarity *in vitro*, reflecting the situation *in vivo* [26,29]. Another actin-crosslinking protein, fimbrin, was identified from *A. thaliana*. The recombinant fimbrin bound to actin filaments *in vitro* [30] and in cells [31]. However, its function and intracellular localization has not yet been elucidated.

Although plant villins *per se* have no $Ca^{2+}$-sensitivity, their actin-bundling activity is inhibited in the presence of $Ca^{2+}$ and calmodulin at physiological concentrations [26,32]. It is possible that this $Ca^{2+}$-associated regulation of plant villin relates to $Ca^{2+}$-dependent dynamic organization of actin filaments at the tip region of pollen tubes.

## Microtubule-based transport

Organelles isolated from pollen tubes move along microtubules *in vitro* and kinesin-related motor proteins are involved in this motility. The speed of motility induced by the microtubule system is ~150 nm/sec [33•], far slower than that of streaming induced by the actin–myosin system in pollen tubes (several micrometers per second). The slower microtubule-dependent transport of organelles may be masked by the dramatic and rapid transport facilitated by the actin–myosin system in microscopic observation of pollen tubes. This may be the case in other plant cells where the actin–myosin system is the main machinery of cytoplasmic streaming.

In various plant cells, chloroplasts change their position in response to light conditions in plant cells. In protonemata of the moss *Physcomitrella patens*, microtubules provide tracks for rapid movement in a longitudinal direction and microfilaments for slow movement in any direction in the dark. Microtubules are concerned with chloroplast movement regulated by phytochrome, and both microtubules and microfilaments are associated with chloroplast movement regulated by blue light [34]. Thus, dynein, kinesin or related motor protein(s) may be involved in the transport of organelles in plant cells.

## Conclusions and perspectives

Biochemical isolation of myosin and actin-bundling protein made it possible to analyze the function of these proteins *in vitro*. Once a protein was isolated, the gene could be easily identified. On the other hand, molecular biological studies in *A. thaliana* revealed the presence of myosins VIII and XI. Physiological, biochemical and biophysical approaches to genetically identified myosins are needed. It seems that each myosin identifies and binds to its target organelle. Elucidation of the molecular

mechanism for specific binding between myosin and organelle is one of the most urgent problems to be solved. Analysis using myosin mutants will be one of the most fruitful approaches. It is expected that such comprehensive studies can elucidate not only the molecular mechanism of the actin–myosin system but also the biological role of cytoplasmic streaming in plant cells.

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The authors showed that both movement of isolated vesicles along actin filaments and that of actin filaments on a myosin-coated glass surface are inhibited by the protein phosphatase inhibitor okadaic acid but activated by the protein kinase inhibitor staurosporine. Treatment of myosin with protein kinase C (PKC) greatly diminished motility, supporting the suggestion that myosin phosphorylation by PKC regulates $Ca^{2+}$-dependent inhibition of cytoplasmic streaming in *Chara corallina*.

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Endoplasmic streaming of characean cells of *Nitella* or *Chara* is known to be in the range 30–100 μm/second. The sliding velocity of myosin along actin filaments equals the step size (i.e. the displacement generated by myosin per ATP hydrolysis cycle) divided by the dwell time of the step (i.e. the time the myosin is bound to actin per ATP hydrolysis cycle). These authors, using single-molecule analysis, showed that the 19 nm step size and the relatively short dwell time observed could not explain the fast movement seen in these cells. They speculate that dwell time decreases if multiple myosin molecules slide on the same actin filament, resulting in an accelerated release of ADP and the fast sliding movement.

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