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The molecular mechanism and physiological role of cytoplasmic streaming

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Cytoplasmic streaming occurs widely in plants ranging from algae to angiosperms. However, the molecular mechanism and physiological role of cytoplasmic streaming have long remained unelucidated. Recent molecular genetic approaches have identified specific myosin members (XI-2 and XI-K as major and XI-1, XI-B, and XI-I as minor motive forces) for the generation of cytoplasmic streaming among 13 myosin XIs in *Arabidopsis thaliana*. Simultaneous knockout of these myosin XI members led to a reduced velocity of cytoplasmic streaming and marked defects of plant development. Furthermore, the artificial modifications of myosin XI-2 velocity changed plant and cell sizes along with the velocity of cytoplasmic streaming. Therefore, we assume that cytoplasmic streaming is one of the key regulators in determining plant size.

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Introduction

Animals can move, whereas plants cannot and remain at their place of germination. However, an active intracellular flow called 'cytoplasmic streaming' occurs in the plant cell. This was first reported in 1774 by the Italian physicist Bonaventura Corti, who found the flow of cytoplasm when he observed intermodal cells of algae *Nitella* and *Chara* [1]. Because cytoplasmic streaming occurs widely in plants ranging from algae to angiosperms, it is supposed to be a primitive and essential system in plants [2–4]. However, more than 200 years after its discovery, the molecular mechanism for the force generation in cytoplasmic streaming remains unclear. In 1956, Kamiya and Kuroda achieved a breakthrough pertaining to this problem. They measured the flow velocities in the cells of the algae *Nitella* and found that they were high in proximity to cell membranes and low in areas distant from cell membranes. Based on this observation and hydrodynamic considerations, they proposed the 'sliding theory', by which the motive force in streaming is generated in proximity to cell membranes [5]. Over the past several years, understanding concerning plant myosin has advanced progressively. Here we review recent advances in our understanding of cytoplasmic streaming and plant myosin.

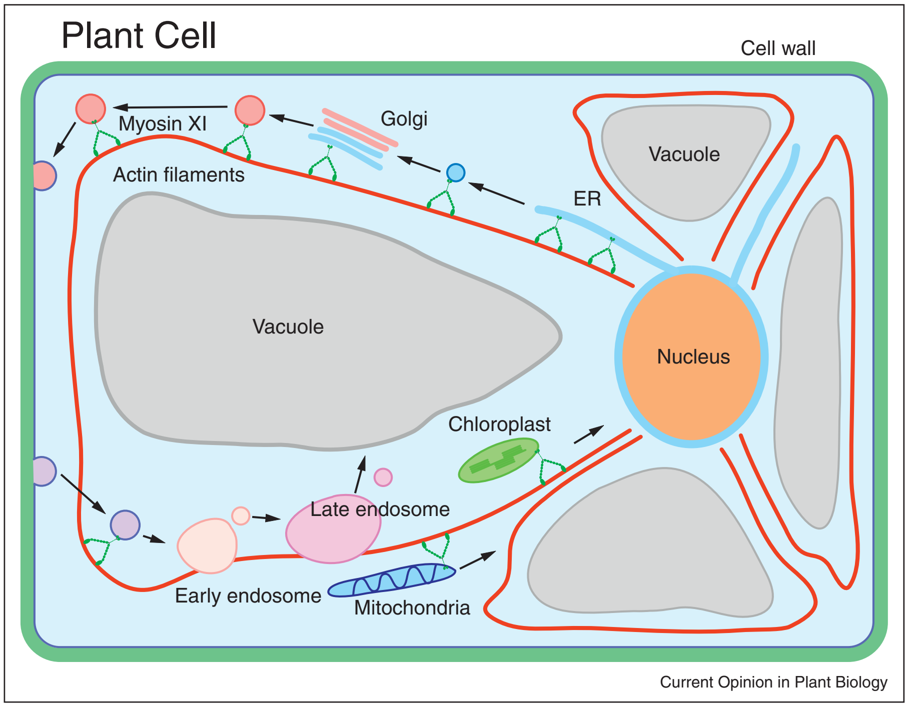
Mechanism underlying cytoplasmic streaming

Actin filaments were found to be located in the proximity of cell membranes in cells of the alga *Chara* [6]. *Chara* is an ancestor of land plants inhabiting ponds and have been used for physiological research of cytoplasmic streaming because of its large cell size (>10 cm). Pharmacological study using cytochalasin, a specific inhibitor of actin polymerization, demonstrated reversible inhibition of cytoplasmic streaming in characean cells [7]. These results strongly suggested that the 'actin–myosin' system in proximity to cell membranes may provide the motive force in cytoplasmic streaming (Figure 1). Although much effort was made to purify plant myosins following the discovery of plant actin, its purification took 20 years owing to the difficulty in isolating active myosin from plant tissue. The *in vitro* motility assay system, in which fluorescently labeled actin filaments glide on a glass surface coated with myosin molecules, has enabled the identification of plant myosins from pollen tubes of lily (*Lilium longiflorum*) [8]. Using the same system, myosin was also purified from intermodal cells of *Chara*. The purified lily and *Chara* myosins moved actin filaments in the *in vitro* motility assay at 7.7 and 40–60 μm s^{-1}, respectively [8–10]. These were astonishing results, given that these velocities are greater than those of animal fast skeletal muscle myosins. Particularly, the velocity of *Chara* myosin was more than 10-fold that of the myosins of all other organisms. Actin sliding velocities with purified plant myosins were consistent with the cytoplasmic streaming velocities observed in plant cells from which myosins had been isolated. Therefore, these results strongly suggested that these myosins indeed evoke cytoplasmic streaming.

Molecular properties of plant myosins

Myosin is a motor protein that converts the chemical energy liberated by ATP hydrolysis into directed

Figure 1



Cytoplasmic streaming is an active intracellular movement generated by organelle-associated plant-specific class XI myosins moving along the actin filaments in plant cells. In *Arabidopsis*, there are 13 myosins in the class XI family. As reviewed in this paper, many studies indicated specific and/or overlapping localization and function among class XI myosins. The localization and function of each class XI myosin are not fully understood.

movement on actin filaments and is generally present in eukaryotes. Phylogenetic analyses of amino acid sequences of myosin have revealed at least 35 classes [11]. Motor functions such as motility and ATP hydrolysis vary greatly among classes, allowing each myosin to be finely tuned for a specific task [12]. Only class VIII and XI myosins are present in higher plants. *Arabidopsis thaliana* harbors four and thirteen genes [13] and *Oryza sativa* (rice) two and twelve genes [14] encoding class VIII and XI myosins, respectively. The first identified and sequenced plant myosin was *Arabidopsis* class VIII myosin (*Arabidopsis thaliana* myosin 1, ATM1) [15]. Recently, the molecular properties and intracellular localizations of class VIII myosin, ATM1, were elucidated [16•]. The actin-activated ATPase activities and actin sliding velocity of ATM1 were very low (V_{max} of actin-activated ATPase activities was 4 Pi head^{-1} s^{-1} and actin sliding velocity was 0.2 μm s^{-1}). Green fluorescent protein (GFP)-fused full-length ATM1 expressed in *Arabidopsis* was localized to plasmodesmata, plastids, newly formed cell walls, and actin filaments in the cell cortex. The low velocity and localization of ATM1 show that class VIII myosin functions as a tension sensor/generator, but not as a generator of cytoplasmic streaming.

The first identified and sequenced class XI myosins were also from *Arabidopsis* (MYosin from *Arabidopsis* 1, MYA-1) [17]. The molecular structure of myosin XI of *Arabidopsis* deduced from its cDNA is composed of an N-terminal motor domain that contains actin-binding and nucleotide-binding sites, a neck domain comprising six tandem repeats of IQ motifs serving as binding sites for six calmodulin-like myosin light chains, a α-helical coiled-coil domain supporting dimer formation, and a globular tail domain (GTD) (Figure 2). This molecular structure resembles that of animal and fungus class V myosins [18]. By immunoscreening using antibodies raised against a purified *Chara* myosin whose velocity was similar to that of cytoplasmic streaming of *Chara* cells, cDNA encoding *Chara* myosin was cloned. The sequence of the cloned cDNA indicated that *Chara* myosin should be classified as a class XI myosin [19,20], showing that class XI myosin is a generator of cytoplasmic streaming. Because yields of native *Chara* myosin that could be purified to homogeneity were very low, biochemical analyses have employed recombinant *Chara* myosin XI. The V_{max} of the actin-activated ATPase activities [21,22] and the ADP dissociation rate from acto–myosin [23] of the recombinant *Chara* myosin XI were 500 Pi head^{-1} s^{-1} and 2800 s^{-1}, respectively.

Intensive molecular characterization of higher plant class XI myosin has been performed using *Nicotiana tabacum* 175-kDa class XI myosin (tobacco myosin XI) purified from cultured tobacco BY-2 cells [24]. Single-molecule