Functions of plant-specific myosin XI: From intracellular motility to plant postures

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Abstract

The plant-specific protein motor class myosin XI is known to function in rapid bulk flow of the cytoplasm (cytoplasmic streaming) and in organellar movements. Recent studies unveiled a wide range of physiological functions of myosin XI motors, from intracellular motility to organ movements. *Arabidopsis thaliana* has 13 members of myosin XI class.
In vegetative organs, myosins XIk, XI1, and XI2 primarily contribute to dynamics and spatial configurations of endoplasmic reticulum that develops a tubular network in the cell periphery and thick strand-like structures in the inner cell regions. Myosin XI-i forms a nucleocytoplasmic linker and is responsible for nuclear movement and shape.
In addition to these intracellular functions, myosin XIf together with myosin XIk is involved in the fundamental nature of plants; the actin-myosin XI cytoskeleton regulates organ straightening to adjust plant posture.

Introduction

Myosin is a motor protein that is widely conserved in eukaryotic cells. The sliding of myosin motors on actin filaments (F-actin) generates a driving force for muscle contraction or transport of cellular components. Plants have evolved specific classes of myosin VIII and myosin XI. In *Arabidopsis thaliana*, the myosin VIII family contains four members, whereas

the myosin XI family contains 13 members [1]. This review focuses on the myosin XI family responsible for the characteristic feature of intracellular motility in plants using conventional nomenclature (myosins XIa–XIk, XI1, and XI2) rather than phylogenetic tree-based nomenclature (Myo11A–H for myosin XI) [2].

Recent studies using gene knockout and dominant-negative inhibition demonstrated the intracellular function of each member of *A. thaliana* myosin XI in the movement of cytoplasmic components including organelles, vesicles, and processing bodies [3-5]. At the cellular and organ levels, myosin XI is required for the expansion and elongation of various types of cells and regulates plant size and fertility [4]. In this review, we discuss recent advances in our understanding of the intracellular and biological functions of myosin XI.

Myosin XI functions in endoplasmic reticulum dynamics

The endoplasmic reticulum (ER) is a membrane-enclosed organelle in eukaryotes. The ER develops an elaborate polygonal network composed of tubules and cisternae in the cell periphery, which is frequently remodeled through tubule growing/shrinking, sliding junctions, and cisternal expansion/contraction [6-9]. In addition, plant ER develops thick strand-like structures (Figure 1a). Previous studies reported rapid ER streaming in the strands [10-12], which is characteristic of plant ER dynamics. Velocity maps depicted by KbiFlow software [13] reveal ER motility with velocities ranging from ~0.7 μm/s (in most peripheral region of cell) to ~4.2 μm/s (in the cell interior) within a single cell (Figure 1b). The ER streams 6-fold faster in the cell interior than in the peripheral region.

ER motility in plant cells is primarily driven by the actin-myosin XI cytoskeleton and secondarily by the microtubule cytoskeleton [14]. The myosin XI motors responsible for ER motility have been identified in recent years. A myosin XI member in tobacco BY-2 cultured cells (175-kD myosin XI) is associated with the ER [15]. In an *in vitro* ER tubule formation assay with the cytosol-and-microsome fraction of BY-2 cells and exogenously added ATP, GTP, and F-actin, depletion of 175-kD myosin XI significantly inhibited tubule formation from small ER vesicles [16]. These results clearly demonstrate that the ER-associated 175-kD myosin XI provides a motive force to elongate ER tubules in tobacco cells.

Reverse genetic analysis of *A. thaliana* identified three myosin XI members responsible for the ER streaming; myosin XIk is a primary contributor, whereas the contributions of myosins XI1 and XI2 are more limited in cotyledonary petioles [13]. The involvement of

myosin XI in ER network dynamics has been analyzed with persistency maps and by expressing a motor-less truncated tail of myosin XI, which is thought to disturb endogenous myosin XI function [17]. Transient expression of truncated tails of *A. thaliana* myosins XIc, XIe, XIk, and XI1 significantly suppresses normal ER dynamics in the cell cortex of tobacco leaves [17,18]. This result is consistent with the phylogenetic data of full-length myosin XI, which indicates that *A. thaliana* myosins XIc, XIe, XIk, and XI1 belong to closely related subfamilies [1,19]. However, a lack of myosin XIc or XIe did not suppress normal ER motility in *A. thaliana* vegetative organs (H. Ueda *et al.*, unpublished), in which myosins XIc and XIe are expressed at low levels [20]. Because of technical restrictions, most of imaging data are obtained from epidermal cells exclusively. We cannot exclude the possibility that other myosin XI members are involved in the ER dynamics in different cell types like the mesophyll or vasculature of the same organ/tissue.

Myosin XIk partially co-fractionates with ER of aerial *A. thaliana* tissues [13,21]. Intriguingly, it is suggested that only a motile ER subdomain is associated with myosin XIk in leaf midvein epidermal cells [21]. However, the distribution of YFP-tagged myosin XIk does not match that of an ER marker at the root hair tip [21,22]. Collectively, multiple members of myosin XI are involved in ER dynamics in *A. thaliana*. Among these myosin XI members, myosin XIk has been demonstrated to be associated with the ER.

Several studies have investigated the mode of association between ER and myosin XI. Direct electrostatic binding is suggested between myosin XI and the ER membrane in *Chara* [23]. In *A. thaliana*, a receptor protein of myosin XI members is identified: a membrane protein family MyoB with a domain of unknown function 593 (DUF593) [24]. MyoB1-GFP co-localizes with myosin XIk-mCherry [24]. A homolog of MyoB (RISAP, RAC5 interacting subapical pollen tube protein) is associated with myosin XI in *Nicotiana tabacum* [25]. The association possibly requires adaptor proteins such as Rab GTPases because myosin XI2 is reported to target peroxisomes through an interaction with AtRabC2a [26].

Three-way interaction between ER, myosin XI, and actin

A. thaliana myosins XIk, XI1, and XI2 are required for spatial ER configurations in addition to the ER streaming. The triple mutant *myosin xik xi1 xi2* has no ER strands, whereas it abnormally develops large ER aggregates and thick ER cisterna-like structures [13]. These features are very similar to the aberrant ER architectures induced by treatment with

latrunculin B [13], an inhibitor of actin polymerization. This morphological conversion of the ER suggests that large amounts of ER associate with actin bundles via myosin XI, which functions in driving ER motility through the cell.

The *myosin xik xi2* [13] and *myosin xik xi1 xi2* [27] mutants exhibit defects in formation of longitudinally-oriented F-actin bundles in the elongated cells. Similar results have been reported in *Physcomitrella patens* [28]. In addition, rapid dynamic rearrangements of actin filaments at the fungal penetration sites are markedly reduced by myosin inhibitors in *A. thaliana* [29]. Therefore, the regulation of F-actin organization is a widely conserved function of myosin XI. Cortical actin arrays in *A. thaliana myosin xik xi1 xi2* cells are much less dense and more bundled than those in wild-type cells [30]. Quantitative analyses show that gene knockout of *myosins XIk*, *XI1*, and *XI2* reduces F-actin turnover and buckling/straightening of single actin filaments and bundles [30]. Furthermore, a reduction in F-actin dynamics has been observed in root hair cells of the single mutant *myosin xik* [22]. Taken together, these results indicate that myosins XIk, XI1, and XI2 contribute to actin remodeling by stimulating actin turnover and generating the force for actin filament shape changes [30].

Based on these reports, we propose a positive-feedback model of ER streaming mediated by a three-way interaction between ER, myosin XI, and F-actin (Figure 1c). Initially, myosin XI slides the ER sub-domains along randomly oriented actin filaments. This ER sliding gradually causes actin filaments to be longitudinally-oriented in the elongated cell. Reiteration of this process results in the formation of longitudinally-oriented, thick F-actin bundles that provide tracks for extensive streaming of ER strands. ER is the largest endomembrane system; therefore, it appears to be a natural candidate for anchoring and aligning multiple actin filaments along the longitudinal axis of elongated cells. The extensive ER network linked to the actin cytoskeleton via myosin XI may also have a role in generating forces for filament shape changes during actin remodeling, which results in appropriate bundling of the F-actin.

The large surface area of unidirectionally streaming ER could act to propel other cytosolic components such as vesicles and particulate organelles. This hypothesis is supported by observations of similar patterns and velocities of ER and cytosolic streaming [13]. This hypothesis could explain why the movement of Golgi stacks [31,32], mitochondria [31], and peroxisomes [31] are largely suppressed in the myosin *xik* mutant. RHD3, an ER-membrane anchored GTPase, is required for ER motility, possibly because it provides ER membrane fluidity [33]. The streaming of Golgi stacks, mitochondria, peroxisomes, and ER are similarly

suppressed in the *rhd3* mutant [33]. A close association between ER and various organelles also has been reported [34,35]. Taken together, these data suggest that myosin XI actively drives ER motility, which induces streaming of these particulate organelles. Additionally, the organelles might have machinery to move independently of ER movements.

Myosin XI-i is a component of a nucleocytoplasmic linker that is required for nuclear shape and movement

A recent study reports that myosin XI-i functions in the movement of the nucleus. Myosin XI-i-dependent nuclear movement is two orders of magnitude slower than the ER movements driven by myosins XIk, XI1, and XI2. A forward genetics approach using *A. thaliana* reveals that a mutation in *KAKU1/myosin XI-i* affects nuclear movement [36]. Bidirectional nuclear movements observed in wild-type root hair cells (2.68 ± 1.37 μm/min) are dramatically suppressed in the *myosin xi-i* mutant (0.52 ± 0.35 μm/min for *kaku1-2*) [36]. GFP-tagged full-length or truncated tail region of myosin XI-i associates with the nuclear envelope [18,36,37]. These results indicate that myosin XI-i is a driver of the nuclear movement.

Nuclei change their positions in response to various conditions such as development, biotic interactions, and abiotic signals [38,39]. For example, nuclei in *A. thaliana* leaves move away from strong light and relocate to the side walls of the cell, whereas they move to the cell bottom toward the leaf center plane during dark adaptation [40]. This light-avoiding nuclear movement depends on the blue-light receptor and F-actin [41]. Recent work proposed that plastid relocation in response to strong blue light, which is mediated by short actin filaments, generates a motive force for light-avoiding nuclear movement [42]. It has been suggested that myosin XI is involved in the light-dependent plastid relocation, but this role is currently controversial [43,44]. Light-induced nuclear movement occurs normally in the *myosin xi-i* mutant, whereas dark-induced nuclear movement is impaired [36]. Therefore, myosin XI-i appears to be involved in dark-induced nuclear movement.

The *myosin xi-i* mutant contains aberrant spherical nuclei with abnormally invaginated nuclear envelope [36]. Among single mutants of the 13 members of myosin XI, only *myosin xi-i* exhibits a spherical nuclear shape, indicating that myosin XI-i is the predominant myosin XI involved in nuclear morphology, which is spindle-shaped in wild-type cells [36]. Consistently, only myosin XI-i is localized to the nuclear envelope [18,36,37]. On the other hand, extremely elongated nuclei are reported in trichome cells of *myosin xik* and *myosin xi2*

mutants [45]. A possible explanation is that defective F-actin organization or dynamics in *myosin xik* and *myosin xi2* may affect the myosin XI-i-dependent nuclear shape.

Myosin XI-i interacts directly with WPP domain-interacting tail-anchored proteins (WIT1 and WIT2), which are integral membrane proteins localized on the outer nuclear membrane [36]. The fluorescent signal of YFP-tagged myosin XI-i tail is not detected on the nuclear envelope in the *wit1 wit2* mutant, indicating that WIT proteins are required to anchor myosin XI-i to the nuclear envelope [36]. WIT interacts with the outer nuclear membrane protein WIP (WPP domain-interacting protein) [36,46], and WIP interacts with the inner nuclear membrane protein SUN [36,47]. The aberrant spherical nuclear shape observed in *wit1 wit2* [36], *wip1 wip2 wip3* [47], and *sun1-knockout sun2-knockdown* [47,48], and the impaired nuclear movement observed in *wit1 wit2* [36], are very similar to those observed in *myosin xi-i.* Therefore, the myosin XI-i-WIT-WIP-SUN complex is proposed as a nucleocytoplasmic linker in plant cells, which regulates nuclear shape and movement (Figure 2) [36]. MyoB7, which is a distant homolog of the myosin XI-k-binding protein MyoB1/2, has been identified as a myosin XI-i-binding protein [24]. YFP-tagged MyoB7 labels motile vesicles but not the nuclear envelope [24], suggesting that MyoB7 is not part of the nucleocytoplasmic linker complex. On the other hand, nucleoplasmic proteins CROWDED NUCLEI 1 (CRWN1)/KAKU2 [49-52], CRWN4 [49-51], NUP136 [53], and KAKU4 [52] affect nuclear shape. These proteins are thought to act on nuclear shape determination by forming or binding lamina-like structures independently of the myosin XI-i-WIT-WIP-SUN complex [54].

Myosin XI functions in straightening to determine plant posture

Among 13 members of *A. thaliana* myosin XI, gene knockout of both *myosins XIf* and *XIk* causes kinked morphologies of various organs including petioles, inflorescence stems, pedicels, and siliques [55]. This phenotype is strongest in the quintuple (*myosin xib xif xik xi1 xi2*) and sextuple (*myosin xib xif xig xik xi1 xi2*) mutants (Figure 3a) [55]. Plant morphology is affected by tropic responses, the bending of various organs by directional growth in response to environmental stimuli such as gravity and light [56,57]. The *myosin xif xik* mutant exhibits extreme bending in various organs responses to gravity and light [55], suggesting that a combination of myosins XIf and XIk has a role in restraining the organ bending.

Organ segments that have undergone gravitropic bending once can straighten during the

course of gravitropic responses [58]. This straightening phenomenon is more evident when bent organs are subjected to clinorotation, which simulates microgravity conditions by counteracting the Earth's unilateral gravitational pull [58]. This nature is called autostraightening, automorphogenesis, and autotropism [58,59]. Here, we use the term straightening. Clinostat analysis reveals that wild-type inflorescence stems steadily straighten the bended shape, whereas stems of the *myosin xif xik* and *myosin xif xik xi2* mutants fail to straighten and continue to bend and coil under dark and simulated microgravity conditions (Figure 3b) [55]. The *myosin xif xik* mutant offers the first genetic evidence for the role of a straightening system to stop or restrain bending during tropic responses [55].

These results are compatible with a model proposed by Bastien *et al.*, in which plant posture resulting from a gravitropic response is determined by a balance between bending (by graviceptive sensing) and straightening (by proprioceptive sensing) [60]. Proprioceptive sensing is the ability to sense local curvature of the plant organs. The observed straightening deficiency in *myosin xif xik* is possibly due to defective proprioception.

The marked contrast between the kinked posture of *myosin xif xik* and the straight posture of *myosin xib xik xi1 xi2*, in which ubiquitously and highly expressed *myosins XIk*, *XI1*, and *XI2* are eliminated, implies that myosin XIf must be a key factor in the straightening system [55]. Only limited information is available about myosin XIf; however, DNA microarray data suggest that myosin XIf is specifically expressed in elongating organs and tissues [20]. The *myosin XIf* promoter is active in elongating organs including inflorescence stem, root, and hypocotyl [55]. In inflorescence stem, *myosin XIf* is specifically expressed in fiber cells, which are parallel to the stem and encircle it in a thin band [55]. Fiber cells are extremely long (~1 mm) and develop long, thick F-actin bundles [55], which are thought to be suitable for sensing curvature. F-actin involvement in proprioception is supported by observations in the actin-related mutants *cyclase-associated protein1* (*cap1*) [61], *actin2 actin7* [62], *actin8*/*frizzy1* (*fiz1*) [63], *villin2 villin3* [64], and *actin2*/*wavy1* [65], all of which exhibit kinked gross morphology similar to that in *myosin xif xik*. The *fiz1* mutant is unable to straighten on a clinostat [55]. These results provide insight into the molecular mechanism of proprioception, in which an actin-myosin XI cytoskeleton might act as a bending sensor in elongated cells. Actin cables under tension might activate mechanosensitive channels to trigger the straightening system, as actin stress fibers can activate mechanosensitive channels in animal cells [66].

Conclusions

Table 1 summarizes the intracellular motilities, other intracellular functions, and biological functions at the single-cell level, and biological functions at the organ level of *A. thaliana* myosin XI members based on analyses of gene knockout mutants. Compared with other myosin family proteins, the myosin XI family in angiosperms comprises powerful motors with velocities of several μm per second [67-69]. The powerful myosin XI motors associated with organelles propel the cytoplasm by sliding on unidirectional F-actin bundles, which develop in large plant cells. The cytoplasmic streaming velocity is positively correlated with the motor activity of myosin XI expressed in transgenic plants and with the size of transgenic plants [70]. It should be noted that the cytoplasmic streaming velocity is a plant size determinant [70]. Myosin XI motors might be involved in a broad range of physiological processes, not only at the subcellular level but also at the organ level.

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Figure Legends

Figure 1. Positive-feedback model of ER streaming. **(a)** Peripheral ER network labeled with luminal GFP. **(b)** ER streaming-velocity maps depicted by KbiFlow software (upper panels) and maximum-intensity projections of time-lapse images of GFP-labeled ER (lower panels). Seven-day-old *A. thaliana* cotyledonary petioles were observed by spinning-disk confocal microscopy; 100 images of GFP-labeled ER were captured at ~50-ms intervals in four optical planes from the cell periphery to an inner plane. Arrow lengths and colors in the velocity map indicate ER streaming velocities. Scale bars=10 μm. **(c)** Proposed positive-feedback model of ER streaming mediated by a three-way interaction between ER, myosin XI, and F-actin. (i) ER sub-domains slide along randomly oriented F-actin via myosin XI. (ii) ER sliding gradually causes actin filaments to be longitudinally-oriented in the elongated cell by aligning adjacent actin filaments through the myosin XI-coated ER. (iii) Reiteration of this process results in the formation of longitudinally-oriented, thick F-actin bundles that provide tracks for extensive streaming of ER strands.

Figure 2. Proposed model of a new type of nucleocytoplasmic linker in plants. Myosin XI-i binds to both the actin cytoskeleton and the outer nuclear membrane WIT protein. WIT interacts with the SUN-WIP bridge. The resulting nucleocytoplasmic linker regulates nuclear shape and movement.

Figure 3. Myosin XI is required for straightening, which determines plant posture. **(a)** Kinked gross morphology of the *myosin xi1 xi2 xib xif xik* quintuple mutant. **(b)** Representative images of wild-type plants and the *myosin xif xik xi2* triple mutant during clinorotation.

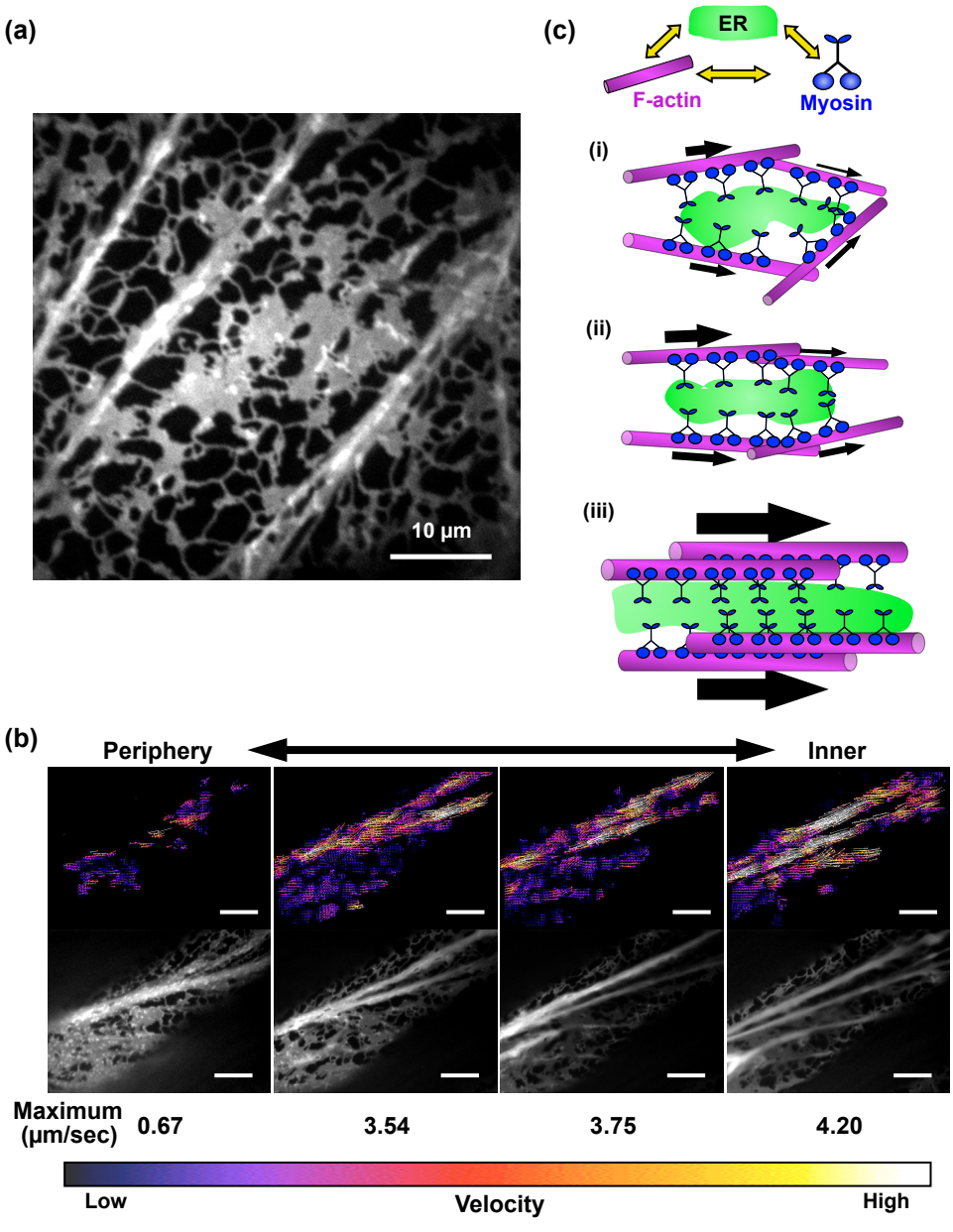
Table 1
Myosin XI functions based on analyses of single and multiple knockout mutants of *Arabidopsis thaliana*

	Myosin XI members in *Arabidopsis thaliana* [2]					
Conventional nomenclature	**myosin XI1**	**myosin XI2**	**myosin XIk**	**myosin XIf**	**myosin XI-i**	**myosin XIb**
Alternative nomenclature[1]	**Myo11F**	**Myo11B2**	**Myo11E**	**Myo11H**	**Myo11G**	**Myo11B1**
Intracellular motilities						
Golgi	*xi1* [71]	*xi2* [31]	*xik* [31,32]			
Peroxisome	*xik xi1* [71]	*xi2* [31]	*xik* [31]			
Mitochondrion	*xik xi1* [71]	*xi2* [31]	*xik* [31]			
ER	*xik xi1 xi2* [13]	*xik xi2* [13]	*xik* [13]			
Nucleus					*xi-i* [36]	
Plastid[3]			*xif xik* [55]	*xif xik* [55]		
P-body			*xik* [72]			
Other intracellular functions						
ER morphology	*xik xi1 xi2* [13]	*xik xi2* [13]	*xik* [13]			
Nuclear shape		*xi2* [45]	*xik* [45]		*xi-i* [36]	
Actin organization	*xik xi1 xi2* [27]	*xik xi2* [13]	*xik xi2* [13]			
		xik xi1 xi2 [27]	*xik xi1 xi2* [27]			
Actin dynamics	*xik xi1 xi2* [30]	*xik xi1 xi2* [30]	*xik* [22]			
			xik xi1 xi2 [30]			
Biological functions at the single cell level						
Leaf cell expansion/elongation	*xik xi1* [71]	*xik xi1 xi2* [27]	*xik xi1* [71]		*xi-i xik xi1 xi2* [27]	*xib xik xi2* [27]
					xib xi-i xik xi2 [27]	
Root hair elongation		*xi2* [31]	*xik* [22,31,73]			*xib xik xi2* [27]
Stigmatic papillae elongation	*xik xi1 xi2* [45]	*xik xi1 xi2* [45]	*xik xi1 xi2* [45]			
Trichome morphology	*xik xi1 xi2* [45]	*xik xi1 xi2* [45]	*xik* [73]			
Pavement cells lobing	*xik xi1 xi2* [45]	*xik xi1 xi2* [45]	*xik xi1 xi2* [45]			
Biological functions at the organ level						
Plant size	*xik xi1 xi2* [27]	*xik xi1 xi2* [27]	*xik xi1 xi2* [27]	*xif xik xi2* [55]	*xi-i xik xi1 xi2* [27]	
		xif xik xi2 [55]	*xif xik xi2* [55]			
Straightening		*xif xik xi2* [55]	*xik* [55]	*xif xik* [55]		
Fertility	*xik xi1* [71]	*xik xi1 xi2* [27,45]	*xik xi1* [71]		*xi-i xik xi2* [27]	
	xik xi1 xi2 [27,45]	*xi-i xik xi2* [27]	*xik xi1 xi2* [27,45]			
			xi-i xik xi2 [27]			

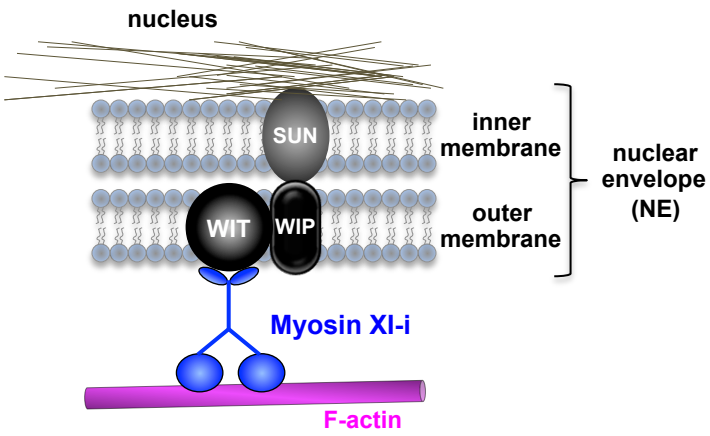
[1]The phylogenetic-tree-based nomenclature is proposed in [2].

[2]Currently, no significant abnormal phenotypes of a single mutant lacking myosin XIa, XIc, XId, XIe, XIg, XIh, or XIj has been reported.

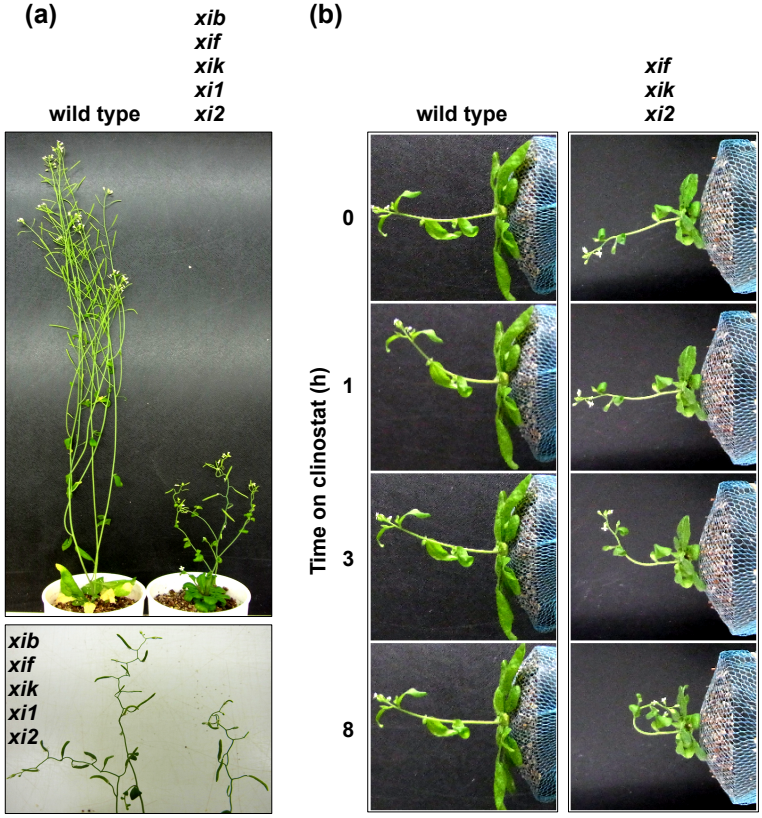
[3]Plastid streaming was observed in fiber cells of inflorescence stems.



Hara-Nishimura, Figure 1



Hara-Nishimura, Figure 2



Hara-Nishimura, Figure 3