TITLE:

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

AUTHOR(S):

Ueda, Haruko; Tamura, Kentaro; Hara-Nishimura, Ikuko

CITATION:

Ueda, Haruko ...[et al]. Functions of plant-specific myosin XI: from intracellular motility to plant postures.. Current opinion in plant biology 2015, 28: 30-38

ISSUE DATE:

2015-09-29

URL:

<http://hdl.handle.net/2433/202506>

RIGHT:

© 2015. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <http://creativecommons.org/licenses/by-nc-nd/4.0/>; The full-text file will be made open to the public on 30 September 2017 in accordance with publisher's 'Terms and Conditions for Self-Archiving'. ; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。 ; This is not the published version. Please cite only the published version.

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

Haruko Ueda, Kentaro Tamura, and Ikuko Hara-Nishimura

Department of Botany, Graduate School of Science, Kyoto University, Kyoto 606-8502,
Japan

Corresponding author: Ikuko Hara-Nishimura

Tel.: +81 (75) 753-4142; E-mail: ihnishi@gr.bot.kyoto-u.ac.jp

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 XI_k, XI_l, 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 XI_f together with myosin XI_k 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 $\sim 0.7 \mu\text{m/s}$ (in most peripheral region of cell) to $\sim 4.2 \mu\text{m/s}$ (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 *KAKUI/myosin XI-i* affects nuclear movement [36]. Bidirectional nuclear movements observed in wild-type root hair cells ($2.68 \pm 1.37 \mu\text{m}/\text{min}$) are dramatically suppressed in the *myosin xi-i* mutant ($0.52 \pm 0.35 \mu\text{m}/\text{min}$ 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 XIi* 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 XIi 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 XI_f must be a key factor in the straightening system [55]. Only limited information is available about myosin XI_f; however, DNA microarray data suggest that myosin XI_f is specifically expressed in elongating organs and tissues [20]. The *myosin XI_f* promoter is active in elongating organs including inflorescence stem, root, and hypocotyl [55]. In inflorescence stem, *myosin XI_f* 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.

Acknowledgement

This work was supported by Specially Promoted Research of Grant-in-Aid for Scientific Research to I.H.-N. (no. 22000014) and Grants-in-Aid for Scientific Research to H.U. (nos. 25440132 and 15KT0151), to I.H.-N. (no. 15H05776), and to K.T. (nos. 15K14545 and 26711017) from the Japan Society for the Promotion of Science (JSPS).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Reddy AS, Day IS: **Analysis of the myosins encoded in the recently completed *Arabidopsis thaliana* genome sequence.** *Genome Biol* 2001, **2**:RESEARCH0024.
 2. Muhlhausen S, Kollmar M: **Whole genome duplication events in plant evolution reconstructed and predicted using myosin motor proteins.** *Bmc Evolutionary Biology* 2013, **13**.
 3. Sparkes I: **Recent advances in understanding plant myosin function: life in the fast lane.** *Mol Plant* 2011, **4**:805-812.
 4. Madison SL, Nebenfuhr A: **Understanding myosin functions in plants: are we there yet?** *Curr Opin Plant Biol* 2013, **16**:710-717.
 5. Buchnik L, Abu-Abied M, Sadot E: **Role of plant myosins in motile organelles: is a**

direct interaction required? *J Integr Plant Biol* 2015, **57**:23-30.

6. Lichtscheidl IK, Baluska F: **Motility of endoplasmic reticulum in plant cells.** In *Actin: A Dynamic Framework for Multiple Plant Cell Functions*. Edited by Staiger CJ, Baluska F, Volkmann D, Barlow P: Kluwer Academic Publishers; 2000:191-201. vol Developments in Plant and Soil Sciences , Vol. 89]
7. Quader H, Zachariadis M (Ed): *The Morphology and Dynamics of the ER* Berlin / Heidelberg: Springer; 2006.
8. Westrate LM, Lee JE, Prinz WA, Voeltz GK: **Form follows function: the importance of endoplasmic reticulum shape.** *Annu Rev Biochem* 2015, **84**:791-811.
9. Joensuu M, Jokitalo E: **ER sheet-tubule balance is regulated by an array of actin filaments and microtubules.** *Exp Cell Res* 2015.
10. Quader H, Schnepf E: **Endoplasmic reticulum and cytoplasmic streaming: Fluorescence microscopical observations in adaxial epidermis cells of onion bulb scales.** *Protoplasma* 1986, **131**:250-252.
11. Allen NS, Brown DT: **Dynamics of the endoplasmic reticulum in living onion epidermal cells in relation to microtubules, microfilaments, and intracellular particle movement.** *Cell Motil Cytoskeleton* 1988, **10**:153-163.
12. Boevink P, Cruz S, Hawes C, Harris N, Oparka KJ: **Virus-mediated delivery of the green fluorescent protein to the endoplasmic reticulum of plant cells.** *Plant J* 1996, **10**:935-941.
13. Ueda H, Yokota E, Kutsuna N, Shimada T, Tamura K, Shimmen T, Hasezawa S, Dolja VV, Hara-Nishimura I: **Myosin-dependent endoplasmic reticulum motility and F-actin organization in plant cells.** *Proc. Natl. Acad. Sci. USA* 2010, **107**:6894-6899.
14. Hamada T, Ueda H, Kawase T, Hara-Nishimura I: **Microtubules contribute to tubule elongation and anchoring of endoplasmic reticulum, resulting in high network complexity in Arabidopsis.** *Plant Physiol* 2014, **166**:1869-1876.
15. Yokota E, Ueda S, Tamura K, Orii H, Uchi S, Sonobe S, Hara-Nishimura I, Shimmen T: **An isoform of myosin XI is responsible for the translocation of endoplasmic reticulum in tobacco cultured BY-2 cells.** *J Exp Bot* 2009, **60**:197-212.
- 16. Yokota E, Ueda H, Hashimoto K, Orii H, Shimada T, Hara-Nishimura I, Shimmen T: **Myosin XI-dependent formation of tubular structures from endoplasmic reticulum isolated from tobacco cultured BY-2 cells.** *Plant Physiol* 2011, **156**:129-143.

This paper establishes an *in vitro* assay for ER network reconstitution using tobacco BY-2 cell extracts. The authors demonstrate that the 175 kDa myosin XI generates a motive force to elongate ER tubules.

17. Sparkes I, Runions J, Hawes C, Griffing L: **Movement and remodeling of the endoplasmic reticulum in nondividing cells of tobacco leaves.** *Plant Cell* 2009, **21**:3937-3949.
- 18. Griffing LR, Gao HT, Sparkes I: **ER network dynamics are differentially controlled by myosins XI-K, XI-C, XI-E, XI-I, XI-1, and XI-2.** *Front Plant Sci* 2014, **5**:218.

The authors demonstrate multiple members, which belong to closely related subfamilies of myosin XI in *A. thaliana*, are involved in ER dynamics by using persistency maps and

expression system of a motor-less truncated tail of myosin XI.

19. Wang G, Wang F, Wang G, Wang F, Zhang X, Zhong M, Zhang J, Lin D, Tang Y, Xu Z, et al.: **Opaque1 encodes a myosin XI motor protein that is required for endoplasmic reticulum motility and protein body formation in maize endosperm.** *Plant Cell* 2012, **24**:3447-3462.
20. Peremyslov VV, Mockler TC, Filichkin SA, Fox SE, Jaiswal P, Makarova KS, Koonin EV, Dolja VV: **Expression, splicing, and evolution of the myosin gene family in plants.** *Plant Physiol* 2011, **155**:1191-1204.
21. Peremyslov VV, Klocko AL, Fowler JE, Dolja VV: **Arabidopsis Myosin XI-K Localizes to the Motile Endomembrane Vesicles Associated with F-actin.** *Front Plant Sci* 2012, **3**:184.
22. Park E, Nebenfuhr A: **Myosin XIX of Arabidopsis thaliana accumulates at the root hair tip and is required for fast root hair growth.** *PLoS One* 2013, **8**:e76745.
23. Nunokawa SY, Anan H, Shimada K, Hachikubo Y, Kashiyama T, Ito K, Yamamoto K: **Binding of chara Myosin globular tail domain to phospholipid vesicles.** *Plant Cell Physiol* 2007, **48**:1558-1566.
- 24. Peremyslov VV, Morgun EA, Kurth EG, Makarova KS, Koonin EV, Dolja VV: **Identification of myosin XI receptors in Arabidopsis defines a distinct class of transport vesicles.** *Plant Cell* 2013, **25**:3022-3038.

The authors identify a new family of transmembrane proteins as myosin XI receptors.

25. Stephan O, Cottier S, Fahlen S, Montes-Rodriguez A, Sun J, Eklund DM, Klahre U, Kost B: **RISAP is a TGN-associated RAC5 effector regulating membrane traffic during polar cell growth in tobacco.** *Plant Cell* 2014, **26**:4426-4447.
26. Hashimoto K, Igarashi H, Mano S, Takenaka C, Shiina T, Yamaguchi M, Demura T, Nishimura M, Shimmen T, Yokota E: **An isoform of Arabidopsis myosin XI interacts with small GTPases in its C-terminal tail region.** *J Exp Bot* 2008, **59**:3523-3531.
27. Peremyslov VV, Prokhnovsky AI, Dolja VV: **Class XI myosins are required for development, cell expansion, and F-Actin organization in Arabidopsis.** *Plant Cell* 2010, **22**:1883-1897.
28. Vidali L, Burkart GM, Augustine RC, Kerdavid E, Tuzel E, Bezanilla M: **Myosin XI is essential for tip growth in Physcomitrella patens.** *Plant Cell* 2010, **22**:1868-1882.
29. Yang L, Qin L, Liu G, Peremyslov VV, Dolja VV, Wei Y: **Myosins XI modulate host cellular responses and penetration resistance to fungal pathogens.** *Proc Natl Acad Sci U S A* 2014, **111**:13996-14001.
- 30. Cai C, Henty-Ridilla JL, Szymanski DB, Staiger CJ: **Arabidopsis myosin XI: a motor rules the tracks.** *Plant Physiol* 2014, **166**:1359-1370.

The authors use high spatiotemporal imaging of live cells to provide genetic evidence that three *Arabidopsis* class XI myosins contribute to actin remodeling by stimulating F-actin turnover and generating a force for actin filament shape changes.

31. Peremyslov VV, Prokhnovsky AI, Avisar D, Dolja VV: **Two class XI myosins function in organelle trafficking and root hair development in Arabidopsis.** *Plant Physiol.* 2008, **146**:1109-1116.

32. Avisar D, Abu-Abied M, Belausov E, Sadot E: **Myosin XIK is a major player in cytoplasm dynamics and is regulated by two amino acids in its tail.** *J Exp Bot* 2012, **63**:241-249.

•33. Stefano G, Renna L, Brandizzi F: **The endoplasmic reticulum exerts control over organelle streaming during cell expansion.** *J Cell Sci* 2014, **127**:947-953.

The authors use an *Arabidopsis* ER membrane protein mutant to show that ER membrane fluidity and motility are important for motilities of cytoplasmic organelles.

34. Stefano G, Hawes C, Brandizzi F: **ER - the key to the highway.** *Curr Opin Plant Biol* 2014, **22**:30-38.

35. Hawes C, Kiviniemi P, Kriechbaumer V: **The endoplasmic reticulum: a dynamic and well-connected organelle.** *J Integr Plant Biol* 2015, **57**:50-62.

•36. Tamura K, Iwabuchi K, Fukao Y, Kondo M, Okamoto K, Ueda H, Nishimura M, Hara-Nishimura I: **Myosin XI-i links the nuclear membrane to the cytoskeleton to control nuclear movement and shape in *Arabidopsis*.** *Curr. Biol.* 2013, **23**:1776-1781.

The authors demonstrate that *Arabidopsis* myosin XI-i is responsible for relatively slow movement in plant cells. This paper proposes a plant-specific nucleocytoplasmic linker that regulates nuclear shape and movement.

37. Avisar D, Abu-Abied M, Belausov E, Sadot E, Hawes C, Sparkes IA: **A comparative study of the involvement of 17 *Arabidopsis* myosin family members on the motility of Golgi and other organelles.** *Plant Physiol* 2009, **150**:700-709.

38. Griffis AH, Groves NR, Zhou X, Meier I: **Nuclei in motion: movement and positioning of plant nuclei in development, signaling, symbiosis, and disease.** *Front Plant Sci* 2014, **5**:129.

39. Higa T, Suetsugu N, Wada M: **Plant nuclear photorelocation movement.** *J Exp Bot* 2014, **65**:2873-2881.

40. Iwabuchi K, Sakai T, Takagi S: **Blue light-dependent nuclear positioning in *Arabidopsis thaliana* leaf cells.** *Plant Cell Physiol* 2007, **48**:1291-1298.

41. Iwabuchi K, Minamino R, Takagi S: **Actin reorganization underlies phototropin-dependent positioning of nuclei in *Arabidopsis* leaf cells.** *Plant Physiol* 2010, **152**:1309-1319.

42. Higa T, Suetsugu N, Kong SG, Wada M: **Actin-dependent plastid movement is required for motive force generation in directional nuclear movement in plants.** *Proc Natl Acad Sci U S A* 2014, **111**:4327-4331.

43. Sattarzadeh A, Krahmer J, Germain AD, Hanson MR: **A myosin XI tail domain homologous to the yeast myosin vacuole-binding domain interacts with plastids and stromules in *Nicotiana benthamiana*.** *Mol Plant* 2009, **2**:1351-1358.

44. Suetsugu N, Dolja VV, Wada M: **Why have chloroplasts developed a unique motility system?** *Plant Signal Behav* 2010, **5**:1190-1196.

45. Ojangu EL, Tanner K, Pata P, Jarve K, Holweg CL, Truve E, Paves H: **Myosins XI-K, XI-1, and XI-2 are required for development of pavement cells, trichomes, and stigmatic papillae in *Arabidopsis*.** *BMC Plant Biol* 2012, **12**:81.

46. Zhao Q, Brkljacic J, Meier I: **Two distinct interacting classes of nuclear envelope-associated coiled-coil proteins are required for the tissue-specific nuclear envelope targeting of Arabidopsis RanGAP.** *Plant Cell* 2008, **20**:1639-1651.
47. Zhou X, Graumann K, Evans DE, Meier I: **Novel plant SUN-KASH bridges are involved in RanGAP anchoring and nuclear shape determination.** *J Cell Biol* 2012, **196**:203-211.
48. Oda Y, Fukuda H: **Dynamics of Arabidopsis SUN proteins during mitosis and their involvement in nuclear shaping.** *Plant J* 2011, **66**:629-641.
49. Dittmer TA, Stacey NJ, Sugimoto-Shirasu K, Richards EJ: **LITTLE NUCLEI genes affecting nuclear morphology in Arabidopsis thaliana.** *Plant Cell* 2007, **19**:2793-2803.
50. Sakamoto Y, Takagi S: **LITTLE NUCLEI 1 and 4 regulate nuclear morphology in Arabidopsis thaliana.** *Plant Cell Physiol* 2013, **54**:622-633.
51. Wang H, Dittmer TA, Richards EJ: **Arabidopsis CROWDED NUCLEI (CRWN) proteins are required for nuclear size control and heterochromatin organization.** *BMC Plant Biol* 2013, **13**:200.
52. Goto C, Tamura K, Fukao Y, Shimada T, Hara-Nishimura I: **The Novel Nuclear Envelope Protein KAKU4 Modulates Nuclear Morphology in Arabidopsis.** *Plant Cell* 2014, **26**:2143-2155.
53. Tamura K, Fukao Y, Iwamoto M, Haraguchi T, Hara-Nishimura I: **Identification and characterization of nuclear pore complex components in Arabidopsis thaliana.** *Plant Cell* 2010, **22**:4084-4097.
54. Zhou X, Groves NR, Meier I: **Plant nuclear shape is independently determined by the SUN-WIP-WIT2-myosin XI-i complex and CRWN1.** *Nucleus* 2015, **6**:144-153.
- 55. Okamoto K, Ueda H, Shimada T, Tamura K, Kato T, Tasaka M, Morita TM, Hara-Nishimura I: **Regulation of organ straightening and plant posture by an actin-myosin XI cytoskeleton.** *Nat. Plants* 2015, **1**:2015.2031.

This paper provides the first genetic evidence of a straightening system that quickly responds to environmental changes.

56. Darwin C: *The Power of Movements in Plants*. New York: D. Appleton and Company; 1880.
57. Gilroy S, Masson PH: *Plant Tropisms*. Oxford: Blackwell; 2008.
58. Stankovic B, Volkmann D, Sack FD: **Autotropism, automorphogenesis, and gravity.** *Physiol. Plant.* 1998, **102**:328-335.
59. Iino M: **Toward understanding the ecological functions of tropisms: interactions among and effects of light on tropisms.** *Curr. Opin. Plant Biol.* 2006, **9**:89-93.
- 60. Bastien R, Bohr T, Moulia B, Douady S: **Unifying model of shoot gravitropism reveals proprioception as a central feature of posture control in plants.** *Proc. Natl. Acad. Sci. USA* 2013, **110**:755-760.

The authors determine the gravitropic kinematics of different organs from 11 angiosperms and provide a mathematical model to explain the final organ shapes. This paper shows

that sensing of local curvature (called proprioceptive sensing) is as important as gravity sensing in gravitropic control.

61. Deeks MJ, Rodrigues C, Dimmock S, Ketelaar T, Maciver SK, Malho R, Hussey PJ: **Arabidopsis CAP1 - a key regulator of actin organisation and development.** *J Cell Sci* 2007, **120**:2609-2618.
62. Kandasamy MK, McKinney EC, Meagher RB: **A single vegetative actin isovariant overexpressed under the control of multiple regulatory sequences is sufficient for normal Arabidopsis development.** *Plant Cell* 2009, **21**:701-718.
63. Kato T, Morita MT, Tasaka M: **Defects in dynamics and functions of actin filament in Arabidopsis caused by the dominant-negative actin fiz1-induced fragmentation of actin filament.** *Plant Cell Physiol* 2010, **51**:333-338.
64. van der Honing HS, Kieft H, Emons AM, Ketelaar T: **Arabidopsis VILLIN2 and VILLIN3 are required for the generation of thick actin filament bundles and for directional organ growth.** *Plant Physiol* 2012, **158**:1426-1438.
65. Lanza M, Garcia-Ponce B, Castrillo G, Catarecha P, Sauer M, Rodriguez-Serrano M, Paez-Garcia A, Sanchez-Bermejo E, T CM, Leo del Puerto Y, et al.: **Role of actin cytoskeleton in brassinosteroid signaling and in its integration with the auxin response in plants.** *Dev Cell* 2012, **22**:1275-1285.
66. Hayakawa K, Tatsumi H, Sokabe M: **Actin stress fibers transmit and focus force to activate mechanosensitive channels.** *J Cell Sci* 2008, **121**:496-503.
67. Tominaga M, Nakano A: **Plant-Specific Myosin XI, a Molecular Perspective.** *Front Plant Sci* 2012, **3**:211.
68. Henn A, Sadot E: **The unique enzymatic and mechanistic properties of plant myosins.** *Curr Opin Plant Biol* 2014, **22**:65-70.
69. Diensthuber RP, Tominaga M, Preller M, Hartmann FK, Orii H, Chizhov I, Oiwa K, Tsiavaliaris G: **Kinetic mechanism of Nicotiana tabacum myosin-11 defines a new type of a processive motor.** *FASEB J* 2015, **29**:81-94.
- 70. Tominaga M, Kimura A, Yokota E, Haraguchi T, Shimmen T, Yamamoto K, Nakano A, Ito K: **Cytoplasmic streaming velocity as a plant size determinant.** *Dev Cell* 2013, **27**:345-352.

The authors use transgenic plants expressing chimeras of *Arabidopsis* myosin XI2 with the high-speed *Chara* myosin XI motor or the low-speed human myosin V motor and show that plant size positively correlates with cytoplasmic streaming velocity.

71. Prokhnevsky AI, Peremyslov VV, Dolja VV: **Overlapping functions of the four class XI myosins in Arabidopsis growth, root hair elongation, and organelle motility.** *Proc Natl Acad Sci U S A* 2008, **105**:19744-19749.
72. Steffens A, Jaegle B, Tresch A, Hulskamp M, Jakoby M: **Processing-body movement in Arabidopsis depends on an interaction between myosins and DECAPPING PROTEIN1.** *Plant Physiol* 2014, **164**:1879-1892.
73. Ojangu EL, Jarve K, Paves H, Truve E: **Arabidopsis thaliana myosin XIK is involved in root hair as well as trichome morphogenesis on stems and leaves.** *Protoplasma* 2007, **230**:193-202.

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

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

²Currently, no significant abnormal phenotypes of a single mutant lacking myosin XIa, XIc, XI_d, XI_e, XI_g, XI_h, or XI_j has been reported.

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

Hara-Nishimura, Figure 1