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Understanding myosin functions in plants: are we there yet?

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Myosins are motor proteins that drive movements along actin filaments and have long been assumed to be responsible for cytoplasmic streaming in plant cells. This conjecture is now firmly established by genetic analysis in the reference species, *Arabidopsis thaliana*. This work and similar approaches in the moss, *Physcomitrella patens*, also established that myosin-driven movements are necessary for cell growth and polarity, organelle distribution and shape, and actin organization and dynamics. Identification of a mechanistic link between intracellular movements and cell expansion has proven more challenging, not the least because of the high level of apparent genetic redundancy among myosin family members. Recent progress in the creation of functional complementation constructs and identification of interaction partners promises a way out of this dilemma.

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Introduction

Myosins form a large family of motor proteins that convert the energy released by ATP hydrolysis into mechanical force to move cargo along actin filaments. Myosin genes are found in almost all eukaryotes and can be grouped into 35 different classes, of which class VIII and class XI are found in plants [1]. A recent comprehensive analysis of all known plant myosins has provided a definitive picture of myosin evolution in plants [2]. On the basis of this analysis a new, unified myosin nomenclature was proposed [2] which removes previous inconsistencies and makes interspecies comparisons easier by clearly identifying orthologs (Table 1). Both types of plant myosins have a similar domain architecture (Figure 1) that is related to class V myosins in animals and fungi [3], with which they share a common ancestry [1]. The cellular function of myosins is generally assumed to be the movement of cellular components along actin filaments, which in the case of flowering plants takes the form of cytoplasmic streaming, that is, the rapid movement of organelles and cytosol throughout the cell (Figure 2) [4]. The biological function of this energetically very expensive process is largely unknown, although it has been proposed that the large size of plant cells necessitates a constant 'mixing' of cytoplasm to ensure rapid distribution of metabolites [5,6]. Comparisons with the related class V myosins in animals and fungi, which are involved in establishing and maintaining cell polarity [3], suggest that plant myosins may also participate in other cellular processes, but evidence for such activities has been slow to emerge. Recent progress, primarily in the model species *Arabidopsis thaliana* and *Physcomitrella patens*, has shed new light on these questions and allows us to draw preliminary conclusions about myosin functions in plants.

Loss of myosin VIII leads to growth defects in moss

Relatively little is known about class VIII myosins. Myosin VIII localization to the cell surface, endosomes, plasmodesmata and the forming cell plate, as determined by immunolocalization or expression of fluorescently labeled tail constructs, implicated these motors in endocytosis, cell–cell communication and/or transport, and cell division [7–12]. The myosin VIII gene family in angiosperms is relatively small with typically two to four paralogs [2], but a genetic analysis has not been described so far, making it difficult to assess the relevance of myosin VIII activity to any of these processes. By contrast, all five myosin VIII genes in the moss, *P. patens*, have recently been disrupted to test the effect of these genes on gametophyte growth [13•]. Interestingly, the resulting quintuple mutants were found to be viable albeit of smaller size than wild-type. This implies that this class of myosins modulates growth by facilitating a process that is limiting for cell expansion.

Curiously, the smaller and slower growth of the quintuple mutants was accompanied by developmental defects such as increased branching and earlier gametophore development. Additional experiments also revealed defects in hormone responses and nutrient utilization, suggesting that class VIII myosins can have pleiotropic effects on the physiology of plants [13•]. It remains to be seen whether these developmental defects are indirect effects of the reduced cell growth in the quintuple mutants, or whether a fundamental problem in hormone homeostasis in the mutants causes all the observed phenotypes. The excellent genetic tools available in *P. patens* [14,15] will be instrumental in resolving this problem.

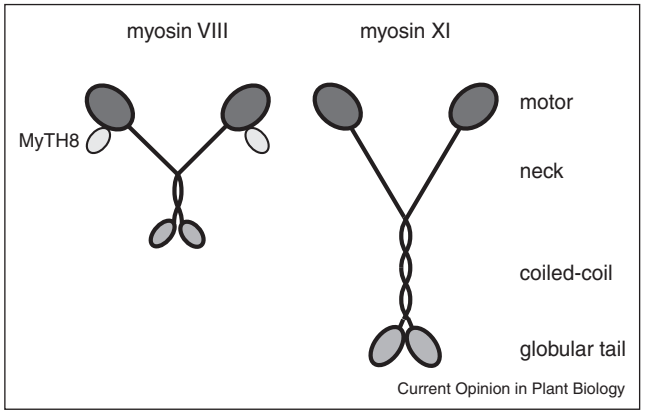
Table 1

New nomenclature of myosin XI genes based on their phylogenetic relationships

Subtype	*Arabidopsis thaliana*		*Oryza sativa*	
	New	Old	New	Old
Myo11A	*Myo11A1*	*XIA, XI-A*	*Myo11A1*	*OsMyoXID*
	Myo11A2	*XID, XI-D*	*Myo11A2*	*OsMyoXIG*
			Myo11A3	*OsMyoXIB*
Myo11B	*Myo11B1*	*XIB, XI-B*		
	Myo11B2	*MYA2, XI-2*		
	Myo11B3	*XIH, XI-H*		
	Myo11B4	*XIG, XI-G*		
Myo11C	*Myo11C1*	*XIC, XI-C*	*Myo11C*	*OsMyoXIJ*
	Myo11C2	*XIE, XI-E*		
Myo11D	*Myo11D*	*XIJ, XI-J*		
Myo11E	*Myo11E*	*XIK, XI-K*	*Myo11E1*	*OsMyoXIH*
	Myo11E2	*HDK*[a]	*Myo11E2*	*OsMyoXIA*
			Myo11E3	*OsMyoXIF*
			Myo11E4	(pseudogene)
Myo11F	*Myo11F*	*MYA1, XI-1*		
Myo11G	*Myo11G*	*XI-I*	*Myo11G1a*	*OsMyoXIC*
			Myo11G1b	*OsMyoXIK*
			MyoG2	*OsMyoXIL*
Myo11H	*Myo11H*	*XIF, XI-F*	*Myo11H*	*OsMyoXIE*

Letters identify the eight major subtypes and numbers (and lower-case letters) indicate taxon-specific duplications. Subtypes B, D, and F resulted from a relatively recent whole genome duplication in eudicots and are paralogous to subtypes A, C, and E, respectively. One member each of the subtypes A, C, E, G, and H is present in the earliest branching angiosperm, *Amborella trichopoda*, thus defining the minimal complement of myosin XI in flowering plants [2]. Old nomenclature for Arabidopsis [16,49,50] and rice [51] is included for comparison.
[a] *AtMyo11E2* (*HDK*) is a headless derivative of *AtMyo11E* (*XIK*) that lacks the motor and IQ domains [50].

Figure 1



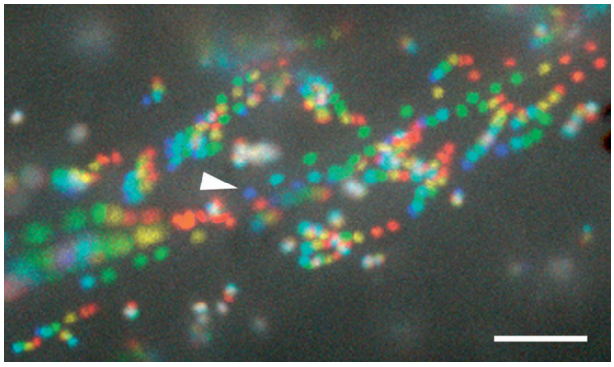
Schematic representation of the domain organization of myosin VIII and myosin XI of plants. The molecules are drawn to scale based on crystal structures of homologous myosins, except for the N-terminal extension (MyTH8) [2] and globular tail domain of myosin VIII. Not shown are the (small) SH3-like domains that precede the motor domain in many plant myosins. The motor domain binds actin and hydrolyzes ATP to generate movement [36]. The neck domain is bound by calmodulin-like proteins (not shown) and acts as a lever arm that amplifies the small movement of the motor domain [52]. The coiled-coil domain serves as dimerization interface to create a processive motor [53]. The globular tail functions as the cargo binding domain [32].

Myosin XI activity is required for expansion of many different cell types

Most of the effort towards understanding myosin function in plants has been directed at class XI myosins since it is generally assumed that these motors are responsible for cytoplasmic streaming [4]. Even though cytoplasmic streaming is absent from moss gametophytes, *P. patens* again demonstrated its usefulness as a model organism since the myosin XI gene family is very small in this species with only two expressed paralogs, compared to around 10 genes in angiosperms [2,16,17•]. Targeted silencing of both myosin XI transcripts in *P. patens* resulted in small round cells that failed to undergo polarization or initiate tip growth. The resulting plants were severely stunted, emphasizing that myosin XI is required for setting up or maintaining proper cell polarity and growth [17•].

It is not known whether a similar situation exists for class XI myosins in angiosperms since the large size of the gene family (typically seven to thirteen [2]) has so far prevented elimination of all myosin XI function from these plants. However, several publications have examined the effects of T-DNA insertions in highly expressed genes of *A. thaliana*, primarily *Myo11E* (*XIK*) and *Myo11B2* (*MYA2*) [18,19,20•,21–23]. A common

Figure 2



Movement of Golgi stacks in an onion epidermal cell. Eight images were taken in 1 s intervals and color coded from blue to red to illustrate the movement of individual Golgi stacks. The arrowhead indicates the starting point (blue) of a Golgi stack that moves rapidly to the upper right. Size bar = 10 μm.

theme that emerged from these studies is that myosin XI genes seem to act redundantly to enhance cell expansion in many different tissues. This is most readily visible in root hairs, where single mutants of *Myo11E* (*XIK*) and *Myo11B2* (*MYA2*) genes already show an effect [18,19]. Higher order mutants involving these and other genes show progressively more stunting that coincides with reduced cell expansion in leaves [20•,21,22] and trichomes [20•]. A recently published detailed study on the *myo11f myo11b2 myo11e* (*mya1 mya2 xik*) triple mutant revealed that pavement cells in the epidermis of leaves are not only smaller than in wild-type but also showed reduced lobing [20•], again emphasizing the function of myosin XI in normal cell growth. Interestingly, this study also demonstrated that the placement of trichome branches is altered in the *myo11f myo11b2 myo11e* (*mya1 mya2 xik*) triple mutant, suggesting that myosin function is also required for normal branch point selection.

In addition to these cell expansion defects, it was also found that fertility was reduced in the *myo11f myo11b2 myo11e* (*mya1 mya2 xik*) triple mutant [20•,21]. Reciprocal crosses revealed that the reduced number of seeds per silique resulted from a female defect [20•] which was consistent with the mutated genes showing very low expression levels in pollen but high levels in the stigma. Scanning electron microscopy demonstrated that the low fertility rate of the triple mutant correlated with delayed elongation of stigmatic papillae, which in turn resulted in poor pollination [20•]. Thus, the reduced fertility of higher order mutants could also be traced back to a cell expansion defect. The mechanism(s) by which class XI myosins mediate their effects on cell growth are still largely unknown, although recent research has identified several aspects of cell physiology that are affected in myosin XI mutants.

Subcellular defects linked to myosin XI function

Myosin XI motors have been thoroughly examined for their effect on organelle motility and cytoskeletal elements by employing two different approaches. In the first approach, various fluorescent markers were expressed in myosin knockout mutants. This demonstrated that Golgi stacks [19,21,22,24], peroxisomes [19,21,22], mitochondria [19,22], and the endoplasmic reticulum [23] move at reduced speeds in *Arabidopsis* mutants of class XI myosins. The second approach employed by several groups was to overexpress the tail portion of myosins in cells expressing various organelle markers. These dominant-negative tail fragments resulted in reduced motility of Golgi stacks [24–27], peroxisomes [25,26], mitochondria [25–27], endoplasmic reticulum [28,29,30••], *trans*-Golgi network [24], prevacuolar compartment [24], endosomes [24], and exocytic vesicles [24].

Interestingly, these results did not reveal a simple one-to-one relationship between myosin isoforms and specific organelles. While assigning unique functions to individual myosin XI genes is inherently difficult with the dominant negative approach due to potential off-target effects [31], this also proved to be difficult for knockout mutants. Single class XI myosin mutants have been shown to influence the motility of multiple organelles, and the motility of a particular organelle was affected by more than one myosin mutant (e.g. *myo11b2* [*mya2*] and *myo11e* [*xik*] [19]). It is not clear how this apparent promiscuity arises since various tail constructs of the different myosin XI isoforms were shown to localize to different organelles [26,27,32–34] and hence are expected to move only these subcellular compartments. It is possible that at least some organelle movements observed in wild-type occur passively, that is, as a result of the general hydrodynamic flow caused by movement of other organelles [35]. A reduction of this hydrodynamic flow in a given myosin mutant would then cause all organelles to display reduced movements even though they are not directly moved by this myosin. Alternatively, myosin XI mutations may affect other, more general aspects of intracellular dynamics that then lead to an overall reduction in organelle movement. A candidate for such a 'common factor' has emerged recently in the form of the actin cytoskeleton.

Actin filaments are well established as the tracks along which myosin motors move [4,36], but interestingly, these filaments can also be moved by myosins. This feed-back effect became evident from work in *P. patens* where loss of all myosin XI activity resulted in a complete disorganization of the normally highly polarized actin array [17•].

Similarly in *A. thaliana*, triple *myo11f myo11b2 myo11e* (*mya1 mya2 xik*) and quadruple *myo11f myo11b2 myo11e myo11g* (*mya1 mya2 xik xi-i*) myosin XI mutants displayed actin cables that were oriented more transversely while cables in equivalent wild-type cells were primarily longitudinal [21]. This latter result was later expanded to *myo11b2 myo11e* (*mya2 xik*) double mutants that showed a subtle phenotype of more skewed actin filaments that did not align as well with the long axis of the cell [23]. Recently, a reduction in actin filament dynamics was described in the *myo11e* (*xik*) single mutant that did not show a difference in actin organization [37•], thus confirming a direct effect of myosin motors on actin filaments that had previously been inferred from inhibitor treatments [38]. Presumably, the movement of myosin motors along actin filaments introduces tension in the filament network which ultimately leads to alignment of the filaments with the long axis of the cell. It should be noted, however, that actin dynamics do not appear to be affected by the near total loss of myosin XI activity in *P. patens* [17•]. In this case, however, an interdependence of myosin and actin localization at the tip was found that again highlights the mutual regulation of tracks and motors [39]. Thus, the situation in plants is similar to yeast and mammalian cells where myosin V has been found to influence actin organization and thereby influence cell polarity [40,41].

An exciting addition to the repertoire of myosin functions was described in *Zea mays*. Cloning of the *Opaque1* gene revealed it to be *ZmMyo11G1*, one of two close homologs of *AtMyo11G* (*XI-I*) in *A. thaliana* [30••]. The *opaque1* phenotype of non-translucent seed kernels was caused by accumulation of abnormally small, non-spherical protein bodies in the endosperm. These protein bodies are subdomains of the endoplasmic reticulum. Interestingly, Opaque1 myosin appears to be associated with ER membranes based on cell fractionation studies, and ER dynamics are reduced in *opaque1* mutants [30••]. Similar effects on ER dynamics and organization have also been described for myosin mutants in *A. thaliana* [21,23]. How these myosin driven movements of the ER lead to the formation of appropriately sized zein protein bodies in the lumen of the ER is still not clear, but this discovery highlights the variety of myosin functions that go beyond classical cytoplasmic streaming.

Another example of 'alternative' myosin functions was revealed when a mutant screen for abnormal nuclear shape in *A. thaliana* identified a defect in *Myo11G* (*XI-I*) [42••]. Loss of this myosin also resulted in greatly reduced nuclear motility, which is consistent with the subcellular localization of this motor to the nuclear envelope [27,42••]. Other myosin single mutants did not affect nuclear shape, thus demonstrating a unique functional specialization of this myosin in *A. thaliana*. At the same time, however, *Myo11G* (*XI-I*) appears to contribute to overall growth since loss of this gene in the *myo11f myo11b2 myo11e* (*mya1 mya2 xik*) triple mutant exacerbates the phenotype [21]. Whether this effect depends on redundant (i.e. overlapping) functions of the involved myosins or on separate contributions of the myosins to different growth-limiting processes is currently not known. It is also noteworthy that two *Myo11G* type myosins appear to perform vastly different functions in *A. thaliana* and *Z. mays* [30••,42••]. Apparently, the simple assumption that orthologous genes perform the same or similar functions is not always correct.

Myosin localization and interacting partners provide further insight

A crucial aspect in understanding how myosins exert their function is to identify their molecular interactions inside cells. While binding of the myosin motor domain to actin filaments is often considered a given and hence rarely investigated [43], it is the interaction of the tail domain with myosin cargo that is of central importance in this respect [44]. Considerable effort has been extended towards identification of the subcellular localization of various tail constructs tagged with fluorescent proteins [26,27,32–34]. Unfortunately, the functional relevance of these localizations cannot be assessed since the tail constructs appear to exert their dominant negative effect on organelle movements non-selectively. In other words, a given tail construct either inhibits movement of all tested organelles, as is the case for Myo11E (XIK) [24,27], or of none, as is the case for Myo11A1 (XIA) [27]. It is therefore necessary to create full-length fusion constructs that can functionally complement well-documented mutant phenotypes.

This was first accomplished in *P. patens* [17•], but recently two groups succeeded in this endeavor in *A. thaliana* and rescued the mutant phenotypes with YFP-tagged transgenes. In the first case, a Myo11E-YFP (XIK-YFP) construct driven by the native *Myo11E* (*XIK*) promoter was transformed into the *myo11f myo11b2 myo11e* (*mya1 mya2 xik*) triple mutant [45•], while in the second case, the YFP tag was fused to the N-terminus of Myo11E (YFP-XIK) and introduced into the *myo11e* (*xik*) single mutant, again under control of the native promoter [37•]. Interestingly, both *A. thaliana* constructs localized to the tip of growing root hairs as well as to motile vesicles in the shank of root hairs and leaf midrib epidermal cells [37•,45•]. The identity of the small vesicles or the nature of the accumulation at the root hair tip could not be identified, but it is tempting to speculate that they correspond to secretory vesicles as suggested by cell fractionation studies [45•], even though their distribution at the tip does not overlap completely with that of Myo11E (XIK) [37•].

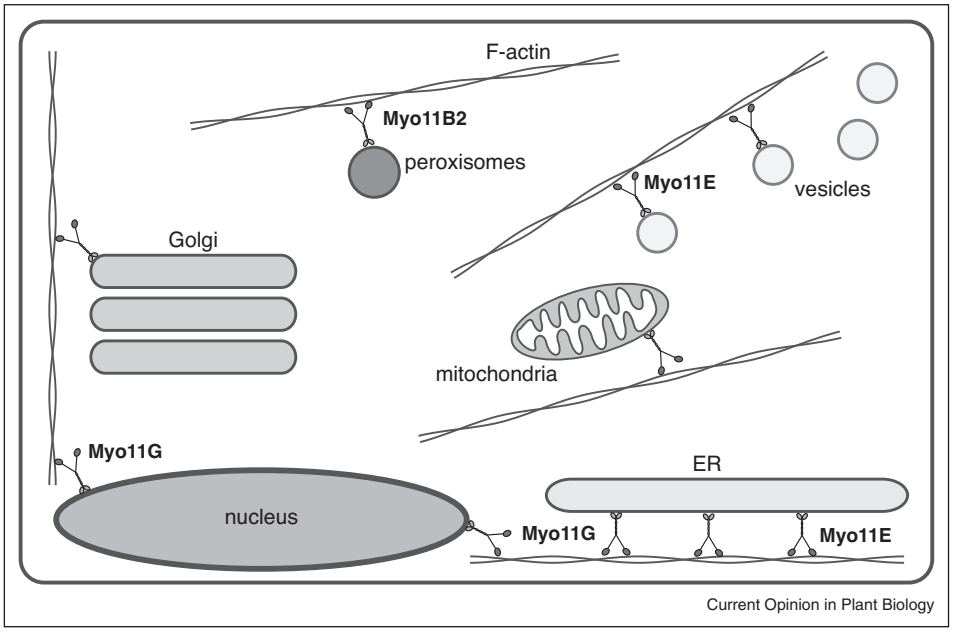
The pool of Myo11E (XIK) at the root hair tip depends on actin filaments [37•] and is constantly replenished

from other sources in the cell [45•]. This situation is similar to that described for myoXIa in *P. patens* which also accumulated at the tip of caulonemal cells during growth [17•,39]. It remains to be seen whether this pattern is found for all myosin XI motors or whether some of the isoforms function far away from the growing tip. Importantly, the ability to complement mutant phenotypes will also allow new experimental manipulations that take advantage of targeted alterations of myosin sequences.

Another approach to determine the functions of individual myosins is to identify interacting partners for each myosin. An earlier study using a yeast two-hybrid screen identified RabC2a and RabD1 as interacting partners for *Arabidopsis* Myo11B2 (MYA2) [46]. RabC2a was shown to localize to peroxisomes suggesting it may recruit Myo11B2 (MYA2) to peroxisomes [46]. A second yeast two-hybrid screen identified HSP70-interacting protein (HIP) as an interacting partner for *Z. mays* Opaque1 (ZmMyo11G1) [30••]. The C-terminus of HIP bound Opaque1 while the N-terminus of HIP contains a tetratricopeptide repeat domain that could be involved in other protein–protein interactions [30••], raising the possibility that HIP could act as an adaptor between Opaque1 and the ER. Further studies are necessary to confirm the biological relevance of these interactions and their regulation.

A recent study identified, also by a yeast two-hybrid screen, a family of plant-specific proteins containing the DUF593 domain of unknown function as interactors of several myosin XI isoforms in *A. thaliana* [47•]. These proteins appear to localize to a previously unidentified class of small vesicles that cannot be distinguished from the ER in density gradients, some of which colocalized with Myo11E-YFP (XIK-YFP) [47•]. In addition, different myosin XI isoforms showed various levels of selectivity for different members of the DUF593 family [47•], thus possibly explaining the observed mixture of genetic redundancy and specificity among myosin mutants. The detected genetic interaction with *myo11e* (*xik*) [47•] furthermore is compatible with the concept that DUF593 proteins act in the same pathway as myosins. Curiously, the DUF593 domain was previously identified as responsible for zein binding in the maize protein Floury1 [48]. While these two findings are clearly incompatible with each other, it is tempting to speculate that the similarity of the *opaque1* and *floury1* phenotypes actually results from the direct interaction of the

Figure 3



Myosin XI-dependent organelle movements. Most organelles in angiosperms display rapid, myosin-dependent movements that are thought to be driven by direct association of myosin motors with the organelle surfaces (active movement). It is also possible that at least some organelle movements are passive, that is, dependent on the hydrodynamic flow generated by other, actively moving organelles [35]. Several truncated myosins have been seen associated with various organelles in transient expression experiments, but the functional association of these motors with these organelles has not been verified. Direct motor–cargo interactions have been functionally and/or molecularly demonstrated for nuclei [42••], peroxisomes [46], and small vesicles [47•], and probably the ER [23]. The relevant myosins from *Arabidopsis thaliana* are indicated in the diagram. Interestingly, a Myo11G homolog in *Zea maize* is implicated in ER movement and protein storage body formation [30••], suggesting either multiple functions for individual myosin XI subtypes, or species-dependent specialization of myosin subtypes. It is generally assumed that the delivery of secretory vesicles to the plasma membrane is rate limiting for growth and that reduced directed movement of these vesicles in myosin mutants leads to reduced growth.

ZmMyo11G1 motor with its cognate DUF593 receptor on the ER membrane.

Another type of myosin interactor was discovered in *Arabidopsis* using a biochemical approach of immunoprecipitating plant expressed Myo11G (XI-I) tails and identifying co-precipitated proteins by mass spectrometry [42••]. In this case, a WPP domain-interacting tail-anchored protein (WIT2) was identified that localizes to the nuclear envelope. Importantly, loss of WIT2 and the related WIT1 in *wit1 wit2* double mutants resulted in mislocalization of YFP-Myo11G (XI-I) tails, thus demonstrating that WIT1 and WIT2 are required for attachment of this myosin motor to the nuclear envelope [42••]. Furthermore, the *wit1 wit2* double mutant resulted in the same phenotype of immobile round nuclei as the *myo11g* (*xi-i*) mutant, again confirming the close functional interaction of the two proteins. Finally, it could be shown by pull-down from plant extracts that WIT1/2 interacts with WIP, which in turn links to SUN proteins that anchor the inner nuclear membrane to the nuclear lamina [42••], thus beautifully explaining how the forces exerted by Myo11G (XI-I) on the nuclear surface lead to a different shape of the nucleus.

Conclusions

Recent years have seen major progress in our understanding of myosin function in plant cells. It is now firmly established that myosin motors are responsible for the rapid organelle movements that lead to cytoplasmic streaming. It is also clear that these movements are required for normal rapid expansion of cells. In addition, other effects of myosins on cell polarity as well as actin and ER organization and nuclear shape have been described, raising the possibility that the biological functions of these important motors are broader than the previously envisioned 'mixing' role. The distribution of these functions between myosin VIII and myosin XI is beginning to come into focus with recent progress in *P. patens*.

Deeper understanding of the mechanisms by which myosins exert these functions will require identification of the cargo molecules which are being moved by these motors (Figure 3). Knowledge of these interacting partners combined with the availability of functional full-length constructs will enable us to perform more specific experiments as nicely illustrated in the identification of myosin receptors on the nuclear surface [42••]. Additional progress along these lines should help us to overcome the apparent redundancy of myosin genes and establish the biological functions of all myosins in plants.

Acknowledgements

We apologize to our colleagues whose work could not be included due to space constraints.

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- • of special interest
- •• of outstanding interest

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