RESEARCH PAPER

Genome-wide identification, splicing, and expression analysis of the myosin gene family in maize (Zea mays)

Guifeng Wang*, Mingyu Zhong*, Jiajia Wang, Jushan Zhang, Yuanping Tang, Gang Wang† and Rentao Song†

Shanghai Key Laboratory of Bio-Energy Crops, School of Life Sciences, Shanghai University, No. 333 Nanchen Road, Shanghai, PR China

* These authors contributed equally to this work.
† To whom correspondence should be addressed. E-mail: wg@shu.edu.cn and rentaosong@staff.shu.edu.cn

Received 9 August 2013; Revised 17 November 2013; Accepted 20 November 2013

Abstract

The actin-based myosin system is essential for the organization and dynamics of the endomembrane system and transport network in plant cells. Plants harbour two unique myosin groups, class VIII and class XI, and the latter is structurally and functionally analogous to the animal and fungal class V myosin. Little is known about myosins in grass, even though grass includes several agronomically important cereal crops. Here, we identified 14 myosin genes from the genome of maize (Zea mays). The relatively larger sizes of maize myosin genes are due to their much longer introns, which are abundant in transposable elements. Phylogenetic analysis indicated that maize myosin genes could be classified into class VIII and class XI, with three and 11 members, respectively. Apart from subgroup XI-F, the remaining subgroups were duplicated at least in one analysed lineage, and the duplication events occurred more extensively in Arabidopsis than in maize. Only two pairs of maize myosins were generated from segmental duplication. Expression analysis revealed that most maize myosin genes were expressed universally, whereas a few members (XI-1, -6, and -11) showed an anther-specific pattern, and many underwent extensive alternative splicing. We also found a short transcript at the O1 locus, which conceptually encoded a headless myosin that most likely functions at the transcriptional level rather than via a dominant-negative mechanism at the translational level. Together, these data provide significant insights into the evolutionary and functional characterization of maize myosin genes that could transfer to the identification and application of homologous myosins of other grasses.

Key words: Alternative splicing, evolution, expression pattern, headless myosin, maize, myosin.

Introduction

In eukaryotic cells, motor proteins use the energy released from ATP hydrolysis to transport various intracellular cargos, including membranous organelles, protein complexes, and mRNAs, along tracks of cytoskeletal polymers (Lee and Liu, 2004). Of these systems, the actin–myosin system is required for the organization and dynamics of the endomembrane system and transport network in plant cells.

Plants harbour two unique myosin groups: class VIII and class XI. The latter is structurally analogous to class V in metazoans and fungi and contains a dilute domain in the globular tail (Li and Nebenfuhr, 2008). Class V myosin is a processive motor that is responsible for organelle and vesicle transport, and partitioning during cell division, mitotic spindle positioning, mRNA localization, and the establishment of cell polarity (Hammer and Sellers, 2012). Following the first cloned Arabidopsis myosin gene ATMI (Knight and Kendrick-Jones, 1993), many advances have been achieved in understanding plant myosin function over the past decade.

In Arabidopsis, the myosin family contains 17 genes, including 13 class XI (XI-A, -B, -C, -D, -E, -F, -G, -H, -I, -J, -K, -L, -M, -N).

Abbreviations: ER, endoplasmic reticulum; eYFP, enhanced yellow fluorescent protein; ORF, open reading frame; RT-PCR, reverse transcription-PCR.

© The Author 2013. Published by Oxford University Press on behalf of the Society for Experimental Biology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
XIK, and XI-2/MYA2) and four class VIII (VIII-1/ATM1, VIII-2/ATM2, VIII-A, and VIII-B) members (Reddy and Day, 2001). Immunolocalization studies have indicated that ATM1 appears to be localized in plasmodesmata in cress and maize root cells, suggesting possible roles in cell-plate maturation and actin cable re-establishment (Reichelt et al., 1999). Using transient expression of an ATM1 tail–green fluorescent protein fusion protein in tobacco, it was found that the signals co-localized with plasmodesmata and with the endosomal tracer FM4-64, implying a role of ATM1 in endocytosis (Golomb et al., 2008). However, ATM2 could bind to early endosomes and mediate transportation through the endocytic pathway in concert with ATM1 (Golomb et al., 2008; Sattarzadeh et al., 2008). Ectopic expression of the tail domains of tobacco class VIII myosins but not those of class XI could inhibit the plasmodesmal localization of the Hsp70 homologue of beet yellows virus (Avisar et al., 2008a).

In the moss Physcomitrella patens, the class VIII quintuple mutant Amyo8ABCDE is smaller, produces more side branches, and forms gametophores earlier than the wild type; it also generates protonemal patterning defects in the absence of nutrient medium (Wu et al., 2011). Thus, myosin VIII proteins are associated with endocytosis, cytokinesis, plasmodesmal function, and moss protonemal patterning.

In contrast, class XI contains a large number of members that originated from a burst of gene duplication. For example, Arabidopsis has 13 class XI genes with remarkable diversification. Immunolocalization studies have shown that MYA2 localizes on peroxisomes in epidermal and guard cells of Arabidopsis leaves in an actin-dependent manner (Hashimoto et al., 2005), and that overexpression of the tail domains of MYA1, MYA2, XI-C, XI-E, XI-I, and XI-K impair the motility of peroxisomes, Golgi bodies, and mitochondria in tobacco leaves (Avisar et al., 2008b, 2009; Sparkes et al., 2008; Sattarzadeh et al., 2011). Additionally, XI-K is physically associated with the endoplasmic reticulum (ER) and is required for its movement and remodelling (Sparkes et al., 2009; Ueda et al., 2010). An in vitro assay in tobacco BY2 cells indicated that myosin XI is also involved in tubular ER formation (Yokota et al., 2011). Recently, it was found that the functional, full-length XI-K–yellow fluorescent protein (YFP) fusion protein was associated primarily with endomembrane vesicles trafficking along F-actin instead of with larger organelles (Peremyslov et al., 2012).

However, functional dissection of the class XI myosins has largely been hindered owing to the redundancy of different paralogous genes. Only two (XIK and MYA2) of all 13 Arabidopsis class XI myosin mutants exhibit detectable phenotypes such as shorter root hairs under normal growth conditions (Peremyslov et al., 2008). Double mutants of Arabidopsis class XI myosin pairs indicate overlapping and additive effects for XI-K, XI-B, and MYA2 on root hair elongation (Prokhnevsky et al., 2008). Additionally, the xik mya2 mutant was stunted with reduced fecundity. Moreover, triple and quadruple mutants exhibited defects in cells undergoing polarized elongation and diffuse growth (Peremyslov et al., 2010). Simultaneous silencing of moss myosin XI-A and XI-B resulted in severely stunted plants that were composed of small, rounded cells (Vidali et al., 2010). Together, these data indicate that class XI myosins are involved in cytoplasmic streaming, organelle motility, and remodelling, and in plant growth and development.

In Poaceae, only limited progress has been achieved in myosin research, although grasses includes several agronomically important cereal crops with available whole-genome sequences. Twelve, seven, and nine class XI myosin genes exist in rice, sorghum, and brachypodium, respectively, and all contain two class VIII myosin genes (Jiang and Ramachandran, 2004; Peremyslov et al., 2011). Rice myosin XI-B is required for normal pollen development by localizing its protein in a photoperiod-sensitive manner (Jiang et al., 2007). Three myosin genes have been obtained in maize, and one of the XI members is associated with mitochondria, plastids, and the molecular chaperone subunit TCP-16 (Liu et al., 2001; Wang and Pesacreta, 2004). Previously, we functionally characterized opaque1, which encodes a myosin XI protein and influences protein body assembly by affecting ER morphology and motility (Wang et al., 2012a). Here, we employed bioinformatics and publicly available data to identify and analyse maize myosin genes at the genome-wide scale. These findings will provide an important blueprint for future maize myosin functional characterization that will transfer to other grass species for agricultural trait improvement, such as for Opaque1.

Materials and methods

Plant material

Maize inbred-line W22 plants were cultivated in the field at the campus of Shanghai University. Tissues (root, stem, the third leaf, silk, sheath, husk, tassel, and ear) were obtained from at least three healthy plants at the V12 stage as described previously (Wang et al., 2010), and developing kernels were collected at 3, 9, 15, 21, 27 and 33 d after pollination. Tobacco (Nicotiana benthamiana) plants were grown in a greenhouse under a 16/8 h day/night regime at a temperature of 20–25 °C (Wang et al., 2011). Maize callus was induced using F1 seeds of the hybrid Hi II line (Hi II pA×Hi II pB) and grown and maintained in an improved N6 medium.

Isolation and analysis of maize myosin genes

First, the conserved amino acids of the myosin head ATPase domain (Pfam: PF00063) and IQ motif (Pfam: PF00612) were used to search the maize genome database MaizeGDB (http://www.maizegdb.org/). Second, all Arabidopsis and rice myosin protein sequences were used as query sequences to search against the maize genome database and National Center for Biotechnology Information (NCBI) using the BLASTP program. The retrieved sequences were then assembled to remove redundancy. The Pfam (http://pfam.sanger.ac.uk/search) and SMART (http://smart.embl-heidelberg.de/) data bases were used to confirm each predicted maize myosin sequence. For misannotated or split myosins, reverse transcription (RT)-PCR was used to combine the separated cDNA fragments with the primers described in Supplementary Table S1 (at JXB online).

Gene model and splicing analysis of maize myosin genes

The information for annotated maize myosin genes, including accession number, chromosomal location, open reading frame (ORF) length and exon–intron structure, were retrieved directly from the
B73 maize sequencing database (http://www.maizesequence.org/index.html), and the exon–intron organization of our filled, complete myosins was identified in the maize sequence database using the BLASTN program and constructed using the DNAman software. RepeatMasker searching was used to identify repetitive sequences that were present in large introns (>1 kb) (Taraza-Graovac and Chen, 2009). Maize RNA-seq transcriptome data were downloaded from the NCBI Short Read Archive (accession numbers SRX105522, SRX105560, SRX058602, SRX058603, SRX058601, SRX058608, and SRP006965; http://www.ncbi.nlm.nih.gov/sra). RNA-seq reads were mapped to the maize genome assemblies using the TopHat 2.0.9 software (http://tophat.cbcb.umd.edu/; Trapnell et al., 2009).

Phylogenetic analysis

The amino acid sequences of maize myosins, along with those of Arabidopsis thaliana, Oryza sativa, Sorghum bicolor and Saccharomyces cerevisiae, were submitted to the ClustalW program at the BCM search launcher (Baylor College of Medicine, Houston, TX) using their default settings (pairwise alignment options: gap opening penalty 10, gap extension penalty 0.1; multiple alignment options: gap opening penalty 10, gap extension penalty 0.2, gap distance 0.5) to obtain amino acid sequence alignments (http://www.ncbi.nlm.nih.gov/sra). Pairwise alignments were used to compare the amino acid sequences of maize and sorghum (Schnable et al., 2009), maize myosins were mapped on chromosomes by identifying their physical chromosome position, which was provided in the maize sequence database. Gene duplication events of maize myosin genes were investigated according to block pairs, as described previously (Wei et al., 2007).

Chromosomal distribution and myosin gene duplication in maize

Based on a previously constructed syntenic map of maize, rice, and sorghum (Schnable et al., 2009), maize myosins were mapped on chromosomes by identifying their physical chromosome position, which was provided in the maize sequence database. Gene duplication events of maize myosin genes were investigated according to block pairs, as described previously (Wei et al., 2007).

RNA extraction and RT-PCR

Immature kernel RNA was isolated using a previously described protocol (Wang et al., 2012b), and RNA was extracted from tissues using TRIzol RNA extraction reagent (Tiangen). The residual gDNA of total RNA from each sample was reverse transcribed to cDNA using RevertAid H Minus Reverse Transcriptase (Fermentas) according to the manufacturer’s protocol. cDNA was amplified from maize kernel and inserted into the reconstructed pBI121 vector using the EcoRI and SalI sites, respectively. A transient expression assay was performed using a previously described method (Wang et al., 2012a; Gao et al., 2013), and images were obtained using a combination of 514 nm laser excitation and 530–580 nm long-pass emission filters using a Zeiss confocal microscope.

Subcellular localization

First, the enhanced YFP (eYFP) coding sequence was amplified from pB7WGY2.0 and inserted into pBI121 using the EcoRI–SalI and XhoI sites. The O1-head-IQ and O1-head cDNA fragments were amplified from maize kernel and inserted into the reconstructed pBI121 vector using the EcoRI and SalI sites, respectively. A transient expression assay was performed using a previously described method (Wang et al., 2012a; Gao et al., 2013), and images were obtained using a combination of 514 nm laser excitation and 530–580 nm long-pass emission filters using a Zeiss confocal microscope.

Myosin gene family in maize genome

Results

The maize myosin gene family has 14 members

With the complete maize genome sequence available (Schnable et al., 2009), BLASTP and TBLASTN searches were performed in MaizeGDB, using the head domain and full-length amino acid sequences of the rice (O. sativa) and Arabidopsis myosin proteins. We first retrieved 22 sequences encoding myosin homologues (Supplementary Table S2 at JXB online). Fourteen of the sequences were incomplete because they contained only head or tail domains. Of these sequences, six pairs contained separated myosin head and dilute domains at the approximate positions on the chromosomes, which indicated that these coupled sequences were most likely split from a complete myosin gene.

RT-PCR was used to fill the gaps between the coupled sequences, and we successfully obtained the missing cDNA sequences of the six incomplete myosin genes. Therefore, 14 complete myosin genes were identified in the maize genome (Table 1), including the previously reported Opaquel (Wang et al., 2012a, ZMM1, ZMM2, and ZMM3 (Liu et al., 2001). This number was comparable to that of its monocot relatives Sorghum (12) and rice (14), but was less than that in Arabidopsis (17). The genomic region of the maize myosin genes ranged from 8.218 to 85.126 kb and encoded proteins of 990–2641 aa. Noticeably, the average size of maize myosins was 32.918 kb (class VIII, 12.177 kb; class XI, 38.575 kb), which was much larger than those in Sorghum (16.887 kb), rice (16.595 kb), and Arabidopsis (8.981 kb).

Maize myosin genes possess typical domains but complicated intron–exon organization

The Pfam (Punta et al., 2012) and SMART (Letunic et al., 2012) databases were used to identify the putative domains that were present in the 14 complete maize myosins (Fig. 1, Supplementary Table S3 at JXB online). The myosins all contained a large, ATPase motor domain and several IQ motifs, which were used for ATP hydrolysis and binding calmodulin, respectively. Except for the 990 aa member (GRMZM2G460396), maize myosins could apparently be divided into two classes (VIII and XI) according to the
Table 1. Myosins identified from the completed maize genome sequence

| Myosin name/new nomenclature | Chromosome | Position (bp) | Strain | Length (kb) | Exon | Gene accession no. | No. of aa | MW (kDa) | pl | Evidence | Arabidopsis best hit |
|----------------------------|------------|---------------|--------|-------------|------|--------------------|----------|----------|----|----------|---------------------|
| ZmVIII-1/ ZmMyo8A1        | 1          | 229190070     | -      | 15.850      | 23   | GRMZM2G113202      | 1191     | 133.472  | 8.53 | Y(M3)    | VIII-1              |
| ZmVIII-2/ ZmMyo8A2        | 5          | 25983026      | -      | 11.034      | 23   | GRMZM2G139583      | 1194     | 134.072  | 8.50 | Y         | VIII-1              |
| ZmVIII-3/ ZmMyo8B         | 7          | 157571319     | -      | 9.647       | 24   | GRMZM2G057390      | 1238     | 139.825  | 9.13 | Y         | VIII-2              |
| ZmXI-1/ ZmMyo11A2         | 1          | 300108722     | -      | 40.839      | 38   | GRMZM2G471108      | 1506     | 170.675  | 7.70 | Y         | XI-B                |
| ZmXI-2/ ZmMyo11E2*        | 3          | 203463334     | +      | 44.509      | 52   | KF493895           | 2641     | 299.535  | 5.93 | Y         | XI-K                |
| ZmXI-3/ ZmMyo11E3         | 3          | 220883061     | -      | 19.056      | 39   | AC155377.1_FG001   | 1529     | 173.465  | 8.53 | Y(M1)    | XI-K                |
| ZmXI-4/ ZmMyo11G1         | 4          | 176870473     | -      | 26.818      | 38   | GRMZM2G449909      | 1520     | 173.204  | 8.85 | Y(O1)    | XI-I                |
| ZmXI-5/ ZmMyo11H          | 5          | 7359267       | -      | 8.218       | 34   | GRMZM2G460396      | 990      | 113.532  | 8.01 | Y         | XI-F                |
| ZmXI-6/ ZmMyo11G2         | 5          | 36445589      | +      | 85.126      | 38   | KF493897           | 1522     | 174.373  | 8.83 | Y         | XI-I                |
| ZmXI-7/ ZmMyo11A3         | 5          | 215701287     | +      | 13.163      | 39?  | KF493892           | 1554     | 175.882  | 8.48 | Y(M2)    | XI-B                |
| ZmXI-8/ ZmMyo11E1         | 6          | 161483674     | +      | 63.910      | 45   | KF493894           | 1973     | 224.613  | 6.87 | Y         | XI-K                |
| ZmXI-9/ ZmMyo11A1         | 7          | 36208341      | +      | 52.790      | 39?  | KF493893           | 1506     | 171.027  | 8.04 | Y         | XI-B                |
| ZmXI-10/ ZmMyo11E2b       | 8          | 154701271     | -      | 59.851      | 45?  | KF493896           | 1880     | 213.827  | 7.07 | Y         | XI-K                |
| ZmXI-11/ ZmMyo11C         | 9          | 4288567       | -      | 10.042      | 39   | GRMZM2G435294      | 1529     | 173.418  | 8.92 | Y         | XI-E                |

*The new nomenclature of myosin genes proposed by Madison and Nebenfuhr (2013).*
remaining domains. Compared with class VIII, the class XI myosins were much longer and had an N-terminal SH3-like domain and a tail dilute domain. Similar to that in Arabidopsis and rice, the maize myosin head domain was located more towards the N terminus in class XI than in class VIII. Moreover, the class VIII myosins contained a coiled-coil domain and four IQ motifs, whereas a variable number of both domains were present in class XI members.

The intron–exon structures of the maize myosin genes were determined by comparison of the cDNA with genomic sequences. The results revealed that maize myosin genes consisted of 23 (ZmVIII-1 and ZmVIII-2) to 52 (ZmXI-2) exons, and the sizes varied from 14 to 1199 bp (Fig. 2, Table 1 and Supplementary Table S4 at JXB online). The class VIII myosin genes contained 23–24 exons, whereas class XI genes harboured 38–52 exons. Noticeably, the length of most exons appeared to be conserved in the same order in the different maize myosin genes.

Compared with the conserved exon sizes in the same order, the introns were more divergent in length (57 bp to 22,499 kb) and caused the large sizes of the maize myosin genes. RepeatMasker searching was used to identify repetitive sequences that were present in large introns (>1 kb) (Tarailo-Graovac and Chen, 2009). As shown in Supplementary Table S5 (at JXB online), retroelements (L1/CIN4, Ty1/Copia, SINEs, RTE/Bov-B, and Gypsy/DIRS) and DNA transposons (hobo-Activator, Tourist/Harbinge, and Tcl-IS630-Pogo) were abundant in maize myosin introns. This finding revealed that transposable elements play an essential role in maize myosin size increase and intron–exon organization.

Maize myosins are less duplicated than those of Arabidopsis

The available myosin gene family in Arabidopsis, rice, and sorghum allowed us to investigate the evolutionary relationship between dicot and monocot myosin proteins. A neighbour-joining tree was constructed using the full-length protein sequences of 17 Arabidopsis, 13 rice, 9 sorghum, and 14 maize myosins, with two yeast class I myosins as an outgroup (Fig. 3). The results indicated that these myosins were divided distinctly into two groups, class VIII and class XI, which supports the suggestion that two myosin ancestors existed in planta before lineage-specific expansions (Peremyslov et al., 2011). Eleven myosins from maize, 13 from Arabidopsis, 11 from rice and seven from sorghum in class XI were found, whereas the smaller VIII group contained three maize, four Arabidopsis, and two rice and sorghum members. This result was generally consistent with the conclusion that was made previously using myosin motor domains (Peremyslov et al., 2011). The class VIII myosins were divided into two distinct subgroups (VIII-A and VIII-B), and class XI was split into the I, G, F, K, and E subdivisions (Peremyslov et al., 2011; Wang et al., 2012a). Interestingly, Arabidopsis XI-J was grouped alone into a clade without any close homologues.

It was clearly shown that class VIII myosins had undergone a single-gene duplication but that a burst of duplication occurred in class XI (Fig. 3). Two paralogous branches were formed within subclasses VIII-A and VIII-B in the dicot Arabidopsis compared with only one in monocots, excepting for the two in maize VIII-B. The largest subgroup, XI-G, contained six members in Arabidopsis but only three in the monocots rice, sorghum, and maize. Moreover, two paralogues were found within XI-K and XI-E in Arabidopsis, compared with only one in monocots. Consistently, subgroup XI-F was the only subdivision that was not duplicated in any of the plant lineages (Peremyslov et al., 2011). Noticeably, three maize and two rice myosins comprised a separate clade that was previously designated XI-K (Jiang and Ramachandran, 2004).

Chromosomal localization and myosin gene duplication in grass

To investigate the chromosomal distribution of the myosin family in maize, the loci were determined by directly
identifying their physical positions provided in MaizeGDB. As shown in Fig. 4, the 14 myosins were mapped on eight out of the 10 maize chromosomes, excluding chromosomes 2 and 10. The largest number of myosin members (four) was present on chromosome 5, followed by two genes each on chromosomes 1, 3, and 7. The remaining chromosomes (4, 6, 8, and 9) each had a unique myosin.

Maize originated from an ancient allotetraploid and has undergone several rounds of whole-genome duplication events during its gene evolution (Wei et al., 2007; Schnable et al., 2009). Among these maize myosin genes, four sister pairs (ZmVIII-1 and ZmVIII-2, ZmXI-1 and ZmXI-5, ZmXI-2 and ZmXI-10, and ZmXI-4 and ZmXI-7) appeared to be generated from segmental duplication events due to their position on the same duplicated gene blocks within maize (Fig. 4). To confirm this possibility, we further analysed myosin gene evolution among maize, rice, and sorghum (Table 2). Thirteen of 14 maize myosins had collinear genes in rice (the exception was ZmXI-9), while all had syntenic members in sorghum. Two of the sister pairs (ZmVIII-1 and ZmVIII-2, and ZmXI-2 and ZmXI-10) each had a unique, syntenic myosin in rice and sorghum, which indicated that they were generated from segmental duplication after the divergence between maize and its relatives (rice and sorghum). The remaining two pairs (ZmXI-1 and ZmXI-5, and ZmXI-4 and ZmXI-7) were each located on the same duplicated blocks, but coupled, syntenic myosins were found in rice and sorghum. It is possible that the ancestor of grasses already contained two myosin genes at this region and that one copy of each duplicate myosin pair was lost at the duplicated blocks after the whole-genome duplication in maize (Schnable et al., 2011). In addition, three rice myosin genes (OsXI-D, OsXI-K, and OsXI-L) had no syntenic member in maize or sorghum. OsXI-L was most likely duplicated from OsXI-I, whereas OsXI-D and OsXI-K appeared to be duplicated or redistributed from the original loci to the current positions, and the original gene was partially or fully lost (Salse et al., 2008).

Expression pattern of maize myosin genes
To investigate the spatial patterns of myosin gene expression in maize, we detected their transcripts using RT-PCR in eight representative tissues, i.e. sheath, stem, leaf, tassel, husk, silk, root, and ear (Fig. 5A). The results revealed that the majority of maize myosin genes (excluding ZmXI-2 and ZmXI-10) were expressed at varying levels in all tested tissues. The ZmXI-2 transcripts were particularly abundant in root but absent in silk and tassel. In comparison, its duplicated sister, ZmXI-10, was expressed at low levels in all tested tissues and was undetectable in tassel. Three myosin
We also investigated the expression levels of maize myosins in a broad range of tissues and organs of the inbred line B73 using a NimbleGen microarray (Sekhon et al., 2011). For the currently misannotated full-length myosin genes, we used probes corresponding to their head and tail domain for further analysis. According to their expression levels, maize myosin genes could be clustered into three categories (Fig. 5C). The first category was composed of eight genes (ZmVIII-1, -2 and -3; ZmXI-3, -4, -7, -9-tail and -10-tail) with high and ubiquitous expression levels, suggesting comprehensive roles in plant growth and development. All maize class VIII myosins belonged to this category; however, their biological functions remain to be elucidated. Five class XI myosins (ZmXI-2, -5, -8, -9-head and -10-head) formed the second category with intermediate and differential transcription levels. The third category included ZmXI-1, -6 and -11, for which the expression levels were relatively low. However, all three genes were expressed preferentially in anther, especially ZmXI-11 (Fig. 5C), implying that these myosins are required for anther and/or pollen growth and development.

Alternative splicing is a common character of the maize myosin genes

Alternative splicing plays an essential role in regulating gene expression during growth and development, as well as in response to biotic and abiotic stresses (Stamm et al., 2005; Syed et al., 2012). RNA-seq analysis has revealed that more than 61% of intron-containing genes in Arabidopsis undergo alternative splicing under normal growth conditions (Marquez et al., 2012). Due to their large sizes and the complicated gene models of myosins, we could speculate that maize myosin genes underwent extensive alternative splicing events. We then detected the splicing transcripts of the full-length cDNA for each maize myosin gene in leaf and kernel in two randomly selected windows. Six out of the 14 myosin genes had splicing transcripts in the given cDNA fragment in the two samples (Fig. 6 and Supplementary Fig. 1 at JXB online). Of these genes, four (ZmXI-2, -5, -7 and -10) possessed at least three splicing transcripts, while the remaining had two transcripts. Interestingly, ZmXI-11 was spliced in a tissue-dependent manner. A 375 bp cDNA fragment of ZmXI-11 was unique to tassel, while the others had an unspliced, 637 bp transcript (Fig. 6). It is unknown whether this alternative splicing affects the subsequent translation. In the case of the maize ZmXI-11 gene, the spliced transcript encoded a complete myosin (1529 aa), whereas the unspliced transcript generated a truncated myosin of 1454 aa in length that lacked a portion of the DIL domain. Together with its preferential expression pattern, this finding suggests that ZmXI-11 most likely plays an essential role in microsporogenesis.

To evaluate further the gene models for maize myosins, transcript evidence created using the high-throughput RNA-seq transcriptome data from developing kernels was compared with all publicly available myosin genomic and transcript data using the TopHat 2.0.9 software (Trapnell et al., 2009). According to this method, we revised the gene models for 13 maize class VIII and class XI myosins, except for ZmXI-11 with few reads in the

Fig. 3. Phylogenetic relationships between the entire myosins of maize, rice, sorghum, and Arabidopsis. The phylogenetic tree was constructed using MEGA 5.0 software, and yeast class I myosins were used as outgroups (see Materials and methods). At, Arabidopsis thaliana; Os, Oryza sativa; Sb, Sorghum bicolor; Sc, Saccharomyces cerevisiae; Zm, Zea mays.

XI genes, ZmXI-3, ZmXI-5, and ZmXI-8, exhibited high transcription levels in silk. Noticeably, ZmXI-11 was highly expressed in tassel, whereas the remaining myosins were less abundant in tassel when compared with the other tissues. Interestingly, the short ZmXI-11 transcript was unique to tassel, while the long, unspliced transcript was common in the remaining tissues (Fig. 6). Furthermore, the two duplicated myosin gene pairs shared similar expression patterns in all tissues. We also unravelled the expression profiles of the maize myosin genes during kernel development (Fig. 5B). Transcripts of all 14 maize myosins were detectable with dynamic patterns. Most were expressed preferentially at the early stage, and peaked approximately 3 and 9 d after pollination. ZmXI-3, ZmXI-6, and ZmXI-7 exhibited a distinct pattern, with two expression peaks at the early and late stages of kernel development. The duplicated gene pairs also showed similar expression profiles.
RNA-seq data (Supplementary Fig. 2 at JXB online). In the case of ZmXI-2, the RNA-seq transcript evidence supported the results that ZmXI-2 was a complete myosin gene (Fig. 7A) and our RT-PCR detected splicing transcripts did exist in the transcriptomic data (Fig. 7B). In addition, the RNA-seq data revealed a novel exon splicing event in annotated exon 19 (Fig. 7B). All these splicing variants resulted in truncated proteins that only harboured the myosin head domain (Fig. 7C).

Fig. 4. Chromosomal localization and segmental duplication of myosin genes in the maize genome (adapted from Schnable et al., 2009). S and R represent the syntenic blocks between maize and sorghum and rice, respectively. D indicates oriented homologous sites of duplicated gene blocks within maize. The coupled colours in the maize myosins represent genes that were located in the same block. (This figure is available in colour at JXB online.)

O1, a functional myosin, is responsible for ER streaming and morphology that ultimately affects endosperm texture

Previously, we isolated the maize endosperm mutant opaque1 (o1) using map-based cloning and found that it encoded a class XI myosin protein (Wang et al., 2012a). O1 was identical to ZmXI-4, and resulted in a transcript of 5131 bp, encoding a 173 kDa protein of 1520 aa. To study the association of ZmXI-4/O1 with actin filaments, we transiently expressed the ZmXI-4 (Head-6IQ/Head)–eYFP reporters, which harboured the head domain with/without the IQ motif fused to eYFP at its C terminus (Fig. 8A), for direct, microscopic observation. The signal of the ZmXI-4 head–neck domain protein showed a clear pattern of actin filaments throughout the entire cell (Fig. 8B) that also decorated the nucleus (Fig. 8C). When only the ZmXI-4 head domain was expressed, the YFP signal was similarly associated with actin filaments (Fig. 8D), indicating that the head domain was essential for myosin binding to actin filaments and that the IQ motif had no effect on this process. Together with overexpression of the ZmXI-4/O1 tail
Table 2. Analysis of gene duplication in maize myosins

The light grey and light and medium brown shading represent maize, rice, and sorghum myosin genes, respectively (from top to bottom); the other coupled shading represents the myosin genes in the same duplication block in maize.

| Maize myosin | Physical block | Chr | Block pairs | Rice syntenic region | Physical position | Chr | Sorghum syntenic region | Physical position |
|--------------|----------------|-----|-------------|----------------------|------------------|-----|------------------------|------------------|
| ZmVIII-1     | 46             | 1   | 10La        | Os10g34710 (OsVIII-A/B²) | 18.53            | 10  | Sb01g018770 (SbVIII-1) | 19.62            |
| ZmVIII-2     | 212            | 5   |             |                      |                  |     |                        |                  |
| ZmXII-1      | 212            | 5   |             |                      |                  |     |                        |                  |
| ZmXII-2      | 322            | 7   | 7L          | Os07g43750 (OsVIII-B/A²) | 22.48            | 7   | Sb02g038390 (SbVIII-2) | 70.79            |
| ZmXII-3      | 67             | 3   | 3L          | Os03g64220 (OsXI-G) | 36.33            | 3   | Sb01g009330 (SbXI-1) | 0.18             |
| ZmXII-4      | 206            | 5   |             | Os03g53660 (OsXI-F/E) | 30.76            | 3   | Sb01g008180 (SbXI-2) | 7.05             |
| ZmXII-5      | 143            | 3   | 1L          | Os01g51630, Os01g51632, Os01g51634 (OsXI-A) | 29.65* | 1 | Sb03g032760, Sb03g032770 (SbXI-5) | 61.20* |
| ZmXII-6      | 359            | 8   |             |                      |                  |     |                        |                  |
| ZmXII-7      | 150            | 3   | 1L          | Os01g40200 (Headless) | 22.68            | 1   | Sb03g026110 (SbXI-4) | 52.49            |
| ZmXII-8      | 182            | 4   | 2Lb         | Os02g53740 (OsXI-I/C²) | 32.90            | 2   | Sb04g034830 (SbXI-6) | 64.68            |
| ZmXII-9      | 254            | 5   |             | Os02g57190 (OsXI-B) | 35.02            | 3   |                        |                  |
| ZmXII-10     |                |     |             |                      |                  |     |                        |                  |
| ZmXII-11     | 217            | 5   | 10La        | Os10g25565 Os10g25560 (OsXI-C/K²) | 13.25* | 10 | Sb01g023160, (SbXI-10) | 30.58* |
| ZmXII-12     | 287            | 6   | 5L          | Os05g46030 (OsXI-H) | 26.68            | 5   | Sb09g026840, (SbXI-8) | 56.01* |
| ZmXII-13     |                |     |             |                      |                  |     |                        |                  |
| ZmXII-14     | 300            | 7   | 7S          | Os02g34390 (OsXI-D) | 20.36            | 2   | Sb02g010040 (SbXI-3) | 14.57            |
| ZmXII-15     | 368            | 9   | 6S          | Os09g23950 (OsXI-E/U²) | 16.77            | 6   | Sb10g098210 (SbXI-9) | 8.22             |
| ZmXII-16     |                |     |             | Os10g19860 (OsXI-L) | 9.90             | 10  |                        |                  |

a Maize FPC contigs (http://www.genome.arizona.edu/fpc/maize/WebFPC/) have been numbered sequentially along each chromosome.
b Regions on chromosomes that have contiguous contigs that are syntenic with rice as defined by SyMAP.
c The physical position on each chromosome.
d The former letter represents the rice myosin gene name that was proposed by this study; the latter indicates that proposed by Jiang and Ramachandran (2004).
e The misannotated myosin genes.
Fig. 5. Expression patterns of maize myosin genes. (A) RNA expression levels of maize myosin genes in various tissues. (B) Expression profiles of myosin genes during maize kernel development. (C) Development- and anatomy-specific expression profiles of maize myosin genes. Public microarray data sets were obtained from PLEXdb. The genes are located on the right and the tissues are indicated at the bottom of each column. The colour bar represents the expression values. Head and tail represent the splicing cDNAs encoding myosin head and tail domains, respectively. (This figure is available in colour at *JXB* online.)
domain significantly inhibiting ER streaming in tobacco cells and the phenotypes of the opaque1 mutant. O1 was characterized as a functional myosin XI protein that was responsible for protein body assembly and endosperm texture determination (Wang et al., 2012a).

We further explored whether functional myosin headless variants were present in the genome and their roles during plant growth and development. Apart from the longest 5131 bp, mature transcript of ZmXI-4/O1, we also identified a 2380 bp transcript that consisted of the second half of the longest transcript at the same locus (Fig. 9A) using two independent, 5′ RACE experiments (Supplementary Fig. 3 at JXB online). This short transcript contained an ORF of 1935 bp that conceptually encoded a 73 kDa headless myosin. Domain prediction analysis revealed that the deduced protein contained coiled-coil and DIL domains, and completely lost the N-terminal SH3-like domain, head domain, and IQ motif (Fig. 9B). To verify whether this headless myosin was a novel gene model or an alternative splicing variant of O1, the approximately 2 kb genomic regions that were located upstream of the transcription start sites of O1 (P1) and the headless myosin (P2) were fused to the GUS ORF. After bombardment into maize callus, substantial GUS staining was observed for the O1pro::GUS construct, while the headless myosin promoter showed no activity in the callus or developing kernel (Fig. 9C, D). Curiously, the predicted headless myosin protein was also undetectable in all samples using an immunoblot assay (Fig. 9E). This finding suggested that this short transcript was a headless derivative of O1 and most likely functioned at the transcriptional level rather than in regulation of O1 activity via dominate-negative suppression in vivo.

Discussion

Myosins are cytoskeletal molecular motors that use the energy released from ATP hydrolysis to move along actin filaments. In a genome-wide screen, we identified 14 complete myosin genes that harboured conserved domains in maize. A comparable number of myosin genes exists in maize (14), rice (14), and Arabidopsis (17), although the maize genome size (2300 Mb) (Schnable et al., 2009) is ~5.3 and ~18.4 times larger than those of rice (430 Mb) (Burr, 2002) and Arabidopsis (125 Mb) (Arabidopsis, 2000), respectively. This huge discrepancy might partially be compensated by the larger average size of the maize myosin genes, which are nearly two and four times longer than those in rice and Arabidopsis, respectively. Moreover, the exon sizes of the maize myosin genes are similar to those in rice and Arabidopsis, but maize myosin genes contain larger introns because of the insertion of transposable elements (Supplementary Table S5). Overall, transposable elements comprise approximately 85% of the B73 maize genome (Schnable et al., 2009) and play important roles in genome organization and evolution (Fedoroff, 2012), as well as in gene-family expansion (Janousek et al., 2013), which is most likely what is occurring in the maize myosin gene family.

Phylogenetic reconstruction analyses using the sequence information of several complete genomes of the green algae, mosses, and higher plants indicate that two types of myosin (VIII and XI) are present in planta (Avisar et al., 2008b) and that three class VIII and 11 class XI myosin members are present in maize. In most cases, subgroups are duplicates of at least one lineage (Fig. 4). For example, subgroup VIII-A and VIII-B were duplicated after the separation between dicot...
Arabidopsis and monocots, while VIII-A was duplicated after the divergence between maize and its relatives. Prominently, only XI-F has not been duplicated within any lineage. The Arabidopsis T-DNA mutant of XI-F showed no discriminable phenotypes in vegetative growth (Peremyshl et al., 2008), and some unexpected clades, such as Arabidopsis XI-J and the maize- and rice-specific XI-K, were found. Thus, the specific function of these myosin genes still requires further experimental elucidation.

Two rounds of whole-genome duplications have occurred during cereal genome evolution: the first occurred before the divergence of maize, rice, and sorghum, whereas the second took place in the specification of the maize lineage (Salse et al., 2009). It appears that relatively more family members exist in maize than in other cereals; however, this is not the case for the maize myosin gene family (Fig. 5, Table 2). Overall, most of the myosins were present before the specification of maize, rice, and sorghum, but they were subjected to gene loss and redistribution within a specific lineage. For example, the rice syntenic gene (OsXI-D) of ZmXI-9 was lost in the syntenic region 7S but was redistributed to a new locus in rice chromosome 2. Only two myosin pairs (ZmVIII-1 and ZmVIII-2, and ZmXI-2 and ZmXI-10) were segmentally duplicated after the divergence of maize and its relatives.

**Fig. 7.** Transcript evidence and splicing for maize myosin ZmXI-2. RNA-seq reads were mapped to the maize genome assemblies using TopHat 2.0.9 software. (A) RNA-seq reads distribution and the empirical gene models of ZmXI-2. A box and an arrow mark alternative splicing events. Reads coloured in red and blue represent the sense and antisense sequences, respectively. (B) Validation of splicing details of ZmXI-2 detected in Fig. 6, I, intron 17–18 retention; II, intron 18–19 retention; III, exon 19 splicing. Intron 17–18 and Intron 18–19 unspliced correspond to the 543 bp band in Fig. 6; intron 17–18 unspliced corresponds to the 463 bp band. (C) Deduced protein models of ZmXI-2 and its splicing transcripts. All the splicing variants encode truncated proteins only having the myosin head domain.
Myosin gene family in maize genome

relatives. Additionally, the *Arabidopsis* myosins have been amplified in most of the subgroups. Therefore, the events that triggered these lineage-specific duplications of the myosin genes and the subfunctionalization or neofunctionalization of their paralogous myosins following the duplications are becoming known.

Fig. 8. O1 is associated with actin filaments. (A) Schematic diagrams of the CaMV35S::O1(Head-IQ or Head)–eYFP vectors in which eYFP was fused at the C terminus. (B–D) Representative tobacco leaf pavement cells are shown by confocal microscopy 2 d after agroinfiltration. O1(Head-IQ) is present in actin filaments (B) and decorates the nucleus (C); the signals of the O1(Head) are similarly present in actin filaments (D). (This figure is available in colour at JXB online.)

Fig. 9. Identification and analysis of the headless derivative of O1. (A) A putative gene model of the maize headless myosin. TSS, transcription start site; P1 and P2, promoter regions of O1 and the headless myosin. (B) Conserved domains present in the maize headless myosin, which lose the N-terminal SH3-like domain, head domain, and IQ motif. (C) GUS staining of the O1 pro::GUS construct observed in the maize callus. Red arrowheads indicate the blue signals. (D) No activity is shown for the headless myosin promoter in the callus and developing kernel. (E) Immunoblot analysis of O1 and the headless myosin proteins in developing maize kernels at 20 d after pollination using an antibody described previously (Wang et al., 2012). Lanes: 1, W22 inbred line; 2, o1-ref; 3, o1-N1478A; 4, o1-N1243; 5, the wild type. The arrows on the right indicate the expected protein weights of O1 and the headless myosin proteins. (This figure is available in colour at JXB online.)
Due to the global overlapping expression patterns of the *Arabidopsis* myosin genes (Peremyslov et al., 2011), only two (*MYA2* and *XI-K*) of all 13 class XI myosin single mutants exhibited detectable defects under normal growth conditions (Peremyslov et al., 2008), whereas their double, triple, and quadruple mutants displayed similar but more severe phenotypes when compared with the single mutants (Prokhnevsky et al., 2008; Peremyslov et al., 2010; Ueda et al., 2010; Ojangu et al., 2012). Transcripts of all myosin family members were detected in all tested tissues and organs of maize, although their abundance was considerably varied (Fig. 6). It has been shown that the global expression patterns of a fraction of orthologous genes are conserved in animals (Zheng Bradley et al., 2010) and plants (Davidson et al., 2012). In the case of orthologous myosin genes in maize and *Arabidopsis*, most display similar expression patterns. For example, the *Arabidopsis* orthologues (XI-C and XI-E) of the maize anther-expressed ZmXI-II displayed extremely low levels in the entire plant but exclusively high levels in the stamen/anther (Peremyslov et al., 2011), suggesting their important roles in pollen growth. In addition, the segmental duplicated maize myosin pairs shared correlated expression patterns when compared with other paralogues.

In humans, alternative splicing of myosin Va occurs in a region lying between the coiled-coil region of the IQ neck and the globular tail region in a tissue-specific manner (Hodi et al., 2006; Wagner et al., 2006; Roland et al., 2009; Wu et al., 2002), and usually these exons in this region are subject to alternative splicing. For example, exon B is required for the dynein light chain 2 (DLC2)–myosin Va interaction (Hodi et al., 2006; Wagner et al., 2006), whereas exon D is essential for Rab10 binding to myosin V tails in vivo (Roland et al., 2009). In the present study, we found that many maize class XI myosin genes had several splicing transcripts within the randomly selected cDNA fragment in leaf and kernel (Fig. 7). Further sequence analysis showed that most of these fragments mapped to the region between the IQ motif and the tail domain, suggesting a conserved mechanism that regulates myosin function in human and plants.

*Opaquel* is the first characterized maize class XI myosin that is required for ER motility and protein body formation (Wang et al., 2012a). In addition to the full-length O1 cDNA, we isolated a short O1 transcript that conceptually encoded a truncated, headless O1 at the O1 locus (Fig. 9). *Arabidopsis* and *Brachypodium* possess and express a headless variant of XI-K that emerged via partial myosin XI duplication (Peremyslov et al., 2011). In comparison with *HDK*, the short myosin transcript is not an independent gene model but rather a headless variant of O1 and appears to function at the transcription level. It is well known that overexpression of the myosin XI tail domain can inhibit its corresponding, endogenous, complete myosin function via the competitive binding of adaptors that mediate the interaction between myosin and its cargo. However, in the case of the headless derivative of O1, whether another mechanism other than dominant-negative regulation of the function of myosin exists remains to be addressed.
Gao Y, Bian L, Shi J, Xu J, Xi M, Wang G. 2013. Expression of a conifer COBRA-like gene CICOB1 from Chinese fir (Cunninghamia lanceolata) alters the leaf architecture in tobacco. *Plant Physiology and Biochemistry* **70**, 483–491.

Golomb L, Abu-Abied M, Belausov E, Sadot E. 2008. Different subcellular localizations and functions of Arabidopsis myosin VIII. *BMC Plant Biology* **8**, 3.

Hammer JA III, Sellers JR. 2012. Walking to work: roles for class V myosins as cargo transporters. *Nature Reviews Molecular Cell Biology* **13**, 13–26.

Hashimoto K, Igarashi H, Mano S, Nishimura M, Shimmen T, Yokota E. 2005. Peroxisomal localization of a myosin XI isoform in Arabidopsis thaliana. *Plant and Cell Physiology* **46**, 782–789.

Hodi Z, Nemeth AL, Radnai L, Hetenyi C, Schlett K, Bodor A, Perczel A, Nyitrai L. 2006. Alternatively spliced exon B of myosin Va is essential for binding the tail-associated light chain shared by dynein. *Biochemistry* **45**, 12582–12595.

Janousek V, Karn RC, Laucatikis CM. 2013. The role of retrotransposons in gene family expansions: insights from the mouse Abp gene family. *BMC Evolutionary Biology* **13**, 107.

Jiang S, Ramachandran S. 2004. Identification and molecular characterization of myosin gene family in *Oryza sativa* genome. *Plant and Cell Physiology* **45**, 590–599.

Jiang SY, Cai M, Ramachandran S. 2007. ORYZA SATIVA MYOSIN XI B controls pollen development by photoperiod-sensitive protein localizations. *Developmental Biology* **304**, 579–592.

Knight AE, Kendrick-Jones J. 1993. A myosin-like protein from a higher plant. *Journal of Molecular Biology* **231**, 148–154.

Lee YR, Liu B. 2004. Cytoskeletal motors in Arabidopsis. Sixty-one kinesins and seventeen myosins. *Plant Physiology* **136**, 3877–3883.

Leticia I, Doerks T, Bork P. 2012. SMART 7, recent updates to the protein domain annotation resource. *Nucleic Acids Research* **40**, D302–D305.

Li JF, Nebenfuhr A. 2008. The tail that wags the dog: the globular tail domain defines the function of myosin V/XI. *Traffic* **9**, 290–298.

Liu L, Zhou J, Pesacreta TC. 2001. Maize myosins: diversity, localization, and function. *Cell Motility and the Cytoskeleton* **48**, 130–148.

Madison SL, Nebenfuhr A. 2013. Understanding myosin functions in plants: are we there yet? *Current Opinion in Plant Biology* http://dx.doi.org/10.1016/j.pbi.2013.10.004 (in press).

Marquez Y, Brown JW, Simpson C, Barta A, Kalyna M. 2012. Transcriptome survey reveals increased complexity of the alternative splicing landscape in Arabidopsis. *Genome Research* **22**, 1184–1195.

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

Peremyslov VV, Klocko AL, Fowler JE, Dolja VV. 2012. Arabidopsis myosin XI-K localizes to the motile endomembrane vesicles associated with F-actin. *Frontiers in Plant Science* **3**, 184.

Peremyslov VV, Mockler TC, Filichkin SA, Fox SE, Jaiswal P, Makarova KS, Koonin EV, Dolja VV. 2011. Expression, splicing, and evolution of the myosin gene family in plants. *Plant Physiology* **155**, 1191–1204.

Peremyslov VV, Prokhnevsky AI, Dolja VV. 2010. class XI myosins are required for development, cell expansion, and F-Actin organization in Arabidopsis. *Plant Cell* **22**, 1883–1897.

Peremyslov VV, Prokhnevsky AI, Avisar D, Dolja VV. 2008. Two class XI myosins function in organelle trafficking and root hair development in Arabidopsis. *Plant Physiology* **146**, 1109–1116.

Prokhnevsky AI, Peremyslov VV, Dolja VV. 2008. Overlapping functions of the four class XI myosins in Arabidopsis growth, root hair elongation, and organelle motility. *Proceedings of the National Academy of Sciences, USA* **105**, 19744–19749.

Punta M, Coggill PC, Eberhardt RY, et al. 2012. The Pfam protein families database. *Nucleic Acids Research* **40**, D290–D301.

Reddy AS, Day IS. 2001. Analysis of the myosins encoded in the recently completed Arabidopsis thaliana genome sequence. *Genome Biology* **2**, RESEARCH0024.

Reichelt S, Knight AE, Hodge TP, Baluska F, Samaj J, Volkmann D, Kendrick-Jones J. 1999. Characterization of the unconventional myosin VIII in plant cells and its localization at the post-cytokinetic cell wall. *The Plant Journal* **19**, 555–567.

Roland JT, Lapierre LA, Goldenring JR. 2009. Alternative splicing in class V myosins determines association with Rab10. *Journal of Biological Chemistry* **284**, 1213–1223.

Salse J, Abrouk M, Bolot S, Guilhot N, Courcelle E, Faraut T, Waugh R, Close TJ, Messing J, Feuillet C. 2009. Reconstruction of monocotelydoneous proto-chromosomes reveals faster evolution in plants than in animals. *Proceedings of the National Academy of Sciences, USA* **106**, 14908–14913.

Salse J, Bolot S, Throude M, Jouffe V, Piegu B, Quraishi UM, Calcagno T, Cooke R, Delseny M, Feuillet C. 2008. Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. *Plant Cell* **20**, 11–24.

Sattarzadeh A, Fransen R, Schmelzer E. 2008. The Arabidopsis class VIII myosin ATM2 is involved in endocytosis. *Cell Motility and the Cytoskeleton* **65**, 457–468.

Sattarzadeh A, Schmelzer E, Hanson MR. 2011. Analysis of organelle targeting by Dil domains of the Arabidopsis myosin XI family. *Frontiers in Plant Science* **2**, 72.

Schnable JC, Springer NM, Freeling M. 2011. Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. *Proceedings of the National Academy of Sciences, USA* **108**, 4069–4074.

Schnable PS, Ware D, Fulton RS, et al. 2009. The B73 maize genome: complexity, diversity, and dynamics. *Science* **326**, 1112–1115.

Sekhon RS, Lin H, Childs KL, Hansey CN, Buell CR, de Leon N, Kaeppler SM. 2011. Genome-wide atlas of transcription during maize development. *The Plant Journal* **66**, 553–563.

Sparkes I, Runions J, Hawes C, Griffling L. 2009. Movement and remodeling of the endoplasmic reticulum in nondividing cells of tobacco leaves. *Plant Cell* **21**, 3937–3949.

Sparkes IA, Teanby NA, Hawes C. 2008. Truncated myosin XI tail fusions inhibit peroxisome, Golgi, and mitochondrial movement in tobacco leaf epidermal cells: a genetic tool for the next generation. *Journal of Experimental Botany* **59**, 2499–2512.
Stamm S, Ben-Ari S, Rafalska I, Tang Y, Zhang Z, Toiber D, Thanaraj TA, Soreq H. 2005. Function of alternative splicing. Gene 344, 1–20.

Syed NH, Kalyna M, Marquez Y, Barta A, Brown JW. 2012. Alternative splicing in plants—coming of age. Trends in Plant Science 17, 616–623.

Tarailo-Graovac M, Chen N. 2009. Using RepeatMasker to identify repetitive elements in genomic sequences. Current Protocols in Bioinformatics Chapter 4, Unit 4.10.

Trapnell C, Pachter L, Salzberg SL. 2009. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25, 1105–1111.

Ueda H, Yokota E, Kutsuna N, Shimada T, Tamura K, Shimmen T, Hasezawa S, Dolja VV, Hara-Nishimura I. 2010. Myosin-dependent endoplasmic reticulum motility and F-actin organization in plant cells. Proceedings of the National Academy of Sciences, USA 107, 6894–6899.

Wang G, Gao Y, Wang J, Yang L, Song R, Li X, Shi J. 2011. Overexpression of two cambium-abundant Chinese fir (Cunninghamia lanceolata) α-expansin genes CIEXPA1 and CIEXPA2 affect growth and development in transgenic tobacco and increase the amount of cellulose in stem cell walls. Plant Biotechnology Journal 9, 486–502.

Wang G, Wang F, Wang G, et al. 2012a. Opaque1 encodes a myosin XI motor protein that is required for endoplasmic reticulum motility and protein body formation in maize endosperm. Plant Cell 24, 3447–3462.

Wang G, Wang H, Zhu J, Zhang J, Zhang X, Wang F, Tang Y, Mei B, Xu Z, Song R. 2010. An expression analysis of 57 transcription factors derived from ESTs of developing seeds in maize (Zea mays). Plant Cell Reports 29, 545–559.

Wang G, Zhang X, Wang F, Song R. 2012b. Isolation of high quality RNA from cereal seeds containing high levels of starch. Phytochemical Analysis 23, 159–163.

Wang Z, Pesacreta TC. 2004. A subclass of myosin XI is associated with mitochondria, plastids, and the molecular chaperone subunit TCP-1alpha in maize. Cell Motility and the Cytoskeleton 57, 218–232.

Wei F, Coe E, Nelson W, et al. 2007. Physical and genetic structure of the maize genome reflects its complex evolutionary history. PLoS Genetics 3, e123.

Wu SZ, Ritchie JA, Pan AH, Quatrano RS, Bezanilla M. 2011. Myosin VIII regulates protonemal patterning and developmental timing in the moss Physcomitrella patens. Molecular Plant 4, 909–921.

Wu X, Wang F, Rao K, Sellers JR, Hammer JA III. 2002. Rab27a is an essential component of melanosome receptor for myosin Va. Molecular Biology of the Cell 13, 1735–1749.

Yokota E, Ueda H, Hashimoto K, Orii H, Shimada T, Haranishimura I, Shimmen T. 2011. Myosin XI-dependent formation of tubular structures from endoplasmic reticulum isolated from tobacco cultured BY-2 cells. Plant Physiology 156, 129–143.

Zheng-Bradley X, Rung J, Parkinson H, Brazma A. 2010. Large scale comparison of global gene expression patterns in human and mouse. Genome Biology 11, R124.