Research

Analysis of the myosins encoded in the recently completed *Arabidopsis thaliana* genome sequence

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Abstract

Background: Three types of molecular motors play an important role in the organization, dynamics and transport processes associated with the cytoskeleton. The myosin family of molecular motors move cargo on actin filaments, whereas kinesin and dynein motors move cargo along microtubules. These motors have been highly characterized in non-plant systems and information is becoming available about plant motors. The actin cytoskeleton in plants has been shown to be involved in processes such as transportation, signaling, cell division, cytoplasmic streaming and morphogenesis. The role of myosin in these processes has been established in a few cases but many questions remain to be answered about the number, types and roles of myosins in plants.

Results: Using the motor domain of an *Arabidopsis* myosin we identified 17 myosin sequences in the *Arabidopsis* genome. Phylogenetic analysis of the *Arabidopsis* myosins with non-plant and plant myosins revealed that all the *Arabidopsis* myosins and other plant myosins fall into two groups - class VIII and class XI. These groups contain exclusively plant or algal myosins with no animal or fungal myosins. Exon/intron data suggest that the myosins are highly conserved and that some may be a result of gene duplication.

Conclusions: Plant myosins are unlike myosins from any other organisms except algae. As a percentage of the total gene number, the number of myosins is small overall in *Arabidopsis* compared with the other sequenced eukaryotic genomes. There are, however, a large number of class XI myosins. The function of each myosin has yet to be determined.

Background

Movement of eukaryotic cells, intracellular transport, signaling, cell division and cell shape are functions of the cytoskeleton [1-4]. The cytoskeleton is made up of three types of filaments: actin filaments, intermediate filaments and microtubules. Three groups of proteins called molecular motors utilize energy from the hydrolysis of ATP to move in association with the cytoskeleton: kinesins, dyneins and myosins [1,5,6]. Kinesins and dyneins move along microtubules [5,7] and actin is utilized by myosin for motility [8,9].

Molecular motors in non-plant systems have been extensively characterized but less is known about the presence and functions of these motors in plant cells. Using antibodies to mouse dynein, two 400 kDa proteins were identified in tobacco pollen during pollen germination [10] suggesting the presence of dynein in pollen tubes. To date, no report has been published on the presence of dynein at the molecular level. Using animal dynein sequences to search the *Arabidopsis* database TAIR (The *Arabidopsis* Information Resource) [11], no sequences similar to heavy or intermediate chains

were found. However, some sequences showing similarity to light chains are present in the database. Kinesins have been identified in *Arabidopsis* and other plant systems [12-16] and their movement along microtubules has been analyzed [16-19]. Kinesins are a superfamily of molecular motors containing at least nine subfamilies [7,20]. Plant kinesins are represented in all but two of the families. Using the amino-acid sequence of the motor domain of a plant kinesin, a search of the *Arabidopsis* genome yielded 61 kinesin-like proteins [21]. This is the largest number of kinesins in an organism per thousand genes compared to yeast, *Drosophila melanogaster* and *Caenorhabditis elegans*.

Phylogenetic analysis of known myosins in various organisms has resulted in the classification of myosins into several groups. The Myosin Home Page (MHP) [22] has a phylogenetic tree with 143 myosins classified into 17 classes. However, an analysis of the myosin superfamily in *Drosophila*, concluded that two new mammalian myosins and a *Drosophila* myosin make up a new class of myosins, class XVIII [23]. These myosins have a unique amino-terminal PDZ domain. The classes have been named according to the order in which each class was first discovered except for myosins I and II. Myosin II is the conventional myosin, which was discovered 60 years ago [8]. The next myosin identified was myosin I and then in order of class name. Myosins have three domains in common; a motor domain that interacts with actin and binds ATP, a neck domain that binds light chains or calmodulin and a tail domain. The tail domain varies by class. Phylogenetic analysis is often based on the motor domain of the myosins. However, using the full-length sequence results in nearly the same grouping, indicating that the heads and tails have evolved together [23-26]. A study using the head (motor domain), neck and tail domains separately for phylogenetic analysis or the head and neck/tail showed that this is generally true [27]. The neck domain consists of one or more helical sequences termed the IQ motif, which has the consensus sequence IQXXXRGXXXR [28]. The IQ motif binds the conventional myosin II light chains and calmodulin or calmodulin-like proteins in other myosins [29]. Unlike most calmodulin-binding proteins, myosins bind calmodulin in the absence of Ca^{2+}.

As actin is utilized by myosin for motility, the possible functions of myosin in plants are closely linked to the functions of actin. The actin cytoskeleton has been shown to be involved in many processes in plants including transportation, signaling, cell division, cytoplasmic streaming and morphogenesis [2,3]. Much of the cytoplasmic streaming work has been done in algal cells and the direct involvement of actin and myosin has been shown [30,31]. Genetic, biochemical and cell biological studies with trichomes during the past four years have provided interesting insights into the role of the cytoskeleton in trichome morphogenesis. These studies indicate that actin and the microtubule cytoskeleton play a pivotal role in cell expansion and branching during trichome development [32]. Localization studies and visualization of the actin cytoskeleton in live cells with an actin-binding protein tagged with green fluorescent protein (GFP) indicate that the organization of F-actin changes during trichome morphogenesis [33,34]. Chemicals that promote depolymerization or stabilization of the actin cytoskeleton did not effect branching but produced distorted trichomes. The morphology of these trichomes is similar to that observed in a 'distorted' class of mutants, suggesting that at least some of the affected genes are likely to code for proteins involved in actin organization/dynamics (for example myosins, actin-depolymerizing factors, actin-binding proteins). There is also evidence that the actin cytoskeleton is involved in mitosis and during separation of daughter cells after the successful segregation of chromosomes into daughter nuclei [3]. The actin cytoskeleton is also involved in pollen tube growth, and calcium regulation has also been shown to be involved [35,36].

Myosins have been identified in plants both biochemically [37-40] and at the molecular level [41-43]. Immunological detection of myosins using antibodies against animal myosin identified proteins of various sizes from different plants [44-46]. Immunofluorescence studies localized myosin to the surface of organelles, the vegetative nuclei and generative cells in pollen grains and tubes [39], to the active streaming lanes and cortical surface in pollen tubes [40] and, more recently, to plasmodesmata in root tissues [38,47]. Motility assays [48] and ATPase assays [48-50] using myosin-like proteins isolated from plants have also demonstrated the presence of myosins in plants.

Since 1993, five partial or full-length myosins from *Arabidopsis* have been characterized at the molecular level. Using PCR-based approaches, Knight and Kendrick-Jones [43] cloned a myosin they called ATM (*Arabidopsis thaliana* myosin), Kinkema and Schiefelbein [41] cloned the myosin MYA1 and Kinkema *et al.* [42] cloned another full-length myosin, ATM2, and two partial length myosins MYA2 and MYA3. Kinekema *et al.* [42] also identified three PCR products that coded for unique myosin motor domain sequence. Phylogenetic analysis using these myosins indicated that the ATM myosins were a unique class and they were named class VIII. The MYA myosins are somewhat related to class V myosins but as other analyses have been done, these myosins were also assigned to a new class, class XI [8,42].

Myosins have been identified in *Zea mays,* two of which belonged to class XI and one to class VIII [51]. PCR fragments for fern myosins have been reported [52,53] and sequences are available for myosins from *Helianthus annuus* (O. Vugrek and D. Menzel, unpublished data). Two fern (*Anemia phyllitidis*) PCR products and the *H. annuus* myosins also fall either into class VIII or class XI myosins [22,42]. Two algal myosins are also members of the class XI myosins, one from *Chara corallina* and one from *Chlamydomonas reinhardtii* [22,54]. A third class of myosins (XIII)

is composed of two algal myosins from *Acetabularia cliftonii*. No animal myosins are in any of these classes and no plant myosins are in any other myosin class. However, the cellular slime mold *Dictyostelium discoideum* has one myosin (Dd MyoJ), which is alternatively grouped with class V or class XI [27].

Other organisms have myosins from more than two classes. The yeast *Saccharomyces cerevisiae* has five myosins in three different classes. *Caenorhabditis elegans* has myosins in seven classes and *Drosophila melanogaster* in nine. Do plants have only two classes of myosins? How many myosins are in a plant genome? What are the similarities and differences between plant and non-plant myosins that might help establish a function for the myosins? Until the recent completion of the sequencing of the *Arabidopsis* genome [55], answers to these questions were not known. It is now possible to determine how many myosins are in the *Arabidopsis* genome and to see if any plant myosins fall into other myosin classes. As the myosin motor domain is highly conserved, the sequence from one myosin motor can be used to search a database for all other myosins. We used the motor domain from MYA1 to search the *Arabidopsis* database [11] for sequences with similarity to this domain. We identified 17 *Arabidopsis* myosins, including the 5 reported myosins, in the *Arabidopsis* genome. Phylogenetic analysis using non-plant and plant myosins showed that all 17 fall into either myosin class VIII or XI. Only 4 are in class VIII and 13 in class XI. An analysis of their exon/intron junctions and sequence similarities indicates that all myosins are highly conserved and some may represent gene duplication events.

Results

Identification of *Arabidopsis* myosins

Using the amino-acid sequence of the conserved motor domain of the plant myosin MYA1 [41], databases were searched using BLASTP and TBLASTN at TAIR [11]. Other searches using the amino-acid sequence of motor domains from representatives of other classes of myosins were also done but they did not reveal any other myosin sequences. Sixteen unique sequences were obtained that contain a myosin motor domain as identified by the SMART (Smart Modular Architecture Research Tool) program [56]. The sequences obtained in this search were compared to the Munich Information Center for Protein Sequences (MIPS) [57] list of myosin domains in *Arabidopsis*. MIPS lists 16 *Arabidopsis* sequences showing myosin domains. A check of these showed that 13 of the sequences were myosins identified in our search and one was a myosin not available in the NCBI (National Center for Biotechnology Information) protein database [58]. Two are not full-length myosins. One is a putative helicase (At1g26370) with no myosin motor domain and one is a possible pseudogene (At1g42680) with only 162 amino acids that have some similarity to the myosin motor domain. MIPS does not list three myosins identified in our search (At XIG, At XIF and At XI-I). Table 1 lists the myosins by names as given in the phylogenetic tree constructed by Hodge and Cope [59] and as assigned by us. There are a total of 17 myosin genes in *Arabidopsis*. In comparison, *S. cerevisiae*, *Schizosaccharomyces pombe*, *C. elegans* and *D. melanogaster* have 5, 5, 20 and 13 myosins, respectively (Figure 1) [60,61]. *Arabidopsis* has the lowest percentage (0.068%) of myosin genes out of the total number of genes, as compared to *S. cerevisiae* and *S. pombe* with 0.080% and 0.093%, respectively, *C. elegans* with 0.11% and *D. melanogaster* with 0.096% (see Figure 1).

Only 5 of the 17 *Arabidopsis* myosins have been reported in the literature [41-43]. The other 12 are sequences obtained from the *Arabidopsis* database sequenced as part of the *Arabidopsis* Genome Sequencing Project. These sequences are, therefore, predicted sequences that have not been verified by complete cDNAs. The average sequence length of the *Arabidopsis* myosins is 1,400 residues, with the shortest sequence prediction being 1,085 (At VIIIA) amino acids and the longest 1,730 (At XIA). Some of the intron/exon predictions may not be correct, which could reduce or increase the size of the predicted proteins and so the sizes may change as more characterization is done for each myosin. A case in point is the cDNA that was isolated by Kinkema and Schiefelbein [41] for At MYA1 (At MYA1) which codes for 1,520 amino acids, whereas the predicted protein has 1,599 because of differences in intron prediction.

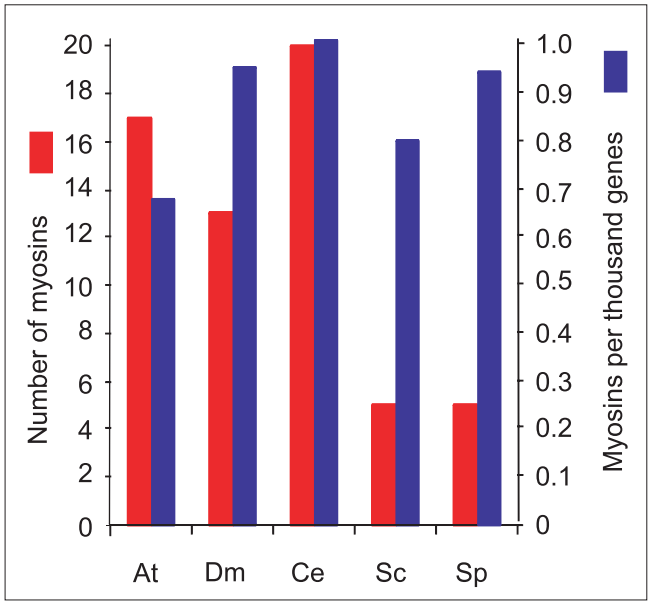

Figure 1
The numbers of myosins in eukaryotic sequenced genomes. The number of myosins in each organism is on the left (red column) and the number per thousand for each organism is on the right (blue column). At, *Arabidopsis thaliana*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*.

Table 1

Myosin-like proteins in *Arabidopsis*

Name	Number of amino acids	Protein ID	Gene code	Old name	Class	Domains	Reference
1. At ATM	1166	479413 11994771	AT3g19960	(ATM1)* MZE19.1	VIII	MD,CC,IQ	[43] AtDB, MIPS
2. At ATM2	1111 1101†	9759501 499045	AT5g54280	MDK4.10 ATM2/AtMYOS1	VIII	MD,CC,IQ	AtDB, MIPS [42]
3. At VIIIA	1085	5734787	AT1g50360	F14I3.6	VIII	MD,CC,IQ	AtDB, MIPS
4. At VIIIB	1126	3269298	AT4g27370	M4I22.180	VIII	MD,CC,IQ	AtDB, MIPS
5. At MYA1	1520 1599‡	1076348 8778462	AT1g17580	(AtMYA1)* F1L3.28	XI	MD,CC,IQ	[41] AtDB, MIPS
6. At MYA2	1505‡ 1515	2129653 8953751	AT5g43900	F6B6.4 (AtMYA2)*	XI	MD,IQ	AtDB, MIPS [42]
7. At XIA	1730	2494118	AT1g04600	T1G11.15	XI	MD,CC,IQ	AtDB, MIPS
8. At XIB	1519	3142302	AT1g04160	F20D22.7	XI	MD,IQ	AtDB, MIPS
9. At XIC	1572	3063460	AT1g08730	F22O13.22	XI	MD,CC,IQ	AtDB, MIPS
10. At XID	1611	2924770	AT2g33240	F25I18.2	XI	MD,CC,IQ	AtDB, MIPS
11. At XIE	1529	3776579	AT1g54560	T22H22.1	XI	MD,CC,IQ	AtDB, MIPS
12. At XIF	1556§	4887746	AT2g31900	F20M17.6	XI	MD,IQ	AtDB, MIPS
13. At XIG	1502	4512706	AT2g20290	F11A3.16	XI	MD,CC,IQ	AtDB, MIPS
14. At XIH	1452§	4218127	AT4g28710	F16A16.180	XI	MD,CC,IQ	AtDB, MIPS
15. At XI-I	1374	4455334	AT4g33200	F4I10.130	XI	MD,CC,IQ	AtDB, MIPS
16. At XIJ	1242 963† 998†	11276963 602328 629533	AT3g58160	F9D24.70 (AtMYOS3)*, (AtMYA3)*	XI	MD,CC,IQ	AtDB, MIPS [42] [42]
17. At XIK	1544		AT5g20490	F7C8.80	XI	MD,CC,IQ	MIPS

*Name as reported in the literature. †Number of amino acids previously reported for partial sequence. ‡Number of amino acids predicted by NCBI. §Edited by authors for full-length sequence: AtDB, *Arabidopsis* database; MIPS, Munich Information Center for Protein Sequences; MD, motor domain; CC, coiled-coil region; IQ, putative calmodulin-binding motif.

Using the *Arabidopsis* Sequence Map Overview of TAIR [62], the location of each myosin was determined (Figure 2). The myosin genes are distributed throughout the *Arabidopsis* genome. The chromosome lengths are based on the centimorgan (cM) scale as shown on the TAIR Map Overview [62]. The maps reported with the announcement of the *Arabidopsis* genome sequence show somewhat different lengths than the TAIR maps [55].

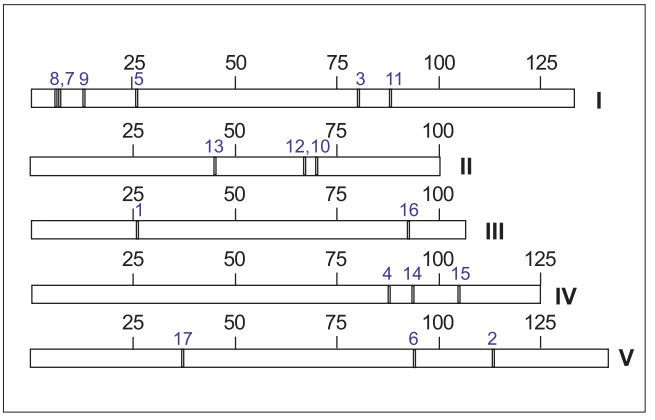

Figure 2
Location of myosins on the *Arabidopsis* chromosomes. Roman numerals represent chromosome numbers. Large numbers indicate chromosome length in cM. Small blue numbers are the myosin numbers from Table 1.

Phylogenetic analysis

All *Arabidopsis* myosins and a selection of myosins from other organisms representing each of the myosin classes were aligned using the motor domain sequence as determined by the SMART program [56]. The alignment was done in Megalign by the CLUSTAL method and a phylogenetic tree was generated using the Bootstrap (100 replicates) method with a heuristic search of the PAUP 4.0b6 program (Figure 3). The *Arabidopsis* myosins all group into two classes along with other plant myosins - class VIII and

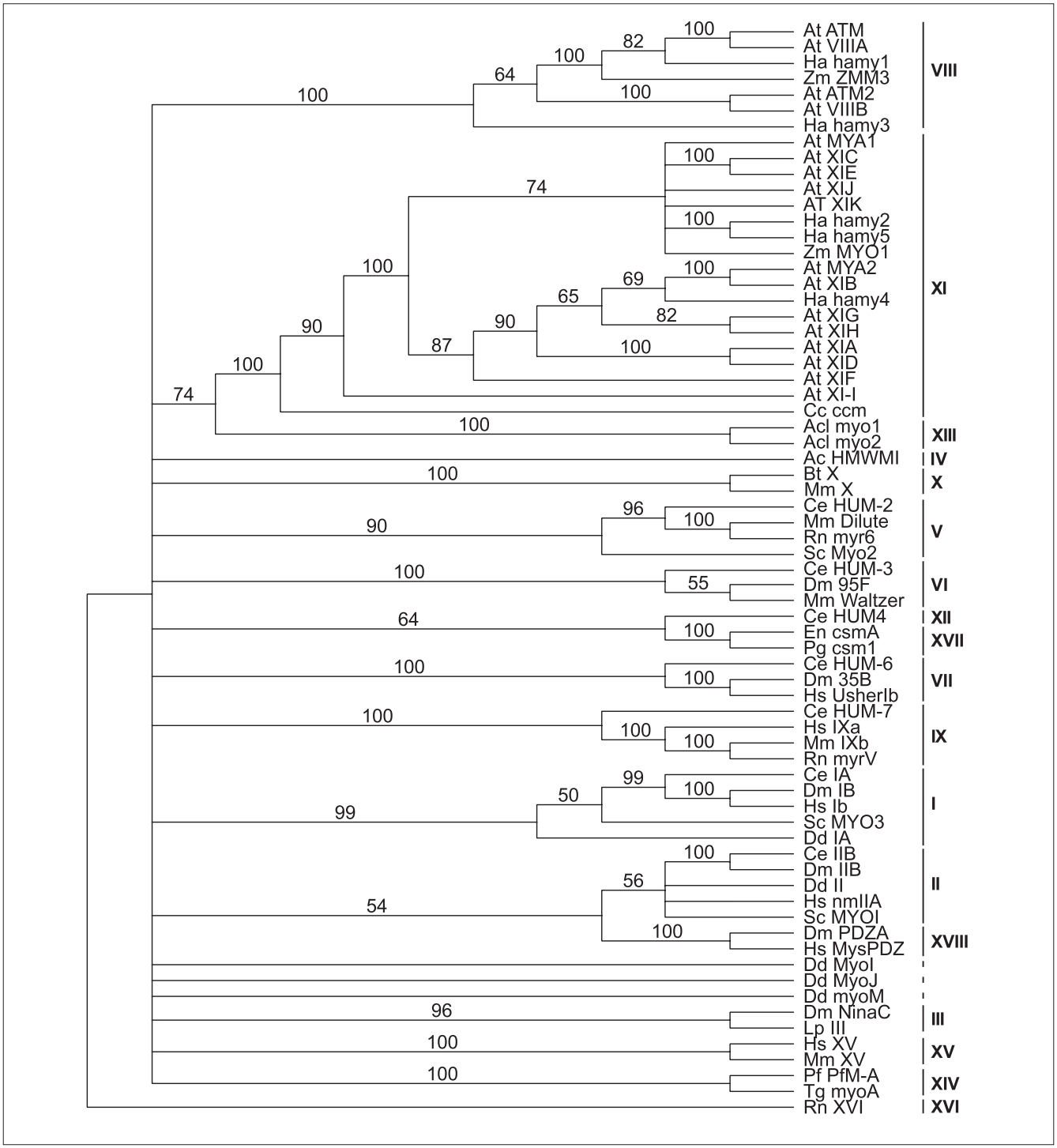

Figure 3
Phylogenetic tree. Alignment of the motor domain of representative myosins and all *Arabidopsis* myosins was done in Megalign by the CLUSTAL method and a phylogenetic tree was generated using the bootstrap method with a heuristic search of the PAUP 4.0b6 program. The myosin groups, as defined by Hodge and Cope [59] and Yamashita *et al.* [23], are identified on the right in roman numerals. Myosins from the following organisms were used: Ac, *Acanthamoeba castellani;* Acl, *Acetabularia cliftoni*; At, *Arabidopsis thaliana*; Cc, *Chara corallina,* Ha, *Helianthus annuus;* Zm, *Zea mays;* Bt, *Bos taurus*; Mm, *Mus musculus;* Ce, *Caenorhabditis elegans;* Dm, *Drosophila melanogaster;* Rn, *Rattus norvegicus*; Sc, *Saccharomyces cerevisiae;* Hs, *Homo sapiens*; Dd, *Dictyostelium discoideum*; Lp, *Limulus polyphemus*; En, *Emericella nidulans*; Pg, *Pyricularia grisea*; Pf, *Plasmodium falciparum*; and Tg, *Toxoplasma gondii*. The number at the branches indicates the number of times the dichotomy was supported out of 100 bootstrap tries.

class XI. No animal myosins group with the plant myosins and no plant myosins group into any of the animal myosins. An algal (*Chara corallina*) myosin, Cc ccm, does group with the plant class XI myosins but is on a separate branch from any other class XI myosin (Figure 3). The *D. discoideum* myosin Dd myoJ did not fall into a class with any of the plant myosins. In fact, three *D. discoideum* myosins (Dd myoI, Dd myoJ, and Dd myoM) did not fall into any of the classes (Figure 3). The phylogenetic trees of Hodge and Cope and the tree on the myosin home page [22,59] show the Dd myoI branching from class VII myosins. A heuristic search without bootstrapping also showed the Dd myoI myosin as a branch from class VII and domain analysis shows that Dd myoI has the MyTH4 domain found in other class VII myosins. Other phylogenetic anaylses have placed Dd myoJ as a branch off class XI myosins from plants [22,59]. However, the phylogenetic tree generated from full-length sequences of plant myosins and Dd myoJ (see below) also shows that Dd myoJ is separate from the plant myosins.

Myosins from another alga, *Acetabularia cliftonii,* are classified into a separate group (XIII) and one myosin each from the fungi *Emericella nidulans* and *Pyricularia grisea* are also assigned to a separate class (XVII). A second alignment was done using the full-length sequences for all *Arabidopsis* and other known full-length plant myosins with a human heavy-chain myosin (Hs lb) as an outgroup. The two classes of plant myosins are clearly seen (Figure 4). Among the class XI myosins the similarity ranges from 40-85% (full length) and 61-91% (motor domain). The similarity between the class VIII myosins ranges from 50-83% (full length) and 64-92% (motor domain). When class VIII myosins are compared to class XI myosins the similarity only ranges from 22-29% (full-length) and 35-42% (motor domain). Thirteen *Arabidopsis* myosins group into class XI. Two subgroups branch off in this class with three outliers (Figure 4). One subgroup consists of two pairs of *Arabidopsis* myosins, At XIB/At MYA2 and At XIG/At XIH, which are most similar to the sunflower myosin Hahamy4 and then another pair of *Arabidopsis* myosins, At XID/At XIA. The other subgroup consists of the *Arabidopsis* myosin pair At XIC/At XIE and two unpaired *Arabidopsis* myosins, At XIK and At MYA1, that are most closely related to sunflower myosins Hahamy2 and Hahamy5 and to the maize myosin ZmMYO1. At XIJ, AT XIF and At XI-I are on separate branches that group with the other class XI myosins but not within the two subgroups. There are four class VIII *Arabidopsis* myosins that form two pairs, At ATM/At VIIIA and At VIIIB/At ATM2. The first pair group with class VIII myosins from *Z. mays* and *H. annuus* whereas the second pair are on a separate branch.

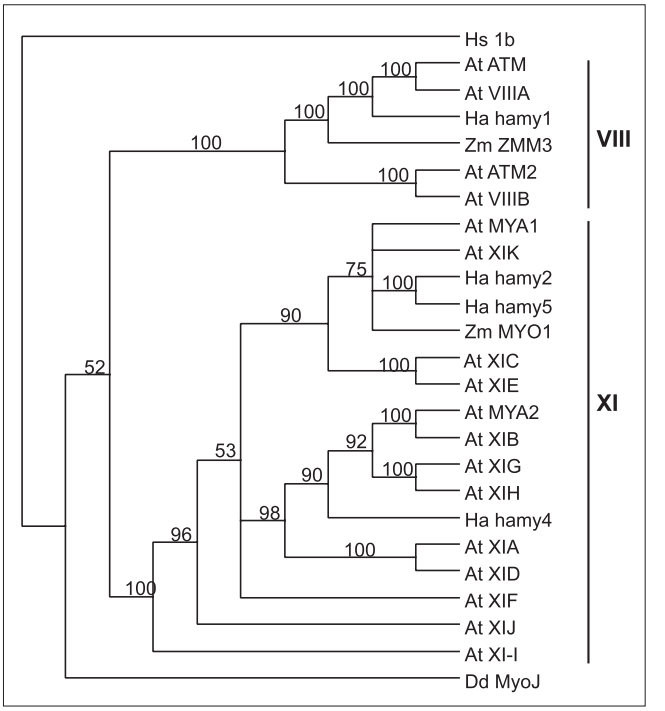

Figure 4
Phylogenetic tree for plant myosins. Alignment of the full-length *Arabidopsis* myosins, other full-length plant myosins available in the NCBI database and Dd myoJ was done in Megalign by the CLUSTAL method and a phylogenetic tree was generated using the bootstrap method with a heuristic search of the PAUP 4.0b4a (PPC) program. A human myosin (Hs 1b) was used as an outgroup. At, *Arabidopsis thaliana*; Dd, *Dictyostelium discoideum;* Ha, *Helianthus annuus;* Zm, *Zea mays.* The number at the branches indicates the number of times the dichotomy was supported out of 100 bootstrap tries.

Characterization of the *Arabidopsis* myosins

Figure 5 shows schematic diagrams of each myosin. The motor domain in all cases is in the amino-terminal region. The motor domain starts at about 50-55 residues for the class XI myosins whereas the class VIII myosins have a longer amino-terminal region before the motor domain (99-159 residues). The IQ domains usually follow right after the motor domain but are separated slightly from the motor domain in At XID, At XI-I, and At XIK. There are three or four IQ domains in class VIII myosins and five or six in class XI, except for At XIK, which has only four. There are coiled-coil domains, that differ in length and number, in all the myosins. They often follow directly after the IQ domains, but in some cases there is intervening sequence. Based on the presence of the coiled-coil domains, the *Arabidopsis* myosins are probably dimeric [26]. The class XI myosins are much longer than the class VIII myosins with the difference being in the length of the carboxy-terminal region following the conserved domains found in myosins.

Besides the motor, IQ and coiled-coil domains, other domains have been identified in myosins from classes other than the plant classes VIII and XI. These include SH3 domains (Src homology 3 domains, that bind to target proteins), MYTH4 (a domain of unknown function found in a few classes of myosins), a zinc-binding domain, a pleckstrin homology

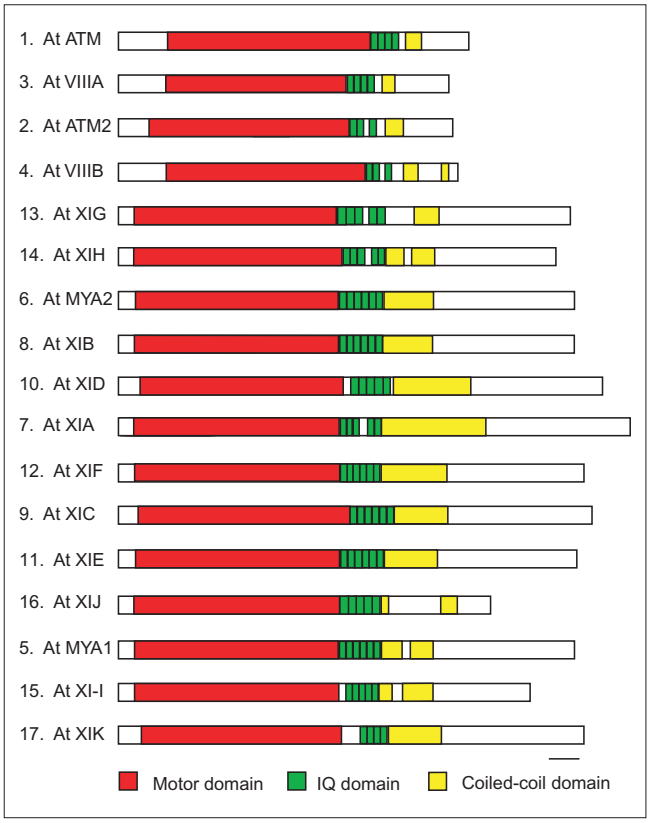

Figure 5
Schematic diagram of *Arabidopsis* myosins. The numbers refer to the number in Table 1. The motor domain, IQ domains, and coiled-coil domains are as indicated in the key. The first four myosins are in class VIII and the following 13 are in class XI. The bar represents 100 amino acids.

domain, FERM/talin (band 4.1/ezrin/radixin/moesin), GPA-rich domains and a protein kinase domain [8,22,26]. These domains are involved in protein interactions and presumably give specificity to the action of the myosin. Except for the IQ and coiled-coil domains, the SMART program used to identify the motor domain of the myosin sequences did not identify any domains other than a few with scores less significant than the required threshold.

Myosins have 131 highly conserved residues spread throughout the motor domain that define a core consensus sequence [26]. Comparison of an alignment of *Arabidopsis* myosin motor domains to these conserved sequences shows a great deal of conservation among them (data not shown). One example is the ATP-binding site which consists of GESGAGKT (179-187 in *Dictyostelium* myosin II, DmyoII) and NxNSSR-FGK (233-241, DmyoII). With the exception of only one residue these are conserved in all 17 *Arabidopsis* myosins. The conformational state of myosin changes with ATP hydrolysis and a very conserved region implicated in this process has the conserved sequence LDIxGFExFxxN(S/G)(F/L)EQxxINxx NExLQQxF (453-482, DmyoII) [26]. The plant sequences are very conserved through this region. The sequence in this region is LDIYGFExFxxNSFEQxCINE(K/R)LQQHF (the first x is S in all but one myosin, the fourth x is F in all but one myosin). Cope *et al.* [26] suggest that release of the γ-phosphate of ATP may be through a hole in the structure centered around an absolutely conserved arginine residue (residue 654, DmyoII) which is also absolutely conserved in all *Arabidopsis* myosins. The presence of these highly conserved residues in plant myosins suggests that they are capable of motor function. In fact, *in vitro* motility studies with a purified myosin from *Chara* (myosin XI, Cc ccm in Figure 3) have confirmed that it is indeed an actin-based motor [54]. A loop present in the motor domain called the HCM (mutations in this loop cause hypertrophic cardiomyopathy) is the location of a phosphorylatable serine (S) or threonine (T) in certain amoeboid myosin I molecules and myosin VI molecules. This S or T residue is 16 residues upstream from the conserved DALAK sequence. The enzyme activity of the amoeboid myosins depends on phosphorylation of this site, but although phosphorylation of the myosin VI T residue has been demonstrated, the regulation of enzyme activity has not [8,63]. Most other myosins have a constitutively negatively charged amino acid, either aspartic acid (D) or glutamic acid (E) at this site. This site has been named the TEDS rule site on the basis of these amino acids [8]. The *Arabidopsis* and other plant myosins all have aspartic acid, glutamic acid or glycine residue at this site, suggesting that they are not regulated by phosphorylation at this site. However, three residues upstream (19 from DALAK), all the class XI myosins have a threonine residue.

The site for each predicted or actual intron was located and is shown schematically in Figure 6. The intron locations were determined from the information at MIPS [57]. The length of each exon and the domain(s) they code for are shown in Tables 2 and 3 for class VIII and class XI myosins, respectively. The exons vary in length from 12 to greater than 672 nucleotides (the length of the beginning and last exons for each gene are not known as the predicted sizes include only the protein-coding nucleotides) with an average of 122 nucleotides. The four class VIII myosins have seven exons of the same length in the same order within the myosin motor domain (Table 2). The motor domain starts in the third exon of each class VIII myosin. The start of the IQ domains and the coiled-coil domains is more variable except for the At ATM2/At VIIIB pair. The class XI myosins also have many exons that are of the same length and in the same order but that differ from the class VIII pattern (Table 3). The exons coding for the motor domain sequence are most conserved in length. Most class XI myosins motor domains start in the third exon and end in the twentieth. Six of the class XI myosins have an intron after the start codon. Most differences in exon length are in the carboxy-terminal regions (Figure 6 and Table 3). However, even in the carboxy-terminal region there are some exon lengths conserved between some or all of the myosins. The two XI

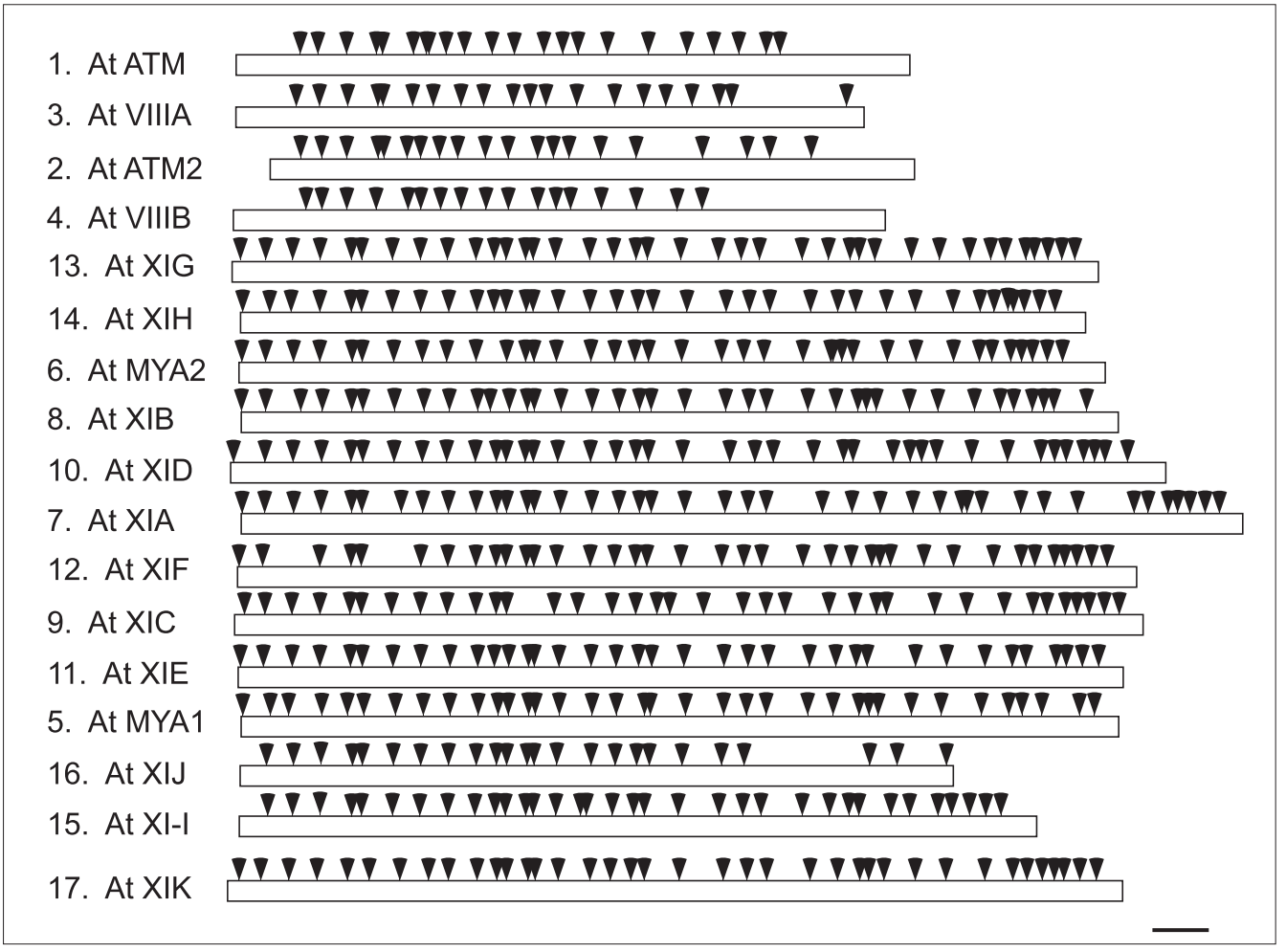

Figure 6
Location of the introns. The numbers refer to the number in Table 1. Arrowheads indicate the location of each intron along the length of the myosin. The bar represents 100 amino acids.

myosins with the closest similarity are At XIB and At MYA2. A Clustal alignment at Pole Bio-Informatique Lyonnais [64] showed 83.88% identity, 8.19% strong similarity and 2.36% weak similarity between these two myosins. Their motor domains are 91.6% identical. Twenty-three of their introns are at the same location in the motor domain area and then following a few different size exons, there are similar sized exons again. They are located on chromosomes I and V, respectively. It is possible that this pair is a result of gene duplication. Class VIII myosins At ATM and At VIIIA have 13 exons of the same length. Their full-length sequences are 79% identical with another 6.72% strongly similar and 3.52% weakly similar. Their motor domains have 93% similarity. At ATM is on chromosome III whereas At VIIIA is on chromosome I. This again may have resulted from a gene duplication. Analysis of the total *Arabidopsis* genome revealed that a whole genome duplication occurred, followed by subsequent gene loss and extensive local gene duplications [55]. The duplicated segments represent 58% of the *Arabidopsis* genome. The *S. cerevisiae* genome has also had a complete ancient genome duplication and 30% of the genes form duplicate pairs. Duplicated genes account for 48% of the total genes of *C. elegans* and *Drosophila* [60].

If the gene pairs are the result of duplication, it is interesting to note that while exon lengths have been conserved, intron lengths have not. The intron lengths are shown in Table 4. No pattern can be seen in intron lengths between any of the myosins. The average intron length is 131 nucleotides with the shortest intron at 47 nucleotides and the longest at 860. At XI-I has the highest average, 272 nucleotides. It contains the 860-nucleotide intron and three others that are over 500 nucleotides. In a study of 998 introns only 3.3% of the introns were longer than 500 nucleotides with sizes ranging from 59 to 1242 nucleotides [65]. This makes At XI-I unusual in having four out of 33 introns (12%) longer than 500 nucleotides. Only two other myosins had an intron over 500 nucleotides. Of the total 557 splice sites that were identified

Table 2

Analysis of exon sizes in class VIII myosins and the domain coded by each exon

	At ATM		At VIIIA		At ATM2		At VIIIB	
Number	Size	Domain	Size	Domain	Size	Domain	Size	Domain
1	339	N	315	N	159	N	333	N
2	102	N	132	N	102	N	118	N
3	144	N,M	144	N,M	144	N,M	131	N,M
4	151	M	151	M	151	M	155	M
5	28	M	28	M	25	M	169	M
6	166	M	158	M	129	M	64	M
7	64	M	104	M	64	M	99	M
8	14	M	139	M	99	M	104	M
9	84	M	119	M	104	M	139	M
10	104	M	153	M	139	M	119	M
11	139	M	90	M	119	M	153	M
12	119	M	78	M	153	M	90	M
13	153	M	159	M	90	M	78	M
14	90	M	207	M	78	M	159	M
15	78	M	144	M	159	M	186	M
16	159	M	114	M	186	M	206	M
17	207	M	130	M,I	342	M	136	M
18	206	M	147	I	244	M,I	130	M,I
19	136	M	68	C	116	I	108	I
20	130	M,I	595	C,T	213	I,C	140	I,C
21	147	I	83	T	480	C,T	189	C
22	68	I,C					375	C,T
23	672	C,T						

N, amino-terminal sequence; M, motor domain; I, IQ domain; C, coiled-coil domain; T, tail domain. The size of the first and last exons in each gene reflects only the size of the coding region.

in the *Arabidopsis* myosins only six (a little more than 1%) were over 500 nucleotides with four out of the six being in one myosin. Hunt *et al*. found that a SV40 small-t intron only 66 nucleotides in length was spliced efficiently in tobacco cells [66]. Several of the introns in the myosins are between 66 and 70 nucleotides and so may be long enough to be spliced. Only one is in a cloned myosin known to be spliced at that site (At XIJ). There is also a predicted intron of only 47 nucleotides in length (At XID) which is thought to be too short for efficient splicing. Brown *et al*. [65] found three introns less than 66 nucleotides in length in known expressed proteins, but none of them was less than 59 nucleotides. Until the expression of At XID is studied, no conclusion can be made as to the validity of this intron prediction. The significance of the range and variability of intron length is not known. In *Arabidopsis*, in general, the range is even greater (47-6,442) [11].

The consensus nucleotide sequences for the 5′ and 3′ splice sites are $A_{-2}G_{-1}$ $G_{+1}T_{+2}A_{+3}A_{+4}G_{+5}T_{+6}$ and $T_{-5}G_{-4}C_{-3}A_{-2}G_{-1}$ $G_{+1}T_{+2}$, respectively [65]. The most conserved sequences are the 5′ consensus G (100%) T (99%) at the +1, +2 positions, respectively, and the 3′ A(100%) G(100%) at the -2, -1 positions, respectively. The splice sites in the reported myosins and the predicted myosins (Table 4) all contain the 5′ GT and 3′ AG sequences. The sequences in the *Arabidopsis* myosins upstream and downstream of these two very conserved sites varied as a reflection of the less conserved nature of these nucleotides (Table 4). However, these predicted sites at the 5′ and 3′ splice sites need to be confirmed experimentally.

Discussion

Only two classes of myosins are present in *Arabidopsis*. A study of myosins in lily and tobacco pollen tubes using antibodies to three animal-type myosins IA and IB, II and V suggested the presence of three types of myosins in these plants [40]. However, no type I, II or V myosins have been found in any plant and only two types (VIII and XI) have been identified. Class XI are somewhat similar to class V myosins [42] and this may explain the reaction with the type V antibody. Possibly the other reactions were due to similarities in the myosin motor domain. Phylogenetic analysis of *Arabidopsis* myosins along with other plant myosins suggests that most

Table 3

Analysis of exon sizes in class XI myosins and the domain coded by each exon

	At XIG		At XIH		At MYA2		At XIB		At XID		At XIA		At XIF	
No.	Size	Domain	Size	Domain	Size	Domain	Size	Domain	Size	Domain	Size	Domain	Size	Domain
1	36	N	3	N	3	N	3	N	3	N	3	N	3	N
2	126	N	139	N	129	N	129	N	171	N	126	N	129	N
3	144	N,M	131	N,M	144	N,M	144	N,M	144	N,M	144	N,M	144	N,M
4	146	M	146	M	146	M	146	M	146	M	146	M	146	M
5	157	M	157	M	157	M	160	M	157	M	157	M	157	M
6	59	M	59	M	59	M	59	M	59	M	59	M	59	M
7	160	M	160	M	160	M	160	M	160	M	160	M	160	M
8	150	M	150	M	150	M	150	M	150	M	150	M	150	M
9	134	M	137	M	137	M	136	M	137	M	137	M	137	M
10	147	M	147	M	147	M	147	M	147	M	147	M	147	M
11	102	M	102	M	102	M	102	M	102	M	102	M	102	M
12	58	M	58	M	58	M	58	M	58	M	58	M	58	M
13	102	M	102	M	102	M	102	M	102	M	102	M	102	M
14	38	M	38	M	38	M	38	M	38	M	38	M	38	M
15	127	M	127	M	127	M	127	M	127	M	127	M	127	M
16	171	M	171	M	168	M	168	M	171	M	171	M	171	M
17	132	M	132	M	132	M	132	M	132	M	132	M	132	M
18	110	M	110	M	110	M	110	M	110	M	110	M	107	M
19	61	M	82	M	61	M	61	M	61	M	61	M	61	M
20	178	M,I	178	M,I	178	M,I	178	M,I	178	M,I	178	M,I	178	M,I
21	194	I	206	I	206	I	206	I	251	I	206	I	206	I
22	120	I	120	I	120	I,C	120	I,C	120	I,C	120	I,C	120	I,C
23	99	U	99	U	99	C	99	C	99	C	99	C	99	C
24	213	C	213	C	213	C	213	C	213	C	288	C	216	C
25	140	C,T	140	C	140	C	140	C	153	C	153	C	140	C
26	112	T	94	C,T	12	C	115	C	54	C	150	C	102	C
27	45	T	168	T	45	C,T	45	C,T	203	C	165	C	109	C,T
28	84	T	144	T	63	T	51	T	94	C,T	140	C	45	T
29	198	T	201	T	171	T	171	T	60	T	115	C,T	60	T
30	144	T	138	T	153	T	150	T	78	T	21	T	171	T
31	162	T	71	T	201	T	192	T	182	T	78	T	156	T
32	111	T	46	T	129	T	129	T	187	T	171	T	207	T
33	71	T	57	T	71	T	71	T	177	T	153	T	150	T
34	100	T	57	T	97	T	97	T	78	T	177	T	71	T
35	57	T	81	T	57	T	57	T	50	T	291	T	100	T
36	57	T	83	T	57	T	57	T	97	T	71	T	57	T
37	81	T	112	T	81	T	164	T	57	T	100	T	57	T
38	65	T			83	T	169	T	57	T	57	T	81	T
39	118	T			112	T			81	T	57	T	83	T
40									77	T	81	T	133	T
41									115	T	77	T		
42											115	T		

	At XIC		At XIE		At XIJ		At MYA1		At XI-1		At XIK	
No.	Size	Domain	Size	Domain	Size	Domain	Size	Domain	Size	Domain	Size	Domain
1	52	N	12	N	126	N	180	N,M	144	N	55	N
2	104	N	129	N	144	N,M	138	M	126	N,M	119	N
3	144	N,M	144	N,M	146	M	146	M	146	M	144	N,M
4	146	M	146	M	157	M	157	M	157	M	146	M
5	157	M	157	M	59	M	92	M	59	M	157	M
6	59	M	59	M	160	M	160	M	156	M	110	M
7	160	M	160	M	150	M	150	M	150	M	160	M
8	150	M	150	M	137	M	137	M	137	M	150	M
9	137	M	137	M	147	M	147	M	147	M	137	M
10	147	M	147	M	102	M	102	M	102	M	111	M
11	102	M	102	M	58	M	58	M	58	M	102	M
12	58	M	58	M	102	M	102	M	102	M	58	M
13	242	M	102	M	38	M	38	M	38	M	102	M

Table 3 (continued)

	At XIC		At XIE		At XIJ		At MYA1		At XI-I		At XIK	
No.	Size	Domain	Size	Domain	Size	Domain	Size	Domain	Size	Domain	Size	Domain
14	127	M	38	M	127	M	127	M	131	M	38	M
15	171	M	127	M	168	M	171	M	122	M	127	M
16	132	M	171	M	132	M	132	M	36	M	159	M
17	110	M	132	M	110	M	110	M	132	M	108	M
18	61	M	110	M	61	M	61	M	110	M	110	M
19	178	M,I	61	M	178	M,I	313	M	61	M	61	M
20	206	I	178	MI	206	I	206	M,I	178	M,I	178	M
21	120	I	206	I	120	I,C	120	I	206	I	239	I
22	99	C	120	I,C	651	C	99	I,C	120	I,C	120	I
23	222	C	99	C	140	C,T	219	C	99	C	99	C
24	140	C	222	C	257	T	140	C	222	C	222	C
25	112	C,T	140	C	53	T	139	C,T	140	C	140	C
26	48	T	112	C,T			51	T	100	C,T	118	C
27	255	T	48	T			51	T	51	T	51	C,T
28	156	T	255	T			171	T	171	T	72	T
29	207	T	156	T			156	T	63	T	171	T
30	144	T	195	T			210	T	177	T	156	T
31	71	T	144	T			147	T	71	T	207	T
32	100	T	71	T			71	T	100	T	138	T
33	57	T	157	T			100	T	81	T	75	T
34	57	T	57	T			114	T	83	T	81	T
35	81	T	81	T			85	T	151	T	57	T
36	83	T	83	T			76	T			57	T
37	124	T	124	T			124	T			81	T
38											83	T
39											136	T
40												
41												
42												

N, Amino-terminal sequence; M, motor domain; I, IQ domain; C, coiled-coil domain; U, undefined; T, tail domain. The size of the first and last exons in each gene reflects only the size of the coding region.

class XI myosins (except three) fall into two subgroups (Figure 4).

The *Arabidopsis* myosins have anywhere from three to six IQ domains. The IQ domain in non-plant myosins has been shown to bind to calmodulin in a calcium-independent manner. The regulation of myosin action is thought to be due to calmodulin interaction. In plants, two myosin heavy chains have been shown to associate with calmodulin [37,67]. A myosin-containing protein fraction from tobacco BY2 cells was used in motility assays with F-actin. Concentrations of Ca^{2+} higher than 10^{-6} M caused a significant reduction in F-actin sliding [37]. Another study with myosin isolated from lily pollen, also demonstrated a co-precipitation of myosin and calmodulin and a similar effect of Ca^{2+} concentration [67]. Not only did concentrations above 10^{-6} M cause inhibition of myosin activity, but the effects of concentrations higher than 10^{-5} M were not reversible upon Ca^{2+} removal. These studies provide evidence that plant myosins bind calmodulin in the absence of Ca^{2+} and are active when calmodulin is bound and inactivated when the Ca^{2+} concentration is increased. They also found that when the myosin fraction was pretreated with $CaCl_2$, calmodulin did not bind the myosin, suggesting that calmodulin dissociates from myosin at high concentrations of Ca^{2+}. The myosins in the above studies have not been cloned, and binding to specific IQ domains has not been established. However, the presence of IQ domains in *Arabidopsis* and other plant myosins suggests that these are the sites of Ca^{2+} regulation. It would be interesting to investigate the possible phosphorylation of the threonine residue which is three residues upstream from the TEDS rule site in class XI myosins and to see if enzyme activity is regulated by phosphorylation of this residue.

Myosins are involved in a wide range of cellular functions. They have been shown to be involved in movement, translocation, cell division, organelle transport, G-protein-linked signal cascade and maintenance of structure within cells [26]. Insight into the function of plant myosins has been gained by studies in algae. Cytoplasmic streaming is responsible for movement of organelles and vesicles and of generative cells and vegetative nuclei in pollen tubes. Physiological studies in *Chara* have shown that an increase in Ca^{2+} concentration causes cytoplasmic streaming to stop [68]. A myosin isolated from the alga *Chara corallina* was shown to be responsible for cytoplasmic streaming [30,69,70]. The myosin was cloned and characterized and found to be a class XI myosin related to the *Arabidopsis* MYA myosins [54].

Table 4

Intron size and sequence of 5′ and 3′ splice sites

	At ATM			At VIIIA			At ATM2			At VIIIB		
No.	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site
1	137	AG GTATTC	TTTAG AT	107	AG GTATTG	TAGAG GC	310	AG GTAATT	TTCAG AA	179	AG GTAAAT	GCCAG AA
2	84	AA GTAAGT	AACAG GT	88	AA GTAAGT	AACAG GT	95	AT GTGAGT	CAAAG GT	81	AA GTTCTT	AGTAG CA
3	124	AT GTAAGT	GCTAG AC	126	AT GTAAAT	GCTAG AC	91	AT GTGAGT	TACAG AG	84	TA GTAAGT	TTTAG AG
4	109	CG GTGGGT	TCCAG AT	92	AG GTTGGA	TTCAG TC	113	AG GTGAGG	AGAAG AG	226	GA GTGAAA	CTTAG TC
5	247	AG GTTAGT	TCCAG CG	302	AG GTTAGT	TCCAG TG	121	AG GTACGG	TATAG AG	159	TC GTGAGT	TGCAG GG
6	114	TT GTAAGC	TACAG GG	643	TT GTAAGC	GACAG GG	152	TT GTGAGA	CACAG GT	194	TT GTAAGA	AGTAG TC
7	103	CT GTAAGT	TGCAG TT	89	AG GTAACT	TTCAG GA	205	TT GTAAGT	GGTAG TC	196	AG GTAACA	TGCAG AG
8	101	AG GTAGCT	AACAG TC	201	AA GTATGG	TCCAG GT	151	AG GTAACA	TGTAG AG	100	TG GTACTT	TATAG GA
9	376	AG GTATGG	TGCAG AG	170	AG GTAGGC	ACCAG GC	102	TG GTAATT	TGCAG GA	98	AG GTAGAG	TACAG CT
10	101	AG GTAATT	TGCAG GA	135	AT GTATGC	TGCAG AA	78	AG GTAGAA	TACAG CT	97	TG GTTTGT	TTCAG GC
11	295	AA GTAAGC	TTCAG GT	114	AG CTAACG	TCCAG GA	94	AG GTAATG	TTAAG GT	75	AG GTTCGT	TTTAG GA
12	326	AG GTATAT	TTCAG GC	207	AG GTAATG	TGCAG AA	89	AG GTTAGT	TTCAG AA	123	TG GTGATC	TTCAG GA
13	197	AT GTATGT	TGCAG AA	146	TG GTAATA	CTCAG GT	82	AG GTGGTT	CTCAG GA	139	TG GTAAGT	TGCAG AA
14	136	AG GTAAAG	TTCAG GA	192	AG GTTGGG	TTCAG GG	95	AG GTAATT	AGCAG AA	126	AG GTCAGT	AATAG GT
15	160	AG GTATAT	TGCAG AA	211	AG GTCGTT	TGGAG AA	125	AG GTCAGT	TACAG GT	111	TG GTGACA	TACAG GC
16	122	AG GTAACA	ATCAG GT	86	TG GTACTT	TGCAG AT	87	AG GTAAAG	TACAG GG	104	TG GTTTGG	AGTAG AT
17	228	AG GTGAGT	TCCAG AG	85	TA GTATTG	TTCAG TT	87	AA GTAAGC	CATAG AT	82	AT GTAAGT	GATAG AT
18	87	AG GTGACA	TGCAG AT	103	TG GTAAAA	TGTAG CA	82	TG GTAAGC	TGCAG CG	109	TA GTAATC	TACAG AT
19	77	AG GTATAA	TGCAG AT	88	TG GTCCTC	TGTAG TG	82	AG GTACTT	TTCAG GA	85	TA GTAAAT	TGTAG TG
20	112	AT GTATAA	TTCAG TT	83	AG GTGGTT	TTGAG AC	88	AG GTCAAA	TGCAG AT	70	GC GTCTCT	TTGAG GT
21	250	AG GTAAAA	TGCAG CA							80	AG GTAAGT	TGCAG AT
22	111	AG GTAAAA	CGCAG AC									

	At XIG			At XIH			At MYA2			At XIB		
No.	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site
1	168	TG GTTATT	TTCAG CG	365	AT GTGAGA	TGCAG GC	330	TG GTAAGA	TACAG GT	618	TG GTAAAA	TGCAG GT
2	103	CG GTATGT	TTCAG GT	135	CA GTTTGA	TAAAG TT	100	AT GTATGT	TTCAG GT	127	AA GTATGT	CACAG GT
3	92	AT GTGAGT	ACTAG AC	137	AG GTGAGT	TCCAG AC	74	AT GTGAGT	TTCAG AC	143	AT GTGAGT	TTCAG AC
4	90	AG GTGCTT	TATAG AC	96	AG GTGCCT	GGTAG AC	102	AG GTAATT	TGCAG AC	87	AG GTAATT	TGCAG AC
5	105	AG GTAACT	TGCAG TC	98	AG GTTATC	TGCAG TC	300	AG GTGAAA	TTCAG TC	201	AG GTGAAA	TACAG TC
6	120	AG GTGAAT	TGCAG TC	123	AG GTGTAT	TGCAG TC	76	AG GTAACC	TATAG TC	101	AG GTAAGG	TATAG TC
7	274	AG GTACAT	GACAG GA	289	AG GTACAT	ATCAG GA	125	AA GTAAGT	TACAG GA	93	AA GTAAGT	TTCAG GA
8	76	AG GTAGTT	GTCAG GA	83	AG GTAACT	GTCAG GA	95	AG GTAGTT	TTCAG GA	81	AG GTACCT	TTTAG GA
9	115	AT GTGTGT	TGCAG GT	101	TA GTGAGT	GTCAG GT	103	AG GTAAAT	TCCAG CT	89	AT GTAAAT	TGCAG GT
10	111	TG GTATGT	TGTAG GA	107	TG GTATGT	TTCAG GA	111	TG GTGGGT	TGCAG GC	125	TG GTGAGT	TGCAG GC
11	300	AG GTGCAT	TTCAG TT	284	AG GTGCTT	TGCAG TT	355	AG GTGCTT	TGCAG TT	417	AG GTGCTT	TGCAG TT
12	84	AG GTTTGT	GGCAG CA	88	AG GTTTGT	GGCAG CA	91	AG GTTTGA	TGCAG CA	91	AG GTTTTG	TGCAG CA
13	97	AG GTAACT	TTCAG AA	80	AG GTTAGT	CTCAG AA	234	GA GTCTGT	TTCAG AA	243	AG GTTATC	TTCAG AA
14	82	TG GTAAGC	TGCAG CA	87	TG GTATGA	TGCAG CA	153	TG GTGAGT	TGCAG CA	123	AG GTGAGT	TGCAG CA
15	99	AT GTGAGT	TTCAG GT	104	TA GTGAGT	TTCAG GT	117	AT GTGAGT	TCCAG GT	121	AT GTGAGC	TCCAG GT
16	85	AG GTGCAG	TGCAG CA	82	AG GTGCAG	TGCAG CA	87	AG GTAAGT	TTCAG CA	91	AG GTGAGT	TGCAG CA
17	92	GG GTGAGA	TTTAG GG	87	GG GTGGGA	TTCAG GG	91	GG GTGCGA	TTTAG GG	98	GG GTGCGA	CACAG GG
18	86	AG GTATGC	GCTAG TT	79	AG GTTCCC	TCTAG TA	77	AA GTAAGA	AATAG CT	88	AA GTAAGA	ACTAG TT
19	75	AG GTACTT	CACAG AT	113	AA GTACGT	TCCAG AT	87	AG GTAATT	TGTAG AT	93	AG GTAATT	TGTAG AT
20	99	AG GTATCT	AACAG GT	86	AG GTACTT	TGTAG GT	117	AG GTATTT	GTCAG GT	88	AG GTATTT	TTCAG GT
21	147	AG GTGGAG	CAGAG CC	147	AG GTGCTG	TACAG AG	159	AG GTACAC	TATAG AC	170	AG GTATGA	TACAG AC
22	130	CG GTGTGC	TGCAG GA	296	TG GTGAGC	TGCAG GC	122	TG GTGAGA	CCTAG GC	150	AG GTGAGA	CACAG GC
23	117	GG GTCAGA	TGTAG GT	120	GG GTAAGT	TTTAG AC	125	GG GTGTGA	TGCAG AC	105	GG GTGAGT	TGCAG AC
24	107	AG GTAGGG	TGCAG TC	119	AG GTAGGA	TTCAG TC	150	AG GTTTGT	TACAG AG	120	AG GTGGGT	TGCAG GG
25	99	AA GTATTC	TGCAG TC	94	GA GTACCC	TGCAG AC	89	TG GTATCC	TCCAG GC	87	AG GTACTG	TGCAG GC
26	84	AG GTAGAC	TTTAG AA	392	CA GTTAAG	AGGAG AA	89	AG GTAGAA	TGTAG AA	90	AG GTAGAA	TGCAG AA
27	85	CA GTGTAA	TGCAG GG	133	AG GTACTG	ATCAG GA	104	AT GTATAT	TCCAG GA	106	TA GTAGGG	TTCAG GA
28	152	AT GTATGT	TGAAG AG	89	TG GTATAT	ACCAG GG	82	TT GTATGT	TGCAG AT	82	TT GTACTG	TGCAG GA

Table 4 (continued)

	At XIG			At XIH			At MYA2			At XIB		
No.	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site
29	85	AG GTACTA	TTTAG GA	105	AG GTCAGC	TCTAG GC	181	AG GTAATT	TTCAG AA	316	TG GTAAAT	TTCAG AA
30	97	AG GTATAT	AACAG GG	73	TT GTATGG	TTCAG GT	103	TG GTTTGT	ACCAG AG	86	TG GTATTT	ACCAG AG
31	83	AG GTGACA	TCTAG GC	81	AG GTGAGA	TGTAG CC	95	AG GTTCCT	TTCAG GC	158	TG GTTTCA	TTCAG GC
32	78	TT GTATGT	TACAG GT	150	TT GTAAAA	TGCAG TA	85	AT GTAAGG	TCCAG GT	77	AT GTAAGG	TACAG GT
33	91	AG GTGAGA	TGCAG CC	128	TG GTATGT	AACAG GT	78	AG GTAAGT	TACAG TC	169	AG GTAAAT	AATAG CC
34	81	AG GTAATC	GATAG TA	100	CT GTGAGT	TGCAG AT	95	AA GTAAAA	GGCAG TA	74	AA GTAAGT	TGCAG TA
35	104	TG GTATGT	AACAG CT	92	AT GTATGC	AACAG GT	165	AG GTATGT	TGCAG GT	90	TG GTATGT	ATCAG GT
36	88	CA GTAAGT	CTCAG AA	101	AG GTAACA	CTTAG CA	88	CG GTAAGG	TACAG GT	83	CG GTAAAG	TACAG AT
37	89	AT GTAAGC	AATAG GT				103	AA GTACCT	TGCAG GT	86	AG GTAACT	AATAG AC
38	108	AG GTAAGT	CACAG CA				156	AG GTGAAA	GACAG CA			

	At XID			At XIA			At XIF			At XIC		
No.	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site
1	228	TG GTACGA	ATCAG GC	430	TG GTACGA	TGCAG GC	89	TG GTAAGC	GTTAG GG	143	AG GTTAGT	TGTAG GT
2	47	AG GTACCT	TGTAG GT	215	CG GTAAGA	CTTAG GT	169	CA GTAAGA	TACAG GT	93	AG GTCCAG	TATAG GT
3	173	AT GTACGC	TACAG AC	134	TA GTAAGC	TCCAG AC	100	TA GTCAGT	CGCAG AC	82	AT GTTTTG	GACAG AC
4	89	AG GTAATC	TTTAG AA	91	AG GTAACT	TTCAG GA	81	TG GTAAAA	ACTAG GG	95	AG GTGAGT	CTCAG GG
5	109	AG GTAGAT	TGCAG TC	112	AG GTAATG	TGCAG TC	71	AG GTGAGT	TATAG TC	93	AA GTAATG	TCCAG TC
6	90	AG GTGGAA	TGCAG TC	93	AG GTGGAG	TGCAG TC	96	AG GTGGTG	GACAG TC	83	AG GTGAAG	CTCAG TC
7	117	AG GTAAAC	TTCAG GA	101	AG GTAAGC	TTCAG GA	84	AG GTAAGT	TTCAG GA	72	AG GTACGT	AGCAG GA
8	68	AG GTACCT	TGTAG GA	66	AG GTACTT	TGTAG GA	76	TG GTTTGT	TTTAG GA	101	AG GTCAGT	AACAG GA
9	84	AT GTATAT	GGTAG GT	86	TA GTAAAT	TGCAG GT	79	TG GTATCT	CGTAG GT	174	AT GTAAAA	TTCAG GT
10	90	GG GTAGGT	CCCAG GC	80	TG GTAGAT	TTAAG GA	264	TG GTATGT	GACAG GA	74	TG GTAAGT	TCTAG TA
11	309	AG FTFCTT	TGCAG TT	297	AG GTGCTT	TGCAG TT	79	AG GTAGAC	CAAAG TT	76	AG GTAAAT	TGCAG TT
12	93	AG GTTGGA	TACAG CA	74	AG GTTGGA	TACAG CA	72	AG GTAGAA	TGCAG CA	71	AG GTATTG	TTCAG CA
13	113	AG GTAAGT	GTCAG AA	99	AG GTTAGT	GTCAG AA	97	AG GTATAA	TTCAG AA	84	TG GTAAAG	TTCAG CA
14	86	TG GTAATG	TACAG TA	84	TG GTAATG	TGCAG CA	106	TG GTAAGT	TGCAG CA	74	AA GTAGGT	TCCAG GT
15	105	AT GTTAGT	TTCAG GT	82	AT GTTAGT	TCCAG GT	78	AT GTGAGA	TCCAG GT	154	AG GTAGGG	TGCAG CT
16	78	AG GTCTAC	TACAG CA	214	AG GTCTGA	TACAG CA	70	AG GTAAGC	CCCAG CA	135	GT GTAAGT	TCTAG GG
17	102	GG GTAAGC	CTCAG GG	105	GG GTAAGC	TTCAG GG	90	GA GTAAGC	AACAG GG	92	AG GTAAGT	AACAG CT
18	111	AG GTAGAT	TATAG CT	128	AG GTAGCT	AATAG CT	102	GG GTAAAA	GACAG AT	120	AG GTAACG	TGCAG AT
19	152	AG GTGCGT	CACAG AT	202	AG GTGCAG	CATAG AT	101	AG GTATGT	TTCAG AT	114	AG GTGAGC	TGTAG GA
20	92	AG GTAATA	TTCAG GA	83	AT GTTATA	TTTAG GT	175	AG GTTTTT	TGTAG CA	88	AG GTTTAG	GGCAG GC
21	69	TC GTATCT	CACAG AG	113	AA GTAAGT	CGCAG AG	292	AG GTACTA	AACAG AG	296	TG GTACAA	TTCAG GC
22	280	TG GTGACT	TCCAG GC	256	TG GTAATC	TTCAG GC	148	TG GTAAGT	CAAAG GC	79	GG GTATTT	TATAG GG
23	86	GG GTACAC	TGCAG AT	126	GG GTACAC	TGCAG AT	73	AG GTATTG	ATTAG GC	114	AG GTACTT	AACAG GT
24	72	AG GTAAGG	CTAAG GA	122	AG GTTAGT	AAAAG GT	68	AG GTAAGT	TGTAG GT	105	AG GTAAGA	ATCAG GA
25	120	CC GTCATT	CGTAG GC	114	AG GTAAGA	CTTAG GC	86	AG GTATAC	TCCAG AT	96	AG GTAAAC	TACAG AG
26	432	AC GTAACA	TACAG GA	117	AG GTAATC	CTTAG GC	176	AG GTACGG	ATCAG CC	92	TG GTAAAT	ATCAG GA
27	118	AG GTTATC	TTTAG GC	87	TA GTTAGT	AACAG GA	84	AG GTGCAA	TGCAG AA	88	AG GTTGGC	CTCAG AC
28	77	AG GTGTCA	TCAAG AA	120	AG GTTTTG	TTTAG GC	70	AG GTACGA	TTCAG GA	113	AG GTGATG	ATTAG AG
29	96	AT GTAAGT	TACAG GA	79	CG GTAAAT	TGCAG CC	121	AG GTATTA	GACAG GA	87	AG GTATGC	AATAG GC
30	86	AT GTATGT	TGCAG GA	105	AG GTAAGT	TACAG GA	93	AG GTAATA	AGAAG GG	85	AT GTGAGT	TTTAG GT
31	78	AA GTTTAA	CTCAG AA	88	TA GTATGT	AGCAG GA	75	AA GTAAGC	TGTAG GG	103	AG GTTTTT	AACAG CC
32	121	AG GTAACA	TTTAG GG	164	AG GTAACC	TTCAG AA	93	AT GTTAGT	AACAG GC	70	AG GTATCT	TTCAG TA
33	360	AG GTAGAA	CTGAG GA	147	TG GTAACG	TTTAG GG	85	AT GTAAAA	TCCAG GT	79	TG GTAACC	TACAG GT
34	109	AC GTAAGA	CTCAG AA	92	TG GTATAC	TTCAG AG	82	AG GTACAA	GGCAG TT	148	CG GTAAGT	GACAG GT
35	97	AG GTAAAA	TGCAG CC	67	AC GTAAGA	TTCAG GT	97	AG GTAGGC	TACAG GC	97	AC GTAAGT	AATAG GT
36	87	AT GTAAGT	TGCAG TT	97	TG GTTATT	TGCAG TC	84	TG GTATAG	TACAG GT	74	AG GTTGTT	TGCAG CA
37	98	TG GTCAGT	TCCAG GT	76	AG GTAAAA	TGCAG TT	230	CG GTAAAG	CTCAG GT			
38	125	CG GTAACT	CTCAG GC	78	TG GTTTGT	TTCAG GT	123	AG GTAAGT	AATAG GT			
39	84	AC GTATGT	TGCAG GT	206	CG GTAAGT	GTCAG GT	70	AG GTACGC	TTCAG CA			
40	91	AG GTATTG	CTCAG CA	79	AG GTACAT	TGCAG GT						
41				84	AG GTACTG	AACAG CA						

Table 4 (continued)

	At XIE			At XIJ			At MYA1			At XI-I		
No.	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site	Size	5′ site	3′ site
1	111	CA GTGACT	TGCAG GG	120	AT GTAAA	GTCAG GT	330	TG GTAAGA	TACAG GT	134	AG GTCTGA	AAAAG CT
2	86	AG GTGAGT	TGTAG AT	117	AT GTAAGA	GACAG AC	100	AT GTATGT	TTCAG GT	860	AT GTGAAC	TTCAG AC
3	80	AT GTTAGT	GACAG AC	85	AG GTGATT	AACAG GG	74	AT GTGAGT	TTCAG AC	95	AG GTGATC	CCCAG AG
4	80	AG GTGCTC	TTCAG GG	292	AA GTAAGT	TACAG TC	102	AG GTAATT	TGCAG AC	181	AA GTAAGA	TGCAG TC
5	116	AA GTATGA	GGCAG TC	135	AG GTAAAC	TACAG CC	300	AG GTGAAA	TTCAG TC	241	AG GTGGGT	TTCAG CC
6	85	AG GTGAAA	GTCAG AT	72	AG GTAGGT	TGCAG GA	76	AG GTAACC	TATAG TC	149	AT GTAATT	CTTAG GA
7	75	AG GTATAC	ACTAG CA	88	AG GTTTGC	TTCAG GA	25	AA GTAAGT	TACAG GA	90	AG GTATAA	ATCAG GA
8	79	AG GTAAGC	AACAG GA	67	AT GTAATA	TTTAG GT	95	AG GTAGTT	TTCAG GA	91	AA GTACAT	ATCAG GT
9	76	AT GTAAGT	TTTAG GT	91	TG GTAAAT	TCCAG GT	103	AG GTAAAT	TCCAG CT	94	TG GTTTGC	GTCAG GC
10	101	TG GTAAGT	TGCAG GT	315	AG GTGATG	TGCAG TT	111	TG GTGGGT	TGCAG GC	135	AG GTTAGC	TGCAG TT
11	86	AG GTAAGG	TGCAG TT	81	AG GTATGA	TACAG CA	355	AG GTGCTT	TGCAG TT	83	AG GTAATA	TTCAG CA
12	88	AG GTAATT	TTCAG CA	440	AG GTTTGT	TGCAG AA	91	AG GTTTGA	TGCAG CA	717	AG GTCGTT	TGCAG AA
13	115	AG GTTATT	AGCAG AA	110	TG GTATAA	TGCAG CA	234	GA GTCTGT	TTCAG AA	85	TG GTACAA	TGCAG CA
14	91	TG GTAATA	TTCAG CA	88	AT GTAAGT	TTCAG GT	153	TG GTGAGT	TGCAG CA	98	AA GTCTTG	TGAAG CC
15	103	AA GTAAGT	TTCAG GT	138	AG GTGACT	TGCAG CT	117	AT GTGAGT	TCCAG GT	127	AG GTAGAG	TTTAG CA
16	70	AG GTAGAT	GATAG TT	75	GG GTCTGT	TGCAG GG	87	AG GTAAGT	TTCAG CA	547	GG GTTAGT	GATAG CC
17	107	GT GTAAGT	TGTAG GG	106	GA GTATGT	ATCAG GT	91	GG GTGCGA	TTTAG GG	302	AG GTACGA	TGCAG CA
18	85	AA GTAAGT	AACAG CT	154	AG GTAAAG	TGCAG AT	77	AA GTAAGA	AATAG CT	95	AG GTATGG	CACAG CT
19	92	AG GTTTTT	TGCAG GT	99	AG GTGAGG	TTTAG GA	87	AG GTAATT	TGTAG AT	269	AG GTTCCT	GCAAG GA
20	157	AG GTGAAC	TATAG GA	99	AG GTTCTA	TGCAG GC	117	AG GTATTT	GTCAG GT	180	AG GTACTT	TTTAG GC
21	88	AG GTTTTA	TGCAG GC	119	AG GTATTG	TATAG GC	159	AG GTACAC	TATAG AC	96	AG GTATGA	TGCAG GT
22	184	TG GTACGT	TTCAG GC	134	AG GTAATG	TTCAG GC	122	TG GTGAGA	CCTAG GC	80	GA GTATGT	TACAG AC
23	90	GG GTATTT	GTCAG GT	130	AG GTATTA	TCCAG GT	125	GG GTGTGA	TGCAG AC	701	AG GTAATT	CACAG AA
24	164	AG GTACTC	AACAG GC	197	AG GTCAGT	TGCAG GA	150	AG GTTTGT	TACAG AG	88	AG GTTTGT	TTCAG TC
25	125	AG GTAAGT	GTCAG GC				89	TG GTATCC	TCCAG GC	277	AA GTATGT	AGCAG AA
26	95	AG GTACGG	AACAG GT				89	AG GTAGAA	TGTAG AA	620	TT GTAAGT	ATCAG GA
27	101	TG GTAAGT	ATCAG GA				104	AT GTATAT	TCCAG GA	220	AG GTGATC	TGCAG AG
28	91	AG GTTTGT	TTCAG AC				82	TT GTATGT	TGCAG AT	129	AT GTGAGT	ACCAG GG
29	85	AG GTGTGT	TCTAG AG				181	AG GTAATT	TTCAG AA	466	AG GTGAGA	GATAG GT
30	90	AG GTATAT	AATAG GC				103	TG GTTTGT	ACCAG AG	89	AG GTAAAT	TTCAG TC
31	86	AC GTGAGT	CTTAG GT				95	AG GTTCCT	TTCAG GC	399	AG GTACAC	TATAG GT
32	79	AG GTCTGT	TACAG TC				85	AT GTAAGG	TCCAG GT	88	AG GTGAGT	TGTAG GT
33	92	AG GTACAT	TGCAG GT				78	AG GTAAGT	TACAG TC	326	AG GTATTA	TGCAG CA
34	78	CG GTAAGT	TGCAG GT				95	AA GTAAAA	GGCAG TA			
35	80	AC GTAAGT	GATAG GT				165	AG GTATGT	TGCAG GT			
36	99	AG GTTAGT	GGCAG TA				88	CG GTAAGG	TACAG GT			
37							103	AA GTACCT	TGCAG GT			
38							156	AG GTGAAA	GACAG CA			

	At XIK								
No.	Size	5′ site	3′ site	No.	Size	5′ site	3′ site	No.	Size	5′ site	3′ site
1	237	AA GTGAGT	CCCAG TC	14	157	TG GTAGGC	TGCAG TA	27	98	CG GTAAGG	CACAG GA
2	269	CC GTAAGT	TTCAG GT	15	87	AG GTATAA	ATCAG GC	28	110	AG GTATCA	TGCAG GA
3	105	AT GTAAGT	CGCAG AC	16	319	AG GTATGC	TTCAG GT	29	118	AA GTAAGT	ACCAG GT
4	102	AG GTTATT	GGTAG GG	17	148	AC GTAATT	TTAAG GG	30	99	AA GTAAGA	AATAG GG
5	115	TG GTGAGG	GAGAG GC	18	150	AA GTAAGT	TGCAG TT	31	276	AG GTAATT	TATAG GC
6	356	AG GTACGT	TGCAG AC	19	87	AA GTAAGC	TCCAG TT	32	90	TA GTGAGT	TACAG GC
7	105	AG GTATTG	TGTAG GA	20	193	AG GTATCT	TGGAG TT	33	110	TA GTTTCA	GTGAG TG
8	85	AG GTCAGT	ATCAG GA	21	125	AG GTAATT	TTTAG GC	34	91	AA GTAAGC	TACAG TA
9	84	AG GTATGT	AAAG GT	22	84	AG GTTCGG	ATCAG GC	35	93	TG GTAAAA	TTCAG GT
10	229	GC GTTAGC	TTCAG GC	23	74	GA GTAAGT	TATAG TC	36	94	CG GTATTT	TTCAG GT
11	81	AG GTAAAG	CTCAG CT	24	121	AG GTATGT	TACAG GC	37	79	AT GTATGT	CATAG GT
12	87	AG GTCCGT	AACAG CA	25	202	AG GTTCGT	TTCAG AC	38	81	AG GTAACC	CGCAG CA
13	91	AG GTGTCC	TTCAG AA	26	97	CG GTGCCT	TTCAG AG				

Myosins in plants have also been shown to be involved in cytoplasmic streaming. Using immunofluorescence, myosin was localized to vesicles, organelles and generative cells and vegetative nuclei in grass pollen tubes [39]. A myosin isolated from lily pollen has been shown to be responsible for cytoplasmic streaming in pollen tubes and two myosins were identified in tobacco cell cultures that are also thought to participate in cytoplasmic streaming [37,71]. Antibodies to the myosins recognized a protein in vegetative cells as well as pollen tubes. Liu *et al.* [51] suggest that class XI myosins are likely candidates for transport of large vesicles because of the number of IQ domains (5-6). Previous studies showed that translocational step size produced by a myosin motor is proportional to the number of IQ domains and the larger the step the faster or more efficiently they are able to transport vesicles [9]. However, the kinetic properties of the motor domain are also involved in speed and there is a wide range of movement speeds for myosin II molecules [2,72,73].

An antibody specific to a *Z. mays* class XI myosin was used to localize this myosin in fractions of maize proteins and maize root tip cells [51]. The nuclear/cell wall fraction and the plastid fraction contained relatively small amounts of antigen while the mitochondrial fraction and the low density membrane fraction had most of the antigen. The root tip cells showed particulate staining in the cytoplasm, but neither the vacuole membrane nor plasma membrane were stained, although in some cells the staining was too bright to distinguish if the plasma membrane was stained or not. There are 13 class XI myosins in *Arabidopsis* that could be involved in vesicle and organelle transport. The large number could reflect redundancy of function or differential expression. Patterns of expression were different for the cloned *Z. mays* and *Arabidopsis* myosins that have been analyzed [42,51].

Immunolocalization studies have also detected myosin associated with plasmodesmata. Plasmodesmata are interconnections between contiguous plant cells that allow direct cell-to-cell transport of ions and proteins. A recent study using an antibody to a cloned class VIII *Arabidopsis* myosin ATM1 (At ATM) localized this myosin to the plasmodesmata and the plasma membrane regions involved in the assembly of new cell walls [47]. Earlier work suggested that actin was involved in regulation of plasmodesmal transport [74]. Other studies using antibodies to animal myosins in root tissues of *Allium cepa, Z. mays* and *Hordeum vulagare* have also indicated the presence of myosin in the plasmodesmata [38]. However, immunolocalization studies with antibodies to animal myosins need to be interpreted with caution as there are no plant myosins that group with animal myosins.

The recent work by Reichelt *et al.* [47] is more convincing because they used antibody to plant myosin. The myosin was localized mainly to the transverse walls with some punctate labeling of the longitudinal walls. During cell division the anti-class-VIII myosin staining remains confined to the transverse cell walls and is strongest in the newly formed cell wall. Immunogold electron microscopy showed labeling of class VIII myosin associated with the plasma membrane and plasmodesmata. These studies suggest that class VIII myosins may be involved in new cell wall formation and transport in the plasmodesmata. Reichelt *et al.* [47] suggest that myosin VIII could act to bring islands of membrane plate material together or could trigger exocytosis of new cell wall material, or alternatively as an anchor for actin along the transverse walls. The role of myosin in the plasmodesmata was studied further by pretreating tissue with 2,3-butanedione 2-moxoxime (BDM), an inhibitor of actin-myosin motility. The pretreatment resulted in a strong constriction of the neck region of plasmodesmata [38]. Myosin VIII in the plasmodesmata could be a part of a gating complex that is thought to control the opening of the plasmodesma neck [74]. There are four class VIII myosins in *Arabidopsis* that could be involved in these types of functions.

A recent study of the effect of BDM on the distribution of myosins, F-actin, microtubules and cortical endoplasmic reticulum (ER) suggests that myosins may link together microtubules and actin filaments involved in structural interactions [75]. This study used antibody to myosin II from animals and *Arabidopsis* myosin VIII for immunofluorescence studies. BDM treatment disrupted normal cellular distributions of maize myosins and the characteristic distribution of F-actin was also affected. Myosin may participate in the intracellular distribution of actin filaments as was proposed for myosin XV [76]. Microtubule arrangements in cortical root cells were altered, as was the normal ER network. Post-mitotic cell growth was inhibited by BDM, specifically in the transition zone and the apical parts of the elongation region. The study suggested that actin fibers and microtubules interact together via myosins and that myosin-based contractility of the actin cytoskeleton is essential for the developmental progression of root cells [75]. However, BDM has only been shown to inhibit a few myosins *in vitro* [77] and is known to be a nonspecific inhibitor; so these results must be viewed with caution.

Conclusions

As the classification system of myosins now stands, plant myosins fall only into two classes - class VIII and class XI. All animal cells examined contain at least one myosin II gene and usually multiple myosin I genes [8], but this is not true for *Arabidopsis* specifically and possibly for all plants. Also, no animal myosins of type VIII or XI have been identified. Plant and animal cells have some common tasks such as vesicular and organelle movement, but plant cells are unique in many ways and the presence of specific plant myosins is probably a reflection of that uniqueness.

There are 4 class VIII and 13 class XI *Arabidopsis* myosins. The large number of myosins in class XI could be the result

of gene duplication or specialization of function in different tissues or different life cycle times. This work identifies the *Arabidopsis* myosins, their domains and gene intron/exon structure. The task ahead is to analyze the protein products biochemically and try to establish the function of each myosin.

Materials and methods

Using the conserved motor domain of the plant myosin At MYA1 [41] database searches were performed using BLASTP and TBLASTN at TAIR [11]. The sequences were evaluated for the presence of a myosin motor domain using the SMART program [56]. All sequences with a myosin domain had BLASTP scores greater than 100 and E values less than 10^{-20}. The motor domains of representative myosins from other groups were also used to search the *Arabidopsis* domain but the searches did not reveal any new myosin genes. The SMART program also identified the IQ and coiled-coil domains and the location of the domains. The sequences found at TAIR were checked against the MIPS database [57]. Sequences identified at MIPS as myosins but not at TAIR were evaluated as above. The sizes of the exons/introns were determined using the exon/intron data for each myosin sequence using the MIPS predictions for myosins not previously cloned. Two sequences (At XIF, At XIH) were edited by comparing the upstream genome sequence translation to conserved sequences present in the other myosins but missing in the predicted sequences.

Sequences of myosins other than the *Arabidopsis* myosins for phylogenetic analysis were obtained from MHP [22] or NCBI [58]. The names are as in the tree of Hodge and Cope [59]. The motor domain sequences were determined using the SMART program [56]. The motor domain sequences were used for alignment of the plant and non-plant myosins using the Megalign program. The alignment was saved as a PAUP file and the phylogenetic analysis was done using PAUP 4.0b4a (PPC). We performed a bootstrap analysis with 100 replicates using the heuristic method. Full-length sequences were used for analysis of the plant myosins using the same methods as above.

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