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SEQUENCE NOTE

A Myosin from a Higher Plant has Structural Similarities to Class V Myosins

Mark Kinkema and John Schiefelbein

Department of Biology, University of Michigan, Ann Arbor, MI 48109, U.S.A.

In plant cells, myosin is believed to be the molecular motor responsible for actin-based motility processes such as cytoplasmic streaming and directed vesicle transport. In an effort to characterize plant myosin, a cDNA encoding a myosin heavy chain was isolated from *Arabidopsis thaliana*. The predicted product of the MYA1 gene is 173 kDa and is structurally similar to the class V myosins. It is composed of the highly-conserved NH_2-terminal "head" domain, a putative calmodulin-binding "neck" domain, an alpha-helical coiled-coil domain, and a COOH-terminal domain. Northern blot analysis shows that the *Arabidopsis* MYA1 gene is expressed in all the major plant tissues (flower, leaf, root, and stem). We suggest that the MYA1 myosin may be involved in a general intracellular transport process in plant cells.

Keywords: Arabidopsis thaliana; myosin heavy chain; cytoplasmic streaming; IQ motif; coiled-coil

Although plants are immobile, their cells display active intracellular motility, including a variety of actin-based processes (Staiger & Schliwa, 1987). A prominent example is cytoplasmic streaming, the rapid intracellular transport of endoplasmic reticulum along actin filaments (reviewed in Kuroda, 1990), which has been characterized in characean algae (Kachar & Reese, 1988; Grolig *et al.*, 1988) and the cells of higher plants (Seagull & Heath, 1980; Tang *et al.*, 1989). Another example of actin-based motility in plants is the directed transport of secretory vesicles, a process that is particularly apparent in tip-growing cells such as pollen tubes and root hairs (Mollenhauer & Morre, 1976; Schnepf, 1986; Steer, 1991). Other forms of actin-based intracellular motility include chloroplast movement in *Bryopsis* (Menzel & Schliwa, 1986), basipetal nuclear migration in legume root hairs (Lloyd *et al.*, 1987), and wound-induced cell contraction in coenocytic green algae (La Claire, 1989).

The motor proteins responsible for actin-based motility in eukaryotic cells are collectively known as myosin; a diverse group of large mechanoenzymes that use the chemical energy stored in ATP to generate force along actin filaments (Kiehart, 1990). Several lines of evidence indicate that myosin exists in higher plants and may account for the observed actin-based intracellular motility. Immunoblotting using animal myosin antibodies has led to the identification of cross-reacting polypeptides of various sizes (158 to 205 kDa) from different flowering plants (Parke *et al.*, 1986; Qiao *et al.*, 1989; Tang *et al.*, 1989). In addition, immunofluorescence studies with these myosin antibodies led to the decoration of organelles in pollen tubes of tobacco (Tang *et al.*, 1989) and grasses (Heslop-Harrison & Heslop-Harrison, 1989). The presence of a myosin-like activity in pollen tubes has been demonstrated by the use of motility assays. Organelles from pollen tubes were shown to move on characean actin bundles *in vitro*, indicating that myosin is associated with cytoplasmic organelles in these cells (Kohno & Shimmen, 1988; Kohno *et al.*, 1990). In another *in vitro* motility assay, muscle actin filaments moved on a surface coated with a crude pollen extract (Kohno *et al.*, 1991). Finally, biochemical assays based on ATPase activity and actin-binding properties have been used to purify myosin-like polypeptides from tomato (Vahey *et al.*, 1982) and pea tendrils (Ma & Yen, 1989).

Although there is evidence for the existence of myosin-like polypeptides in higher plants, very little is known about their molecular structure. Recently, a classification system based on phylogenetic analysis has been devised that separates all known myosins into seven classes (Espreafico *et al.*, 1992; Cheney *et al.*, 1993). The classification is based on the primary sequence of the highly conserved NH_2-terminal "head" domain present in all myosins. The myosin head is approximately 80 kDa and possesses the ATP and actin-binding properties. Following the conserved head domain is a highly variable "tail" region. Among different classes of myosin, the tail varies in both length and structure and may possess regions which bind to actin or membranes or adopt an alpha-helical coiled-coil

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conformation. All known myosins also contain one or more repeats of a sequence known as the IQ motif, which represents a putative calmodulin-binding regulatory region.

Knight & Kendrick-Jones (1993) recently reported the first sequence of a myosin from a higher plant (*Arabidopsis*). This myosin appears to represent a new (eighth) class of myosin-like proteins. In this paper, we describe the structure of a novel *Arabidopsis* myosin (MYA1). MYA1 appears to be related to a distinct class of myosins (class V) that have been identified in mouse, chicken, and yeast and have been proposed to function in directed organelle/vesicle transport.

Isolation of the MYA1 cDNA

Isolation of an *Arabidopsis* myosin cDNA was facilitated by the amino acid sequence similarity present in the head region of animal and protozoal myosins (Pollard *et al.*, 1991). Degenerate oligonucleotides were synthesized to two conserved amino acid motifs in the myosin head (ERNFHIFY and LDIYGFE) and used in a polymerase chain reaction (PCR) of *Arabidopsis* genomic DNA. Five PCR products, ranging in size from 600 to 1100 nucleotides, were generated and cloned.

One of these PCR products was used to screen a size-selected (3 to 6 kb) cDNA library constructed in the lambdaZap II vector from poly(A)+ RNA of etiolated, three-day-old *Arabidopsis* seedlings. Approximately 20 cDNA clones were identified and purified. The complete nucleotide sequence was determined for two cDNA clones that differed in length (5·2 and 4·6 kb), but were identical in sequence in the overlapping region. The gene encoding this sequence has been designated MYA1 (for MYosin from *Arabidopsis*). The one long open reading frame in the cDNA clones is 4560 nucleotides in length and potentially encodes a polypeptide of 1520 amino acids with a predicted molecular mass of 173 kDa (Figure 1A).

Domain structure of the MYA1 protein

Espreafico *et al.* (1992) have used the Clustal V sequence analysis program (Higgins *et al.*, 1992) to divide all known myosins into seven distinct classes based on the primary sequence of the head domain. Using this program, the *Arabidopsis* MYA1 myosin does not clearly fall into any of the seven known classes. MYA1 is also distinct from the other *Arabidopsis* myosin, ATM1 (Knight & Kendrick-Jones, 1993). Analysis of the entire protein, however, indicates that MYA1 is similar in structure to the class V myosins (Figure 1B).

The NH_2-terminal portion of the MYA1 polypeptide (amino acid residues 1 to 730) displays extensive sequence identity to the head domain of all myosins. It is most similar to the head domains of the class V myosins, showing 46% identity to the mouse Dilute, chicken p190, yeast MYO2, and yeast MYO4 head regions (Figures 1B and 2). MYA1 and the other *Arabidopsis* myosin, ATM1, are 45% identical in this portion of the head domain, but they differ at the NH_2-terminus; ATM1 possesses an unusual 90 amino acid NH_2-terminal extension (Knight & Kendrick-Jones, 1993).

Directly following the MYA1 head domain are six imperfect tandem repeats of a 23 to 25 amino acid sequence known as the IQ motif (Cheney *et al.*, 1991). As displayed in Figure 1B, these motifs are present in the Dilute, p190, MYO2, and MYO4 myosins at the same relative position. Although all known myosins have at least one copy of this motif (Cheney & Mooseker, 1992), class V myosins appear unique in that they possess exactly six copies of this element. The IQ motifs have been shown to bind calmodulin in the p190 myosin (Cheney & Mooseker, 1992), the brush border myosin I (Halsall & Hammer, 1990), the NinaC myosin (Porter *et al.*, 1993), and in the non-myosin, neural-specific protein neuromodulin (Chapman *et al.*, 1991).

Based on the program of Lupas *et al.* (1991), the region of the MYA1 polypeptide (amino acids 871 to 946 and 968 to 1048) that follows the putative calmodulin-binding sites has a strong potential for forming an alpha-helical coiled-coil structure. Unlike the coiled-coils of class II myosins, which extend along the entire length of the tail, the MYA1 coiled-coil only spans a portion of the tail. The presence of this secondary structure suggests that the MYA1 polypeptide may be able to self associate to form dimers. The 22 amino acids in the center of this domain have a lower probability (<90%) of forming a coiled-coil, and may represent a "hinge" region like that proposed to exist in the coiled-coils of myosin heavy chains (Harrington & Rodgers, 1984). Segments of amino acids with a predicted coiled-coil structure are also found in analogous positions (directly following the IQ motifs) in the class V myosins (Figure 1B), as well as the class VI (Cheney *et al.*, 1993) and class VIII (Knight & Kendrick-Jones, 1993) myosins.

The amino acid sequence of the distal portion of the MYA1 tail (amino acids 1049 to 1520) does not display a strong similarity to any known protein sequence. Interestingly, a data base search reveals that the distal tail regions of MYA1, Dilute, p190, and MYO2 are all most similar to a protein reported to be a glutamate decarboxylase (Huang *et al.*, 1990). Figure 3 shows a comparison of a portion of the MYA1 distal tail domain (amino acids 1318 to 1442) to a region of the glutamate decarboxylase polypeptide and the distal tail domains of class V myosins. The relevance of the similarity between these myosin tail regions and a glutamate decarboxylase is not clear, but has been discussed by Espreafico *et al.* (1992). The entire distal tail domain of MYA1 shows 18 to 22% identity to the distal tail regions of Dilute, p190, MYO2 and MYO4 (Figure 1B).

Tissue expression of the MYA1 gene

To determine in which tissues the MYA1 gene is expressed, a portion of the cDNA clone was used

A

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MAAPVIIVGSHVWVEDPHLAWIDGEVTRIDGINVHVKTKKGKTVVTNVYFPKDTEAPSGG      60
VDDMTKLSYLHEPGVLRNLETRYELNEIYTYTGNILIAVNPFQRLPHIYETDMMEQYKGI     120
ALGELSPHVFAIGDAAYRAMINEGKNNSILVSGESGAGKTETTKMLMRYLAFLGGRSGVE     180
GRTVEQQVLESNPVLEAFGNAKTLRNNNSSRFGKFVEIQFDKNGRISGAAIRTYLLERSR     240
VCQISDPERNYHCFYLLCAAPPEDIKKYKLENPHKFHYLNQSSCYKLDGVDDASEYLETR     300
RAMDVVGISNEEQEAIFRVVAAILHLGNIDFGKGEEIDSSVIKDKDSRSHLNMAAELLMC     360
NAQSLEDALIRRVMVTPEEIITRTLDPDNAIASRDTLAKTIYSHLFDWIVNKINTSIGQD     420
PRSKSIIGVLDIYGFESFKCNSFEQFCINFTNEKLQQHFNQHVFKMEQEEYTKEEIAWSY     480
IEFIDNQDVLELIEKKPGGIISLLDEACMFPKSTHETFSQKLFQTFKEHERFAKPKLSRT     540
DFTISHYAGEVTYQSNHFIDKNKDYIVAEHQALFTASNCKFVAGLFHALHEDSSRSSKFS     600
SIGSRFKQQLHSLMESLNGTEPHYIRCIKPNNVLKPGIFENFNVIHQLRCGGVLEAIRIS     660
CAGYPTRLAFYDFLDRFGLLAPEVLEGNYDDKVACQMILDKKSLTDYQIGKTKIFLRAGQ     720
MAELDARRAEVLGNAARVIQRQFRTCMARKNYRSIRNAAIVLQSFLRGEIARAVHKKLRI     780
EAAALRVQKNFRRYVDRKSFVTTRSSTIVLQTGLRAMIARSEFRLRRQRKAAIVLQAHWR     840
GRQAFSYYTRLQKAAIVTQCAWRCRLARRELRMLKMAARDTGALKDAKNKLEQRVEELSL     900
RLHLEKRLRTDLEEAKVQEVAKLQEALHTMRLQLKETTAMVVKEQEAARVAIEEASSVNK     960
EPVVVEDTEKIDSLSNEIDRLKGLLSSETHKADEAQHAYQSALVQNEELCKKLEEAGRKI    1020
DQLQDSVQRFQEKVFSLESENKVLRQQTLTISPTTRALALRPKTTIIQRTPEKDTFSNGE    1080
TTQLQEPETEDRPQKSLNQKQQENQELLLKSISEDIGFSEGKPVAACLIYKCLIHWRSFE    1140
VERTSIFNRIIETIASAIEMQENSDVLCYWLSNSATLLMFLQRTLKAGATGSITTPRRRG    1200
MPSSLFGRVSQSFRGSPQSAGFPFMTGRAIGGGLDELRQVEAKYPALLFKQQLTAFLEKI    1260
YGMIRDKMKKEISPLLASCIQVPRTPRSGLVKGRSQNTQNNVVAPKPMIAHWQNIVTCLN    1320
GHLRTMRANYVPSLLISKVFGQIFSFINVQLFNSLLLRRECCSFSNGEYVKTGLAELEKW    1380
CHDATEEFVGSAWDELKHIRQAVGFLVIHQKPKKSLKEITTELCPVLSIQQLYRISTMYW    1440
DDKYGTHSVSTEVIATMRAEVSDVSKSAISNSFLLDDDSSIPFSLDDISKSMQNVEVAEV    1500
DPPPLIRQNSNFMFLLERSD                                            1520
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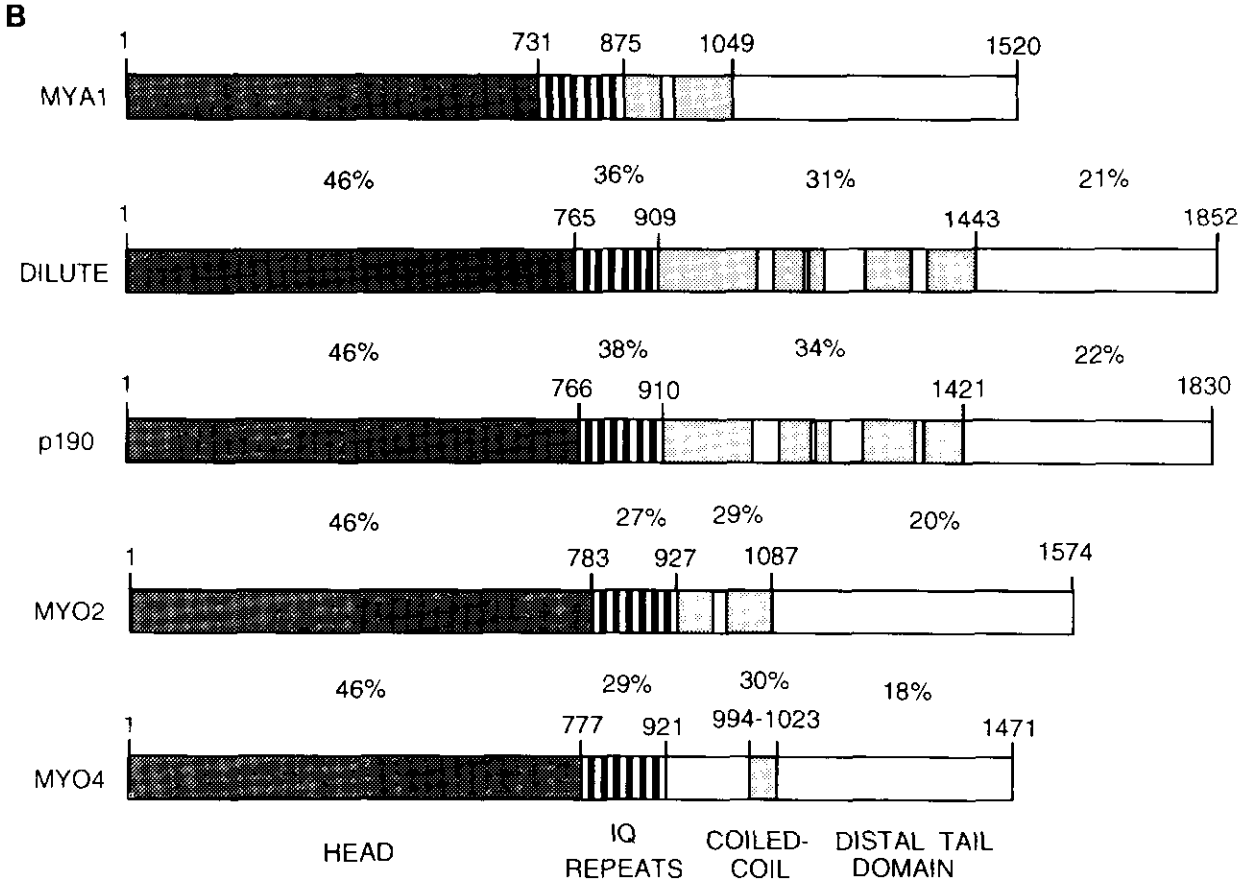

Figure 1. A, Deduced amino acid sequence (single-letter notation) of the MYA1 cDNA. The regions used to design the oligonucleotide primers for the PCR are underlined. The six IQ motifs are indicated by a double-underline. The nucleotide sequence of the MYA1 cDNA has been submitted to the European Molecular Biology Laboratory (EMBL) Data Library under accession number Z28389. B, Schematic comparison of the predicted domains of the myosins from *Arabidopsis* (MYA1), mouse (Dilute), chicken (p190), and yeast (MYO2 and MYO4). The percent amino acid identity of the various domains as compared to MYA1 is indicated (calculated by BESTFIT; Devereux *et al.*, 1984).

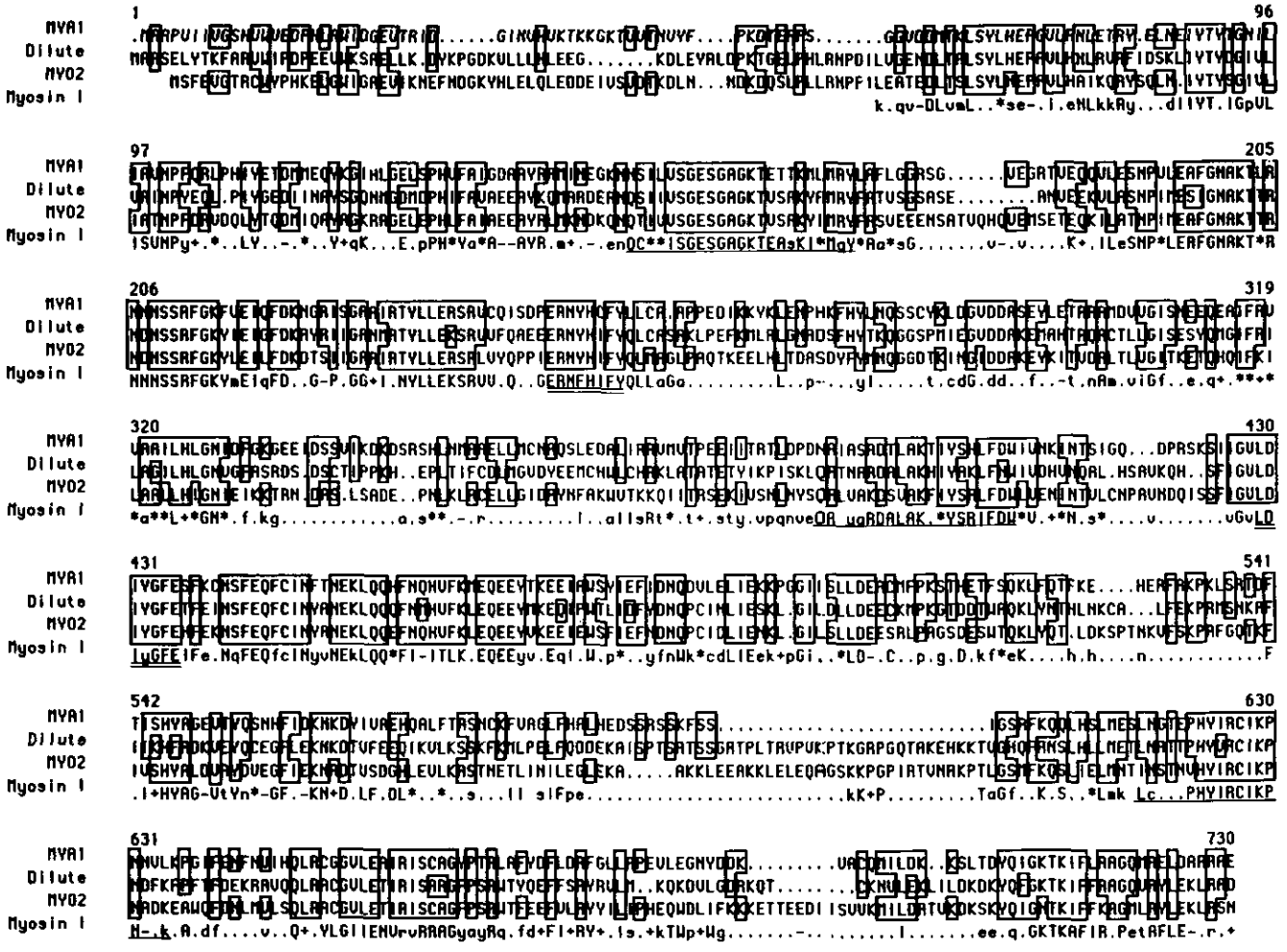

Figure 2. Comparison of the deduced amino acid sequences of the NH_2-terminal region of MYA1 (amino acids 1 to 730) and the head domains from the mouse Dilute (Mercer *et al.*, 1991), chicken p190 (Cheney & Mooseker, 1992), and yeast MYO2 (Johnston *et al.*, 1991) heavy chains. Residues identical to the MYA1 sequence are boxed and all sequences are aligned with a myosin I consensus sequence (Pollard *et al.*, 1991). In the consensus sequence: capital letters represent residues that are highly conserved, small letters represent residues that are moderately conserved, and a period represents a position with no clear consensus residue. Symbols: * represents A, I, L, or V; − represents D or E; + represents H, K, or R. The putative ATP-binding sequence, myosin I diagnostic sequence, and actin-binding sequence are underlined in that order (Pollard *et al.*, 1991). The residues used to derive the oligonucleotide primers for the PCR are indicated by a double-underline.

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MYA1    1318 cLNghlrtMranyVpslLIskVfgQiFsfInVqlfNsLLLRREcCSfSnGeyVKtgLaeLEkW
GAD      535 geNSFhtVlCdqgLDpEIIlqVfkQlFymInAVtlNnLLLRKDaCSWStGmQLRYNIsqLEEW
Dilute  1664 qLNSFhsVMCqhgmDpELIkqVvkQmFyiVgAItlNnLLLRKDmCSWSkGmQIRYNVsqLEEW
p190    1642 qLNSFhsVMCqhgmDpELIkqVvkQmFyiIgAVtlNnLLLRKDmCSWSkGmQIRYNVsqLEEW
MYO2    1361 ffNSiywcMksfhIEnEVfhaVvttllnyVdAIcfNeLImKRnflSWkrGlQLnYNVtrLEEW
MYO4    1279 fLNeFdaVlCkfqVvdsmhtkIfndtlkyLnVmlfNdLItKcpalnWkyGyeVdrNIerLvsW

MYA1     cHdateefvGsAwDeLkhIrQAvgfLVihqKpkkslkEIttelCpVLsiqQLyRIstmYwdd 1442
GAD      lRgKnLhqsG.AvqtmepLIQAA.qLLQLKKkThEDAEAIcslCtsLstqQIvKILnlYtPL  657
Dilute   lRdKnLmnsG.AkEtLepLIQAA.qLLQVKKkTdDDAEAIcsmCnALttAQIvKVLnlYtPV 1786
p190     lRdKnLmnsG.AkEtLepLIQAA.qLLQVKKkTdEDAEAIcsmCnALttAQIvKVLnlYtPV 1764
MYO2     cKtHgL.tdG..tEcLqhLIQtA.kLLQVRKyTiEDIDILrgiCysLtpAQLqKLIsqYqvA 1481
MYO4     fepRie..dv..rpnLiqIIQAv.kILQLKisnlnEfkLLfdfwyALnpAQIqaILlkYkPA 1398
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Figure 3. Comparison of a portion of the myosin distal tail domains (MYA1, Dilute, p190, MYO2, and MYO4) and the glutamate decarboxylase (GAD) polypeptide. Bold, capitalized letters represent conserved residues present in all six sequences. Capitalized letters represent conserved residues present in at least four of the sequences. Lower case letters represent residues that are not conserved in at least four of the sequences. Conservative substitutions: A, I, L, and V; H, K, and R; D and E.

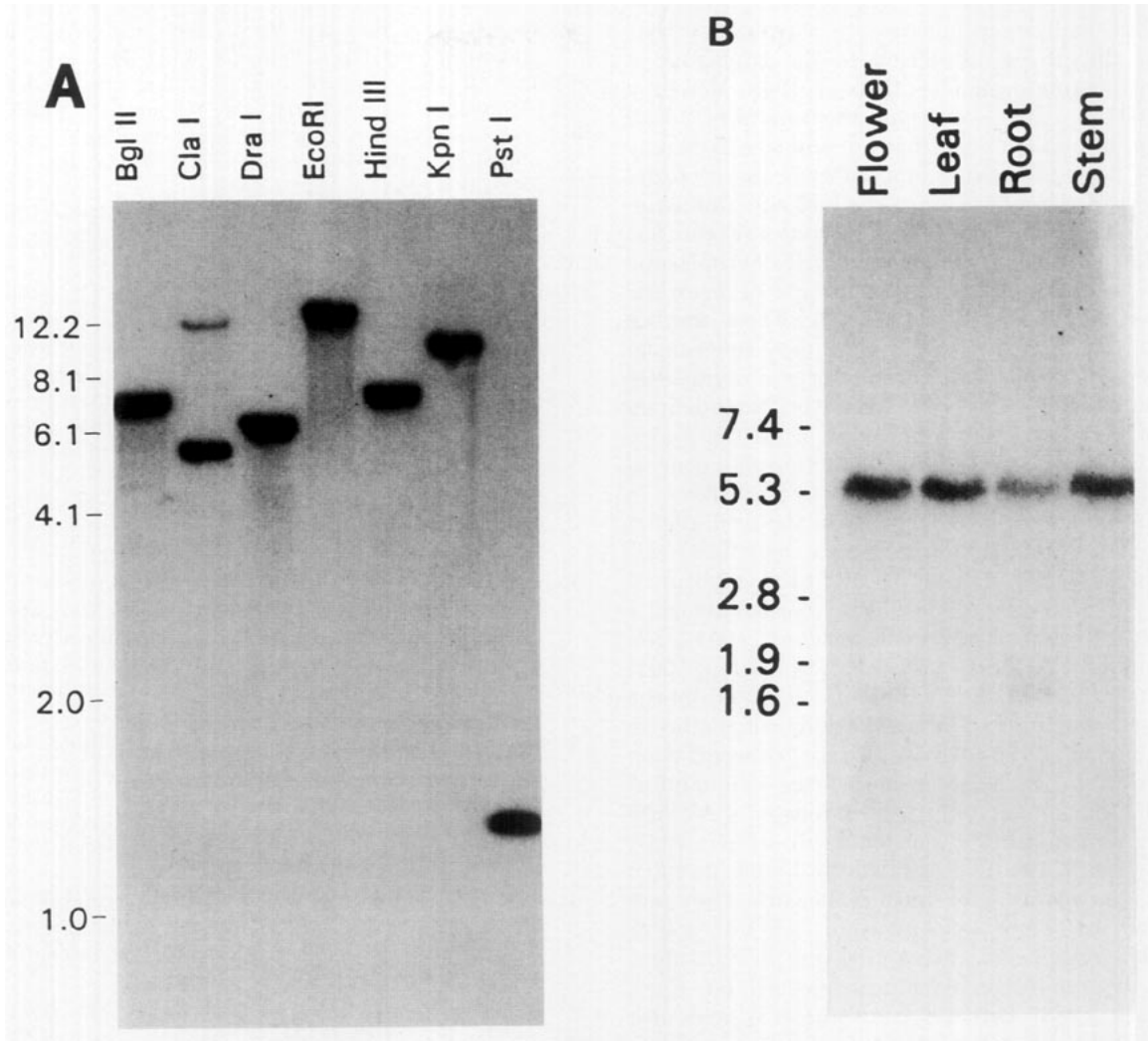

Figure 4. A, Southern (DNA) blot analysis. *Arabidopsis* genomic DNA was digested with the indicated restriction enzymes, separated in a 0·7% (w/v) agarose gel, and blotted to nylon membrane. The filter was hybridized with a random-primed ^{32}P-labeled 316 bp *Eco*RV/*Bam*HI fragment from the 3′ end of the MYA1 cDNA clone. Note that this probe (and therefore the genomic DNA) possesses a *Cla*I site. Positions of the DNA molecular weight markers are indicated in kilobases. B, Northern (RNA) blot analysis. Approximately 20 μg of total cellular RNA from the indicated *Arabidopsis* tissues were separated on a 1% (w/v) agarose-formaldehyde gel essentially as described (Sambrook *et al.*, 1989). Flowers from 4 to 6 week-old plants; leaves from 3 week-old plants; roots from 4 day-old seedlings; stems from 4 to 6 week-old plants; RNA was blotted to nylon membrane and probed with the 316 bp MYA1-specific fragment described above. Positions of the RNA molecular weight markers are indicated in kilobases.

as a probe on a Northern blot. A 316 bp fragment from the 3′ end of the MYA1 cDNA clone was found to be specific for the MYA1 gene. As shown in Figure 4A, this probe hybridizes to a single genomic DNA fragment in each restriction enzyme digest. The two hybridizing fragments in the *Cla*I digest are the result of a *Cla*I restriction site in the 316 bp probe fragment. On a Northern blot of total RNA isolated from various *Arabidopsis* tissues (flower, leaf, root, and stem), a single RNA species of approximately 5·3 kb is detected in each lane (Figure 4B).

Although the *Arabidopsis* MYA1 myosin does not clearly fall into any of the known classes of myosin based solely on the head domain sequence, it displays a strong similarity in overall structure to members of the class V myosins. MYA1 and class V myosins have a distinct structure which consists of an NH_2-terminal myosin head domain, followed by a series of six putative calmodulin-binding (IQ) repeats, a domain with a strong potential for forming an alpha-helical coiled-coil, and a distal tail domain with similarity to a protein reported to be a glutamate decarboxylase. This strong similarity in overall structure suggests that MYA1 represents a higher plant myosin that is related to the class V myosins.

Insights into the possible function of class V myosins have been obtained by the analysis of mutations in the Dilute and MYO2 genes and through immunolocalization studies of the p190 protein. The mouse Dilute mutations are associated

with abnormalities in melanocyte morphology that may be due to an inhibition in the transport of pigment-bearing organelles (Russel, 1949; Mercer *et al.*, 1991). In yeast, mutations affecting MYO2 lead to large, unbudded cells that accumulate secretory vesicles, perhaps because of a defect in the vectorial transport of these vesicles to the bud site (Johnston *et al.*, 1991). Immunolocalization studies reveal that the p190 protein is associated with cytoplasmic vesicles and is abundant at the tips of neuronal processes (Espreafico *et al.*, 1992). These studies indicate that myosins in this class may function in the directed transport of various sorts of organelles/vesicles. Since MYA1 resembles this well-defined class of myosins and is expressed in all the major plant tissues, this myosin may function in a general organelle/vesicle transport process in plant cells.

The presence of six IQ repeats in the neck region of MYA1 suggests that this protein may bind calmodulin. The IQ motifs in the p190 myosin V (Cheney & Mooseker, 1992), brush border myosin I (Halsall & Hammer, 1990; Mercer *et al.*, 1991), and neuromodulin (Wakim *et al.*, 1987; Chapman, 1991) are associated with the binding of calmodulin in the absence of calcium. In detailed studies of the brush border myosin I, an increase in Ca^{2+} concentration was found to dissociate some of the calmodulins from the myosin and cause an inhibition of ATPase activity and motility (Coluccio & Bretscher, 1987; Collins *et al.*, 1990). Several actin-based motility systems in plants are unusual because they are inhibited by high concentrations of Ca^{2+}. For example, cytoplasmic streaming in pollen tubes, *in vivo*, is inhibited by increasing cellular Ca^{2+} (Shimmen, 1988). Directed transport of vesicles also appears to be sensitive to Ca^{2+}; in tip-growing cells, a high concentration of Ca^{2+} is present at the tip, the site of secretory vesicle deposition and fusion with the plasma membrane (Schnepf, 1986; Steer & Steer, 1989). The effects of high Ca^{2+} concentrations on these intracellular motility processes may be explained if the activity of the MYA1 polypeptide (and related plant myosins) is regulated, like the brush border myosin I, through calcium-induced dissociation of calmodulin.

The further characterization of myosin in plants will help expand our understanding of the structure and function of this diverse group of proteins. The power of molecular genetic analyses in *Arabidopsis* should make it a model plant system for such studies.

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