Organelle Targeting of Myosin XI Is Mediated by Two Globular Tail Subdomains with Separate Cargo Binding Sites

Received for publication, January 23, 2007, and in revised form, April 27, 2007. Published, JBC Papers in Press, May 11, 2007, DOI 10.1074/jbc.M700645200

Jian-Feng Li and Andreas Nebenführ

From the Department of Biochemistry, Cellular and Molecular Biology, University of Tennessee, Knoxville, Tennessee 37996

Myosin XI are actin-based molecular motors that are thought to drive organelle movements in plants, analogous to myosin V in animals and fungi. Similar domain structure of these myosins suggests that binding to organelles may occur via the globular tail domain in both types of motors, even though sequence similarity is low. To address this hypothesis, we developed a structure homology model for the globular tail of MYA1, a myosin XI from Arabidopsis, based on the known structure of yeast myosin V (Myo2p) globular tail. This model suggested an interaction between two subdomains of the globular tail which was verified by yeast two-hybrid assay and by in vivo bimolecular fluorescence complementation (BiFC). Interface mapping demonstrated that this subdomain interaction depends critically on the C terminus of helix H6 as well as three specific residues in helices H3 and H15, consistent with the structural prediction. The reconstituted globular tails of several Arabidopsis myosin XIs in BiFC assays targeted to peroxisomes in plant cells, identifying this domain as sufficient for cargo binding. Unlike myosin V, either subdomain of myosin XI alone was targeting-competent and responsible for association with different organelles. In addition, our data suggest that organelle binding is regulated by an allosteric interaction between two tail subdomains. We conclude that the globular tail of myosin XI shares a similar structure with that of myosin V, but has evolved plant-specific cargo binding mechanisms.

Myosins are efficient molecular motor proteins which convert chemical energy from ATP hydrolysis to physical force to move along cytoskeletal actin filaments (1). Based on sequence comparison of myosin motor domains, these nearly ubiquitous eukaryotic motors can be grouped into 24 subfamilies designated as class I to XXIV (2). Plants from green algae to flowering plants encode only two classes, myosin VIII and XI (3). A number of myosin XI isoforms have been identified from a variety of organisms by biochemical isolation (4, 5), molecular cloning (6–8), or genome-wide sequence analysis (9, 10). Little is known about the specific cellular function of class XI myosins in plants, but it is generally assumed that they are responsible for force generation for organelle transport during cytoplasmic streaming (11).

This assumption is based on three lines of evidence. First, disruption of organelle movements in plant cells by anti-actin drug or myosin ATPase inhibitor treatment strongly indicated that organelle motility depends on both actin and myosin (12–16). Second, myosin XI was found to associate with a diverse array of organelles in plant cells by immunostaining (17–20), YFP labeling (21), and co-purification studies (22). Finally, there is an overall structural similarity between class XI and class V myosins, the latter being responsible for organelle movement in fungi and animals (23). In particular: (i) both classes have the same domain arrangement, including a catalytic motor in the head, six copies of calmodulin binding IQ motifs in the neck, a coiled-coil region in the stalk and a globular tail at the C terminus (6); (ii) this conserved domain arrangement is reflected in similar morphologies in rotary metal-shadowing electron microscopy and similar mechanochemical activities in in vitro motility assays (5); (iii) one segment of the globular tails of both class V and class XI myosins shows clear sequence conservation and is taken as the signature element of these myosins (annotated as “dilute domain,” Pfam ID: PF01843). Based on these similarities and the well-studied cargo binding paradigm of myosin V globular tail (23), it is tempting to speculate that the globular tail of myosin XI is also responsible for organelle targeting in plant cells.

The large evolutionary distance of more than one billion years between plants on the one hand and animals and fungi on the other makes direct comparisons difficult. The sequences of class V and XI myosins have diverged significantly (24). There are also indications that the functional roles of these myosins may have shifted during their evolution. For example, it is thought that the ancestral myosin V/XI was involved in cytokinesis and that this function was later taken over by the derived myosin II in animals and fungi (25). There is evidence that some myosins are still active during cytokinesis in plant cells, although it is not clear whether these are class VIII or XI myosins (26). Moreover, significant variation of direct and indirect cargo binding mechanisms was found among myosin Vs involving a wide variety of unrelated adaptor or receptor proteins (27–30), suggesting that individual motor-cargo interactions evolved independently even within this narrow phylogeny.
Subdomains of the Cargo Binding Domain of Myosin XI

Given these concerns, we wanted to test to what extent the cargo binding paradigm developed for myosin V can be applied to the related plant myosin XI. In particular, we wanted to elucidate the structural basis for the proposed motor-anchoring function of myosin XI globular tail to replace the current speculations with experimental evidence. We have taken advantage of the newly published structure of the budding yeast Myo2p globular tail (31) to establish a homology-based model of the globular tail of MYA1, a myosin XI from Arabidopsis, and tested its validity by yeast two-hybrid (Y2H)2 and biomolecular fluorescence complementation (BiFC) assays. Although the general structure of the globular tail appeared conserved between myosin V and XI, we found novel mechanisms of cargo binding in myosin XI.

EXPERIMENTAL PROCEDURES

Bioinformatic Analysis—The amino acid sequences of the globular tail of myosin XI (MYA1) and myosin V (Myo2p) were initially aligned by MUSCLE (32) and T-coffee (33) programs. Manual refinement was made by considering the known secondary structure of Myo2p globular tail and the secondary structure of MYA1 globular tail predicted by the Protein Homology Recognition Engine server. Based on this sequence alignment and the Myo2p structure template (PDB code 2F6HX), a homology model of MYA1 globular tail was built by the Swiss-Model server (34). The resulting PDB file is available online as supplemental data. The residues buried inside the structure were identified as inaccessible residues by the Swiss-PDBViewer program using default settings. Structural models were drawn with MacPyMOL (DeLano Scientific).

Plasmid Construction—All PCR primers used and recombinant plasmids constructed in this study are listed in supplemental Tables S1 and S2, respectively, which are available online. Standard molecular cloning protocols were followed for plasmid construction. For yeast two-hybrid constructs, the bait plasmids were made by inserting different NcoI/SalI-digested plasmid construction. For yeast two-hybrid constructs, the bait plasmids were cotransformed with the indicated GAL4 BD-bait construct (TRP1 marker) and the indicated GAL4 AD-prey construct (LEU2 marker) and grown on SD/−Trp/−Leu plates. Single colonies of each transformant were patched onto a SD/−Trp/−Leu/−His plate containing 25 mM 3-amino-1,2,4-triazole (3-AT, Sigma-Aldrich) which blocks leaky expression of the His3 reporter gene. The plate was then placed at 30 °C for 3 days, and β-galactosidase activities for each colony were analyzed by colony-lift filter assay.

Transient Expression in Plant Cells—Dark green leaves (about 1-cm long) were detached from 4–8-week-old Arabidopsis thaliana Col-0 plants for tungsten particle-mediated bombardment using a PDS-1000 system (Bio-Rad). A construct encoding CFP with a C-terminal type 1 peroxisome targeting signal was co-bombarded with BiFC constructs as transformation indicator and peroxisome marker. Alternatively, a construct encoding CFP with an N-terminal Golgi-resident mannosidase transmembrane domain (13) was co-bombarded as transformation indicator and Golgi marker. After bombardment, the leaves were kept at 28 °C in a moist chamber in darkness for 12–16 h prior to microscopic examination.

Fluorescent Imaging—Fluorescence microscopy was performed using an Axiovert 200 m microscope (Zeiss) equipped with filters for YFP and CFP fluorescence (Chroma, filter set 52017). Transformed cells were first identified under a ×20 objective before representative cells were observed with a ×63 (1.4 NA) plan-apo oil immersion objective. Images were captured with a digital camera (Hamamatsu Orca-ER) controlled by Openlab software (Improvision). Photographs were subsequently processed for optimal presentation with Photoshop 7.0 (Adobe).

RESULTS

The Structure of MYA1 Globular Tail Resembles That of Myo2p—The first high resolution structure of a myosin V tail was recently determined for Myo2p, an essential myosin V isoform in Saccharomyces cerevisiae (31). Because MYA1 and Myo2p share identical domain organization (6), we reasoned that these two classes of myosins might fold similarly. However, the sequence similarity of the globular tails of MYA1 and Myo2p is quite low, and various alignment algorithms recognized only short segments of homology, largely confined to the dilute domain (data not shown). Manual re-alignment based on predicted secondary structures elevated the sequence identity to 15% (36% similarity) and, more importantly, revealed conserved residues throughout the entire tail except the very C-ter-

2 The abbreviations used are: Y2H, yeast two-hybrid; BiFC, bimolecular fluorescence complementation; GAL4 AD, GAL4 DNA activation domain; GAL4 BD, GAL4 DNA binding domain; GT, globular tail; YN, N-terminal half of YFP; YC, C-terminal half of YFP; YFP, yellow fluorescent protein; PDB, Protein Data Bank.
Subdomains of the Cargo Binding Domain of Myosin XI

minal region (Fig. 1A). Notably, 78% of the conserved residues are buried inside in Myo2p structure (Fig. 1B), suggesting that purifying selection was directed at maintaining the folding pattern of the globular tail domain in these myosin classes.

Based on this optimized alignment, we used the Swiss-Model server to predict the likely structure of MYA1 globular tail. The resulting model appeared to be stereochemically robust as judged by Ramachandran plot (supplemental Fig. S1A) (36). The root mean square (RMS) deviation of the predicted structure relative to the template was small (1.91 Å), and larger deviations were all confined to loop regions (supplemental Fig. S1B). The predicted general architecture of MYA1 globular tail is very close to that of Myo2p (Fig. 1C and supplemental data), although the MYA1 globular tail model is composed of 18 α-helices compared with 15 α-helices for Myo2p. The predicted extra three helices (H5a, H5b, and H6a) in MYA1 are caused by two long insertions (residues 1194–1224 and residues 1298–1304) relative to the Myo2p sequence. On the other hand, two insertions in Myo2p (residues 1179–1193 and residues 1507–1519) are absent from MYA1 so that MYA1 is predicted to have shorter loops between helices H2 and H3 and between helices H13 and H14. It is important to emphasize that these novel features of MYA1 globular tail are purely predicted and that their actual structure needs to be verified independently.

In this study, we provide experimental support for the overall conformation of the calculated MYA1 globular tail structure.

Myosin XI Tail Subdomains Interact with Each Other in Yeast Cells—It has been reported that the globular tail of Myo2p could be divided into two distinct subdomains, which tightly associate with each other (37, 38). The predicted structural similarity suggests that these subdomains, GT1 (helices H1 to H6) and GT2 (helices H6a to H15), also exist in myosin XI and associate similarly. Indeed, the interaction between GT1 and GT2 of MYA1 could be detected in a Y2H system (Fig. 2A). Similar interactions between the corresponding GT1 and GT2 subdomains from other myosin XI isoforms, i.e. MYA2, XI-C, and XI-K, could also be observed (Fig. 2A). Interestingly, heterologous interactions between GT1 from MYA1 and GT2 from MYA2, XI-C, or XI-K were also detected in Y2H assay (Fig. 2B). No interaction between GT2 and an intact tail or between two intact tails (Fig. 2B) was found in Y2H assays, which is consistent with a previous report for Myo2p (39). Taken together, our data imply that the myosin XI globular tail subdomains can associate with each other in a conserved manner as predicted, and that the interaction between two tail subdomains occurs only within an individual myosin polypeptide.

Two Regions in MYA1 Tail Subdomains Are Indispensable for the Intra-tail Interaction—To further verify the structure prediction in detail, we created targeted mutations to test specific elements in the MYA1 globular tail model. According to the structure model, the C-terminal end of helix H6 from GT1 inserts into a pocket formed by a bundle of GT2 helices (H6a to H9 and possibly H10), and this interaction may play a pivotal role in the association of the two subdomains. This prediction was tested with two truncations at the C terminus of GT1. The first removed the last twelve amino acids of helix H6 after M1263 (GT1Δ38C) while the second was truncated right after helix H6 (GT1Δ22C). Both constructs were tested by Y2H assay for interaction with GT2. As expected, keeping H6 intact in GT1 retained the interaction between GT1 and GT2 while the loss of residues 1264–1276 blocked the interaction (Fig. 3A), indicating that the C-terminal end of helix 6 is required for the association of GT1 and GT2.

We speculated that the long C-terminal extension of GT2 after helix H13, which loops back and wraps around GT1, would also be necessary to maintain the interaction between GT1 and GT2. A deletion series (GT2Δ53C-GT2Δ3C), which respectively lacked 53, 35, 11, 7, or 3 amino acids from the wild-type GT2 C terminus was created and tested for the interaction with GT1 by Y2H. Intriguingly, all truncations except for the shortest one (GT2Δ3C) prevent interaction with GT1 (Fig. 3A), suggestive of a crucial role for the tetrapeptide FLLE1517.

Based on the predicted structure, this tetrapeptide from helix H15 may interact with its neighboring helices H1 and H3 (Fig. 3B). Removal of helices H1, H2, and H3 (GT1Δ39N) from GT1 disrupted the interaction with GT2 whereas the truncation of helix H1 alone (GT1Δ5N) did not impair the interaction with GT2 (Fig. 3A). We concluded that helix H3 is the probable interacting partner of the tetrapeptide FLLE1517 in helix H15. Based on the structural model, Tyr1130 and Arg1137 from helix H3, and Phe1514 and Glu1517 from helix H15 could participate in the interface within this region (Fig. 3B). To test this prediction, we introduced point mutations to replace Tyr1130, Arg1137, or Phe1514, respectively, with alanine. These point mutations could be expressed normally in yeast cells as shown by protein gel blot analysis (supplemental Fig. S2). Only the F1514A mutation resulted in a loss of interaction, indicative of a key role of Phe1514 in the association of GT1 and GT2 (Fig. 3C). In contrast, the removal of E1517 from GT2Δ3C still permitted the interaction to occur (Fig. 3C). Furthermore, double mutation of Y1130A and R1137A on helix H3 also disrupted the interaction, indicating that Tyr1130, Arg1137, and Phe1514 form an interacting triad in this region. This was further supported by a negative interaction in a triple mutation of Y1130A, R1137A, and F1514A (Fig. 3C), as well as the conservation of these three residues in all Arabidopsis myosin XI isoforms (supplemental Fig. S3).

Interaction of Globular Tail Subdomains Also Occurs in Plant Cells—To confirm our results from yeast two-hybrid assays we checked for subdomain interactions in the cytoplasm of plant cells by bimolecular fluorescence complementation (BiFC). The BiFC approach relies on the reconstitution of a fluorophore when two halves of the fluorescent protein (YN and YC) are brought together by an interaction between their fusion partners (40). Reconstituted YFP fluorescence was visualized in a punctate pattern for YN- and YC-tagged MYA1 globular tail subdomains in Arabidopsis leaf cells, irrespective of the fusion orientation. Both the GT1-YC + YN-GT2 combination (Fig. 4A) and the YN-GT1 + GT2-YC combination (Fig. 4B) resulted in clear YFP signals. However, no YFP fluorescence was observed for the YN-GT2 + GT2-YC combination within 100 cells expressing a transformation indicator, ruling out the possibility of a spontaneous interaction between YN and YC (Fig. 4D). Similarly, restored YFP fluorescence was also visualized in the BiFC assay for three other myosin XI isoforms, MYA2 (Fig. 4C), XI-I (Fig. 4E), and XI-K (Fig. 4F). These findings confirmed that the two tail subdomains of myosin XI inter-
Subdomains of the Cargo Binding Domain of Myosin XI

A

B

C

H1
H2

H3
H4
H5
H5a
H5b

H6
H6a
H7

H8
H9
H10
H11

H12
H13
H14

H15
Subdomains of the Cargo Binding Domain of Myosin XI

Myosin XI Globular Tail Requires Upstream Sequences for Proper Folding—We sought to confirm the specific targeting of myosin XI with full-length globular tail constructs (YFP-MYA1GT and YFP-MYA2GT), but only diffuse cytoplasmic labeling was detected (Fig. 6A and data not shown), resembling the distribution of YFP alone (Fig. 6B). This unexpected distribution could have resulted from incorrect folding of the globular tails. This interpretation was supported by a folding reporter (YN-MYA1GT-YC), which should mimic the situation of the YN-1GT1 + 1GT2-YC BiFC combination (Fig. 4B), but that did not result in any detectable YFP signal within 120 cells containing the transformation indicator (Fig. 6C). This result may indicate that the intact globular tail does not fold easily into the predicted conformation. Interestingly, a longer fusion construct that included the entire coiled-coil region in addition to the globular tail domain of MYA1 resulted in localization to organelles (Fig. 6D), some of which could be identified as Golgi stacks (data not shown). This result suggested that additional sequences outside the globular tail may be involved in its proper folding.

DISCUSSION

Myosin XI and Myosin V Globular Tails Have Similar Conformations—In this study, we have created a homology structure model of MYA1 globular tail based on the deciphered action with each other in living plant cells, thus corroborating the structure homology model of MYA1 globular tail. Importantly, the reconstituted YFP signal in the BiFC assays localized specifically to CFP-labeled peroxisomes for MYA1, MYA2, XI-I, and XI-K (Fig. 4). This result thus established that the reconstituted globular tail is functional and sufficient for organelle binding in plant cells.

**Global Tail Subdomains Can Target Different Organelles Independently**—YFP-tagged GT1 or GT2 of MYA1 when expressed separately labeled punctate structures which coincided with the CFP peroxisome marker (Fig. 5, A and B). Peroxisome targeting was not affected by the point mutations (e.g. Y1130A/R1137A and F1514A) that disrupted subdomain interactions in Y2H assays, suggesting that these residues are only important for intramolecular interactions (supplemental Fig. S4). Curiously, the YFP-GT2 chimera of MYA1 targeted to Golgi stacks (Fig. 5C) instead of peroxisomes in about one-fifth of the cells. Independent targeting of tail subdomains could also be confirmed in MYA2 where GT1 targeted to unknown organelles which were neither peroxisomes (Fig. 5D), mitochon-

chondria, Golgi stacks nor peroxisomal compartments (data not shown), while GT2 bound to peroxisomes (Fig. 5E). Localization of MYA2 subdomains to small organelles or peroxisomes, respectively, is fully consistent with the distribution of immunodetected native protein (20) and YFP-labeled tail constructs (21). None of these constructs prevented movement of the targeted organelles (data not shown), which is similar to the lack of a dominant negative effect of other YFP-tail fusions described previously (21). Taken together, our results indicated that either subdomain alone could target to organelles, thus possessing independent cargo binding sites.

**FIGURE 2.** Yeast two-hybrid assays show that the intra-tail interaction is ubiquitous for myosin XI. A, auxotrophic and β-galactosidase activity assays for yeast cells co-expressing globular tail subdomain 1 (GT1) and subdomain 2 (GT2) from a single MYA1 isoform. Each GT1 or GT2 moiety is expressed with its N terminus fused to GAL4 AD (prey) or BD domain (bait), respectively. Yeast cells were grown on both low-stringency (SD-Trp/−Leu) plates and high-stringency (SD−Trp/−Leu/His/3-AT) plates. The β-galactosidase activity was tested by colony lift assay and the positive or negative result in each assay is individually noted by plus or minus symbol. B, auxotrophic and β-galactosidase activity assay for yeast cells co-expressing GT1 (or GT2) from a single myosin XI isoform. Each GT1 or GT2 moiety is expressed with its N terminus fused to GAL4 AD (prey) or BD domain (bait), respectively. Yeast cells were grown on both low-stringency (SD−Trp/−Leu) and high-stringency (SD−Trp/−Leu/His/3-AT) plates. The β-galactosidase activity was tested by colony lift assay and the positive or negative result in each assay is individually noted by plus or minus symbol.

**FIGURE 1.** Homology structure modeling of myosin XI globular tail based on sequence similarity to myosin V. A, sequence alignment of the globular tail of myosin XI (AtMYA1) and myosin V (ScMyo2p). Identical or similar residues in the alignment are highlighted in red or blue, respectively. Green lines indicate predicted α-helical regions, whereas black bars indicate helices in the solved structure (ScMyo2p) or the structural model (AtMYA1). Helices are numbered H1 to H15 according to the ScMyo2p structure (31). The residues not resolved in the crystal structure of ScMyo2p tail are colored in dashes. The interacting triad identified within MYA1 globular tail is marked by arrowheads, and the residues that might interact with the motor domain are labeled with dots. B, three-dimensional structure of the complete ScMyo2p globular tail as a ribbon diagram. Purple regions have not been solved by x-ray crystallography and are modeled by Swiss-Model in this study. Red and blue colors mark the conserved residues from the alignment. C, predicted three-dimensional structure of the AtMYA1 globular tail. The backbone is colored by secondary structure succession in rainbow colors from blue (N terminus) to red (C terminus). Individual helices are labeled H1 to H15 where space was available.
structure of the equivalent region of budding yeast Myo2p. This model allowed us to make specific predictions about the inter-
face between MYA1 GT1 and GT2 that could be verified exper-
imentally. Interface mapping experiments revealed that this
intra-tail interaction requires the orchestrated action of the C
terminus of helix H6 and its neighboring helices along with the
residues Tyr1130, Arg1137, and Phe1514. In this study, five of
the thirteen myosin XI isoforms in Arabidopsis (9), namely MYA1,
MYA2, XI-K, XI-C, and XI-I, were tested for the intra-tail inter-
action and positive interactions were detected in all cases (Figs.
2 and 4). Given the high degree of sequence similarity of myosin
XI globular tails (supplemental Fig. S3), it is reasonable to pro-
pose that the predicted overall structure of MYA1 globular tail
can be generalized to other myosin XI isoforms.

Despite the similar conformation of myosin XI and V globu-
lar tails, it is evident that these two protein domains are not
simple carbon copies of each other. Several insertions and dele-
tions created differences in loop regions and led to additional helices
in myosin XI. Notably, the predicted three extra α-helices in myosin XI
globular tail (H5a, b and H6a, Fig. 1) may introduce plant-specific mech-
nisms in the motor-cargo interaction. Even the sections where a
gap-free alignment is possible revealed clear differences between these
myosins. Most of the conserved res-
dues were found within the interior
of the protein. This suggests that
these residues were maintained
because of purifying selection
because internal residues are often
involved in establishing the overall
folding pattern. At the same time,
the surface residues showed very
little conservation suggesting dif-
ferent interactions with adaptor
proteins. Consistent with this
interpretation, the residues known
to be necessary for binding of
Myo2p to vacuoles or secretory ves-
icles (31) were not conserved in any
plant myosin XI (supplemental Fig.
S3). Furthermore, several known
myosin V adaptor proteins have no
apparent homologs in the Arabi-
dopsis genome.3 Hence a simple
one-to-one relationship between
cargo interactions of myosin V and
of myosin XI apparently does not
exist.

The Globular Tail of Myosin XI Is
Sufficient for Organelle Targeting in
Plant Cells—We detected myosin
XI globular tail constructs targeted
to specific organelles in both BiFC
assays and YFP labeling experi-
ments (Figs. 4 and 5). The localization of MYA2 globular tail
constructs to peroxisomes and unknown organelles in this
study agrees with previous reports based on antibody or YFP
labeling (20, 21). Thus, our results proved that the globular tail
is sufficient for organelle binding in class XI myosins as in class
V myosins. Interestingly, a recent study employing YFP fusions
to various truncations of several myosin XI proteins did not find
reliable labeling of organelles with shorter constructs although
some distinct spots were seen for a shortened YFP-MYA1 globu-
lar tail construct (21). We speculate that this discrepancy to
our results stems from the use of different fusion sites. For
example, the dilute and ½-tail constructs in that study corre-
sponded to helices H8 through H15 and helices H5a through
H15, respectively, thus, incorporating only parts of the two

3 J.-F. Li, and A. Nebenführ, unpublished data.
globular tail subdomains. These differences may lead to incorrect folding of the fusion proteins, which could explain the failure of those globular tail truncations to achieve stable targeting.

A caveat to these conclusions is that we found that a MYA1 full-length globular tail construct failed to target to specific organelles (Fig. 6A), presumably because of improper folding of the construct as shown by the folding reporter, YN-1GT-YC (Fig. 6C). In contrast, a longer construct containing the coiled-coil region in addition to the globular tail domain routinely targeted to organelles (Fig. 6D). Similar results were also obtained for other myosin XIs (21). This suggests that the coiled-coil region may play a role in targeting; however, this domain by itself cannot bind to organelles.3 Thus, we conclude that the coiled-coil region may facilitate organelle binding by assisting in the correct folding of the globular tail domain. It should be emphasized that we have tested only the effect of the full coiled-coil region, but evidence has been published that shorter segments may already confer this effect (21). This property of myosin XI is clearly different from myosin V where the globular tail alone was found to exert dominant negative effects on vacuolar inheritance and secretion (41), demonstrating that this domain can function as a separate unit and, by inference, fold independently. The physical basis for this difference is not known, but might be related to the different mechanisms of cargo binding.

Myosin XI Isoforms Have Overlapping Functions—Our finding that different myosin XI isoforms can target to specific organelles independently (A, YFP-1GT1 from MYA1 targeted to CFP-labeled peroxisomes. B, YFP-1GT2 from MYA1 targeted to CFP-labeled peroxisomes. C, YFP-1GT2 from MYA1 occasionally targeted to CFP-labeled Golgi stacks. D, YFP-2GT1 from MYA2 targeted to unidentified organelles that are not peroxisomes. E, YFP-2GT2 from MYA2 targeted to CFP-labeled peroxisomes.)

Subdomains of the Cargo Binding Domain of Myosin XI

JULY 13, 2007 • VOLUME 282 • NUMBER 28
JOURNAL OF BIOLOGICAL CHEMISTRY 20599
Subdomains of the Cargo Binding Domain of Myosin XI

![Image](image)

FIGURE 6. Full-length globular tail of MYA1 does not fold normally without upstream sequences. A, YFP-1GT did not bind to any organelle and remained soluble in the cytoplasm. B, cytoplasmic localization of YFP on its own for comparison. C, no restored YFP fluorescence was detected with the folding reporter YN-1GT-YC. Inset shows CFP fluorescence in the same cell as transformation marker at half-size. D, YFP-1CCGT constructs (coiled-coil + globular tail) targeted to organelles.

gene knockouts did not show any obvious morphological and developmental abnormalities (20). Targeting of multiple myosins to the same organelle might also explain the lack of dominant negative effects of overexpressed myosin XI tail constructs on organelle motility in this and other studies (21). This functional redundancy appears to be specific for myosin XI, because no functional redundancy among myosin Vs has been reported so far.

Myosin XI Globular Tails Can Bind to Several Organelles—Our data also indicate that individual myosin XI isoforms have the potential to target to several locations (Fig. 5), thus possibly mediating the movement of different organelles. Promiscuous targeting has previously been described for MYA2 which was detected by antibody staining on peroxisomes and other smaller organelles (20). In another study, three myosin XI isoforms were found to target to both Golgi vesicles and mitochondria in tobacco pollen tubes (22). Interestingly, this versatile binding to different organelles appears to be tightly regulated. For example, a recent study employing YFP-MYA2 fusion constructs extending beyond the globular tail detected only targeting to peroxisomes but not to smaller organelles (21). Similarly, we detected YFP-MYA1GT2 either on peroxisomes (Fig. 5B) or on Golgi stacks (Fig. 5C) but never on both organelles within a given cell. Longer constructs containing both coiled-coil and globular tail domains of MYA1, XI-I, or XI-K also did not target to peroxisomes (Fig. 6D, Ref. 21), again suggesting that organelle targeting of these myosins can be controlled at the cellular level. Elucidation of the events underlying these differential localizations will likely reveal important mechanisms regulating alternative motor-cargo interactions.

The observation that myosin XI isoforms can target to several organelles is consistent with results for myosin V where, for example, Myo2p is involved in moving vacuoles, secretory vesicles, peroxisomes, late Golgi and mitochondria (41). The multiple targeting of myosin XI is based on different binding sites that we could assign to the two globular tail subdomains (Fig. 5). This feature is again similar to Myo2p, where the vacuole and secretory vesicle binding sites are spatially separated within the globular tail (31). However, we could observe organelle targeting of individual myosin XI subdomains, indicating that the organelle binding sites are functional in these truncated proteins. This is apparently different from yeast myosin V (Myo2p) in which each subdomain alone has no dominant negative effects on organelle inheritance (38). Taken together, our results suggest that myosin XI and myosin V, though sharing similar structure, have evolved different mechanisms for tail-organelle interaction. Intact Globular Tail of Myosin XI Exhibits Dynamic Conformation—Our results demonstrating multiple targeting of individual myosin proteins (Fig. 5, also see Ref. 20) seem to be contradicted by the BiFC results of reconstituted tails that only target to a single type of organelles (Fig. 4). This paradox is reminiscent of results obtained with Myo2p where overexpression of full-length globular tail impaired both vacuole and secretory vesicle movements whereas co-overexpression of two separate tail subdomains disrupted only vacuolar targeting (38). To reconcile these conflicting observations, we propose that the reconstituted globular tail of myosin V/XI cannot fully compensate for all functions of an intact globular tail. The physical defect in the reconstituted globular tail, namely disruption of the loop region between helices H6 and H7, may compromise some regulatory function of these myosins in cargo selection (Fig. 7). In particular, we envision that this loop region destabilizes the globular tail structure and allows it to dynamically alternate between different states. Initial and weak binding of an organelle-specific adaptor protein would then select one of these states and induce a conformational change that would in effect lock the globular tail in a high affinity binding for that organelle. Disruption of the loop between H6 and H7 would in this model prevent the labile structure adopted by the intact tail and instead allow the reassembled globular tail to assume a rigid structure that corresponds to only one of the possible binding conformations (Fig. 7). Interestingly, it was not possible to grow crystals for the full-length Myo2p globular tail but crystals of the reassembled Myo2p globular tail after proteolytic cleavage at the H6-H7 loop (shown in Fig. 1B in purple as a predicted loop on the lower left) could routinely be obtained (31). Based on this model, we would suspect that the structures shown in Fig. 1 represent only one of the possible conformations of myosin V/XI globular tails.

Activity Inhibition by Motor-Tail Interaction May Be Conserved in Myosin V and Myosin XI—Recently, the inhibition of myosin V activity by an intramolecular interaction between the motor domain and the globular tail has been reported (42). Based on the structural and functional similarity between myosin XI and myosin V, we postulate that myosin XI may be reg-
ulated in a similar way. If this were the case, we would expect that the regions involved in this motor-tail interaction should be conserved between myosin V and XI. It has been proposed that this head-tail binding is based on ionic interaction of specific acidic residues in the motor domain with basic residues in the tail domain (43). Remarkably, we found two positively charged residues (Arg1359 and Arg1434 in MYA1, Fig. 1A) that are positioned in close proximity at the surface of the distal globular tail, and that are highly conserved between yeast and animal myosin V as well as plant myosin XI. Further research is required to confirm the involvement of those residues in the motor-tail interaction of myosin XI.

CONCLUSIONS

In this report, we have used a combination of computational and experimental approaches to establish that the globular tail of myosin XI can take on a similar conformation as that of myosin V. We have shown that this domain is responsible for organelle targeting in myosin XI and that its two subdomains can target organelles independently. However, our data indicated that the regulation of motor binding to a diverse range of cargoes is dependent on a dynamic allosteric interaction between two tail subdomains of myosin XI, which is switched by the loop region between them. Despite the structural and functional conservation between the myosin classes demonstrated here, the particular mechanisms of cargo binding appear to be different. Further analysis of these mechanisms for myosin XI and their comparison across the large evolutionary distance between myosin XI of plants and myosin V of animals and fungi is likely to reveal fundamental insights into cargo binding and myosin function.

Acknowledgments—We thank Star N. Loar for assistance with the Arabidopsis plants, Ian Wallace for helpful suggestions on the homology model, and Dr. Barry Bruce for critical reading of the manuscript. We are grateful to Dr. John Schieffelin (University of Michigan) for the generous gift of MYA1 and MYA2 cDNAs.

REFERENCES

1. Krendel, M., and Mooseker, M. S. (2005) Physiology 20, 239–251
2. Foth, B. J., Goedecke, M. C., and Soldati, D. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 3681–3686
3. Bezanilla, M., Horton, A. C., Sevener, H. C., and Quatrano, R. S. (2003) J. Mol. Evol. 57, 229–239
4. Yokota, E., and Shimmen, T. (1994) Protoplasma 177, 153–162
5. Tominaga, M., Kojima, H., Yokota, E., Orii, H., Nakamori, R., Katayama, E., Anson, M., Shimmen, T., and Oiwa, K. (2003) EMBO J. 22, 1263–1272
6. Kinkema, M., and Schieffelin, J. (1994) J. Mol. Biol. 239, 591–597
7. Kashiwaba, T., Kimura, N., Mimura, T., and Yamamoto, K. (2000) J. Biochem. 127, 1065–1070
8. Hamada, S., Sekimoto, H., Tanabe, Y., Tsuchikane, Y., and Ito, M. (2006) J. Plant Res. 119, 105–113
9. Reddy, A. S., and Day, I. S. (2001) Genome Biol. 2, 0024.1–0024.17
10. Jiang, S., and Ramachandran, S. (2004) Plant Cell Physiol. 45, 590–599
11. Shimmen, T., and Yokota, E. (2004) Curr. Opin. Cell Biol. 16, 68–72
12. Liebe, S., and Menzel, D. (1995) Biol. Cell 85, 207–222
13. Nebenführ, A., Gallacher, L. A., Dunahay, T. G., Frohlich, J. A., Mazurkiewicz, A. M., Meehl, J. B., and Staehelin, L. A. (1999) Plant Physiol. 121, 1127–1141
14. Jedd, G., and Chua, N. H. (2002) Plant Cell Physiol. 43, 384–392
15. van Gestel, K., Köhler, R. H., and Verbelen, J. P. (2002) J. Exp. Bot. 53, 659–667
16. Higaki, T., Kutsuna, N., Okubo, E., Sano, T., and Hasezawa, S. (2006) Plant Cell Physiol. 47, 839–852
17. Yokota, E., McDonald, A. R., Liu, B., Shimmen, T., and Palevitz, B. A. (1995) Protoplasma 185, 178–187
18. Liu, L., Zhou, J., and Pesacreta, T. (2001) Cell Motil. Cytoskeleton 48, 130–148
19. Wang, Z., and Pesacreta, T. C. (2004) Cell Motil. Cytoskeleton 57, 218–232
20. Hashimoto, K., Igarashi, H., Mano, S., Nishimura, M., Shimmen, T., and Yokota, E. (2005) Plant Cell Physiol. 46, 782–789
21. Reisen, D., and Hanson, M. R. (2007) BMC Plant Biol. 7, 6
22. Romagnoli, S., Cai, G., Faleri, C., Yokota, E., Shimmen, T., and Cresti, M. (2007) Plant Cell Physiol. 48, 345–361
23. Fehrenbacher, K. L., Boldogh, I. R., and Pon, L. A. (2003) Trends Cell Biol. 13, 472–477
24. Korn, E. D. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12559–12564
25. Richards, T. A., and Cavalier-Smith, T. (2005) Nature 436, 1113–1118
26. Molchan, T. M., Valster, A. H., and Hepler, P. K. (2002) Planta 214, 685–693
27. Wu, X. S., Rao, K., Zhang, H., Wang, F., Sellers, J. R., Matesic, L. E., Copeland, N. G., Jenkins, N. A., and Hammer III, J. A. (2002) Nat. Cell Biol. 4, 271–278
28. Lapierre, L. A., Kumar, R., Hales, C. M., Navarre, J., Bhartur, S. G., Burnett, J. O., Provance, D. W., Mercer, J. A., Bahler, M., and Goldenring, J. R. (2001) Mol. Biol. Cell 12, 1843–1857
29. Tang, F., Kauffman, E. J., Novak, J. L., Nau, J. J., Catlett, N. L., and Weisman, L. S. (2003) Nature 422, 87–92
30. Fagarasanu, A., Fagarasanu, M., Itzen, G. A., Aitchison, J. D., and Rachubinski, R. A. (2006) Dev. Cell 10, 587–600
31. Pashkova, N., Jin, Y., Ramaswamy, S., and Weisman, L. S. (2006) EMBO J. 25, 693–700
32. Edgar, R. C. (2004) Nucleic Acids Res. 32, 1792–1797
33. Notredame, C., Higgins, D., and Heringa, J. (2000) J. Mol. Biol. 302, 693–700
Subdomains of the Cargo Binding Domain of Myosin XI

205–217
34. Schwede, T., Kopp, J., Guex, N., and Peitsch, M. C. (2003) *Nucleic Acids Res.* **31**, 3381–3385
35. Colosimo, A., Xu, Z., Novelli, G., Dallapiccola, B., and Gruenert, D. C. (1999) *BioTechniques* **26**, 870–873
36. Ramachandran, G. N., Ramakrishnan, C., and Sasisekharan, V. (1963) *J. Mol. Biol.* **7**, 95–99
37. Catlett, N. L., Duex, J. E., Tang, F., and Weisman, L. S. (2000) *J. Cell Biol.* **150**, 513–525
38. Pashkova, N., Catlett, N. L., Novak, J. L., Wu, G., Lu, R., Cohen, R. E., and Weisman, L. S. (2005) *J. Cell Biol.* **168**, 359–364
39. Pashkova, N., Catlett, N. L., Novak, J. L., and Weisman, L. S. (2005) *Eukaryot. Cell* **4**, 787–798
40. Hu, C. D., Chinenov, Y., and Kerppola, T. K. (2002) *Mol. Cell* **9**, 789–798
41. Weisman, L. S. (2006) *Nat. Rev. Mol. Cell Biol.* **7**, 243–252
42. Wang, F., Thirumurugan, K., Stafford, W. F., Hammer III, J. A., Knight, P. J., and Sellers, J. R. (2004) *J. Biol. Chem.* **279**, 2333–2336
43. Thirumurugan, K., Sakamoto, T., Hammer III, J. A., Sellers, J. R., and Knight, P. J. (2006) *Nature* **442**, 212–215