The ATPase Activity of Myr3, a Rat Myosin I, Is Allosterically Inhibited by Its Own Tail Domain and by Ca\(^{2+}\) Binding to Its Light Chain Calmodulin*

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Myosins are mechanoenzymes that convert chemical energy stored in the molecule ATP into directed force along actin filaments. They are heterooligomers consisting of one or two heavy chains and up to six light chains per heavy chain. The heavy chains exhibit an N-terminal head region, a light chain ATPase binding region, and a C-terminal tail region. The head region is relatively well conserved in all myosins and comprises ATP- and actin-binding sites and exhibits actin-activated ATPase activity (1).

Based on phylogenetic analysis of the head domain sequences myosins can be divided into more than 10 classes. Class I myosin molecules are single headed with relatively short tail-domains that neither dimerize nor form filaments. This class contains numerous members that by sequence comparison can be grouped into subclasses. Members of the "amoeboid" subclass were first identified in Acanthamoeba castellanii (2) and Dictyostelium discoideum (3). More recently, myosin molecules of this subclass have been identified from yeast to man (Ref. 4–7 and Knight and Kendrick-Jones, GenBank® accession number X70400). So far, only members of this subclass from A. castellanii and D. discoideum have been characterized biochemically. Their actin-activated Mg-ATPase activity and their ability to produce force is regulated by phosphorylation of a single serine/threonine residue located in the head region. The recently identified vertebrate members of this subclass lack this phosphorylation site. Therefore, their ATPase and motor activities are likely to be regulated in a different manner. Vertebrate members also differ in their tail regions from the biochemically characterized protozoan members in that they do not contain an extended GPA/GPQ (glycine, proline, and alanine/glutamine)-rich region. This region was reported to exhibit actin binding activity (8, 9). Such an ATP-insensitive actin-binding site was used to explain the peculiar triphasic Mg-ATPase activation observed as a function of F-actin concentration. At low concentrations of F-actin, the Mg-ATPase reaches near maximal velocity. This is followed by a decrease of velocity at intermediate F-actin concentrations before getting reactivated to maximal velocity again at high F-actin concentrations (10). Examination of the actin-activated Mg-ATPase activity of vertebrate amoeboid myosin I molecules could lead to a further clarification of the mechanism responsible for this complex triphasic activation kinetics.

The heavy chain of the rat myosin I molecule Myr3, a vertebrate member of the amoeboid subclass of myosin I molecules, was recently identified by molecular cloning (7). Based on sequence analysis, the heavy chain of Myr3 contains a single light chain binding motif. This light chain binding motif was proposed to bind calmodulin. Myr3 is expressed in many tissues and cell lines. Due to its localization in cell-cell contact regions Myr3 was suggested to play a role associated with cell-cell contacts (7).

To further define the function of Myr3 we attempted to characterize its enzymatic properties and their regulation. We now report the purification of Myr3 from rat liver, the characterization of its enzymatic activities, and their regulation by actin, free Ca\(^{2+}\), tail binding antibodies, and proteolysis.

**EXPERIMENTAL PROCEDURES**

**Purification of Myr3**—Myr3 was purified from rat liver. The livers of 40 adult, male Sprague-Dawley rats were homogenized in TBS (150 mM NaCl, 50 mM Tris/HCl, pH 7.4, final volume: 2 liters) using a tight fitting motor driven Dounce homogenizer. To minimize proteolysis the protease inhibitors Pefabloc (50 mg/liter) and apronin (7.6 trypsin inhibitor units/liter) were added to the homogenate, and all purification steps were carried out at 4 °C. The homogenate was centrifuged in a Sorvall GS3 rotor (E. I. du Pont de Nemours, Bad Homburg, Federal Republic of Germany) at 8500 rpm for 60 min. Ammonium sulfate was added to the supernatant to a final saturation of 35% and stirred for 20 min. The pellet resulting after centrifugation (GS3-rotor, 8500 rpm, 30 min) was resuspended in 400 ml of low-salt buffer (50 mM NaCl, 20 mM
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Hepes, pH 7.4, 2 mM MgCl₂, 0.2 mM EGTA, 1 mM β-mercaptoethanol) and dialyzed against the same buffer overnight to obtain an actomyosin pellet. Sometimes the resuspended pellet was incubated in low-salt buffer for 1 h and the dialysis step omitted. This shortened procedure reduced the amount of contaminating proteins in the actomyosin pellet but the actomyosin pellet was not consistently formed. The actomyosin pellet was resuspended in 150 mM KCl. Myr3 was then extracted from the pellet with sodium pyrophosphate. The pellet was resuspended in 150 mM KCl. Myr3 was then extracted from the pellet with sodium pyrophosphate, washed twice with 400 mM of low-salt buffer supplemented with 100 mM KCl, 2 mM MgCl₂, 20 mM Hepes, pH 7.4, 0.5 mM-β-mercaptoethanol, and 2 mM NaCl. The actomyosin pellet was not consistently formed. The actomyosin pellet was then extracted with sodium pyrophosphate. The pellet was resuspended in 150 mM KCl. Myr3 was then extracted from the pellet with sodium pyrophosphate, washed twice with 400 mM of low-salt buffer supplemented with 100 mM KCl, 2 mM MgCl₂, 20 mM Hepes, pH 7.4, 0.5 mM-β-mercaptoethanol, and 2 mM NaCl. F-actin binding assays were performed as described previously (12) except that bovine serum albumin (1 mg/ml) was added to the assay mixture and centrifuge tubes were Provided with 5% non-fat dry milk.

Electron Microscopy—F-actin (50 μg/ml) incubated either with different amounts of purified Myr3 or buffer alone was adsorbed for 30 s on copper grids coated with a glow-discharged carbon film. Grids were negatively stained with 1% uranyl acetate and examined with a Philips CM10 electron microscope at an accelerating voltage of 60 kV.

Amino Acid Sequence Determination—Purified Myr3 was separated by SDS-PAGE (14) and stained with Coo massie Blue. The light chain was excised from the gel and digested with endoproteinase Lys-C. Digested peptides were separated by reversed-phase HPLC on a Supersph 60 RP select B column (Merck, Darmstadt, Federal Republic of Germany) and sequenced by N-terminal degradation using an automated sequencer (Perkin Elmer, Beckman Instruments).

Miscellaneous Techniques—Polyclonal antibodies against Myr3 were raised and purified as described (7). Fab fragments were produced by papain digestion as described by Wallimann and Szent-Györgyi (13). A C-terminally truncated fragment of Myr3 was obtained by limited proteolysis of purified Myr3 with mercuripapain. The proteolysis was stopped by the addition of 5 mM iodoacetamide. Protein concentrations were determined using the Bradford colorimetric assay (14). Immuno blotting was performed according to Towbin et al. (15). Primary antibodies were diluted in 5% non-fat dry milk to 2 μg/ml. Alkaline phosphatase-coupled secondary antibody was diluted in 5% non-fat dry milk and 0.1% Triton X-100. The phosphatase reaction was visualized by the Protoblot system (Promega Corp., Madison, WI). Gel electrophoresis was performed according to Laemmli (16). For quantitative protein analysis, Coo massie-stained SDS gels were scanned and analyzed using the Image Master gel quantification system (Amersham Pharmacia Biotech).

RESULTS

Purification of Myr3—To characterize the biochemical properties of Myr3 we established a purification scheme for Myr3 from rat liver. Rat liver was chosen as starting material, because it contains high levels of Myr3, and the proteolytic breakdown of Myr3 in liver homogenate is less pronounced than in homogenates from other tissues. Myr3 was readily soluble at salt concentrations of 150 mM (>80%) and could be precipitated from the liver extract with ammonium sulfate reaching between 25 and 35% saturation. Since the bulk of proteins precipitated only at higher ammonium sulfate concentrations, the
precipitation of Myr3 at 35% saturation represented a useful first purification step. Myr3 was further purified by the formation of an actomyosin pellet from which it could be quantitatively extracted by sodium pyrophosphate. The use of sodium pyrophosphate as opposed to Mg-ATP reduced the amount of conventional myosin present in the extract. Myr3 was further purified by gel filtration and cation-exchange column chromatography. Purified Myr3 eluted from a Mono S cation-exchange column as a single sharp peak at relatively high salt concentrations of about 850–900 mM (Figs. 1A and 1B). Attempts to concentrate and/or desalt purified Myr3 consistently failed, because Myr3 exhibited a strong tendency to adhere to plastic surfaces. Therefore, we directly used the fractions from the Mono S column in most of our assays. The described purification protocol yielded between 150 and 300 mg of purified Myr3 from 40 rat livers.

Myr3 Contains a Single Calmodulin Light Chain—Together with the Myr3 heavy chain a protein of 17 kDa was copurified (Fig. 1A). The stoichiometry of the 17-kDa protein to the Myr3 heavy chain was determined to be 0.82 ± 0.03 by gel densitometry. This number is in good agreement with the prediction of a single light chain which binds to the single “IQ motif” in the neck region of the Myr3 heavy chain. We previously reported that calmodulin was present in Myr3 heavy chain immunoprecipitates and that calmodulin bound to the Myr3 heavy chain in a gel-overlay assay (7). Indeed, the 17-kDa light chain comigrated with authentic calmodulin on SDS gels (Fig. 1B). Furthermore, the Myr3 light chain was resistant to boiling and demonstrated a calcium-dependent shift in electrophoretic mobility, two properties that characterize authentic calmodulin (Fig. 1B). Additional proof for the identity of the Myr3 light chain with calmodulin was obtained by peptide sequencing. Two peptide sequences derived from the Myr3 light chain (9 and 16 amino acid residues in length) were identical to corre-

### TABLE I

| ATPase activity | − F-actin | + F-actin |
|----------------|----------|----------|
| K/EDTA         |          |          |
| Magnesium (12 mM KCl) | 722 ± 22 |          |
| Magnesium (90 mM NaCl) | 51 ± 21  | 209 ± 36 |
| Magnesium (590 mM NaCl) | 659 ± 29 | 615 ± 28 |

FIG. 2. Increasing salt concentrations raise the Mg-ATPase activity of Myr3 in the absence and presence of F-actin. The Mg-ATPase activity of Myr3 was determined at increasing salt concentrations in the absence (closed circles, broken line) and presence (open circles, solid line) of F-actin. The inset shows a more extended range of NaCl concentrations.

FIG. 3. Mg-ATPase activity of Myr3 as a function of actin concentration. Purified Myr3 was incubated with different concentrations of F-actin, and the Mg-ATPase activity was determined. Open and closed circles represent data from two different preparations. These assays were performed in the presence of 12 (open circles) and 35 mM KCl (closed circles). Data points were fitted by combining the Michaelis-Menten equation with a polynomial function (solid line). The Michaelis-Menten term is indicated by the broken line.

precipitation of Myr3 at 35% saturation represented a useful first purification step. Myr3 was further purified by the formation of an actomyosin pellet from which it could be quantita-

FIG. 4. Myr3 is able to cross-link F-actin. F-actin (50 µg/ml) was incubated together with purified Myr3 in the presence of 200–250 mM salt and viewed by negative stain in the electron microscope. Large bundles of F-actin laterally cross-linked by Myr3 were noticed. The inset shows two actin filaments bundled by Myr3 at a higher magnification. Bars: 50 nm.

FIG. 5. Binding of purified Myr3 to F-actin. The F-actin binding of Myr3 was determined at 150 mM salt by cosedimentation in the absence (−) or presence (+) of actin (18.7 mM) and ATP (2 mM), respectively. Supernatants (S) and pellets (P) were analyzed for their content of Myr3 by immunoblotting with antibody Tu58.
sponding sequences in rat calmodulin (Fig. 1C). By all these criteria, the Myr3 light chain is identical to calmodulin.

ATPase Activity—To analyze myosin-like activities of purified Myr3, K/EDTA-ATPase and the physiologically relevant actin-activated Mg-ATPase activities were determined. Myr3 exhibited a K/EDTA-ATPase activity of 722 ± 22 nmol/min/mg and a Mg-ATPase activity that was stimulated by F-actin (Table I). Both, basal and actin-activated Mg-ATPase, activities of Myr3 increased with increasing salt concentrations (Fig. 2, Table I). However, up to a salt concentration of approximately 200 mM NaCl the slope of the actin-activated Mg-ATPase activity was smaller than the slope of the basal Mg-ATPase activity. At this salt concentration, there was no longer any actin activation of the Mg-ATPase detectable. Upon increasing the salt concentration further, the Mg-ATPase activity increased irrespective of the presence or absence of F-actin (Fig. 2). This salt-dependent increase in Mg-ATPase activity of Myr3 was reversible as Myr3 was diluted into the assay mixtures from a buffer containing 900 mM NaCl. Addition of exogenous calmodulin to the assay mixtures did not influence the Myr3 ATPase activities.

Next we determined the effect of the actin concentration on the Mg-ATPase activity of Myr3. As shown in Fig. 3, the Mg-ATPase activity of Myr3 was activated by F-actin at low concentrations of salt (12 and 35 mM KCl, respectively) in a triphasic manner. Measurements under standard conditions (90 mM NaCl) revealed no qualitative differences except for a higher basal activity and a smaller activation by F-actin due to the higher salt concentration. The Mg-ATPase activity increased up to a concentration of ~1 μM actin, then decreased up to a concentration of ~3.5 μM actin and finally increased again until it reached a plateau at actin concentrations higher than 7 μM.

**FIG. 6.** Free Ca²⁺ in the micromolar range inhibits the ATPase activity of Myr3. A, Mg-ATPase activity of Myr3 was determined either in the absence (black bars) or presence (gray bars) of F-actin. Activities were measured in the presence of EGTA (Mg) and 3 μM free Ca²⁺ (Mg/Ca). To assess the reversibility of the Ca²⁺ inhibition, Myr3 was preincubated for 20 min in 3 μM free Ca²⁺ before chelating free Ca²⁺ with EGTA (Mg/Ca + EGTA). B, purified Myr3 was incubated in various free Ca²⁺ concentrations ranging from nanomolar to millimolar using a Ca/EGTA buffer system. The ATPase activity was measured in the absence (closed circles, broken line) or presence (open circles, solid line) of F-actin (8 μM).
Myr3 Can Cross-link F-actin, and It Binds to Its Light Chain Calmodulin—As mentioned above, the triphasic F-actin activation of the Mg-ATPase of amoeboid myosin I molecules from protozoa is explained by a mechanism that involves cross-linking of F-actin. To test whether Myr3 can cross-link actin filaments, we examined mixtures of F-actin and purified Myr3 by electron microscopy (Fig. 4). Bundles of cross-linked F-actin were observed in the presence of purified Myr3. This finding indicates that Myr3 is able to cross-link actin filaments, although it lacks a “GPA” region in its tail domain. To determine directly whether Myr3 contains an ATP-independent high affinity actin-binding site, which could explain the triphasic activation of its ATPase activity by increasing F-actin concentrations, we performed actin cosedimentation experiments at low Myr3 concentrations. Myr3 was found to specifically cosediment with actin filaments in the absence, but not in the presence, of ATP (Fig. 5), indicating that Myr3 does not contain a nucleotide-independent high affinity actin binding site.

Ca^{2+} Negatively Regulates the Mg-ATPase Activity of Myr3 by Binding to Its Light Chain Calmodulin—Since we have shown that the Ca^{2+}-binding protein calmodulin serves as the single light chain of Myr3, we hypothesized that the activity of Myr3 might be regulated by Ca^{2+}. Therefore, we examined the effect of free Ca^{2+} ions on its Mg-ATPase activity (Fig. 6A). Addition of micromolar free calcium ions reduced both the basal and actin-activated Mg-ATPase activity of Myr3 by a factor of 2–3. This inhibitory effect of free calcium on the Mg-ATPase activity of Myr3 was reversible. Chelation of free calcium ions with EGTA after a 20-min incubation of purified Myr3 with free Ca^{2+} increased the Mg-ATPase activity again to the initial level (Fig. 6A). A detailed analysis of the free calcium concentration needed to inhibit the Myr3 Mg-ATPase activity revealed a marked inhibition for both the basal and actin-activated Mg-ATPase activity between a concentration range of 0.1–1 μM free Ca^{2+} (Fig. 6B). This concentration range exactly coincides with the affinity of calmodulin for calcium, strongly supporting the notion that the observed inhibition is due to the binding of Ca^{2+} to the Myr3 light chain calmodulin. This Ca^{2+}-dependent inhibition of the Myr3 Mg-ATPase activity could be explained either by an allosteric effect or by dissociation of calmodulin from the Myr3 heavy chain. To discriminate between these two possibilities, we performed an actin cosedimentation assay in the absence and presence of micromolar free Ca^{2+} (Fig. 7). This experiment allows for the separation of free calmodulin from calmodulin bound to the Myr3 heavy chain. Comparable amounts of calmodulin were found to cosediment with Myr3 and F-actin irrespective of the free calcium concentration (Fig. 7). This result demonstrates that calmodulin remains bound to the Myr3 heavy chain irrespective of the free Ca^{2+} concentration and hence that the regulation of the Myr3 Mg-ATPase by Ca^{2+} is of allosteric nature. In further support of this notion, addition of exogenous calmodulin had no effect on the Mg-ATPase activity of Myr3 (data not shown).

The Myr3 Tail Domain Inhibits the Mg-ATPase Activity of the Myr3 Head Domain—As a substitute for a physiological Myr3 tail binding partner, the antibody FML 6, which was raised against a fusion protein of the Myr3 tail domain (encompassing amino acids 821–1107; Ref. 7), was tested for potential regulatory effects on the Mg-ATPase of the Myr3 head domain. Interestingly, the antibody FML 6 caused in a concentration-dependent manner an increase of both basal and actin-activated Mg-ATPase activity (Fig. 8A). The basal Mg-ATPase activity was raised to values that were comparable to the actin-activated Mg-ATPase activity. The actin-activated Mg-ATPase activity was also increased, but to a somewhat smaller degree. The observed activation of the Myr3 Mg-ATPase activity by antibody FML 6 was not simply a result of cross-linking of Myr3 molecules by the antibodies. Monovalent Fab fragments also stimulated the Myr3 Mg-ATPase activity and that to a similar extent as the FML 6 IgG antibodies (Fig. 9). This activation of the Myr3 Mg-ATPase activity was not observed...
with control antibodies (data not shown) and the previously described polyclonal antibody Tu 58 (7) that recognizes a region in the myosin head domain of Myr3 (amino acids 326–342) corresponding to the so called ordered loop in the conventional myosin head (amino acids 400–416; Ref. 34). Antibody Tu 58 specifically inhibited the F-actin activation of the Myr3 Mg-ATPase (Fig. 8B). Additive effects were observed when the two antibodies, Tu 58 and FML 6, were added simultaneously. ATPase measurements in the presence of both antibodies demonstrated an increase in the basal Mg-ATPase activity but no longer any activation by F-actin (data not shown). These results suggest that the two antibodies were affecting two independent regulatory mechanisms and they demonstrate the specificity of the activation of the Mg-ATPase by the Myr3 tail antibodies. Interestingly, a similar activation of the Myr3 Mg-ATPase activity as with the tail binding antibodies was noticed upon proteolytic cleavage of the very C terminus of the Myr3 molecule (Fig. 10). Limited digestion of Myr3 with mercuripapain produced a Myr3 fragment truncated at its C terminus by limited digestion with mercuripapain (lane 2) were separated on SDS-PAGE and stained with Coomassie Blue (A). The corresponding aliquots were used for determination of Mg-ATPase activity (B).

**DISCUSSION**

The establishment of a purification procedure for the rat myosin I molecule Myr3 allowed us to initiate its biochemical characterization. Preparations of purified Myr3 contained in addition to the heavy chain as predicted from its sequence a single light chain in a 1:1 stoichiometry. This light chain was identified as calmodulin based on its electrophoretic mobility, heat stability, and partial peptide sequence. Calmodulin has been found to be associated with other vertebrate myosin I molecules (12, 17–19) but not with amoeboid myosin I molecules from protozoa, which are the closest homologues of Myr3 (20, 21). This difference in the identity of the light chain between Myr3 and other amoeboid myosin I molecules might reflect a difference in regulatory properties. This notion is supported by the fact that Myr3 does not contain the regulatory phosphorylation site present in the head domain of amoeboid myosin I molecules from protozoa.

Myr3 displayed K/EDTA-ATPase and actin-activated Mg-ATPase activities that are characteristic of bona fide myosins. The K/EDTA-ATPase activity of Myr3 was higher than for other vertebrate myosin I molecules such as chicken brush border myosin I (17, 22), myr1 (23), or myr2 (23–25), but lower than for myosin I molecules from protozoa (20). The actin-activated Mg-ATPase activity of Myr3 was comparable with that of other myosin I molecules from vertebrates (17, 25) and the dephosphorylated myosin I molecules from protozoa (20). However, it was considerably lower as compared with the actin-activated Mg-ATPase activity of the phosphorylated myosin I molecules from protozoa.

Myr3, like the amoeboid myosin I molecules from protozoa, exhibited a peculiar triphasic activation of its Mg-ATPase by F-actin. For the myosin I molecules from protozoa this complex kinetic behavior has been proposed to be due to a second ATP-insensitive high affinity F-actin binding site in their tail domains (26, 27). An ATP-insensitive F-actin binding site has been mapped to the GPA/GPQ-rich region in these myosin I molecules (8, 9, 28). The tail domain of Myr3 lacks such a GPA/GPQ-rich region. Nevertheless, in electron micrographs we observed bundling of actin filaments by Myr3. Similar bundling activity has also been reported for brush border myosin I (17, 29), myr1, and myr2 (23), which also lack a GPA/GPQ-rich region. Therefore, in vitro bundling of actin filaments by myosin I molecules does not necessarily require the presence of a GPA/GPQ-rich region. On the other hand, bundling does not automatically mean complex triphasic activation kinetics, because brush border myosin I and myr2 exhibit simple hyperbolic activation kinetics (17, 25, 30). At present, we do not know whether cross-linking of actin filaments by Myr3 is due to a nucleotide-insensitive actin binding site or rather self-association. This question is of relevance for understanding the mechanism of its complex activation by F-actin. However, in accordance with the lack of a GPA/GPQ-rich region in Myr3, we have not obtained any evidence for a high affinity ATP-insensitive F-actin binding site in actin binding experiments performed with purified Myr3. Therefore, alternative mechanisms for explaining the complex triphasic kinetics cannot be excluded.
Surprisingly, the ATPase activity of Myr3 was found to be negatively regulated by micromolar free Ca$^{2+}$ concentrations. This is exactly the opposite of what has been reported for other vertebrate myosin molecules, which contain calmodulin molecules associated as light chains. Micromolar free Ca$^{2+}$ concentrations have been shown to activate the Mg-ATPase activities of brush border myosin I, myr1, myosin Iβ, and myosin V (25, 30–32). In brush border myosin I and myosin V elevation of free Ca$^{2+}$ concentrations also led to a partial dissociation of calmodulin light chains (30, 32). However, we demonstrated that calmodulin is associated with the Myr3 heavy chain irrespective of the free Ca$^{2+}$ concentration and, therefore, conclude that binding of Ca$^{2+}$ to the light chain calmodulin regulates the Myr3 Mg-ATPase activity allosterically. This conclusion is supported by the lack of any modulation of the Mg-ATPase by an excess of exogenously added calmodulin.

Binding of the antibody FML 6 or of monovalent Fab fragments to the tail-domain of Myr3 caused an increase in its Mg-ATPase activity. To explain how the binding of an antibody to the tail-domain of a myosin can affect its ATPase activity, further experimentation will be needed. However, one clue comes from the observation that a C-terminally truncated Myr3 fragment also exhibits increased Mg-ATPase activity. Therefore, it seems likely that both the tail binding antibody FML 6 and the truncation at the very C terminus of the tail domain neutralize an inhibitory constraint imposed by the tail domain. This inhibitory constraint might be imposed by the C-terminal SH3 domain that is missing in the truncated Myr3 molecule (7). This SH3 domain could bind intramolecularly to proline-rich motifs present in the Myr3 tail domain and thereby inhibit Mg-ATPase activity. An inhibitory function for SH3 domains has already several precedents in protein kinases (33).

We reported previously that intact Myr3 in lung homogenates did not bind significantly to F-actin, whereas a C-terminally truncated Myr3 degradation product did bind to F-actin in an ATP-regulated manner (7). We now report that intact purified Myr3 binds in an ATP-regulated manner to F-actin. These seemingly contradictory findings might either be explained by removal of a factor(s) during purification interacting with or modifying the C terminus of Myr3 or by disruption of inhibitory intramolecular interactions. These previously reported results, however, are in agreement with our present findings of an inhibitory function of the Myr3 tail domain.

Our results suggest that caution should be exercised when comparing data obtained from in vitro motility assays in which the tail domains have been immobilized with data obtained in solution. The mode of immobilization of the tail domain might critically affect the motor activity. Furthermore, these results suggest that in vivo binding partners of myosin tail domains might regulate the motor activity and suggest a novel approach for identifying myosin tail binding partners.

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