Actin-facilitated Assembly of Smooth Muscle Myosin Induces Formation of Actomyosin Fibrils

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Abstract. To identify regulatory mechanisms potentially involved in formation of actomyosin structures in smooth muscle cells, the influence of F-actin on smooth muscle myosin assembly was examined. In physiologically relevant buffers, AMPPNP binding to myosin caused transition to the soluble 10S myosin conformation due to trapping of nucleotide at the active sites. The resulting 10S myosin-AMPPNP complex was highly stable and thick filament assembly was suppressed. However, upon addition to F-actin, myosin readily assembled to form thick filaments. Furthermore, myosin assembly caused rearrangement of actin filament networks into actomyosin fibers composed of coaligned F-actin and myosin thick filaments. Severin-induced fragmentation of actin in actomyosin fibers resulted in immediate disassembly of myosin thick filaments, demonstrating that actin filaments were indispensable for mediating myosin assembly in the presence of AMPPNP. Actomyosin fibers also formed after addition of F-actin to nonphosphorylated 10S myosin monomers containing the products of ATP hydrolysis trapped at the active site. The resulting fibers were rapidly disassembled after addition of millimolar MgATP and consequent transition of myosin to the soluble 10S state. However, reassembly of myosin filaments in the presence of MgATP and F-actin could be induced by phosphorylation of myosin P-light chains, causing regeneration of actomyosin fiber bundles. The results indicate that actomyosin fibers can be spontaneously formed by F-actin-mediated assembly of smooth muscle myosin. Moreover, induction of actomyosin fibers by myosin light chain phosphorylation in the presence of actin filament networks provides a plausible hypothesis for contractile fiber assembly in situ.

How smooth muscle cells form contractile actomyosin fibrils is unknown. Studies to date have focused on the assembly properties of isolated smooth muscle myosin. These investigations have consistently shown that, unlike skeletal muscle myosin, the assembly and enzymatic properties of smooth muscle myosin are closely linked (5, 7, 16, 27, 34, 36, 38, 39, 40). In particular, binding and hydrolysis of MgATP promotes depolymerization of nonphosphorylated smooth muscle myosin filaments in vitro (5, 7, 16, 27, 34, 36, 40). The effect is intimately linked to stabilization of the folded 10S myosin monomeric conformation resulting from active site trapping of ATP hydrolysis products (ADP-Pi) (7, 8). Phosphorylation of myosin P-light chains, which is required for actin-activated MgATPase of myosin (reviewed in 29, 35), destabilizes the 10S conformation (8) and thereby promotes myosin assembly in the presence of MgATP (5, 16, 27, 36, 38, 39).

The presence of myosin thick filaments in both relaxed and contracting smooth muscle (32) excludes regulated assembly of myosin as a primary factor governing smooth muscle contractility. Nevertheless, the reversible assembly of smooth muscle myosin in vitro has prompted speculation that a pool of soluble 10S myosin may be present in smooth muscle cells (8). Activation of the cell and subsequent phosphorylation of the P-light chains could result in recruitment of soluble myosin into existing thick filaments, thereby modulating the contractile response (8). However, inferences based on the assembly properties of myosin in vitro are complicated by the comparatively high concentrations of actin in smooth muscles (4, 22). A relevant question concerns the potential regulatory effects of F-actin on smooth muscle myosin assembly and 10S myosin stability. Previous studies by Ikebe and Harts- horne (13), employing the nonhydrolyzable ATP analogue, AMPPNP, provide suggestive evidence of interaction between F-actin and 10S myosin. While AMPPNP promoted transition of smooth muscle myosin to the 10S conformation, myosin cosedimented with F-actin in the presence of AMPPNP. An equally important question concerns possible cytoskeletal rearrangements triggered by smooth muscle myosin assembly in the midst of actin filament networks. For example, Mahajan et al. (21) have shown that F-actin ac-

1. Abbreviation used in this paper: AMPPNP, adenosine 5'-(β,γ-imidophosphate).
celerates Dictyostelium myosin II assembly in vitro in a process resulting in formation of actomyosin fibers. In this work, we have investigated the ability of F-actin to regulate smooth muscle myosin assembly and promote formation of actomyosin structures. Combining soluble 10S myosin containing bound AMPPNP with F-actin instigated coordinate myosin assembly and formation of actomyosin fibers. Simultaneous myosin and actomyosin fiber assembly required full-length actin filaments, since neither structure could be sustained when F-actin was fragmented. Thick filaments and actomyosin fibers containing nonphosphorylated myosin were disassembled by millimolar MgATP, but the effect was reversed upon myosin F-light chain phosphorylation. Coassembly of myosin and actomyosin fibers could therefore be controlled by light chain phosphorylation, suggestive of a mechanism for formation of both smooth muscle and nonmuscle actomyosin fibrils.

**Materials and Methods**

**Protein Isolation**

Proteins were isolated using the following procedures: myosin (12) and myosin light chain kinase (15) from smooth muscle of chicken gizzards; actin (33) from rabbit skeletal muscle; and severin (2) from Dictyostelium discoideum.

**Analytical Gel Filtration**

Fast protein liquid chromatography (FPLC; Pharmacia Fine Chemicals, Piscataway, NJ) gel filtration was performed as described by Cross and Sobieszek (6). A threefold molar excess of [α-32P]-AMPPNP (ICN, Irvine, CA) was added to nonphosphorylated myosin (0.5 mg/ml) in 10 mM imidazole (pH 7.3), 1 mM MgCl₂, 1 mM EGTA, 0.5 mM DTT, containing either 0.15 or 0.6 M KCl. Soluble myosin was passed through a 0.2-μm filter, and a 500-μl aliquot was applied to an HR 10/30 column of Superose 6 pre-equilibrated with buffer containing either 0.15 or 0.6 M KCl. Elution was performed at room temperature at 0.5 ml/min and 0.5-ml fractions were collected. Aliquots of eluted fractions were counted in a liquid scintillation counter, and protein concentrations were determined according to Lowry et al. (20). Myosin eluted in positions corresponding to the 10S and 6S monomeric conformation, under low and high salt conditions, respectively. In either case, the myosin peaks were well separated from excess AMPPNP. The purity of [α-32P]-AMPPNP was >95% as determined by analysis on a MonoQ FPLC column. Contaminating ATP was <3% and in no case did we detect 32P counts in the peak corresponding to ATP.

**AMPPNP Exchange Assay**

The rate of AMPPNP exchange from nonphosphorylated 10S myosin was measured using rapid gel filtration for the separation of free and bound products. A threefold molar excess of [α-32P]-AMPPNP was added to 2-ml samples of nonphosphorylated myosin (0.5 mg/ml) in 0.15 M KCl, 10 mM imidazole (pH 7.3), 1 mM MgCl₂, 1 mM EGTA, and 0.5 mM DTT (assembly buffer). After mixing, which was accompanied by a rapid, visible loss of turbidity, 2 mM ATP was added. At various times, 100 μl of the reaction mixture was removed and quickly applied to a 1-ml column of Sephadex G-50 equilibrated with the same buffer. Free nucleotides were separated from myosin and bound nucleotides during a 1-min centrifugation in a table top clinical centrifuge according to the method of Penefsky (23). Protein concentrations were determined according to Lowry et al. (20) and liquid scintillation counting was used to determine concentrations of bound AMPPNP.

**Light Scattering Measurements**

90° light scattering of protein samples at 20°C was measured at 450 nm in a Perkin-Elmer 650-10S fluorescence spectrophotometer equipped with a water-jacketed cuvette.

**Preparation of the 10S Myosin—ADP·P·Complex**

10S myosin with ATP hydrolysis products trapped at the active sites, designated M₃₀₆-ADP·P·, was prepared as described by Applegate (1). Briefly, nonphosphorylated myosin filaments in assembly buffer were disassembled by addition of sixfold molar excess of MgATP. Excess ATP and free hydrolysis products were removed by rapid gel filtration, as described above. Controls, described previously (1), demonstrated that >95% of myosin eluted as M₃₀₆-ADP·P·.

**Electron Microscopy**

In most cases, 10-20 μl aliquots of samples were pipetted directly onto nitrocellulose/carbon-coated grids and stained with seven drops of 0.22 μm filtered 1% uranyl acetate. The grids were blotted on edge and allowed to dry. For P-light chain phosphorylation studies, 25-μl aliquots were diluted with an equal volume of ice-cold 5% vol/vol glutaraldehyde in assembly buffer containing 1 mM MgATP. After 5 min, the fixed samples were diluted fourfold with ATP-assembly buffer and samples were negatively stained as described above. Grids were viewed in a JEOL 100CXII electron microscope operating at 80 kV accelerating voltage.

**Treatment of Actomyosin Complexes with Severin**

Actomyosin fibers in AMPPNP-assembly buffer were diluted with varying concentrations of purified severin to yield a 1:1 to 1:20 severin/actin molar ratio. Fragmentation was initiated by addition of CaCl₂ to 1 μM to activate severin (2, 41). Comparable dilutions in the absence of severin did not affect the morphology of actomyosin complexes.

**Other Techniques**

Spectrophotometric determinations of protein concentrations were made using E₅₅₀ = 4.5 for gizzard myosin and E₅₅₀ = 11 for actin. MgATPase activity of myosin was measured by monitoring liberated inorganic phosphate using ferrous sulfate as a reducing agent. The progress of P-light chain phosphorylation was assessed by autoradiography with [γ-32P]ATP (DuPont-New England Nuclear, Boston, MA). SDS-PAGE of actomyosin samples was according to Laemmli (18), and dried gels were exposed to Kodak XAR-5 film at -80°C using Lightning Plus intensifying screens (DuPont-New England Nuclear).

**Results**

**Trapping of AMPPNP by 10S Myosin**

In accordance with the observation of Ikebe and Hartshorne (13), AMPPNP in greater than or equal to twofold molar excess disassembled filaments of nonphosphorylated chicken gizzard smooth muscle myosin maintained in assembly buffer. After disassembly, a large fraction of myosin remained in the supernatant following centrifugation at 100,000 g. When subjected to FPLC gel filtration, solubilized myosin eluted in the 10S conformation (Fig. 1A) and experiments employing [α-32P]-AMPPNP revealed that AMPPNP coeluted in nearly stoichiometric ratios to the active sites of myosin (Fig. 1A). In contrast, insignificant amounts of AMPPNP coeluted when myosin was restricted to the extended 6S monomeric conformation by inclusion of 0.6 M KCl in assembly buffer (Fig. 1B). ATP chase experiments demonstrated that AMPPNP bound by 10S myosin was slowly exchanged from the active site (Fig. 2). The fraction of [α-32P]-AMPPNP remaining bound to 10S myosin after addition of 1 mM ATP declined with apparent single exponential kinetics (Fig. 2). The t₁/₂ for AMPPNP exchange, 48 min, was similar to that previously determined for release of trapped ATP hydrolysis products (1, 7). Taken together, gel filtration and ATP chase experiments indicated that the 10S conformation was stabilized by trapping 2 mol of AMPPNP per mole of myosin.
Effect of F-Actin on 10S Myosin Containing Trapped AMPPNP

Myosin assembly did not occur when 10S myosin containing trapped AMPPN, designated M10S(AMPPNP), was incubated in assembly buffer containing a 2-μM excess of AMPPNP. Myosin remained in the supernatant after airfuge centrifugations and light scattering signals were invariant with time (Fig. 3 A). However, when F-actin was present, light scattering increased progressively, eventually reaching a plateau (Fig. 3, A and B). The 1/2 of the light scattering increases ranged from ~40 min for a 2:1 molar ratio of actin to myosin, to 8 min for a 10:1 molar ratio. Light scattering profiles were distinctly different when myosin was restricted to the 6S monomeric conformation. Experiments conducted in 0.35 M KCl, revealed that after an immediate increase reflecting the binding of myosin to actin, light scattering signals remained constant (data not shown).
Morphology of Actomyosin Complexes

The progressive increase in light scattering observed after addition of 10S myosin to actin suggested formation of actomyosin complexes. To determine the morphology of these complexes, aliquots were taken for negative staining electron microscopy at regular intervals after addition of M\textsuperscript{10S} (AMPPNP)\textsubscript{2} to F-actin. The most striking time-dependent change after addition of M\textsuperscript{10S}(AMPPNP)\textsubscript{2} was progressive formation of actomyosin fibers containing myosin thick filaments (Figs. 4D and 5). Loose bundles of actin filaments together with monomeric myosin in the background were observed within five min of addition (data not shown). By 20 min, monomeric myosin was no longer evident and most actin filaments were coaligned in tight bundles with diameters ranging from 50 to 100 nm. Based on an F-actin diameter of 5 nm, from 10 to 20 actin filaments could be laterally packed to form the fiber thickness. No free myosin thick filaments were observed at any stage of the reaction.

Close examination of fibers revealed myosin thick filaments of uniform 11-nm diameter alongside and within bundles of actin filaments (Fig. 5C, arrowheads). Fully visible myosin thick filaments measured ~0.3 μm in length, although uniformity of length could not be rigorously established due to the masking of thick filaments within tight fibers. In the absence of F-actin, EM revealed only monomeric myosin (Fig. 4C). Therefore, F-actin clearly promoted myosin filament assembly in the presence of AMPPNP. Furthermore, induction of myosin assembly by F-actin, resulting in actomyosin fiber formation, appeared to be a coherent process producing uniformly assembled thick filaments embedded in a coaligned actin filament matrix.

Disassembly of Actomyosin Fiber Bundles by Severin

Treatment of actomyosin fibers with severin, an actin filament severing and disassembly protein isolated from Dicystostelium discoideum (2, 3, 4), caused an immediate decrease in light scattering. A 1:20 molar ratio of severin to F-actin caused fiber disruption, revealing numerous myosin thick filaments intercalated within the fiber infrastructure (Fig. 6A). Severin-induced fragmentation of actin filaments appeared to relax actomyosin fibers, facilitating visualization of myosin thick filaments (Fig. 6A, arrows). Thick filaments in severin-relaxed fibers were uniform in both length (0.3 μm) and diameter (11 nm). Further addition of severin to a 1:1 molar ratio of severin/F-actin caused complete disassembly of fiber complexes within 60 s. EM revealed short actin fragments, ~1/10 the length of untreated F-actin, amidst a background of monomeric myosin (Fig. 6B). Characteristic chevrons on many F-actin fragments indicated partial decoration by myosin. Surprisingly, myosin thick filaments were not present, having undergone complete disassembly to myosin monomers. The stability of nonphosphorylated myosin thick filaments in the presence of AMPPNP was absolutely dependent on full-length actin filaments. Hence, reversible assembly and disassembly of actin filament networks could regulate myosin assembly in the presence of AMPPNP.

Effect of F-Actin on the M\textsuperscript{10S}. ADP-P\textsubscript{i} Complex

The novel effect of actin filaments on M\textsuperscript{10S}(AMPPNP)\textsubscript{2} prompted examination of assembly induced by combination of F-actin with the 10S myosin ADP-P\textsubscript{i} complex (M\textsuperscript{10S}. ADP-P\textsubscript{i}). In agreement with previous studies, myosin isolated as M\textsuperscript{10S}. ADP-P\textsubscript{i} slowly assembled in the absence of F-actin (1, 7). The resulting thick filaments, measuring 20 nm in diameter and 0.3 μM in length, were similar in morphology to those formed by dilution of 6S myosin into assembly buffer (Fig. 4A).
When F-actin was present, all myosin thick filaments were incorporated into actomyosin fibers (Fig. 4E). No free myosin thick filaments were apparent at any stage, suggesting that myosin assembly and fiber formation were coincident events. The coaligned myosin thick filaments embedded in actin bundles were indistinguishable from those formed from $M_{105}$-ADP-P$_i$ (Fig. 5C). The lengths of thick filaments in actomyosin fibers were similar to those assembled in the absence of F-actin, but the diameter was smaller, uniformly measuring 11 nm. It is important to note that mixtures of preformed myosin thick filaments and F-actin did not form actomyosin fibers, indicating that fiber formation required myosin assembly among actin filaments.

Fig. 7 compares the light scattering increase associated with $M_{105}$-ADP-P$_i$ myosin assembly in the absence of F-actin (Fig. 7A) with that accompanying actomyosin fiber formation in the presence of a fourfold molar excess of F-actin (Fig. 7B). In the absence of F-actin, following a short lag, light scattering gradually increased to an equilibrium value (Fig. 7A). The $t_{1/2}$, $\sim$50 min, was in good agreement with values reported by Applegate (1) and by Cross et al. (7). The previous studies demonstrated a close correspondence between myosin assembly and slow release of hydrolysis products from $M_{105}$-ADP-P$_i$ (1, 7). The magnitude of light scattering increase due to actomyosin fiber formation was $\sim$15-fold larger (Fig. 7B). No lag phase was apparent and the $t_{1/2}$, $\sim$25 min, was reduced twofold. The apparent acceleration of myosin assembly by F-actin was consistent with the observation that the fraction of myosin which sedimented after airfuge centrifugation was elevated in the presence of F-actin (data not shown).

Millimolar MgATP caused rapid disassembly of myosin thick filaments and actomyosin fibers (Fig. 7A and B, open arrows). Greater than 95% of myosin remained in the supernatant after airfuge centrifugation, indicating disassembly of myosin filaments as a consequence of MgATP-induced transition to the 10S conformation. EM of actomyosin samples after ATP addition revealed individual actin filaments and monomeric myosin, signifying concomitant disassembly of thick filaments and actomyosin fibers (data not shown). Consequently, in the presence of physiological concentrations of MgATP additional factors or myosin modification are required for stable actomyosin fiber formation.

**Actomyosin Fiber Formation Induced by P-Light Chain Phosphorylation**

Because P-light chain phosphorylation promotes smooth muscle myosin assembly in millimolar MgATP (5, 16, 27, 36, ...
Figure 7. M105-ADP-P, myosin assembly in the presence and absence of F-actin. (A) Light scattering accompanying reassembly of myosin filaments in the absence of F-actin. M105-ADP-P, complex was added to assembly buffer to obtain a final [myosin] = 0.15 μM (filled arrow). After assembly, 1 mM MgATP was added (arrow) resulting in an immediate drop in scattering intensity to baseline value for disassembled myosin. (B) Light scattering increase due to actomyosin fiber formation. M105-ADP-P, complex was added to F-actin in assembly buffer to obtain final concentrations of 0.15 μM myosin and 0.6 μM F-actin (filled arrow). After actomyosin fiber formation, addition of 1 mM MgATP (open arrow) caused immediate disassembly of fibers.

38, 39), it was of prime interest to document the effects of phosphorylation on formation of actomyosin fibers. Addition of myosin light chain kinase to samples of nonphosphorylated myosin in assembly buffer containing MgATP, calcium, and calmodulin, caused progressive light scattering increases in both the presence and absence of F-actin (Fig. 8 A). Concomitant phosphorylation of myosin P-light chain was confirmed by autoradiography of samples taken from parallel experiments employing [γ-32P]-ATP.

As expected, light scattering increases in the absence of F-actin reflected assembly of myosin thick filaments. Phosphorylated myosin thick filaments were uniform in diameter (21 nm) and length (0.6 μm) (Fig. 8 B). When actin was present, phosphorylation of myosin P-light chains was accompanied by formation of actomyosin fibers containing myosin thick filaments (Fig. 8 C). No free myosin thick filaments were observed (Fig. 8 C). ATPase assays indicated that <20% of ATP was depleted over the time course of fiber formation shown in Fig. 8 A. Thus, in millimolar concentrations of MgATP, progressive P-light chain phosphorylation initiated spontaneous formation of actomyosin fibers.

Extension of the phosphorylation reaction beyond 40 min resulted in erratic changes in light scattering signals, indicative of formation of contracted actomyosin complexes. EM confirmed formation of dense foci of contracted actomyosin networks (data not shown). Thus, P-light chain phosphorylation appeared to be sufficient to reverse the MgATP-induced disassembly of fibers containing nonphosphorylated myosin, and to elicit spontaneous assembly of actomyosin fibers capable of contraction.

Discussion

The major findings of this study are that smooth muscle 10S myosin can be induced to assemble by F-actin, and that actin-mediated assembly results in spontaneous formation of actomyosin fibers. Myosin assembly in the midst of actin filament networks therefore appears sufficient to create actomyosin fibers. Actin filaments presumably act as polymer templates for myosin thick filament assembly, resulting in bundles containing coaligned F-actin and myosin thick filaments. Actomyosin fibers were similar in morphology to fibers formed during actin-mediated assembly of Dictyos-
s telium myosin II (21), although the assembly properties of the two types of myosin differ in the absence of actin. Unlike smooth muscle myosin, heavy chain phosphorylation can regulate assembly of Dictyostelium myosin II (25, 26, reviewed in ref. 17) and Dictyostelium myosin II does not assume a stable folded monomeric conformation upon trapping of nucleotides at the active site. Nevertheless, in the presence of F-actin, assembly of both Dictyostelium and smooth muscle myosin filaments can proceed by association of unassembled myosin with actin filaments, forming actomyosin fibers (21).

The influence of F-actin on smooth muscle myosin assembly can be understood within the context of the dynamic equilibrium between the folded 10S myosin monomer and the assembly-competent, extended 6S conformation. For example, active site trapping of AMPPNP stabilizes the 10S conformation, which accounts for AMPPNP inhibition of nonphosphorylated myosin assembly in the absence of F-actin. At any given time, a fraction of myosin unfolds to the 6S conformation, permitting AMPPNP exchange and exposing actin binding sites. Actin binding weakens the affinity of AMPPNP for 6S myosin by at least two orders of magnitude (13), and release of nucleotide accompanying actin binding would prevent reconversion to a stable 10S monomeric conformation. In effect, actin binding "captures" the assembly-competent, extended 6S conformation resulting in the observed actin-promoted reassembly of myosin filaments. Interactions between myosin molecules bound to adjacent actin filaments would cause progressive bundling of actin filaments resulting in actomyosin fiber formation.

A similar scenario accounts for formation of actomyosin fibers of coalesced actin and myosin filaments upon addition of M₆S-ADP-P, to F-actin. Transition of myosin molecules to the 6S conformation permits release of ATP hydrolysis products, as well as actin binding. Even in the absence of actin, release of P_i renders myosin assembly competent (1, 7). The strong affinity of the resulting M₆S-ADP-P for actin (10) explains assembly of myosin filaments exclusively along and between actin filaments. In the proposed scheme, a shift in the 10S to 6S equilibrium resulting from weak binding between M₆S-ADP-P and actin (11, 28, 30) would account for actin-induced acceleration of myosin assembly.

Actomyosin fibers formed in the presence of AMPPNP appeared to partially unravel in the presence of low concentrations of severin, an actin binding protein isolated from Dictyostelium discoideum (2, 3, 41). This is consistent with the ability of severin to "sever" actin filaments along their lengths (3, 41). Extensive fragmentation of actin filaments by higher concentrations of severin caused complete disruption of actomyosin fibers, including thorough disassembly of myosin thick filaments. Reduction in F-actin concentration due to severin-induced disassembly of actin (41) was apparently sufficient to allow reconversion to the stable 10S myosin conformation, leading to disassembly of myosin thick filaments. This surprising observation points to a potentially important regulatory mechanism utilizing the assembly state of actin to simultaneously control myosin thick filament and actomyosin fiber formation.

The major regulatory mechanism of smooth muscle myosin assembly is thought to be phosphorylation of P-light chains of myosin (5, 16, 27, 36, 38, 39). We demonstrate here that P-light chain phosphorylation can also govern actomyosin fiber formation in vitro. Actomyosin fibers containing nonphosphorylation myosin filaments were rapidly disassembled by MgATP. ATP weakens the affinity of 6S myosin for actin by several orders of magnitude (11), effectively abolishing the influence of F-actin on the 6S–10S equilibrium. Phosphorylation of P-light chains of myosin, which shifts the equilibrium in favor of the 6S conformation (5, 16, 27, 36, 38, 39), drove spontaneous formation of actomyosin fibers in the presence of MgATP. A priori, phosphorylation-induced stimulation of the actin activated MgATPase of smooth muscle myosin, and resulting sliding of assembling myosin thick filaments might have been expected to disrupt fibers during initial assembly. However, fiber formation preceded substantial actin activation of the MgATPase rate and consequent contraction. Previous studies have intimated that phosphorylation of a single head is sufficient to promote filament assembly in the presence of MgATP (37), but both myosin heads must be phosphorylated before either head can be activated by actin (14, 24, 31). We hypothesize that actomyosin fiber formation is triggered by singly phosphorylated myosin molecules, with the onset of contraction requiring a threshold of doubly phosphorylated myosin. Work now in progress, aimed at correlating degree of phosphorylation with various stages of actomyosin fiber formation and subsequent contraction, should provide greater insight into the mechanism of phosphorylation-induced formation of actomyosin fibers.

The potential implications of these results for formation of contractile actomyosin fibers in smooth muscle and nonmuscle cells are highly intriguing. Lamb and co-workers (9, 19) have recently suggested a link between the integrity of actin bundles and the state of myosin light chain phosphorylation in fibroblasts. Specifically, dephosphorylation of myosin P-light chains elicited by microinjection of either the catalytic subunit of protein kinase A or antibodies to myosin light chain kinase resulted in disassembly of actin bundles in stress fibers (9, 19). A mechanistic interpretation of these results is provided by the demonstration here of spontaneous reconstitution of actomyosin fibers in vitro. In the presence of millimolar MgATP, fibers containing nonphosphorylated myosin disassembled, but spontaneously reformed upon P-light chain phosphorylation. Hence, regulated assembly/disassembly of smooth muscle myosin in the midst of actin networks appears sufficient to induce reversible formation of contractile actomyosin fibers in vitro. Consequently, reversible formation of intracellular contractile actomyosin fibrils might well be explained by a simple interaction between actin and myosin, elicited by light chain phosphorylation.

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