Monoclonal Antibodies Binding to the Tail of *Dictyostelium discoideum* Myosin: Their Effects on Antiparallel and Parallel Assembly and Actin-activated ATPase Activity

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Abstract. Eight monoclonal antibodies that bind to specific sites on the tail of *Dictyostelium discoideum* myosin were tested for their effects on polymerization and ATPase activity. Two antibodies that bind close to the myosin heads inhibited actin activation of the ATPase either partially or completely, without having an effect on polymerization. Two other antibodies bind to sites within the distal portion of the tail that has been shown, by cleavage mapping, to be important for polymerization. One of these antibodies binds close to the sites of heavy chain phosphorylation which is known to regulate both myosin polymerization and actin-activated ATPase activity. Both antibodies showed strong inhibition of polymerization accompanied by complete inhibition of the actin-activated ATPase activity.

A unique effect was obtained with an antibody that binds to the end of the myosin tail. This antibody prevented the formation of bipolar filaments. It caused myosin to assemble into unipolar filaments with heads at one end and the antibody molecules at the other. Only at concentrations higher than required for its effect on polymerization did this antibody show substantial inhibition of the actin-activated ATPase. These results indicate that, using a monoclonal antibody as a blocking agent, parallel assembly of myosin can be dissected out from antiparallel association, and that essentially normal actin-activated ATPase activity could be obtained after significant reductions in filament size.

During cell aggregation of *Dictyostelium discoideum*, amebas respond chemotactically to gradients of cAMP which guide them towards aggregation centers. They respond within seconds to a local stimulus of the attractant by extending pseudopods (Gerisch et al., 1975; Swanson and Taylor, 1982). In response to a sudden increase in cAMP concentration, amebas change their shape with a contraction-like cringe (Futrelle et al., 1982). Amebas also modulate their net speed of movement when exposed to temporal decreases or increases in cAMP concentrations (Varnum et al., 1985).

Myosin has been implicated to function in these cAMP-induced shape changes and movements of *D. discoideum* amebas because it undergoes changes in distribution and in its state of phosphorylation after a chemotactic stimulus. When cells round up with a cringe, myosin that appeared to be filamentous and located throughout the cytoplasm, redistributes to beneath the membrane (Yumura and Fukui, 1985). Myosin seems to be excluded from pseudopods (Rubino et al., 1984) and concentrated at the rear of cells that are migrating up a concentration gradient of chemoattractant (Yumura et al., 1984).

When a pulse of cAMP is given to cells in suspension, threonine residues on the heavy chains of myosin are first slightly dephosphorylated and then rephosphorylated (Rahmsdorf et al., 1978; Malchow et al., 1981; Maruta et al., 1983; Berlot et al., 1985) and the light chains are phosphorylated (Berlot et al., 1985). Heavy chain phosphorylation has two effects on myosin functions in vitro. It reduces the capability of myosin to polymerize (Kuczmarski and Spudich, 1980) and lowers actin-activated ATPase activity (Kuczmarski and Spudich, 1980; Maruta et al., 1983).

The relationships between heavy chain phosphorylation, polymerization, and actin-activated ATPase activity of myosin have also been studied in *Acanthamoeba*. When *Acanthamoeba* myosin II is phosphorylated at the end of its tail, the actin-activated ATPase activity is inhibited (Collins et al., 1982*a*, *b*). Experiments using monoclonal antibodies (Kiehart and Pollard, 1984*a*) and mild proteolytic digestion (Kuznicki et al., 1985) indicated that tail-bearing myosin II must be filamentous to show actin-activated enzyme activity. However, phosphorylation does not simply inhibit actin-activated ATPase activity by preventing filament formation; phosphorylated myosin II still can polymerize, although the filaments formed are smaller than those of dephosphorylated myosin (Pollard, 1982; Collins et al., 1982*b*).

Using a threonine-specific myosin heavy chain kinase from aggregation competent *D. discoideum* cells (Maruta et al., 1983) and monoclonal antibodies whose binding sites on the myosin tail had been mapped by electron microscopy

(Claviez et al., 1982), we showed that heavy chain phosphorylation sites are located ~150 nm along the length of the 188-nm tail away from the heads and adjacent to a region of the molecule that is important for polymerization of chymotryptic fragments (Pagh et al., 1984). This result suggested that, in *D. discoideum*, heavy chain phosphorylation directly affects the assembly state of myosin. Spudich and co-workers identified three monoclonal antibodies each of which binds to the tail of *D. discoideum* myosin and inhibits one of three myosin functions assayed: polymerization, actin-activation of ATPase activity, or motility of myosin-coated beads (Peltz et al., 1985; Flicker et al., 1985). Here we compare the effects on myosin polymerization and actin-activated ATPase activity of eight monoclonal antibodies that bind at defined sites along the tail. A unique effect was found with an antibody that binds to the end of the tail. This antibody completely blocked antiparallel myosin assembly as it normally occurs in the middle region of a filament. The small unipolar filaments that formed could exhibit substantial actin-activated ATPase activity.

Materials and Methods

Protein Purification

D. discoideum myosin was purified from aggregation-competent cells as was described by Maruta et al. (1983), using an extraction buffer containing 30 mM Tris-HCl, pH 7.5, 30% sucrose, 0.2 mM ATP, 2 mM EGTA, 1 mM dithiothreitol, 0.02% NaN_3, 10 mM benzamidine, and 0.1 mM phenylmethylsulfonyl fluoride, or one in which ATP was at 0.5 mM and EGTA was replaced by 2 mM EDTA. Purified myosin was stored on ice and used within 3 wk after purification for the ATPase assays. The phosphate content of myosin from aggregation-competent cells was shown by Maruta et al. (1983) to be negligible. Contamination of purified myosin with RNA, which has been shown to affect its polymerization (Stewart and Spudich, 1979), was measured spectrophotometrically and found to be undetectable. In addition, RNase treatment according to Stewart and Spudich (1979) had no effect on myosin polymerization as is described here.

F-actin was prepared from rabbit skeletal muscle (Schleicher et al., 1984) and was generously provided to us by Dr. G. Isenberg.

Monoclonal antibodies, designated as mAb 14-26-5, 21-32-3, 21-51-3, 15-153-33, and 21-96-3, were produced against *D. discoideum* whole myosin (Claviez et al., 1982). mAb 29-55-4 was produced against a chymotryptic tail fragment, while mAb 40-253-6 and mAb 56-396-5 were obtained by screening hybridomas from mice immunized with plasma membrane fractions. In this paper, the antibodies are referred to as mAb 26, 32, 51, 153, 96, 55, 253, and 396, respectively. The monoclonal antibodies were purified from hybridoma supernatants on protein A-Sepharose as described by Claviez et al. (1982) and stored on ice in 10 mM Tris-HCl, pH 7.5, 15% sucrose, 50 mM KCl, and 0.02% NaN_3.

ATPase and Sedimentation Assays

Monomeric myosin was preincubated with monoclonal antibody for 2–4 h on ice in high-salt buffer containing 500 mM KCl, 10 mM Tris-HCl, pH 7.5, 0.02% NaN_3, and 1 mM dithiothreitol. For preincubation of preformed myosin filaments with antibody, monomeric myosin was first induced to polymerize by dilution into low-salt buffer containing 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM dithiothreitol, and 0.02% NaN_3 for 10 min on ice. Subsequently, antibody was added for 4–6 h of preincubation on ice. Protein concentrations were determined according to Lowry for myosin, and by absorption at 280 nm for antibody assuming an extinction coefficient of 1.5. Typically, myosin was at 1 mg/ml and antibody was at 1.5–2 mg/ml during preincubations.

After preincubation of either monomeric myosin or filaments with antibody, the mixtures were diluted 6–10-fold to final concentrations of 40–50 μg myosin/ml with polymerization buffer containing 10 mM Tris-HCl, pH 7.5, 0.2 mM ATP and, unless specified otherwise, 10 mM $MgCl_2$ diluted from a 4.8 M stock solution as supplied by Sigma Chemical Co. (St. Louis, MO). KCl was adjusted to a final concentration of 50 mM. ATPase assays and sedimentation analyses were carried out after 1 h on ice.

ATPase activity was measured by the release of ^{32}P inorganic phosphate according to Pollard and Korn (1973). Assays were carried out at 25°C, with 20 μg of myosin in polymerization buffer. To measure actin-activated ATPase activity, 1.5 μg of F-actin was added per 2.0 μg of myosin immediately before assay. For the preparation of F-actin, G-actin from rabbit skeletal muscle was freshly polymerized at room temperature for 1 h by adding 1 mM $MgCl_2$ and 50 mM KCl.

For sedimentation assays, samples in polymerization buffer were centrifuged in a Beckman airfuge (Beckman Instruments, Inc., Palo Alto, CA) and processed for SDS PAGE or fixed with glutaraldehyde for electron microscopy.

SDS Gel Electrophoresis

SDS PAGE of total myosin and fractions was carried out using mini-slab gels with 10% polyacrylamide as described by Schleicher et al. (1984). Densitometry of bands stained with Coomassie Blue R-250 was performed with a Gel Scanner (Camag, Mutteuz, Switzerland). Reliability of the method was confirmed by dot blotting of myosin on nitrocellulose with ^{125}I-labeled mAb 396 according to Stadler et al. (1982).

Electron Microscopy

For negative staining, samples were fixed with 0.025% glutaraldehyde for 1 h on ice if not stated otherwise. This mild treatment prevented myosin filaments from dissociating. Glutaraldehyde concentrations of 0.25% seemed to clump filaments at the myosin concentrations routinely used.

Negative staining was carried out according to Pollard (1982). 5-μl aliquots containing 50 μg/ml of myosin alone or together with antibody were stained with 1% uranyl acetate in water. Carbon films were routinely made hydrophilic as described by Trinick and Elliott (1982).

Rotary-shadowed preparations were made by diluting 7 μl of either unfixed or glutaraldehyde-fixed sample with 7 μl of glycerol. The mixture was sprayed immediately onto freshly cleaved mica. Rotary shadowing, electron microscopy, estimates of dissociation constants, and computer analyses of antibody binding positions were performed essentially as described by Claviez et al. (1982). Magnifications were calculated using catalase crystals photographed at a known objective current. The dimensions of negative-stained filaments were measured on prints magnified to ~100,000×.

Immunogold Labeling

The location of antibodies that labeled myosin filaments was determined with 20-nm gold particles coated with goat anti-mouse antibody (Janssen Pharmaceutica, Beerse, Belgium). The commercial stock solution was centrifuged, resuspended in an equal volume of polymerization buffer containing 50 mM KCl or of 50 mM ammonium formate, and diluted directly into a suspension of myosin filaments that had been formed in the presence of antibody. Incubations with immunogold were carried out for 1 h on ice. The best labeling was achieved when filaments were unfixed and freed of unbound antibody by ultracentrifugation. Samples were applied to carbon-coated electron microscopic grids and washed extensively with water before negative staining.

Results

Identification of Monoclonal Antibodies That Interfere with Myosin Polymerization

To study the polymerization of *D. discoideum* myosin, we used eight out of 45 isolated monoclonal antibodies. These selected antibodies could be shown by electron microscopy to bind to unique sites on the tail of myosin monomers. The binding sites of five of these antibodies have been mapped previously (Claviez et al., 1982). Those of mAb 396, mAb 253, and mAb 55 are shown in Fig. 1, *a–f*. The antibody binding sites are located within diverse regions of the myosin tail, between its proximal end near to the heads and its terminus (Fig. 1 *g*). None of these antibodies competed with one another in solid-phase radioimmunoassay. One antibody, mAb 96, binds near to the sites phosphorylated by a myosin

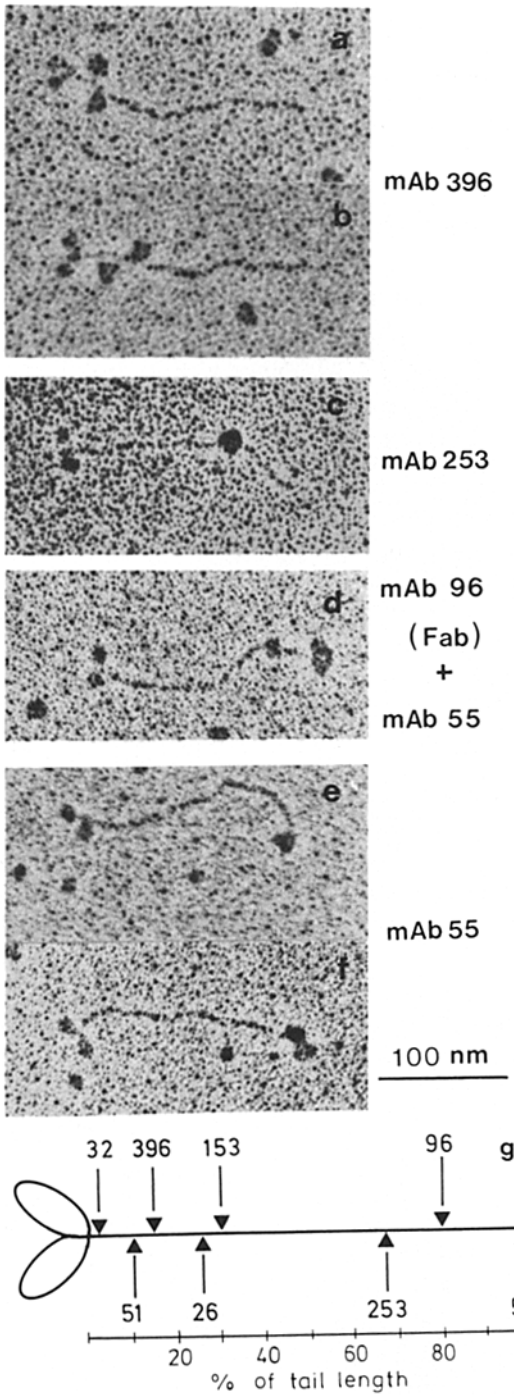

Figure 1. Binding sites of monoclonal antibodies on the tail of *D. discoideum* myosin. Complexes of monomeric myosin and antibody were rotary-shadowed (*a–f*). The map of binding sites shown in *g* has been compiled from previously published (Claviez et al., 1982; Pagh et al., 1984) as well as new data. A new antibody, mAb 396, bound simultaneously to two sites at the same position of the myosin tail (*a* and *b*), indicating that identical epitopes on the two heavy chains of the myosin molecule were accessible. These sites were located 14.4 ± 5.4% of the length of the tail away from the heads. The binding sites of three other antibodies important for this paper are shown in *c–f*. MAb 96 and mAb 55 were distinguishable after double labeling with Fab fragments and IgG, respectively (*d*). MAb 55 bound to the very end of the tail (*e*), and binding sites on both myosin heavy chains were simultaneously accessible (*f*).

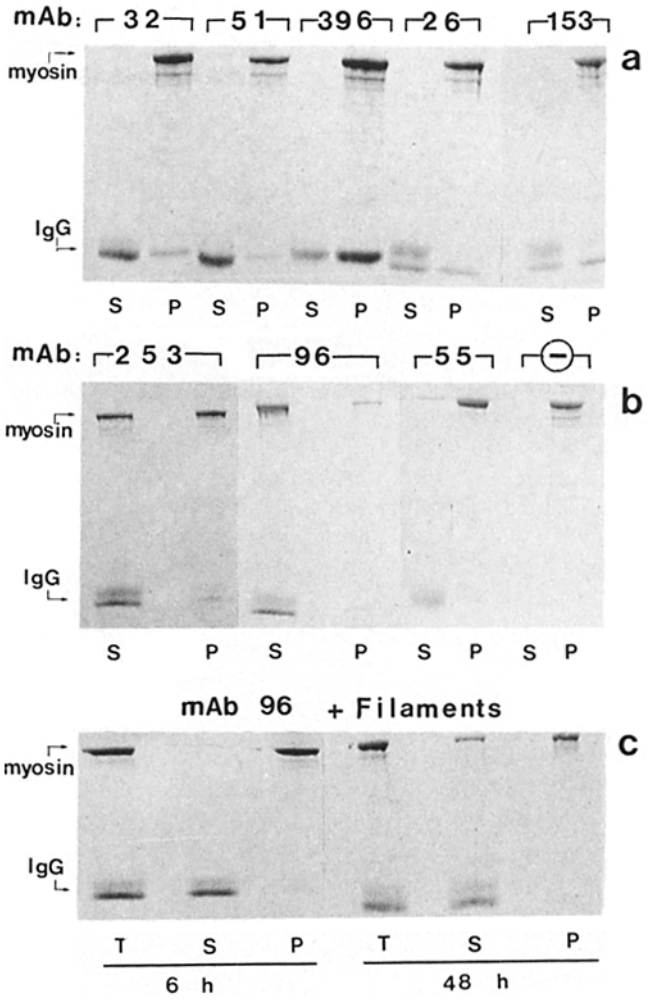

Figure 2. Sedimentation of myosin preincubated with antibodies. Monomers (*a* and *b*) were preincubated with or without (−) antibodies in high-salt buffer. Filaments (*c*) were preincubated with the antibody in low-salt buffer. The samples were then diluted into polymerization buffer. After incubation for 1 h (*a* and *b*) or 6 and 48 h (*c*), they were ultracentrifuged for 15 min at 30 psi in an airfuge. Supernatant (*S*) and pellet (*P*) fractions were subjected to SDS PAGE in minislab gels and the proteins stained with Coomassie Blue. Control samples in *c* for total myosin and antibody (*T*) were not ultracentrifuged. The upper major band is that of the heavy chains of myosin and the lower ones correspond to the heavy chains of the IgG antibody. MAb 396 was poorly soluble under the conditions used; it was the only antibody that pelleted by forming a precipitate, in addition to sedimenting with myosin.

heavy chain kinase (Pagh et al., 1984). Its binding site relative to mAb 55 was demonstrated in a double labeling experiment in which mAb 96 was used as a Fab fragment (Fig. 1 *d*).

The effects of antibodies on myosin polymerization were assessed by sedimentation experiments. Monomeric myosin in a buffer containing 500 mM KCl was preincubated with a fivefold molar excess of antibody, and then induced to polymerize by dilution into a low-salt, high Mg^{2+} buffer ("polymerization buffer") containing final concentrations of 50 mM KCl, 10 mM $MgCl_2$, and 0.2 mM ATP. After ultracentrifugation in an airfuge at 30 psi for 15 min, nearly all of the control myosin was found in the pelleted fraction (Fig. 2). Myosin preincubated with antibody sedimented as in the control

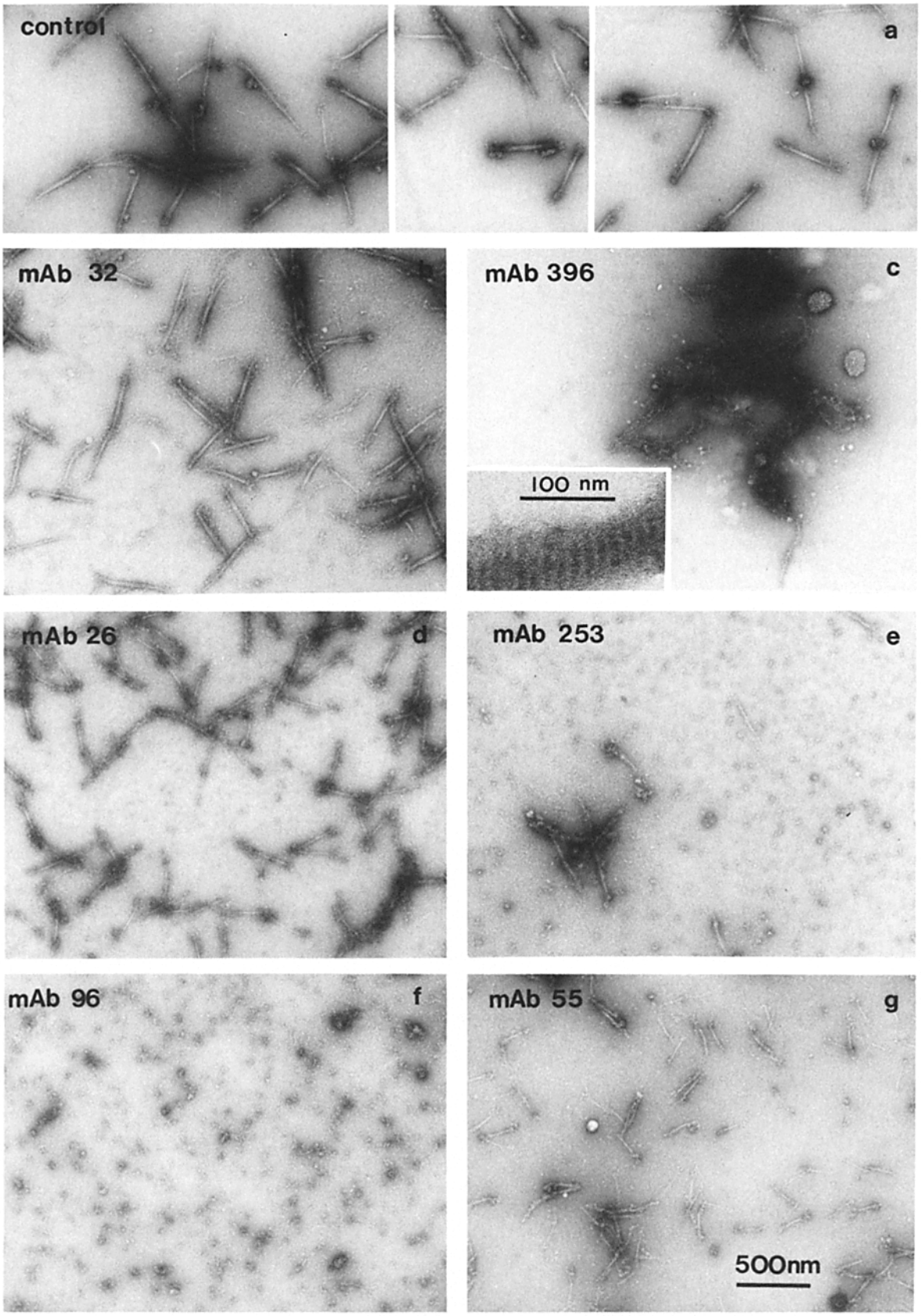

Figure 3. Negative stain preparations of myosin preincubated with a fivefold molar excess of antibodies and induced to polymerize in their presence (*b–g*). In the control (*a*), no antibody was added. After 1 h in the polymerization buffer, samples were fixed with glutaraldehyde and stained with 1% uranyl acetate. The inset in *c* illustrates the enhanced 14.5-nm axial periodicity of preformed filaments after incubation with mAb 396.

in all cases (Fig. 2 *a*) except for three antibodies. About 50 and 96% of the myosin remained in the supernatant with mAb 253 and mAb 96, respectively (Fig. 2 *b*). About 10% of the myosin preincubated with mAb 55 remained in the supernatant fraction. When ultracentrifugation was carried out at 25 psi for 15 min, up to 40% of the myosin preincubated with mAb 55 was found in the supernatant, indicating that the filaments were smaller than in controls, in which only trace amounts of myosin remained in the supernatant under these conditions.

In contrast to their strong effects on the polymerization of monomeric myosin, neither mAb 55 nor mAb 96 disassembled preformed filaments at a fivefold molar excess over myosin. Even after 48 h of incubation with mAb 96, sedimentation analysis showed very little myosin in the supernatant fraction (Fig. 2 *c*).

Effects of Antibodies on the Size and Structure of Myosin Filaments

In negative stain preparations, control myosin formed bipolar filaments (Fig. 3 *a*) that averaged 512 nm in length and formed a bare zone of ~220 nm (Table I), as has been described by Clarke and Spudich (1974). Filaments with sizes resembling the control were found when myosin polymerized in the presence of antibodies that bound nearest to the heads: mAb 32 (Fig. 3 *b*; Table I) and mAb 51. Because mAb 32 co-sedimented significantly with myosin filaments (Fig. 2 *a*), its failure to interfere with polymerization could not be due to a weak affinity for myosin.

With mAb 396, a third antibody that binds relatively near to the heads, the filamentous material that formed had prominent lateral striations with a repeat spacing of 14.5–15.0 nm (Fig. 3 *c*). This is the normal staggering of myosin molecules in filaments (Harrington and Rodgers, 1984). When preformed filaments were incubated with mAb 396, they remained intact and the striations were more clearly seen (Fig. 3 *c*, inset).

Filaments, which were smaller than in the control, formed in the presence of the two antibodies, mAb 26 and mAb 153, that bind one-third of the length of the tail from the heads (Fig. 3 *d*). Filament lengths with mAb 26 averaged 360 nm, which is comparable in size to the filaments formed in controls when $MgCl_2$ was only 0.2 mM, rather than 10 mM (Table I). The "apparent bare zone", i.e., the zone that was free of heads and antibodies, was less than the 188-nm tail length because the antibodies remained bound to the filamentous myosin.

The three antibodies with binding sites within the terminal third of the tail had striking inhibitory effects on myosin polymerization (Fig. 3, *e–g*). Only few filaments were formed in the presence of mAb 253 (Fig. 3 *e*). This antibody also disassembled preformed filaments, as judged from a reduction in the number of filaments in negative stain preparations after 4 h of incubation with a fivefold molar excess of the antibody. With mAb 96, essentially no filaments were observed in negative stain preparations (Fig. 3 *f*). As revealed by rotary shadowing, ~85% of the monomeric myosin molecules had bound mAb 96. The remaining monomers may not have formed filaments because they were below a critical concentration required for polymerization. Essentially no dimers or oligomers of myosin were seen, indicating that mAb 96 inhibits the primary steps required for myosin assembly. With mAb 55, an antibody that binds to the tip of the myosin tail, numerous myosin filaments were visible (Fig. 3 *g*). However, these filaments measured only ~230 nm in length, with an apparent bare zone of 117 nm (Table I), suggesting that they were unusual in structure.

Table I. Sizes of Myosin Filaments Formed at a Fivefold Molar Excess of Antibodies

mAb	Mg^{2+} (mM)	Length (nm)	Apparent bare zone (nm)
None	10	512 ± 58	221 ± 24
32	10	441 ± 69	202 ± 25
26	10	360 ± 34	154 ± 19
55	10	233 ± 20	117 ± 17
None	0.2	360 ± 30	208 ± 21

Myosin was preincubated with one of the antibodies or with buffer alone. Polymerization was induced by dilution into polymerization buffer with $MgCl_2$ concentrations as indicated. The reaction was stopped after 1 h by the addition of glutaraldehyde. Means and standard deviations are given.

Myosin Forms Small Unipolar Filaments in the Presence of mAb 55 That Binds to the Tip of the Tail

In rotary-shadowed preparations of control samples, the heads of myosin molecules were recognizable at both ends of a filament (Fig. 4, *a* and *b*). In contrast to these bipolar filaments, the small filaments formed after incubation with mAb 55 had heads clearly visible at only one end (Fig. 4, *c–e*). At the opposite end were globular particles somewhat larger than myosin heads, which were comparable in size to unbound antibody molecules seen in the background. In some cases, they appeared to be staggered with the periodic 14–15-nm repeat of myosin filaments (Fig. 4 *c*), indicating that the structures observed were not produced by cross-linkage of myosin molecules with the antibody. The number of pairs of heads in these filaments varied between 6 and 12.

Evidence that the globular structures seen at one end of the small filaments were antibody molecules was provided by immunogold labeling. The labeling patterns obtained with various dilutions of gold-conjugated anti-mouse antibody are given in Table II and illustrated in Fig. 5, *a–g*. Most of the labeling was unipolar, i.e., gold particles were located at only one end of the filament. At the highest concentrations of the gold-conjugated antibody used, 90% of all filaments were labeled, and in 90% of these, labeling was unipolar. The remaining 10% of filaments were labeled at both ends although the number of gold particles was always minor at one of these ends (Fig. 5 *d*). Few filaments were associated with gold particles when preformed filaments were incubated with anti-mouse antiserum alone. Association of gold particles with myosin filaments, when it was observed in these controls, occurred with equal probability either somewhere along the bare zone, or at either of the two filament ends (Table II), indicating the absence of any localized labeling without first antibody.

The unipolar organization of filaments formed in the presence of mAb 55 was striking when the filaments had aggregated by their heads into clusters of three or more (Fig. 5, *f* and *g*). Filaments formed in the presence of mAb 32, an antibody that did not alter polymerization, gave a bipolar

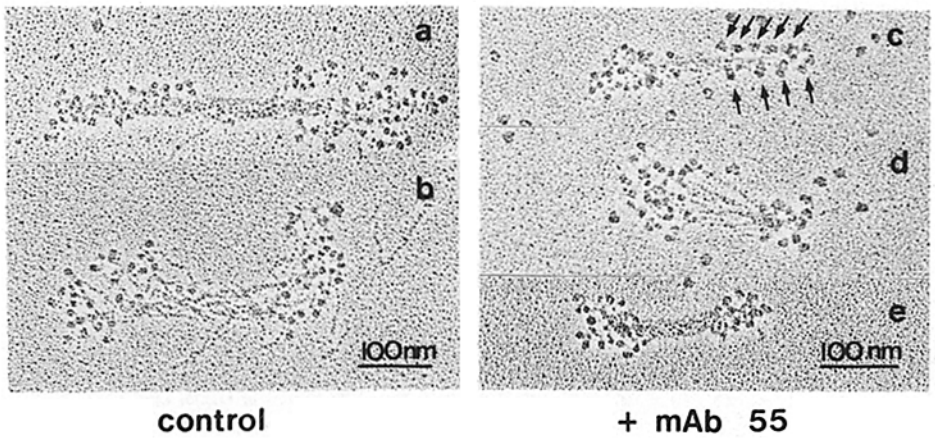

Figure 4. Rotary-shadowed filaments formed by myosin in controls without antibody (*a–b*) and in preparations with mAb 55 (*c–e*). Monomeric myosin was induced to polymerize for 1 h after preincubations without or with a fivefold molar excess of antibody. To reduce background, $MgCl_2$ and ATP were left out of the polymerization buffer for electron microscopy. In *a*, *c*, and *e*, samples were fixed with glutaraldehyde, in *b* and *d* samples were unfixed. In *e*, filaments were sedimented in an airfuge at 30 psi for 15 min before fixation to remove unbound antibody. The periodic substructure of filaments formed in the presence of mAb 55 is indicated by arrows in *c*.

labeling pattern (Fig. 5, *h–j*; Table II), and they aggregated into chains rather than into clusters (Fig. 5 *h*). Even the smaller filaments formed in the presence of mAb 26 were found to be bipolar in labeling (Table II).

Effects of the Antibodies on Actin-activated MgATPase Activity Compared with Their Effects on Myosin Polymerization

Sensitivity of the actin-activated ATPase activity to antibodies was studied under the same conditions as used for polymerization of myosin except that F-actin was added to the polymerization buffer. The actin did not change the inhibitory effects of mAb 96 and mAb 55 on polymerization of the myosin. (For other antibodies this was not tested.) Enzyme activity was near maximal at pH 7.5 and 25°C in our standard polymerization buffer, which contained 10 mM $MgCl_2$ (Fig. 6).

In a first series of experiments, antibody was incubated in high-salt buffer at a fivefold molar excess with monomeric myosin and, after 1 h in the polymerization buffer, samples were assayed for actin-activated MgATPase activities. As shown in Table III (experiments I and II), all antibodies inhibited the enzyme activity to some extent. The degree of inhibition sometimes varied with the amount of F-actin added to the assay mixture. Three antibodies were distinguished from the others by their strong inhibitory effects. Two of them, mAb 253 and mAb 96, bound within the distal portion of the tail of the myosin and were strong blockers of its polymerization (Figs. 2 *b* and 3, *e* and *f*). The third antibody, mAb 396, bound near to the heads and did not block polymerization (Figs. 2 *a* and 3 *c*).

When incubated with myosin filaments, only some of the antibodies showed similarly strong inhibition of the actin-activated ATPase activity as when monomeric myosin was applied (Table III, experiment 3). The two best blockers among these antibodies, mAb 32 and 396, did not inhibit the polymerization of myosin (Figs. 2 *a* and 3, *b* and *c*), although mAb 396 was found to be incorporated into preformed filaments (Fig. 3 *c*, insert). The lack of an effect of mAb 96 on the actin-activated ATPase activity of filaments was in agreement with the inability of this antibody to bind to polymerized myosin (Fig. 2 *c*). The finding that even after 24 h of incubation the ATPase was only weakly inhibited (Table III,

Table II. Localization of Monoclonal Antibodies Associated with Myosin Filaments by the Immunogold Labeling Method

First antibody (mAb)	Immunogold* (rel. concentration)	Labeling pattern‡			Filaments labeled§
		Unipolar	Bipolar	Other	
					%
55	1	92	2	6	<50
55	2	87	11	2	80
55	3.3	85	13	2	>90
55	5	90	8	2	>90
32	2	28	70	2	72
26	2	31	62	7	<50
None	2	47	4	49	<20

Monomeric myosin was incubated with a fivefold molar excess of monoclonal antibody and then induced to polymerize as described in Materials and Methods. For the experiments with mAb 55, filamentous myosin was centrifuged in an airfuge for 30 min at 30 psi. The resuspended filaments were fixed for 10 min with 0.025% glutaraldehyde, ultracentrifuged, and washed with 50 mM ammonium formate, pH 7.2. The fixation greatly reduced the reactivity with mAb 32 and mAb 26; therefore, for these antibodies the fixation step was omitted. We have confirmed that the unipolar labeling pattern obtained with mAb 55 also occurred with unfixed material.

* 20-nm gold particles coated with goat anti-mouse antibody were centrifuged, washed with 50 mM ammonium formate, pH 7.2, and resuspended in the original volume. Numbers denote relative concentrations with 1 representing 10% of the commercial preparation.

‡ "Unipolar" means that gold particles were at one end of a filament and "bipolar" corresponds to labeling at both ends. "Other" designates labeling within the bare zone of filaments. In each experiment, between 50 and 75 labeled filaments were characterized.

§ Percentage of filaments that were labeled with gold particles.

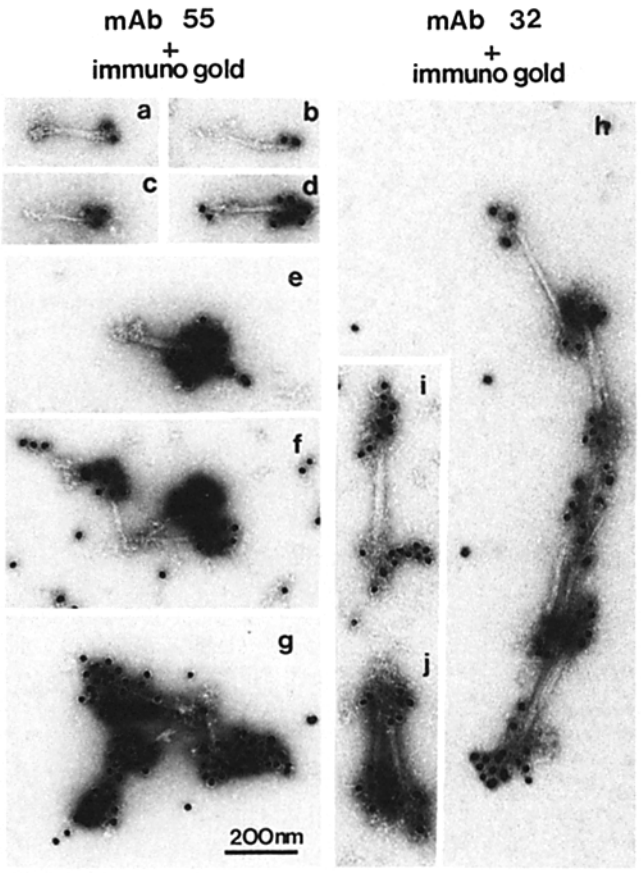

Figure 5. Localization by immunogold labeling of monoclonal antibodies on filaments. Monomeric myosin was induced to polymerize with a fivefold molar excess of either mAb 55 (*a–g*) or mAb 32 (*h–j*). After 1 h, specimens were fixed with glutaraldehyde, ultracentrifuged to remove unbound antibody, and reacted with gold anti-mouse antibody.

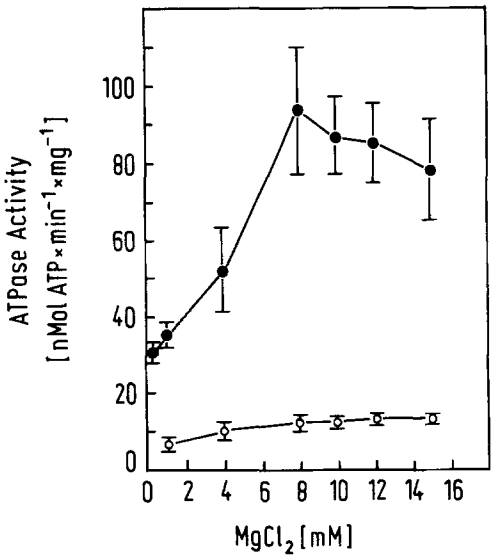

Figure 6. Myosin ATPase activity as a function of $MgCl_2$ concentrations. Basal (○) and actin-activated (●) ATPase activities were measured in polymerization buffer with varying concentrations of $MgCl_2$.

experiment 4) coincided with the low degree of depolymerization observed after even longer incubation of myosin filaments with mAb 96 (Fig. 2 *c*).

To eliminate differences in the inhibitory effects that are caused by varying affinities of the antibodies (Table IV), we have preincubated five antibodies at different molar ratios with monomeric myosin. 1 h after dilution into polymerization buffer, myosin was assayed for basal and actin-activated MgATPase activity, and, in parallel, samples were processed for SDS PAGE (Fig. 7).

Each of the five antibodies showed significant effects on either actin-activated ATPase activity or polymerization of myosin, or on both, at an equimolar concentration relative to myosin. Thus, differences between the effects of these antibodies on myosin were not simply caused by varying affinities. None of the antibodies had a significant effect on the basal MgATPase activity with the possible exception of mAb 96, which seemed to partially inhibit this enzyme activity. Over the entire concentration range examined, no effect of mAb 32 and mAb 396 on myosin polymerization was observed (Fig. 7, *a* and *b*). These antibodies differed in their inhibitory effect on the actin-activated ATPase, which reached a plateau at a level of ~70% inhibition with mAb

Table III. Actin-activated ATPase Activity of Myosin after Preincubation of Monomers (Exps. 1 and 2) or Filaments (Exps. 3 and 4) with Antibodies

	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
mAb	ATPase activity	% Inhibition	ATPase activity	% Inhibition	ATPase activity	% Inhibition	ATPase activity	% Inhibition
32	102 ± 25	49	56 ± 3	55	30 ± 1	77	39 ± 2	73
51	117 ± 16	42	82 ± 3	34	103 ± 5	20	ND	–
396	36 ± 2	82	14 ± 1	89	48 ± 3	63	50 ± 2	65
26	203 ± 9	0	72 ± 1	42	128 ± 4	0	147 ± 5	0
153	179 ± 22	11	56 ± 5	55	129 ± 10	0	ND	–
253	54 ± 5	73	23 ± 1	82	101 ± 3	21	87 ± 1	39
96	32 ± 6	84	14 ± 1	89	137 ± 5	0	128 ± 4	11
55	124 ± 9	38	40 ± 2	68	136 ± 4	0	154 ± 5	0
Control	200 ± 18	–	125 ± 8	–	128 ± 5	–	143 ± 3	–

After preincubation of myosin with a fivefold molar excess of antibody in high-salt buffer the samples were diluted sixfold into polymerization buffer. ATPase activities are expressed in nmoles ATP hydrolyzed per minute and milligram myosin. Means and standard deviations are given. In Exp. 1, 20 μg of myosin was supplemented with 45 μg of F-actin, which resulted in a 20-fold activation over the basal Mg^{2+} ATPase activity of the control. In Exp. 2, 15 μg of F-actin was added, resulting in a 10-fold activation over basal enzyme levels of the control. In Exps. 3 and 4, 20 μg of filamentous myosin was preincubated with a fivefold excess of antibodies, and 15 μg of F-actin was added before assay. In Exp. 3, samples were kept for 1 h and in Exp. 4, for 24 h in polymerization buffer before assay. ND, not determined.

Table IV. Synopsis of the Antibody–Myosin Interactions

No. of antibody	Binding site (% of tail length)	Estimates of K_D*	Inhibition of polymerization	Binding to preformed filaments	Inhibition of actin-activated ATPase‡		Specific properties of the antibody
					Starting with unpolymerized myosin	With preformed filaments	
32	3	$>4 \times 10^{-6}$ $<1 \times 10^{-5}$	−	+	+	++	
51	9	$\geq 3 \times 10^{-7}$ $<2 \times 10^{-6}$	−	ND	+	+	
396	14	ND	−	+	++	+++	Enhances periodic structure of filaments
26	27	ND	Partial	+	− to +	−	Reduce size of bipolar filaments
153	31	1.6×10^{-6}	Partial	+	− to +	−	
253	68	ND	Complete	+	++	+	Block myosin polymerization completely
96	81	$\sim 1 \times 10^{-7}$	Complete	−	++	−	
55	100	7×10^{-7}	Partial	−	+	−	Blocks antiparallel assembly

ND, not determined.
* K_D estimates were obtained by counting antibody-myosin complexes and free myosin after rotary shadowing in the electron microscope (Claviez et al., 1982). K_D was calculated for the interaction of a single binding site on the antibody IgG with a single epitope on the myosin tail. Upper and lower limits refer to cases in which one antibody molecule inhibited the binding of a second, identical one to a myosin molecule.
‡ −, inhibition <20%; +, inhibition 20–70%; ++, inhibition >70%. Variations observed with different concentrations of actin (Table III, Exps. 1 and 2) are included.

32, while mAb 396 inhibited completely. Both mAb 253 and mAb 96 inhibited polymerization and actin-activated ATPase completely at a threefold molar excess over myosin (Fig. 7, *c* and *d*). At lower antibody to myosin ratios, mAb 253 appeared to preferentially inhibit polymerization, while the actin-activated ATPase seemed to be more sensitive to mAb 96. Further work is required to confirm that these minor differences are significant.

The results obtained with mAb 55 are more complicated. The dissociation constant for the interaction of a single antigen binding site of this antibody with a corresponding epitope on one of the myosin heavy chains has been estimated as $K_D = 7 \times 10^{-7}$ M (Claviez, M., unpublished observations). As shown in Fig. 1, *d* and *f*, the ends of both heavy chains of a myosin molecule are accessible to antibody binding. It follows from these data that ~51% of the heavy chain ends should be occupied under our conditions when myosin is preincubated with antibody at a molar ratio of 2:1. At this ratio, mAb 55 appeared to exert its almost maximal effect on myosin polymerization. This is suggested from Fig. 7 *e* where the small decrease in pelleted myosin seen at this ratio remained at almost the same level with increasing antibody concentrations. Moreover, evaluation of the sizes of myosin filaments in negative stain preparations indicated that as little as one mAb 55 molecule per two myosin molecules limited the overall size of the filaments and blocked bipolar filament formation. Even after 24 h of incubation in polymerization buffer, virtually no filaments comparable in size to control filaments were visible. Filament lengths remained essentially constant with molar ratios of mAb 55 to myosin ranging from 0.5 to 8. These results indicate that mAb 55 inhibits bipolar filament formation and reduces the size of the unipolar filaments even if myosin is in excess, and that high excess of antibody still allows the unipolar filaments to be formed. Quite in contrast to the effect of mAb 55 on polymerization, its effect on the actin-activated ATPase, which was negligible at an antibody to myosin ratio of 0.5:1, increased steadily with increasing antibody concentrations (Fig. 7 *e*). Thus, as far as mAb 55 is concerned, the effect on polymerization was not accompanied by significant inhibition of actin-activated ATPase, and strong inhibition of the ATPase at higher antibody concentrations was not paralleled by further inhibition of polymerization.

Discussion

General Conclusions

Monoclonal antibodies with unique binding sites on the tail of *D. discoideum* were found to interfere with filament formation in three distinct ways; by (*a*) suppressing selectively the antiparallel association of myosin monomers, (*b*) inhibiting myosin–myosin polymerization completely, and (*c*) limiting the size but not the normal bipolar structure of filaments.

Two general statements can be made about the effects of antibodies on the actin-activated MgATPase activity of *D. discoideum* myosin. First, any antibody that blocked myosin assembly abolished actin activation, although not every antibody that inhibited actin activation of the ATPase had a concomitant effect on the assembly of myosin. Second, the actin-activated ATPase activity was only marginally affected by reduction in the size of myosin filaments from a length of 500 nm to ~230 nm, even when antiparallel assembly was eliminated. The effects of the eight antibodies examined are summarized in Table IV.

Selective Inhibition of Antiparallel Myosin Assembly

Myosin assembly is thought to start with the association of monomers into dimers and to proceed by antiparallel assem-

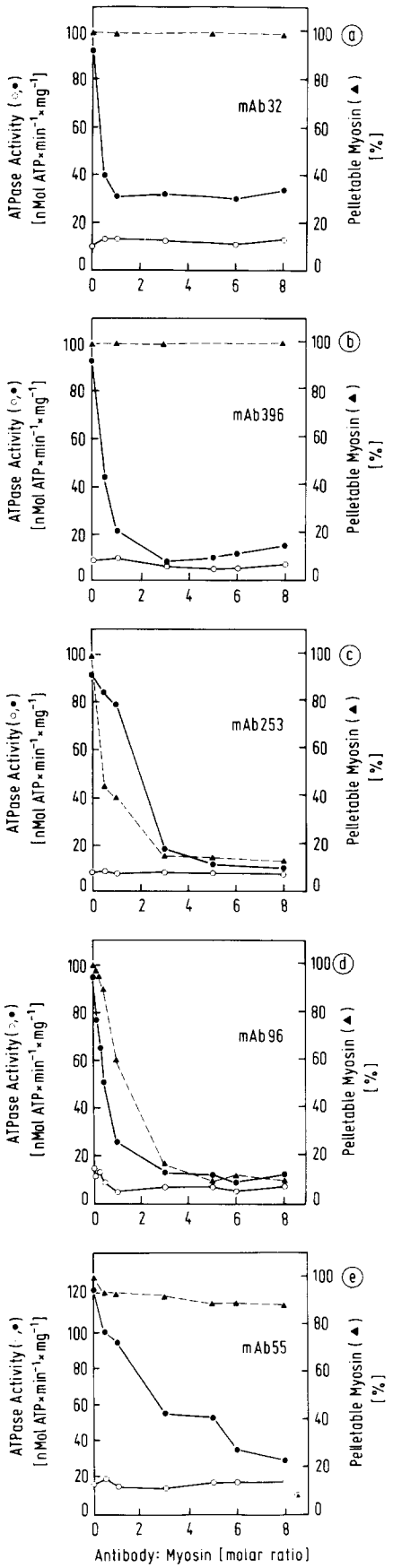

Figure 7. Basal (○) and actin-activated (●) ATPase activities and sedimentation after preincubation of myosin with antibodies at different ratios. Monomeric myosin was preincubated in high-salt buffer in the presence or absence of one of the five antibodies as indicated, and the mixtures were diluted 10-fold into polymerization buffer. After 1 h, ATPase activity was measured with or without the addition of F-actin. In parallel samples, without actin, the myosin heavy chain content was determined in total fractions, and in supernatants and pellets obtained by ultracentrifugation for 15 min at 30 psi in an airfuge. The fractions were subjected to SDS PAGE, Coomassie Blue staining, and densitometry, and the pelleted myosin expressed in percent of the total. In a control, showing that monomeric myosin was not precipitated by mAb 55, a sample was ultracentrifuged at the end of preincubation in high-salt buffer (▲ in *e*).

bly of the dimers. By this process, a bare zone rich in antiparallel interactions is produced (Harrington and Rodgers, 1984). Filaments subsequently increase in length by parallel dimer addition (Davis et al., 1982). One of our antibodies, mAb 55, which binds to the tip of the myosin tail, inhibited selectively all antiparallel associations. The unipolar filaments formed in the presence of mAb 55 seemed to have the typical 14.5-nm periodicity of parallel-packed molecules (Harrington and Rodgers, 1984). We do not as yet know whether mAb 55 inhibits antiparallel assembly because the tail-end is important for this type of assembly, or whether the binding of antibody at this site imposes constraints on myosin interactions that are mediated elsewhere. The same applies to the second inhibitory effect of mAb 55, which is to limit parallel assembly to 6–12 myosin molecules per filament. It appears to be likely that the antibody imposes steric hindrance on monomer–monomer or dimer–dimer interactions, and that the antiparallel association is most susceptible to this. The bare zone, which is rich in antiparallel packing, has proven to be quite resistant to disassembly under various conditions (Niederman and Peters, 1982; Reisler et al., 1982; Trinick and Cooper, 1980). Therefore, antiparallel assembly should not be inhibited by mAb 55 simply because it is less stable than parallel assembly.

It has been proposed that filament size is determined by increases in the off-rate of parallel dimer addition with increasing filament length (Davis, 1985). The limitation in size of the unipolar filaments caused by mAb 55 may be consequently due to antibody-induced increases in the off-rate such that equilibrium is reached at shorter filament lengths.

Complete Inhibition of Myosin Assembly by Antibodies Binding to a Region Important for Polymerization

We defined a region important for polymerization of *D. discoideum* myosin by mapping polymerizable chymotryptic tail fragments with monoclonal antibodies (Pagh et al., 1984). This region spans maximally from ~50 to 80% of the tail length from the heads. The distal portion of this region is adjacent to, and probably covers, the two threonine residues which are phosphorylatable by myosin heavy chain kinase (Maruta et al., 1983).

In the present study, two antibodies were found to completely inhibit myosin–myosin interactions as judged from sedimentation analyses and electron microscopy. These antibodies bind within the region of the tail that we identified as being important for polymerization. One of the two antibodies, mAb 253, binds to a position that is ~68% of the tail length from the heads. The second antibody, mAb 96, binds to a site 81% of the length of the tail from the heads (Claviez

et al., 1982) which is near to the heavy chain phosphorylation sites (Pagh et al., 1984). It has been shown that only exceptionally more than one molecule of mAb 96 binds to a myosin molecule and that this antibody does not cross-link myosin molecules (Claviez et al., 1982). Our results indicate that the 1:1 complexes of myosin and mAb 96 cannot polymerize at all. With decreasing ratios of antibody to myosin, more myosin molecules remain free of antibody and accordingly form normal filaments. Peltz et al. (1985) also identified an antibody that blocks polymerization of *D. discoideum* myosin; its binding site appears to lie in between the two binding sites described here (Flicker et al., 1985).

Based on thermodynamic studies of filament formation, only ~1% of the skeletal muscle myosin molecule is assumed to participate in strong associations (Harrington, 1979). Proteolytic removal of as little as 5–10 kD of the tail tip of skeletal muscle myosin (Lu et al., 1983) or *Acanthamoeba* myosin II (Kuznicki et al., 1985) abolishes polymerization. The finding that antibodies that bind very close to one another on the tail of *Acanthamoeba* myosin II may or may not inhibit filament formation has suggested that the ability of myosin to polymerize may reside in one or two specific sites (Kiehart et al., 1984). Our results together with those of Peltz et al. (1985) suggest that, in *D. discoideum*, sites participating in myosin–myosin association may be more dispersed since three separate binding sites of blocking antibodies were identified within a 13% span of the tail. However, monoclonal antibodies are probably not reliable probes for fine mapping of functional sites on proteins. In addition to blocking specific sites, antibodies may cause conformational changes, impose steric constraints, or induce even grosser shape changes, as was suggested from electron microscopic preparations in which bends were seen at positions where an antibody was bound to the myosin tail (Claviez et al., 1982).

Disassembly of myosin filaments is expected to be rapid when the equilibrium between filaments and monomers is shifted (Josephs and Harrington, 1968). Therefore an antibody like mAb 96, which prevents the reassociation of monomers, should efficiently shift this equilibrium. Under our conditions, dissociation seemed to occur very slowly. Even after 48-h incubation with mAb 96 at concentrations sufficient to block reassembly of monomers, only a small fraction of the polymerized myosin was disassembled. In comparison with conditions thought to be physiological ones, we have used a rather high Mg^{2+} concentration of 10 mM because this was required for an optimal stimulation of actin-activated ATPase, and a higher pH to prevent aggregation of antibodies. It will be of interest to know whether the dissociation rate is faster in vivo such that antibodies that efficiently block polymerization can also disassemble preformed filaments.

Size Constraints on Filaments Imposed by Antibodies Binding Near to the Heads

Two antibodies limited the size but did not alter the structure of myosin filaments. These antibodies, mAb 26 and mAb 153, have binding sites near to one another, at approximately one-third of the length of the tail from the heads. Since an adjacent, more proximal portion of the tail is very sensitive to chymotrypsin (Pagh et al., 1984), these binding sites may correspond to the amino-terminal portion of the light meromyosin fragment formed by proteolytic cleavage of myosins. In skeletal muscle myosin, this portion of the tail is packed into the filament shaft (Harrington, 1979). Therefore, it is likely that these antibodies impose steric constraints on the packing.

The three antibodies that bind to the tail closest to the heads, mAbs 32, 51, and 396, had either undetectable effects or had only marginal ones on filament formation. Both mAb 32 and mAb 396 were demonstrated to bind to filaments, which shows that their binding sites are accessible when myosin is polymerized. (For mAb 51 this has not been tested.) Accessibility of binding sites is particularly evident for mAb 396, which enhances the periodic substructure of preformed filaments. A similar effect has been observed with a monoclonal antibody that binds to skeletal muscle myosin (Shimizu et al., 1985). The portion of the tail near to the heads, to which our three antibodies bind, seems to be flexibly linked to the filament shaft, presumably to allow for movements of the heads (Trinick and Elliott, 1979). It is therefore reasonable that binding of antibody to this portion of the tail does not impose steric constraints on the packing of myosin molecules into filaments.

Effects of Antibodies on Actin-activated ATPase Activity

Antibodies that bind within the proximal 15% of the tail inhibited actin-activated MgATPase activity without preventing myosin from forming large, bipolar filaments. The effect of mAb 32, which binds closest to the heads, saturated without reaching full inhibition of the ATPase, while mAb 396 inhibited completely. The sensitivity of this enzyme activity to monoclonal antibodies binding to the proximal part of the tail has been noted previously (Kiehart and Pollard, 1984*b*; Peltz, et al., 1985), and attention has been drawn to a putative hinge in this region which may function in mechanochemical force transduction (Harrington and Rodgers, 1984).

Two of the antibodies studied, mAb 26 and mAb 153, bind more distal from the heads. Decreases in the length of bipolar myosin filaments from 500 nm to ~360 nm, as caused by these antibodies, had minor effects on the actin-activated ATPase activity.

Strong inhibition of actin-activated MgATPase activity by mAb 96 and mAb 253 paralleled their inhibition of myosin polymerization (Fig. 7). Since these antibodies bind at distances from the heads of 128 and 152 nm, respectively, a direct effect on the ATPase catalytic or F-actin binding sites of the same monomer is unlikely. This finding is in agreement with the work of Kiehart and Pollard (1984*a*) who recognized, for *Acanthamoeba* myosin II, a relationship between the inhibitory effects of monoclonal antibodies on filament formation and actin-activated MgATPase. Accordingly, these authors proposed that myosin must be filamentous to show actin-activated ATPase activity as also was suggested by Kuznicki et al. (1985). However, it has to be taken into account that the ATPase activity of nonpolymerizable fragments of *D. discoideum* myosin is activated by F-actin (Peltz et al., 1981): when myosin is intact, the tail seems to act as a negative regulator, imposing constraints on the activity of the heads.

In *D. discoideum*, phosphorylation has been reported to reduce filament formation and to inhibit the actin-activated ATPase activity of myosin (Kuczmarski and Spudich, 1980). It is therefore of significance that mAb 96, which binds close to the phosphorylation sites on the myosin tail, inhibits poly-

merization, since it suggests that phosphorylation may also inhibit actin-activated ATPase activity by reducing the capability of myosin to form filaments (Pagh et al., 1984). However, we have shown here, using other antibodies, that the filament size must be reduced drastically before substantial inhibition of actin-activated ATPase activity occurs. It has not yet been shown that heavy chain phosphorylation has that strong effect on polymerization. In *Acanthamoeba*, the filament sizes as well as actin-activated ATPase activities are lower in phosphorylated versus dephosphorylated myosin II (Collins et al., 1982*b*). But, by varying a number of conditions, Kuznicki et al. (1983) did not find a strict correlation between filament size and enzyme activity. Thus it is not yet clear, both for *D. discoideum* myosin and *Acanthamoeba* myosin II, to what extent changes in polymerization contribute to the regulation of ATPase activity by heavy chain phosphorylation.

In this context the effects of mAb 55, which binds to the tail at the longest possible distance from the heads, are of interest since its inhibition of bipolar filament formation did not parallel its effect on actin-activated ATPase activity. A sharp decrease in size of filaments from ~500 nm to ~230 nm was accompanied by only slight reductions of actin activation (Fig. 7 *e*). The gradual decrease in actin-activated MgATPase activity with increasing excess of mAb 55 was apparently not due to further reductions in filament size. It is possible that the increase in number of antibody molecules bound to up to a maximum of two per myosin molecule affects the actin-activated ATPase activity by changing the packing of monomers within the filaments. The effect of mAb 55 on actin-activated ATPase activity and also its inhibition of antiparallel myosin assembly demand further investigation.

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References

Berlot, C. H., J. A. Spudich, and P. N. Devreotes. 1985. Chemoattractant-elicited increases in myosin phosphorylation in *Dictyostelium*. *Cell.* 43:307–314.

Clarke, M., and J. A. Spudich. 1974. Biochemical and structural studies of actomyosin-like proteins from non-muscle cells. Isolation and characterization of myosin from amoebae of *Dictyostelium discoideum*. *J. Mol. Biol.* 86:209–222.

Claviez, M., K. Pagh, H. Maruta, W. Baltes, P. Fisher, and G. Gerisch. 1982. Electron microscopic mapping of monoclonal antibodies on the tail region of *Dictyostelium* myosin. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1017–1022.

Collins, J. H., G. P. Côté, and E. D. Korn. 1982*a*. Localization of the three phosphorylation sites on each heavy chain of *Acanthamoeba* myosin-II to a segment at the end of the tail. *J. Biol. Chem.* 257:4529–4534.

Collins, J. H., J. Kuznicki, B. Bowers, and E. D. Korn. 1982*b*. Comparison of the actin binding and filament formation properties of phosphorylated and dephosphorylated *Acanthamoeba* myosin II. *Biochemistry.* 21:6910–6915.

Davis, J. S., 1985. Kinetics and thermodynamics of the assembly of the parallel- and antiparallel-packed sections of synthetic thick filaments of skeletal myosin: a pressure-jump study. *Biochemistry.* 24:5263–5269.

Davis, J. S., J. Buck, and E. P. Greene. 1982. The myosin dimer: an intermediate in the self-assembly of the thick filament of vertebrate skeletal muscle. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 140:293–297.

Flicker, P. F., G. Peltz, M. P. Sheetz, P. Parham, and J. A. Spudich. 1985. Site-specific inhibition of myosin-mediated motility in vitro by monoclonal antibodies. *J. Cell Biol.* 100:1024–1030.

Futrelle, R. P., J. Traut, and W. G. McKee. 1982. Cell behavior in *Dictyostelium discoideum*: preaggregation response to localized cyclic AMP pulses. *J. Cell Biol.* 92:807–821.

Gerisch, G., D. Huelser, D. Malchow, and U. Wick. 1975. Cell communication by periodic cyclic-AMP pulses. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 272:181–192.

Harrington, W. F., and M. E. Rodgers, 1984. Myosin. *Annu. Rev. Biochem.* 53:35–73.

Harrington, W. F. 1979. Contractile proteins of muscle. *In* The Proteins. H. Neurath and R. L. Hill, editors. Academic Press, Inc., New York. IV:245–409.

Josephs, R., and W. F. Harrington. 1968. On the stability of myosin filaments. *Biochemistry.* 7:2834–2847.

Kiehart, D. P., and T. D. Pollard. 1984*a*. Stimulation of *Acanthamoeba* actomyosin ATPase activity by myosin-II polymerization. *Nature (Lond.).* 308:864–866.

Kiehart, D. P., and T. D. Pollard. 1984*b*. Inhibition of *Acanthamoeba* actomyosin-II ATPase activity and mechanochemical function by specific monoclonal antibodies. *J. Cell Biol.* 99:1024–1033.

Kiehart, D. P., D. A. Kaiser, and T. D. Pollard. 1984. Direct localization of monoclonal antibody-binding sites on *Acanthamoeba* myosin-II and inhibition of filament formation by antibodies that bind to specific sites on the myosin-II tail. *J. Cell Biol.* 99:1015–1023.

Kuczmarski, E. R., and J. A. Spudich. 1980. Regulation of myosin self-assembly: phosphorylation of Dictyostelium heavy chain inhibits formation of thick filaments. *Proc. Natl. Acad. Sci. USA.* 77:7292–7296.

Kuznicki, J., J. P. Albanesi, G. P. Côté, and E. D. Korn. 1983. Supramolecular regulation of the actin-activated ATPase activity of filaments of *Acanthamoeba* myosin II. *J. Biol. Chem.* 258:6011–6014.

Kuznicki, J., G. P. Côté, B. Bowers, and E. D. Korn. 1985. Filament formation and actin-activated ATPase activity are abolished by proteolytic removal of a small peptide from the tip of the tail of the heavy chain of *Acanthamoeba* myosin II. *J. Biol. Chem.* 260:1967–1972.

Lu, R. C., L. Nyitray, M. Balint, and J. Gergely. 1983. Localization of a region responsible for the low ionic strength insolubility of myosin. *Biophys. J.* 41:228a. (Abstr.)

Malchow, D., R. Boehme, and H. J. Rahmsdorf. 1981. Regulation of phosphorylation of myosin heavy chain during the chemotactic response of *Dictyostelium* cells. *Eur. J. Biochem.* 117:213–218.

Maruta, H., W. Baltes, P. Dieter, D. Marmé, and G. Gerisch. 1983. Myosin heavy chain kinase inactivated by Ca^{2+}/calmodulin from aggregating cells of *Dictyostelium discoideum*. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:535–542.

Niederman, R., and L. K. Peters. 1982. Native bare zone assemblage nucleates myosin filament assembly. *J. Mol. Biol.* 161:505–517.

Pagh, K., H. Maruta, M. Claviez, and G. Gerisch. 1984. Localization of two phosphorylation sites adjacent to a region important for polymerization on the tail of *Dictyostelium* myosin. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:3271–3278.

Peltz, G., E. R. Kuczmarski, and J. A. Spudich. 1981. *Dictyostelium* myosin: characterization of chymotryptic fragments and localization of the heavy-chain phosphorylation site. *J. Cell Biol.* 89:104–108.

Peltz, G., J. A. Spudich, and P. Parham. 1985. Monoclonal antibodies against seven sites on the head and tail of *Dictyostelium* myosin. *J. Cell Biol.* 100:1016–1023.

Pollard, T. D. 1982. Structure and polymerization of *Acanthamoeba* myosin-II filaments. *J. Cell Biol.* 95:816–825.

Pollard, T. D., and E. D. Korn. 1973. *Acanthamoeba* myosin. I. Isolation from *Acanthamoeba castellani* of an enzyme similar to muscle myosin. *J. Biol. Chem.* 248:4682–4690.

Rahmsdorf, H. J., D. Malchow, and G. Gerisch. 1978. Cyclic AMP-induced phosphorylation in *Dictyostelium* of a polypeptide comigrating with myosin heavy chains. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 88:322–326.

Reisler, E., P. Cheung, C. Oriol-Audit, and J. A. Lake. 1982. Growth of synthetic myosin filaments from myosin minifilaments. *Biochemistry.* 21:701–707.

Rubino, S., M. Fighetti, E. Unger, and P. Cappuccinelli. 1984. Location of actin, myosin and microtubular structures during directed locomotion of *Dictyostelium* amebae. *J. Cell Biol.* 98:382–390.

Schleicher, M., G. Gerisch, and G. Isenberg. 1984. New actin-binding proteins from *Dictyostelium discoideum*. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:2095–2100.

Shimizu, T., J. E. Dennis, T. Masaki, and D. A. Fischman. 1985. Axial arrangement of the myosin rod in vertebrate thick filaments: immunoelectron microscopy with a monoclonal antibody to light meromyosin. *J. Cell Biol.* 101:1115–1123.

Stadler, J., C. Bordier, F. Lottspeich, A. Henschen, and G. Gerisch. 1982. Improved purification and N-terminal amino acid-sequence determination of the contact site A glycoprotein of *D. discoideum*. *Hoppe-Seyler's Z. Physiol. Chem.* 363:771–776.

Stewart, P. R., and J. A. Spudich. 1979. Structural states of *Dictyostelium* myosin. *J. Supramol. Struct.* 12:1–14.

Swanson, J. A., and D. L. Taylor. 1982. Local and spatially coordinated movements in *Dictyostelium discoideum* amoebae during chemotaxis. *Cell.* 28:225–232.

Trinick, J., and J. Cooper. 1980. Sequential disassembly of vertebrate muscle thick filaments. *J. Mol. Biol.* 141:315–321.

Trinick, J., and A. Elliott. 1979. Electron microscope studies of thick filaments from vertebrate skeletal muscle. *J. Mol. Biol.* 131:133–136.

Trinick, J., and A. Elliott. 1982. Effect of substrate on freeze-dried and shadowed protein structures. *J. Microsc.* 126:151–156.

Yumura, S., H. Mori, and Y. Fukui. 1984. Localization of actin and myosin for the study of ameboid movement in *Dictyostelium* using improved immunofluorescence. *J. Cell Biol.* 99:894–899.

Yumura, S., and Y. Fukui. 1985. Reversible cyclic AMP-dependent change in distribution of myosin thick filaments in *Dictyostelium*. *Nature (Lond.)*. 314:194–196.

Varnum, B., K. B. Edwards, and D. R. Soll. 1985. *Dictyostelium* amebae alter motility differently in response to increasing versus decreasing temporal gradients of cAMP. *J. Cell Biol.* 101:1–5.