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ACTIN-BINDING PROTEIN AMPLIFIES ACTOMYOSIN CONTRACTION, AND GELSOLIN CONFERS CALCIUM CONTROL ON THE DIRECTION OF CONTRACTION[1]

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SUMMARY: Cross-linking of muscle actin filaments by low concentrations of actin-binding protein reduces the concentration of muscle myosin required for contraction of actin. Gelsolin, a macrophage protein that divides actin filaments in the presence of calcium, inhibits the amplifying effect of actin-binding protein on contraction of actomyosin. In a calcium gradient, the actomyosin gel moves from high to low calcium concentrations, indicating that calcium-controlled lattice formation can impart directionality to the movement of an isotropic actin network.

The cortical cytoplasm of many nonmuscle cells contains actin filaments (F-actin) and myosin molecules (1), and the idea that the molecular mechanisms of movement in the nonmuscle cell cortex and in the muscle sarcomere are similar is attractive. In striated muscle, shear produced by reciprocal sliding of actin and myosin filaments in the presence of ATP generates force (2), and the direction of the force generated is in the plane of the parallel orientation of these filaments in the sarcomere. In contrast to striated muscle, however, actin filaments of the nonmuscle cell cortex often exist in a three dimensional isotropic meshwork (3). Myosin, randomly distributed throughout such an actin meshwork *in vitro*, can generate a contraction (superprecipitation) of the network in the presence of $MgCl_2$ and ATP (4), but without any orientation except toward the center of the network. How can such an arrangement provide for directional movement?

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Directional movement could occur if the efficiency of contraction differed in one domain of the myosin-containing actin network relative to that in another. In a tug-of-war, envisioned to take place in the lattice, unequal contraction would draw network mass toward a region of stronger contraction. ABP[3] is a potent actin cross-linking agent (5). In addition, a calcium-binding protein called gelsolin was recently isolated from macrophages. It reversibly dissolves networks of actin cross-linked by ABP by reversibly severing actin filaments between the points of cross-linking when the free calcium concentration rises above 2×10^{-8}M (6). In this paper, we document that increases in actin network structure by ABP enhance the efficiency of contraction of actin by myosin, that calcium-activated gelsolin reverses this enhancement, and that these changes can bring about directional movement.

METHODS

The general experimental approach was to observe directional movement of proteins in horizontal capillaries in the presence or absence of a calcium ion gradient. We employed skeletal muscle actin and myosin because these proteins will superprecipitate without added regulatory or activating proteins (7), and because muscle actin interacts with macrophage or gizzard ABP and with macrophage gelsolin (6,8). Rabbit skeletal muscle G-actin (9), 10 mg/ml in 0.2 mM ATP, 0.2 mM Tris-HCl, pH 7.5, was made 0.1 M in KCl and allowed to polymerize by standing at 0°C for 1 hour. Equal volumes of ice cold 0.6 M KCl, 20 mM imidazole-HCl, pH 7.2, containing different concentrations of rabbit skeletal muscle myosin (10) were then added. The actomyosin solutions were then dialyzed at 4°C against 1000 volumes of 0.1 M KCl, 2.5 mM $MgCl_2$, 0.5 mM ATP, 20 mM imidazole-HCl, pH 7.2 for 3 hours. The dialyzed solution was kept at 0°C, and the following solutions added in rapid succession: ATP, pH 7.0, 50 µg of dry charcoal particles (Charcodote; Ormont Drug & Chemical Co., Inc., Englewood, N.J.), to some preparations, lung macrophage ABP (7,9) or chicken gizzard ABP (also called filamin) (11), and to some preparations, macrophage gelsolin (6). The final concentrations of reagents were: actin, 4 mg/ml,myosin, as indicated in the figures, macrophage ABP 50 µg/ml, gizzard ABP, 75 µg/ml, macrophage gelsolin 150 µg/ml, and ATP 1.5 mM. The solution was quickly mixed with a Pasteur pipette and then aspirated into chilled (0°C) 60 x 3.4 mm glass capillaries by suction through a rubber tube attached to one end of the capillary. The capillaries were then mounted horizontally by insertion of each end into the sides of 1.5 ml plastic test tubes (No. 39/10 W. Sarstedt, Inc., Princeton, N.J.) in which holes were punched with an awl. The junctions between the capillary and the tubes were sealed with vacuum grease. The capillary was left standing at room temperature for 15 min. Then the tubes were filled with solutions at room temperature containing 0.1 M KCl, 2.5 mM $MgCl_2$, 1.5 mM ATP and 20 mM imidazole-HCl, pH 7.2. Movement of the protein gel in the capillaries was monitored by measuring its vertical thickness 10 mm from

[3]Abbreviation: ABP, actin-binding protein

each end with a calibrated magnifying eyepiece and averaging the results. Under the conditions of these experiments, the values at each end agreed within 10% of each other. The charcoal aided visualization of the gel boundary and did not influence the extent of contraction, as evidenced by equivalent contractions in the absence of added charcoal.

RESULTS

As shown in Figure 1, muscle actomyosin solutions in capillaries contracted centripetally without a preferred direction, provided that the myosin concentration was above a threshold value (myosin: actin weight ratio about 0.1). Myosin caused a concentration-dependent increase in the extent of con-

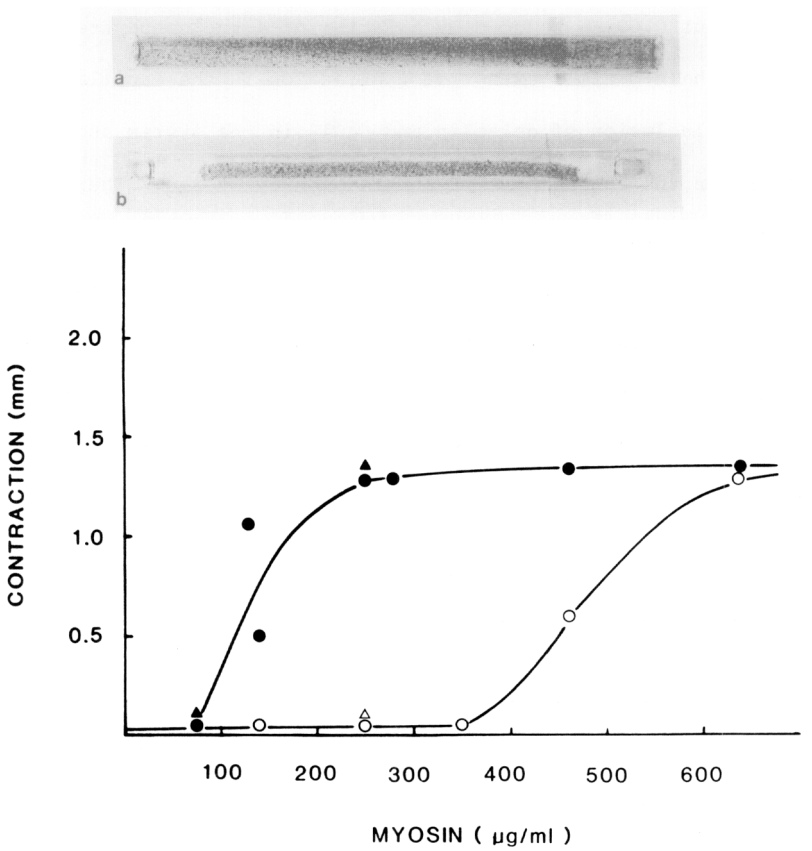

Fig. 1. Effect of myosin concentration on the contraction of F-actin in horizontal capillaries in the absence (0) of ABP, in the presence of macrophage (▲) or gizzard (●) ABP, or in the presence of macrophage ABP plus macrophage gelsolin and 10^{-5}M $CaCl_2$ (Δ). In the figure, capillary (a.) is an actomyosin gel prior to contraction; (b.) is after contraction for 30 min. In the graph, the vertical axis indicates mm of contraction after 30 min. of incubation.

traction up to a myosin:actin weight ratio of ca 0.25 (molar ratio 0.02). Under these conditions, movement of the actomyosin mixture from the ends and sides of the capillary began at about 15 - 30 minutes after the capillaries were loaded and placed at room temperature. This movement continued at a constant rate for approximately one hour, at which time the final volume of the contracted aggregates was approximately 20% of the original volume. Additional ATP did not increase the extent of contraction. Below the threshold myosin concentration, no movement occurred even up to 12 hours.

Addition of either macrophage or gizzard ABP to the system (ABP:actin molar ratio about 0.001) reduced by a factor of 4 the concentration of myosin required for a half-maximal extent of contraction in the capillary in the absence of ABP. Inclusion of gelsolin and $CaCl_2$ in the system abolished the effect of ABP on the enhancement of contraction by myosin. Therefore, cross-linking of actin filaments by ABP amplifies contraction, and breakdown of the cross-linked lattice by gelsolin reverses the amplification. As shown in Table 1, the amplification of contraction by ABP and its reversal by gelsolin is not because of a stimulatory effect of the ABP or an inhibitory effect of gelsolin on the Mg^{2+}-ATPase activity of myosin, the enzymatic basis of force transduction.

TABLE 1: Effect of actin, ABP and gelsolin on the Mg^{2+}-ATPase activity of myosin.

	Mg^{2+}-ATPase activity (nmol P_i/mg myosin protein/min)
Myosin	62 ± 4
Myosin + Actin	1063 ± 8
Myosin + Actin + ABP	900 ± 24
Myosin + Actin + Gelsolin	947 ± 28

The complete system contained, in 125 µl,25 µg muscle myosin, 300 µg muscle F-actin, 20 µg of chicken gizzard ABP, and 20 µg macrophage gelsolin in 60 mM KCl, 2.5 mM $MgCl_2$, 0.05 mM $CaCl_2$, 1 mM ATP and 20 mM imidazole-HCl, pH 7.1. The incubation was carried out at 37°C for 10 min and the liberation of P_i measured as previously described (15).

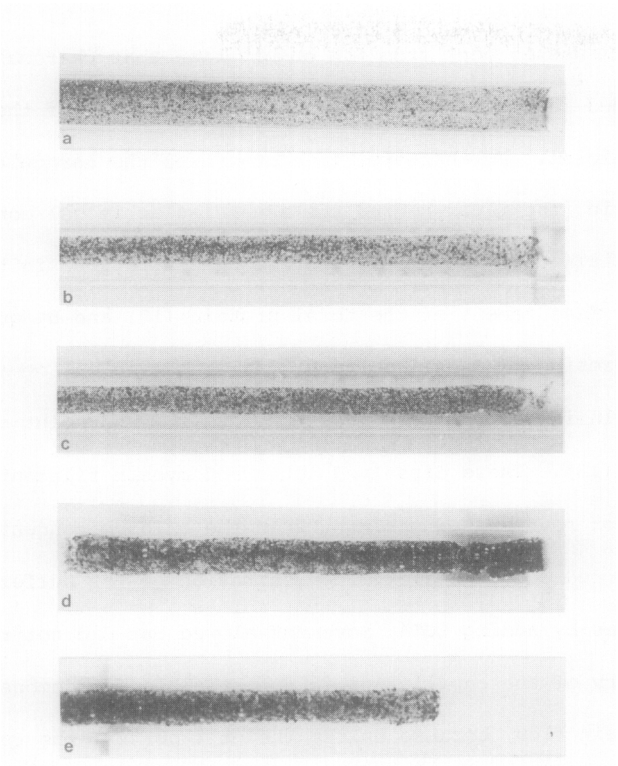

Fig. 2. Effect of gelsolin, 150 µg/ml, and a calcium gradient on the contraction of ABP-actomyosin gels in horizontal capillaries. The reaction conditions were as described in Fig. 1. The myosin concentration was 200 µg/ml. To establish a calcium gradient, the buffer in one of the tubes into which the capillaries were inserted contained 5 x 10^{-5}M calcium. The capillaries shown were photographed after 30 min. of incubation at room temperature. The capillaries show: (a) uncontracted gel (ATP omitted); (b) symmetrical contraction in the absence of gelsolin; (c) lack of an effect of calcium on the unregulated system; (d) no effect of added gelsolin without a calcium gradient; (e) with gelsolin included, there is preferential contraction from the side where calcium was added.

Addition of calcium to either or both sides of the capillaries had no effect on the direction or extent of contraction of actomyosin mixtures, since no regulatory proteins were present. When gelsolin was included, however, the protein gels invariably moved preferentially away from the side with added calcium (Fig. 2), and the time required for the gels to reach their final size approximately halved. The final extent of contraction was similar in the presence or absence of added calcium. Addition of calcium to both ends of the capillaries accelerated the contraction symmetrically from both ends of the capillary (not shown). Gelsolin plus added calcium had no effect on the Mg^{2+}-ATPase activity of actomyosin (Table 1). The solvating effect of calcium on

the actin network was evident from the observation that charcoal particles initially imbedded in the actin gel settled to the bottom of the capillary on the side with calcium. Furthermore, in contrast to the unregulated contraction of actomyosin, the fluid behind the advancing actin gel contained a fraction of the initial actin and myosin protein concentrations initially present, as determined by measurement of the total protein (12) and by quantitative densitometry of actin and myosin polypeptides, resolved on Coomassie-blue stained polyacrylamide gels after electrophoresis of the solutions in sodium dodecyl sulfate (13). These dissolved actin and myosin filaments are potentially available for reversal of movement when the calcium concentration falls, although when we reduced the calcium concentration of the buffers at the ends of the capillaries by adding EGTA, movement slowed but did not reverse. However, the geometry of the capillaries is not suitable for demonstrating reversibility of protein flow, because unlike the cell cortex, the reservoir of available actin network is finite, limited by the boundary at the end of the capillary.

DISCUSSION

We have shown that ABP enhances the contractility of actomyosin solutions. In the absence of ABP, entanglements of actin filaments may add to the mass of each actin filament undergoing contraction by myosin, but many actin filaments are free to slip past one another and do not contribute to net movement (14). ABP, by cross-linking actin filaments, adds them to the contracting mass, and this recruitment is the presumed basis of amplification. We have also shown that directionality of movement of an actomyosin lattice can arise from local changes in the lattice structure of actin. Calcium controls the direction of movement of an actin filament lattice containing myosin and macrophage gelsolin by reversibly decreasing lattice structure of actin filaments cross-linked by ABP. In an actin lattice, filaments interacting with myosin move away from domains of decreased cross-linking because of increased efficiency of contraction in the more cross-linked domains, and any part of a cell attached to the

lattice would move accordingly. Since many cells contain cortical actin meshworks (3), we suggest that our model may be generally applicable to diverse cell surface movements.

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