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Tropomyosin and Gelsolin Cooperate in Controlling the Microfilament System*

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Tropomyosin has been shown to cause annealing of gelsolin-capped actin filaments. Here we show that tropomyosin is highly efficient in transforming even the smallest gelsolin-actin complexes into long actin filaments. At low concentrations of tropomyosin, the effect of tropomyosin depends on the length of the actin oligomer, and the cooperative nature of the process is a direct indication that tropomyosin induces a conformational change in the gelsolin-actin complexes, altering the structure at the actin (+) end such that capping by gelsolin is abolished. At increased concentrations of tropomyosin, heterodimers, trimers, and tetramers are converted to actin filaments. In addition, evidence is presented demonstrating that gelsolin, once removed from the (+) end of the actin, can reassociate with the newly formed tropomyosin-decorated actin filaments. Interestingly, the binding of gelsolin to the tropomyosin-actin filament complexes saturates at 2 gelsolin molecules per 14 actin and 2 tropomyosins, *i.e.* two gelsolins per tropomyosin-regulatory unit along the filament. These observations support the view that both tropomyosin and gelsolin are likely to have important functions in addition to those proposed earlier.

In response to extracellular signals, cells change shape and activity through processes that affect the dynamics of the actin-based microfilament system. The plasticity of this chemo-mechanical transduction system is regulated by a large number of diverse proteins, binding either monomeric or filamentous actin. The regulation involves proteins controlling actin assembly, organizing filaments by cross-linking, and translocating formed actin filament ensembles (1–4).

Gelsolin is an abundant actin binding protein thought to be involved in cell shape regulation by controlling actin assembly at the (+) end (fast growing end) of actin filaments and by severing actin filaments (5). Gelsolin consists of six homologous domains (6, 7), three of which bind to actin (8–11). Domain 1 of gelsolin has a calcium-insensitive high affinity actin-binding site (K_d 5 pM), and domain 4 a calcium-sensitive, lower affinity actin-binding site (K_d 1.8 μM) (12). Domain 2 has been shown to bind to actin filaments with a K_d of 2–7 μM (13, 14). Gelsolin can nucleate filament formation by forming a complex with two actin monomers. In this process, gelsolin binds to the (+) end of the monomers, thereby initiating growth of a filament, where monomers are added to the (−) end of the gelsolin-actin nucleus (15, 16). Gelsolin remains bound as a cap on the (+) end of the filament, inhibiting the further addition of actin monomers at that site. Similarly, gelsolin remains bound to the (+) end of one of the fragments after a severing event. These gelsolin activities can be regulated by Ca^{2+} and polyphosphoinositides (17–19).

Actin filaments in lamellipodia and filopodia at the periphery of cells are organized with their fast growing (+) ends toward advancing cell edges (20, 21), where there appears to be specific protein modules involved in the addition of actin monomers (22–25). Gelsolin has been detected at this location by electron microscopy using gold-labeled gelsolin antibodies (26). With the use of such antibodies in indirect immunofluorescence, gelsolin has also been shown to colocalize with both actin stress fibers in non-muscle cells (27, 28) and with the I-bands in sarcomeres of myofibrils (29). The function of gelsolin in these structures is unknown.

Skeletal muscle tropomyosin is a 40-nm-long coiled-coil heterodimeric protein with seven actin-binding sites (30), displaying strong cooperative binding to actin filaments (31). At saturation, both sides of the actin filament are associated with tropomyosin, creating regulatory units along the filament consisting of 14 consecutive actin monomers. Studies on truncated forms of tropomyosin show that the N- and C-terminal ends of the protein have major influences on its actin binding capacity and its regulatory properties (32, 33). The multiple binding sites and end-to-end binding are the likely basis for the cooperative regulation by calcium via troponin of the actomyosin interaction in skeletal muscle fibers (34).

Myofibers express two isoforms of tropomyosin, whereas non-muscle cells express 6–8 isoforms, whose pattern of expression depends on the type of cell and the physiological conditions (35–37). There is also a variation in the cellular distribution of the tropomyosin isoforms, suggesting different tropomyosins in functionally different actin organizations in the cell (38–40). Tropomyosin has been shown to stabilize and increase the rigidity of actin filaments (41, 42) and to restore the polymerization of a polymerization-defective actin mutant, implying that tropomyosin strengthens the intermonomer bonds in the actin filament (43). Furthermore, tropomyosin inhibits gelsolin severing of α-actin filaments (44) and anneals gelsolin-capped filaments, an effect enhanced by caldesmon (45).

Despite all the available information about tropomyosin, its role in regulating the actomyosin system in both non-muscle and muscle cells is still unclear. Smooth and non-muscle cells do not express troponin; instead, these cells seem to control their actomyosin system by other mechanisms, involving either calcium/calmodulin-regulated caldesmon, a tropomyosin-interacting protein with some homology to troponin (46), or phosphorylation of myosin (47).

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The observations reported here indicate that tropomyosin as well as gelsolin might have important functions in addition to those described before. First, tropomyosin is remarkably effective in transforming gelsolin-actin complexes into long filaments. In fact, even short gelsolin-actin complexes (heterodimers, GA[1]; heterotrimers, GA_2; and heterotetramers, GA_3) can be converted into long filaments by tropomyosin. Second, the appearance of free gelsolin demonstrates that tropomyosin actually dissociates the gelsolin-actin complexes. Finally, tropomyosin-decorated actin filaments were found to bind gelsolin with a distinct stoichiometry of 2 gelsolin molecules per 14 actins and 2 tropomyosins.

MATERIALS AND METHODS

Protein Purification—Bovine β-actin was purified from calf thymus (48). The actin was stored in G buffer pH 7.6 (5 mM Tris-HCl, 0.5 mM ATP, 0.1 mM $CaCl_2$, and 0.5 mM DTT) and used within a week or drop-frozen in (25-μl aliquots) liquid nitrogen. Bovine plasma gelsolin was purified from calf serum utilizing Ca^{2+}-dependent absorption to DEAE-cellulose based on earlier protocols with some modifications (49, 50). Bovine blood obtained fresh from the slaughterhouse was clotted at room temperature for 2 h, and the clot was removed by centrifugation. Five hundred milliliters of serum was dialyzed in 25 mM Tris-HCl, pH 8.0, at 4 °C, 45 mM NaCl, 0.5 mM $CaCl_2$, and 0.1 mM DTT (buffer A) with continuous buffer changes. The dialyzed solution was applied to a DEAE-cellulose (DE52, Whatman) column pre-equilibrated with the same buffer. In the presence of Ca^{2+}, gelsolin is recovered in the flow-through. After the addition of EGTA to the flow-through (2 mM final concentration), the solution was directly applied to a second DEAE-cellulose column equilibrated with buffer A containing 1 mM EGTA, allowing gelsolin to bind to the matrix. Gelsolin was eluted with a 1–4 mM $CaCl_2$ linear gradient in 25 mM Tris HCl, 30 mM NaCl, and 1 mM DTT. Finally, EGTA was added to the eluate to a concentration of 5 mM. The purity was checked by SDS-PAGE, and the protein was dialyzed into a storage buffer containing 10 mM Tris HCl, pH 7.6, at 4 °C, 2 mM EGTA, and 0.2 mM DTT. Rabbit skeletal muscle tropomyosin was purified as described earlier (51) and stored at −20 °C. Protein concentrations were determined spectrophotometrically using the extinction coefficients of 0.63 ml·mg·cm^{-1} for actin at 290 nm, 1.538 ml·mg·cm^{-1} for gelsolin at 280 nm, and 0.3 ml·mg·cm^{-1} for tropomyosin at 280 nm.

Sedimentation Assay—The effect of tropomyosin binding to gelsolin-capped actin filaments was monitored using a sedimentation assay. For this, samples of β-actin (20 or 10 μM) mixed with increasing concentrations of gelsolin (0.1–20 μM) in G buffer was prepared. Polymerization was initiated by the addition of $MgCl_2$ and KCl to final concentrations of 2 and 100 mM, respectively. The mixture was incubated at room temperature for 2 h, then tropomyosin was added to final concentrations of 1.5, 3.0, 5.0, or 10 μM, and the mixtures were incubated for another hour. The samples were ultracentrifuged at room temperature for 30 min at 30 p.s.i. in a Beckman Airfuge. The supernatants were removed, and the pellets were briefly washed with F-buffer and resuspended in equal volumes of G buffer. Pellets and supernatants were analyzed by SDS-PAGE using 10% gels (52). Gel electrophoresis under non-denaturing conditions was performed using 4–15% Tris-HCl precast gels according to the manufacturer's recommendations (Bio-Rad). The proteins were visualized by Coomassie Blue staining, and the amount of protein was estimated by densitometry using ImageQuant 5.0 (Molecular Dynamics). The molar ratio between the actin, gelsolin, and tropomyosin was calculated using values corrected for the differential uptake of Coomassie stain in the proteins. To determine protein concentrations, a standard curve (0.05–0.6 nmol of bovine serum albumin) was included when scanning the gels. The experimental data were fit to a nonlinear curve using the Hill equation (53) (Microcal ORIGIN), which provides a measure of the cooperativity of the reaction. The fraction of sedimented actin was normalized to 1.0 by dividing the amount of actin recovered in the pellet with the total amount of actin in the reaction mix.

Fluorescence Measurements—Polymerization of actin was monitored by recording the increase in fluorescence of co-polymerized 2% pyrenyl-labeled bovine β-actin (54) using a Fluoroscan II plate reader (Labsystems) (55). The nucleation assay studied the conversion of monomeric actin to polymeric actin in samples containing Ca^{2+}-G-actin (2.5 μM) and increasing concentrations of gelsolin (0–120 nM) in G buffer. The polymerization reaction was initiated by the addition of $MgCl_2$ and KCl to 2 and 100 mM, respectively. The salts were added to all wells simultaneously with a Multitip pipette (Finnpipette) with a delay between the addition of salt and the first measurement of 15 s.

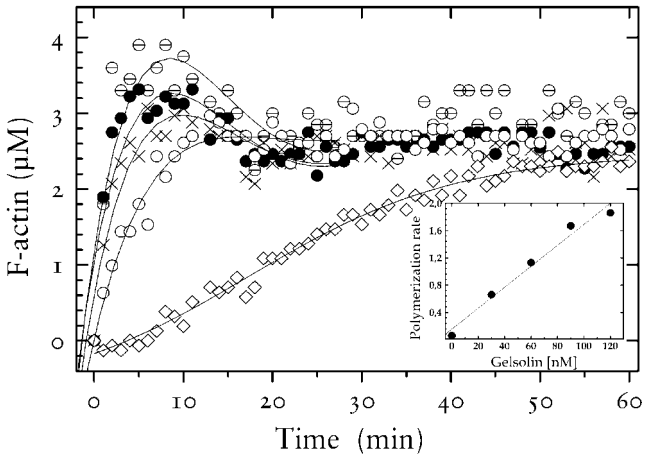

FIG. 1. **Effect of gelsolin on β-actin polymerization.** The rate of actin polymerization in a solutions containing 2.5 μM Ca^{2+}-G-actin mixed with increasing concentrations of gelsolin (0–120 nM) was measured immediately after the addition of polymerizing salts. Actin alone (◇), actin mixed with 30 nM gelsolin (○), 60 nM gelsolin (×), 90 nM gelsolin (●), and 120 nM gelsolin (*circle with horizontal bar*). The polymerization rate (d[F-actin]/dt) was calculated from the slopes of the plots where the increase in the fluorescence intensity with time is approximately linear. The *inset* shows the rate of polymerization (μM F-actin/min) *versus* the concentration of gelsolin (nM), and the on-rate (k_+) was calculated to 0.2 μM^{-1}s^{-1}.

Electron Microscopy—An aliquot (20 μl) of the sample was placed on a glow-discharged carbon-coated Formvar copper grid for 60 s, after which a 15-μl drop of 1% uranyl acetate was placed on the grid for 15 s (repeated seven times). Grids were viewed with a Philips CM120 electron microscope at 80 kV and with 45,000× or 60,000× nominal magnification.

Size Exclusion Gel Chromatography—Actin (30 μM) was mixed with gelsolin (3 μM) and tropomyosin (4 μM) and treated as described above. After ultracentrifugation of the sample, the supernatant was loaded onto a Superdex 200 gel filtration column (Amersham Biosciences) calibrated using standard proteins provided by the manufacturer (Amersham Biosciences). The column was equilibrated with F buffer (5 mM Tris-HCl, pH 7.6, 0.5 mM ATP, 0.1 mM $CaCl_2$, 0.5 mM DTT, 2 mM $MgCl_2$, and 100 mM KCl) and eluted at a rate of 12 cm/h. The protein peaks were analyzed by SDS-PAGE and native PAGE gel electrophoresis. The molecular weight of the complex was determined by interpolation of R_f *versus* M_r using the molecular weight standard curve. Gel filtration chromatography was performed at 4 °C.

RESULTS

Gelsolin Interaction with Non-muscle β-Actin—Previous workers have used skeletal muscle α-actin in their studies of the gelsolin-actin interaction. We have presented evidence that muscle α- and non-muscle β-actin differ in the timing and release of inorganic phosphate from polymerizing actin (56). Here, the interaction between plasma gelsolin and non-muscle β-actin was studied by following the gelsolin-induced nucleation of β-actin polymerization. For this, a series of polymerization experiments were performed by varying the concentration of gelsolin (Fig. 1). The polymerization rate was calculated from the slope of the curves in the region where the increase in fluorescence intensity *versus* time was approximately linear. In the gelsolin-nucleated reaction, the polymerization rate was directly proportional to the concentration of active gelsolin molecules added, and the apparent rate constant for polymerization can be described by the expression d[F-actin]/dt = k_+ [gelsolin][actin monomer]. The gelsolin nucleation of β-actin polymerization gives an apparent rate constant (k_+) of 0.2

[1] The abbreviations used are: GA complex, gelsolin-actin complex; DTT, dithiothreitol; ADF, actin depolymerizing factor.

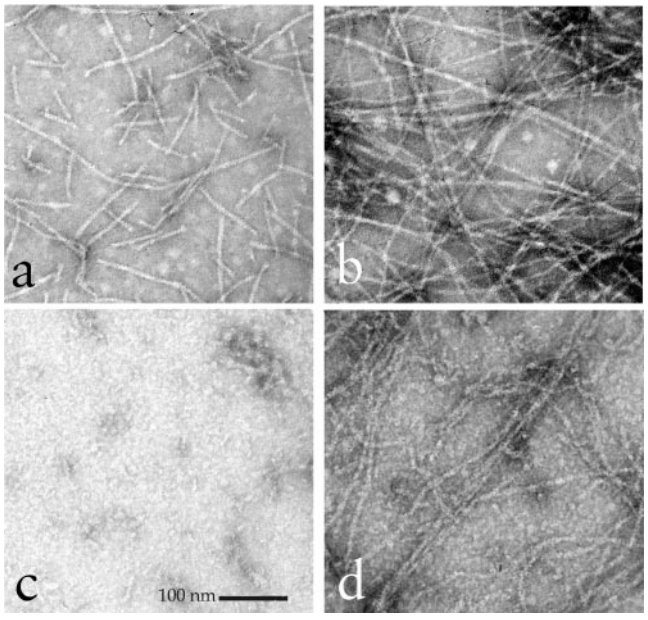

FIG. 2. **Annealing of gelsolin-capped actin filaments analyzed by electron microscopy.** *Panel a* shows gelsolin and actin mixed in a 1:50 molar ratio; *b*, mixture as in *a* after the addition of tropomyosin. *c* shows gelsolin and actin mixed in a 1:3 molar ratio; *d*, mixture as in *c* after the addition of tropomyosin. The *scale bar* represents 100 nm and applies to all panels.

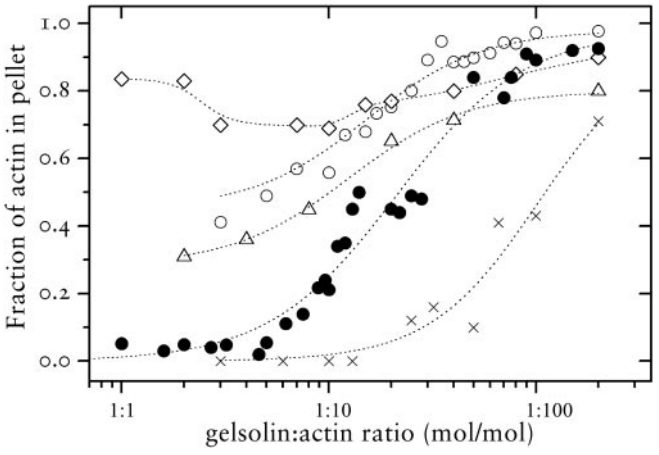

FIG. 3. **Annealing of gelsolin-actin complexes of increasing lengths with increasing concentrations of tropomyosin.** The amount of actin recovered in the pellet after ultracentrifugation was estimated by densitometry of SDS-PAGE gels as described under "Materials and Methods." Fraction of actin in the pellet (of total actin in the sample) was plotted *versus* the molar ratio of gelsolin to actin (1:1–1:200) in the sample mixture in the presence of increasing concentrations of tropomyosin, 1.5 μM (●), 3 μM (△), 5 μM (○), and 10 μM (◇). Sedimentation of gelsolin-actin complexes in the absence of tropomyosin is shown as ×.

$\mu M^{-1}s^{-1}$ for the addition of monomers at the (−) end. Thus, the rate of polymerization relative to gelsolin concentration obtained with non-muscle β-actin was more than three times higher than that previously reported for gelsolin-nucleated polymerization of muscle α-actin (57).

Uncapping of Filament (+) Ends by Tropomyosin and Filament Annealing—It was observed earlier that the addition of tropomyosin to short gelsolin-capped actin filaments resulted in longer filaments (44, 45). The experiments described here establish the highly efficient nature of this process. As shown in Fig. 2*a*, gelsolin and actin mixed in a 1:50 molar ratio gave rise to short actin filaments of an average length of 120 nm, in good agreement with the expected length (50 monomers × 2.7 nm = 135 nm). The electron micrograph in Fig. 2*b* illustrates that tropomyosin added to such gelsolin-actin complexes resulted in the formation of long filaments several micrometers in length. With gelsolin and actin mixed in a 1:3 molar ratio (Fig. 2*c*), the product appeared as short, mainly globular structures. Tropomyosin had the capacity to convert these gelsolin-actin complexes into long actin filaments (Fig. 2*d*). The filaments obtained here are clearly different in appearance compared with those in Fig. 2*b*. Most likely, this is due to the association of the filaments with significant amounts of gelsolin (see below).

The efficiency by which tropomyosin transforms gelsolin-actin complexes was studied by mixing β-actin (10 μM) with decreasing concentrations (10–0.2 μM) of gelsolin to form gelsolin-capped actin oligomers of lengths varying from 1 to 200 monomers. After 1 h of incubation in the presence or absence of tropomyosin, the samples were centrifuged, and the material in the pellets and supernatants was analyzed by SDS-PAGE (see "Materials and Methods"). The addition of 1.5 μM tropomyosin resulted in a sigmoidal increase in sedimentable tropomyosin-decorated actin filaments (Fig. 3, *closed circles*). The transition began at a point where the gelsolin-actin complexes were expected to contain 6–7 actin monomers, implying that each side of the actin oligomers presented only three consecutive binding sites for the seven complementary sites on tropomyosin. The midpoint of the transition occurred at an estimated actin oligomer length of 14 actin monomers. The process was characterized by a Hill coefficient of 2.0 ± 0.4, corresponding to 2 tropomyosins per 14 actin monomers. In control experiments (Fig. 3, ×) involving only β-actin and gelsolin, actin did not appear in the pellets unless the gelsolin-capped actin oligomers contained at least 100 actin monomers.

To further investigate the cooperativity of the process, three series of actin (20 μM) and gelsolin (20–0.1 μM) mixtures were prepared to which 3, 5, and 10 μM tropomyosin was added, respectively. As shown in Fig. 3 (*open symbols*), the higher the concentration of tropomyosin, the higher the yield of sedimentable actin. Remarkably, in the presence of increased concentrations of tropomyosin, 80% of the actin from the shortest gelsolin-actin complexes were recovered in the pellet. This suggests that, in addition to the multi-site cooperative binding of tropomyosin to actin oligomers, there is a novel mechanism of tropomyosin displacing the tightly bound actin from gelsolin.

Further Evidence for Two Mechanisms in the Interaction of Tropomyosin with Gelsolin-Actin Complexes—With 10 μM tropomyosin and increasing concentrations of gelsolin, there was a dip in the recovery of sedimentable actin filaments (Fig. 4, *open circles*), with a minimum reached at a 1:4 GA molar ratio. An indication of such a dip is also seen in Fig. 3 (*diamonds*). Annealing of longer gelsolin-actin oligomers, GA_{80}-GA_8, should represent the effect of multiple-site interactions with tropomyosin. The interaction of tropomyosin with smaller gelsolin-actin complexes (GA_2 and GA) shows that tropomyosin was very efficient also in dissociating gelsolin-actin heterodimers and trimers, resulting in sedimentation of more than 90% of the actin. The addition of gelsolin in molar excess over actin did not decrease the effect of tropomyosin. Furthermore, the corresponding supernatants (Fig. 4, *closed circles*) contained increased amounts of actin up to a maximum, which coincided with the minimum described above. Up to this point, the supernatants contained a 3-fold excess of actin (*closed circles*) to gelsolin (*open diamonds*), indicating the existence of GA_3 complexes. Supernatants obtained after annealing of gelsolin-actin heterodimers and trimers, on the other hand, contained up to a 10-fold molar excess of gelsolin over actin, confirming that tropomyosin released gelsolin from actin and induced actin

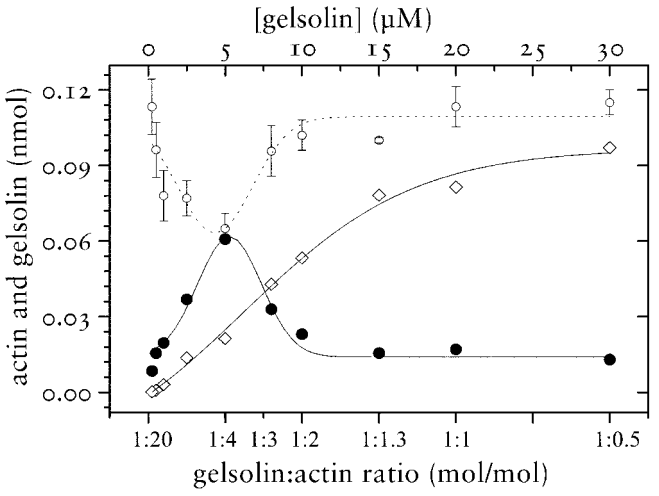

FIG. 4. **Amount of actin and gelsolin recovered in the supernatant and pellet after tropomyosin induced annealing.** Actin (20 μM) was mixed with increasing concentrations of gelsolin (0.25–30 μM) to obtain gelsolin:actin oligomers of decreasing lengths (GA_{80} to $GA_{0.5}$). Tropomyosin was (10 μM) was added to these mixtures, the supernatants and pellets were analyzed by SDS-PAGE, and concentrations of actin and gelsolin were estimated by densitometry. The plot shows actin in the pellets (○), actin in the supernatants (●), and gelsolin in the supernatants (◇). Data points are mean values from three independent experiments.

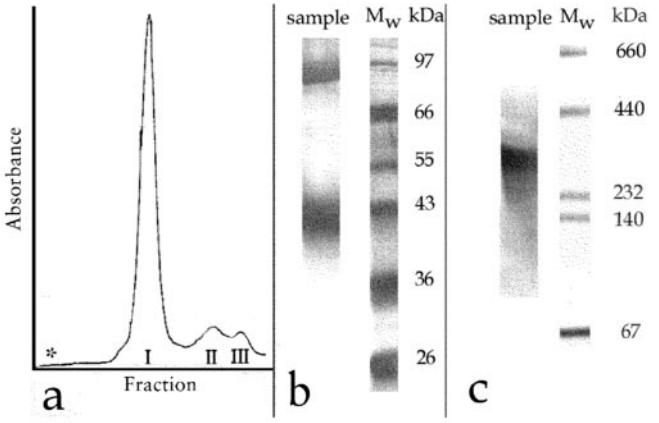

FIG. 5. **Non-sedimentable components obtained after tropomyosin induced annealing.** *Panel a* shows the separation of the components in the supernatant by gel filtration; the *asterisk* indicates the start of elution. A major peak (*I*) of 200 kDa and two smaller peaks containing free gelsolin (*II*) and actin (*III*) are separated. The protein absorbance was followed at 280 nm. *b*, analysis of the protein content from the major peak (*I*) by electrophoresis under denaturing conditions shows that the 200-kDa complex contains one gelsolin (90 kDa) and three actins (42 kDa) in a 1:3 molar ratio. *c*, characterization of the total non-sedimentable material by native polyacrylamide gel electrophoresis showed the presence of 1 component with an approximate molecular mass of 260 kDa.

filament formation. To test whether the excess gelsolin was associated with tropomyosin or appeared in the free form, a corresponding sample was analyzed by gel chromatography on Superdex 200. Analysis of the fractions from the chromatography by SDS-PAGE gave no evidence for complex formation between tropomyosin and gelsolin. In fact, the two proteins appeared in the eluate completely separated from each other (data not shown).

To characterize the status of the proteins remaining in the supernatant, a gelsolin-capped actin oligomer (1:10 molar ratio) was incubated with tropomyosin as described earlier, and the supernatant was collected and analyzed by gel chromatography (Fig. 5). After tropomyosin annealing a gelsolin-actin complex of this length, one major component (*I*) with an approximate molecular mass of 200 kDa was found in the supernatant (Fig. 5*a*). Analysis of this material by SDS-PAGE and subsequent densitometry showed gelsolin and actin in a 1:3 molar ratio (Fig. 5*b*). In addition to the GA_3 peak, there were two smaller peaks containing free gelsolin (*II*) and actin (*III*). Electrophoresis under non-denaturing conditions (Fig. 5*c*) confirmed the existence of one component, whose molecular mass appeared to be 260 kDa.

Gelsolin Binding to Tropomyosin-decorated Actin Filaments—Observations of significant amounts of gelsolin cosedimenting with the tropomyosin-decorated actin filaments prompted a more systematic analysis. For this, mixtures of actin with increasing concentrations of gelsolin were incubated with tropomyosin (10 μM), the mixtures were centrifuged, and pellets were analyzed by SDS-PAGE (Fig. 6*a*). As seen in Fig. 6*b*, increasing the concentrations of gelsolin resulted in increasing amounts of gelsolin cosedimenting with tropomyosin-decorated actin filaments. Saturation of the tropomyosin-actin filaments was reached at 0.15 mol of gelsolin per mol of actin, suggesting 2 gelsolin molecules per 14 actin monomers and 2 tropomyosins. The gelsolin bound to tropomyosin-decorated actin filaments with an apparent K_d of 1.4 μM. Control experiments show that ultracentrifugation of mixtures containing gelsolin and tropomyosin in the absence of actin resulted in the appearance of only small amounts of gelsolin in the pellet.

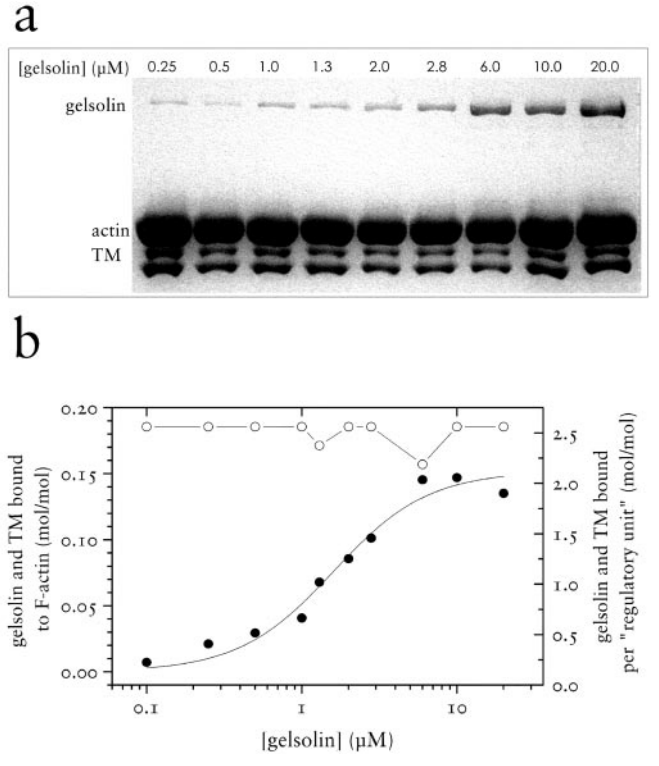

FIG. 6. **Association of gelsolin with tropomyosin-decorated actin filaments.** *Panel a*, cosedimentation of β-actin (20 μM), gelsolin (0.25–20 μM), and tropomyosin (10 μM). Pellets were analyzed by SDS-PAGE, and the concentrations of actin, gelsolin, and tropomyosin (*TM*) were estimated by densitometry. In *panel b*, bound gelsolin (●) and bound tropomyosin (○) are expressed as molar ratios to actin monomers. Bound gelsolin and tropomyosin molecules per actin 14-mer is demonstrated by the right-side *y* axis. Nonlinear least squares fitting to data points (mean values, $n = 7$) shows a maximum binding at 0.15 gelsolin/actin (*i.e.* 2 gelsolin/14 actin) and a K_d of 1.4 μM for gelsolin binding to the tropomyosin-decorated filaments.

DISCUSSION

Control of the Actin Filament (+) End—Gelsolin caps the actin filament (+) end with high affinity (K_d in nM range).

Partial dissociation of gelsolin from actin can be accomplished by the addition of calcium-chelating agents. Efficient dissociation of gelsolin from the (+) end of actin monomers or filaments *in vitro* has been accomplished only by the addition of polyphosphoinositides (18), and evidence for the involvement of polyphosphoinositides in the control of actin polymerization *in vivo* has been reported (5). Our observations that tropomyosin can dissociate GA complexes, including the high affinity-bound actin monomer strongly suggests that tropomyosin is an important element in the control of actin polymerization in the cell. Both the generation of free (+) ends and the tropomyosin-induced polymerization of small GA complexes could be important in this context. Actin filament (+) ends may be capped by other members of the gelsolin/villin family of actin regulatory proteins or by the ubiquitously expressed Cap Z (58, 59). Therefore, the effects of tropomyosin isoforms on filaments of the α-, β-, and γ-actin isoforms, capped by different (+) end binding proteins, need to be systematically investigated.

Mechanisms in Tropomyosin-induced Dissociation of GA Complexes—There appear to be two mechanisms involved in the dissociation of gelsolin from actin oligomers by tropomyosin. One mechanism depends on multi-site association of tropomyosin with gelsolin-capped actin oligomers. It can be detected with gelsolin-actin oligomers containing six or more actin monomers even at low concentrations of tropomyosin. The midpoint of the transition from gelsolin-actin complexes to tropomyosin-bound actin polymers occurred when the complexes were about 14 monomers long. The cooperativity of the interaction seen in this case suggests that there is an underlying conformational change in the actin oligomer that causes the dissociation of gelsolin.

A second mechanism operating at higher concentrations of tropomyosin causes the dissociation of small gelsolin-actin complexes, GA, GA_2, and GA_3, with lesser efficiency. This effect was unexpected, since the affinity of gelsolin for the actin filament (+) ends (K_d <5 nM) is much higher than that of tropomyosin for filamentous actin (K_d 0.2 μM) (60). It has been reported, however, that there is a calcium-dependent interaction between intact gelsolin and tropomyosin *in vitro* (K_d 0.6 μM) (61) and that the actin filament binding domain 2 of gelsolin also binds to tropomyosin (62). It is reasonable therefore to suggest that the tropomyosin effect on small gelsolin-actin complexes is due to tropomyosin causing a conformational change in the gelsolin molecule, resulting in its release from actin.

The appearance of a minimum in the curve describing tropomyosin-driven conversion of GA complexes of varying lengths (Fig. 4) suggested that complexes consisting of gelsolin and three to five actins are more stable than either smaller or larger complexes. Analysis of the supernatants obtained in these experiments showed that GA_3 was the major, non-sedimentable component obtained from mixtures initially containing gelsolin and more than three actins. The explanation to GA_3 being the most stable complex could be related to the fact that three of the domains of gelsolin interact with actin. It is possible that these domains bind to three different actin monomers and that this provides stability to a gelsolin-bound actin trimer. The dip in the curve mentioned above would then reflect the operation of the two mechanisms of tropomyosin-driven dissociation of GA complexes, annealing of longer gelsolin-bound actin complexes through multi-site interactions and annealing of shorter complexes due to tropomyosin-induced conformational change in gelsolin.

The initial phase of the interaction involving both short and long GA complexes might result in the release of free gelsolin. In the case of long GA complexes, released gelsolin would reassociate with actin monomers to form stable GA_3 complexes. Alternatively, multi-site interactions between tropomyosin and longer GA complexes could weaken the actin-actin bonds three monomers away from gelsolin, resulting in the direct dissociation of GA_3 complexes. The observation that GA_3 complexes are particularly stable is in agreement with results obtained in a systematic study of the appearance of stable complexes formed with gelsolin and actin mixed in different proportions and under different conditions.[2]

Conformational Changes in Actin—Several lines of evidence indicate that actin filaments can exist in at least two different conformational states depending on the type of ligand bound to the polymer. Gelsolin binding to the (+) end of an actin filament changes the polymer conformation, stabilizing the filament in a state that binds proteins of the ADF/cofilin family with increased affinity. Biochemical data suggest that this effect propagates 10–20 monomers from the (+) end of the filament (63). Evidence obtained by electron microscopy and time-resolved phosphorescence and absorption anisotropy indicate that the effect of gelsolin extends over longer distances and involves changes in the helicity and torsional flexibility of the actin filament (64, 65). Furthermore, it has been demonstrated that binding of ADF/cofilin to F-actin changes the helical twist of actin filaments (66).

ADF/cofilin and tropomyosin display mutually exclusive binding to actin filaments (67–69). This appears not to be due to steric hindrance, since the two proteins do not have overlapping binding sites on actin filaments (70, 71). Phalloidin can bind to tropomyosin-decorated actin filaments (72), whereas it cannot bind to cofilin-decorated filaments (63), suggesting that cofilin and tropomyosin stabilize different conformers of actin. Also, as mentioned above, the relative affinity of ADF/cofilin for actin filaments is increased by gelsolin (63), whereas phalloidin is displaced by the binding of gelsolin (73). Thus, tropomyosin and phalloidin appear to stabilize one state of the actin filament and gelsolin and ADF/cofilin another.

The observations reported here further support the idea of multiple conformations of actin filaments accessible via cooperative transitions along the length of the filaments induced by binding of various actin-binding proteins. How an interplay between tropomyosin and ADF/cofilin might be used by cells to control actin polymerization is unclear. It is possible that signal transduction pathways controlling the activity of ADF/cofilin (74, 75) in turn control the activity of tropomyosin described here.

Connections between Gelsolin and Tropomyosin in the Generation of Force—Our observation that tropomyosin establishes the position of gelsolin along actin filaments points to an intimate functional connection between these two proteins. This recalls observations that gelsolin is colocalized with actin stress fibers in tissue-cultured cells (27, 28) and in myofibrils (29). Gelsolin was shown to be important for stress fiber contractility *in vivo* (28), and *in vitro* gelsolin enhances the actomyosin Mg-ATPase activity (76, 77), an effect potentiated by tropomyosin and dependent on calcium ions (76, 78).

In muscle cells, the calcium-sensitive troponin complex forms a regulatory unit with tropomyosin, controlling the actomyosin ATPase. It has long been held, dating back to Bremel and Weber (79), that conformational changes propagated along tropomyosin-decorated actin filaments confer cooperativity to the regulation of force development. Stress fibers in non-muscle cells consist of bundles of actin filaments with periodic arrangements of both myosin and tropomyosin, perhaps form-

[2] K. Narayan, M. Nyåhern-Meazza, S. Irischholz, C. E. Schutt, and U. Lindberg, submitted for publication.

ing regulatory units similar to those in sarcomeres of muscle cells (80). A possible mechanistic implication of the observations discussed here is that gelsolin-tropomyosin-decorated actin filaments can sustain the directional propagation of force progressively from one regulatory unit to the next. In this model, gelsolin conditions actin filaments for tension development, a role similar to that of troponin in muscle cells.

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REFERENCES

1. Sheterline, P., Handel, S. E., Molloy, C., and Hendry, K. A. (1991) *Acta Histochem. Suppl.* **41,** 303–309
2. Pollard, T. D., Blanchoin, L., and Mullins, R. D. (2000) *Annu. Rev. Biophys. Biomol. Struct.* **29,** 545–576
3. Pawlak, G., and Helfman, D. M. (2001) *Curr. Opin. Genet. Dev.* **11,** 41–47
4. Small, J. V., Stradal, T., Vignal, E., and Rottner, K. (2002) *Trends Cell Biol.* **12,** 112–120
5. Sun, H. Q., Yamamoto, M., Mejillano, M., and Yin, H. L. (1999) *J. Biol. Chem.* **274,** 33179–33182
6. Burtnick, L. D., Koepf, E. K., Grimes, J., Jones, E. Y., Stuart, D. I., McLaughlin, P. J., and Robinson, R. C. (1997) *Cell* **90,** 661–670
7. Kwiatkowski, D. J., Stossel, T. P., Orkin, S. H., Mole, J. E., Colten, H. R., and Yin, H. L. (1986) *Nature* **323,** 455–458
8. Bryan, J. (1988) *J. Cell Biol.* **106,** 1553–1562
9. Way, M., Gooch, J., Pope, B., and Weeds, A. G. (1989) *J. Cell Biol.* **109,** 593–605
10. McGough, A., Chiu, W., and Way, M. (1998) *Biophys. J.* **74,** 764–772
11. Renoult, C., Blondin, L., Fattoum, A., Ternent, D., Maciver, S. K., Raynaud, F., Benyamin, Y., and Roustan, C. (2001) *Eur. J. Biochem.* **268,** 6165–6175
12. Pope, B., Maciver, S., and Weeds, A. (1995) *Biochemistry* **34,** 1583–1588
13. Sun, H. Q., Wooten, D. C., Janmey, P. A., and Yin, H. L. (1994) *J. Biol. Chem.* **269,** 9473–9479
14. Puius, Y. A., Fedorov, E. V., Eichinger, L., Schleicher, M., and Almo, S. C. (2000) *Biochemistry* **39,** 5322–5331
15. Yin, H. L., Hartwig, J. H., Maruyama, K., and Stossel, T. P. (1981) *J. Biol. Chem.* **256,** 9693–9697
16. Janmey, P. A., Chaponnier, C., Lind, S. E., Zaner, K. S., Stossel, T. P., and Yin, H. L. (1985) *Biochemistry* **24,** 3714–3723
17. Yin, H. L., and Stossel, T. P. (1979) *Nature* **281,** 583–586
18. Janmey, P. A., and Stossel, T. P. (1987) *Nature* **325,** 362–364
19. Kinosian, H. J., Newman, J., Lincoln, B., Selden, L. A., Gershman, L. C., and Estes, J. E. (1998) *Biophys. J.* **75,** 3101–3109
20. Small, J. V., Isenberg, G., and Celis, J. E. (1978) *Nature* **272,** 638–639
21. Wang, Y. L. (1985) *J. Cell Biol.* **101,** 597–602
22. Hoglund, A. S., Karlsson, R., Arro, E., Fredriksson, B. A., and Lindberg, U. (1980) *J. Muscle Res. Cell Motil.* **1,** 127–146
23. Small, J. V., Herzog, M., and Anderson, K. (1995) *J. Cell Biol.* **129,** 1275–1286
24. Reinhard, M., Jarchau, T., and Walter, U. (2001) *Trends Biochem. Sci.* **26,** 243–249
25. Dickinson, R. B., and Purich, D. L. (2002) *Biophys. J.* **82,** 605–617
26. Hartwig, J. H., Chambers, K. A., and Stossel, T. P. (1989) *J. Cell Biol.* **108,** 467–479
27. Dissmann, E., and Hinssen, H. (1994) *Eur. J. Cell Biol.* **63,** 336–344
28. Arora, P. D., Janmey, P. A., and McCulloch, C. A. (1999) *Exp. Cell Res.* **250,** 155–167
29. Gonsior, S., and Hinssen, H. (1995) *Cell Motil. Cytoskeleton* **31,** 196–206
30. Hitchcock-DeGregori, S. E., and Varnell, T. A. (1990) *J. Mol. Biol.* **214,** 885–896
31. Wegner, A. (1979) *J. Mol. Biol.* **131,** 839–853
32. Maytum, R., Geeves, M. A., and Konrad, M. (2000) *Biochemistry* **39,** 11913–11920
33. Moraczewska, J., and Hitchcock-DeGregori, S. E. (2000) *Biochemistry* **39,** 6891–6897
34. Lehrer, S. S., and Geeves, M. A. (1998) *J. Mol. Biol.* **277,** 1081–1089
35. Lees-Miller, J. P., and Helfman, D. M. (1991) *Bioessays* **13,** 429–437
36. Lin, J. J., Warren, K. S., Wamboldt, D. D., Wang, T., and Lin, J. L. (1997) *Int. Rev. Cytol.* **170,** 1–38
37. Gunning, P., Hardeman, E., Jeffrey, P., and Weinberger, R. (1998) *Bioessays* **20,** 892–900
38. Lin, J. J., Hegmann, T. E., and Lin, J. L. (1988) *J. Cell Biol.* **107,** 563–572
39. Schevzov, G., Gunning, P., Jeffrey, P. L., Temm-Grove, C., Helfman, D. M., Lin, J. J., and Weinberger, R. P. (1997) *Mol. Cell. Neurosci.* **8,** 439–454
40. Percival, J. M., Thomas, G., Cock, T. A., Gardiner, E. M., Jeffrey, P. L., Lin, J. J., Weinberger, R. P., and Gunning, P. (2000) *Cell Motil. Cytoskeleton* **47,** 189–208
41. Broschat, K. O., Weber, A., and Burgess, D. R. (1989) *Biochemistry* **28,** 8501–8506
42. Kojima, H., Ishijima, A., and Yanagida, T. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 12962–12966
43. Wen, K. K., Kuang, B., and Rubenstein, P. A. (2000) *J. Biol. Chem.* **275,** 40594–40600
44. Ishikawa, R., Yamashiro, S., and Matsumura, F. (1989) *J. Biol. Chem.* **264,** 7490–7497
45. Ishikawa, R., Yamashiro, S., and Matsumura, F. (1989) *J. Biol. Chem.* **264,** 16764–16770
46. Marston, S. B., and Redwood, C. S. (1993) *J. Biol. Chem.* **268,** 12317–12320
47. Redowicz, M. J. (2001) *J. Muscle Res. Cell Motil.* **22,** 163–173
48. Lindberg, U., Schutt, C. E., Hellsten, E., Tjader, A. C., and Hult, T. (1988) *Biochim. Biophys. Acta* **967,** 391–400
49. Kurokawa, H., Fujii, W., Ohmi, K., Sakurai, T., and Nonomura, Y. (1990) *Biochem. Biophys. Res. Commun.* **168,** 451–457
50. Bryan, J. (1992) *Methods Enzymol.* **215,** 88–99
51. Smillie, L. B. (1982) *Methods Enzymol.* **85,** 234–241
52. Laemmli, U. K. (1970) *Nature* **227,** 680–685
53. Hill, A. V. (1910) *J. Physiol. (Lond.)* **40,** 4–8
54. Kouyama, T., and Mihashi, K. (1981) *Eur. J. Biochem.* **114,** 33–38
55. Schüler, H., Korenbaum, E., Schutt, C. E., Lindberg, U., and Karlsson, R. (1999) *Eur. J. Biochem.* **265,** 210–220
56. Nyman, T., Schuler, H., Korenbaum, E., Schutt, C. E., Karlsson, R., and Lindberg, U. (2002) *J. Mol. Biol.* **317,** 577–589
57. Janmey, P. A., and Stossel, T. P. (1986) *J. Muscle Res. Cell Motil.* **7,** 446–454
58. Kwiatkowski, D. J. (1999) *Curr. Opin. Cell Biol.* **11,** 103–108
59. Witke, W., Li, W., Kwiatkowski, D. J., and Southwick, F. S. (2001) *J. Cell Biol.* **154,** 775–784
60. Hitchcock-DeGregori, S. E. (1994) *Adv. Exp. Med. Biol.* **358,** 85–96
61. Koepf, E. K., and Burtnick, L. D. (1992) *FEBS Lett.* **309,** 56–58
62. Maciver, S. K., Ternent, D., and McLaughlin, P. J. (2000) *FEBS Lett.* **473,** 71–75
63. Ressad, F., Didry, D., Xia, G. X., Hong, Y., Chua, N. H., Pantaloni, D., and Carlier, M. F. (1998) *J. Biol. Chem.* **273,** 20894–20902
64. Orlova, A., Prochniewicz, E., and Egelman, E. H. (1995) *J. Mol. Biol.* **245,** 598–607
65. Prochniewicz, E., Zhang, Q., Janmey, P. A., and Thomas, D. D. (1996) *J. Mol. Biol.* **260,** 756–766
66. McGough, A., Pope, B., Chiu, W., and Weeds, A. (1997) *J. Cell Biol.* **138,** 771–781
67. Bernstein, B. W., and Bamburg, J. R. (1982) *Cell Motil.* **2,** 1–8
68. Nishida, E., Maekawa, S., and Sakai, H. (1984) *Biochemistry* **23,** 5307–5313
69. Bamburg, J. R. (1999) *Annu. Rev. Cell Dev. Biol.* **15,** 185–230
70. McGough, A. (1998) *Curr. Opin. Struct. Biol.* **8,** 166–176
71. Ono, S., and Ono, K. (2002) *J. Cell Biol.* **156,** 1065–1076
72. De La Cruz, E., and Pollard, T. D. (1994) *Biochemistry* **33,** 14387–14392
73. Allen, P. G., and Janmey, P. A. (1994) *J. Biol. Chem.* **269,** 32916–32923
74. Amano, T., Tanabe, K., Eto, T., Narumiya, S., and Mizuno, K. (2001) *Biochem. J.* **354,** 149–159
75. Dan, C., Kelly, A., Bernard, O., and Minden, A. (2001) *J. Biol. Chem.* **276,** 32115–32121
76. Onji, T., Takagi, M., and Shibata, N. (1988) *Biochem. Biophys. Res. Commun.* **155,** 91–99
77. Albanesi, J. P., Coue, M., Fujisaki, H., and Korn, E. D. (1985) *J. Biol. Chem.* **260,** 13276–13280
78. Dabrowska, R., Hinssen, H., Galazkiewicz, B., and Nowak, E. (1996) *Biochem. J.* **315,** 753–759
79. Bremel, R. D., and Weber, A. (1972) *Nat. New Biol.* **238,** 97–101
80. Lazarides, E. (1976) *J. Cell Biol.* **68,** 202–219