Regulation of Actin Binding and Actin Bundling Activities of Fascin by Caldesmon Coupled with Tropomyosin*

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Human fascin is an actin-bundling protein and is thought to play a role in the formation of microfilament bundles of microspikes and stress fibers in cultured cells. To explore the regulation of fascin-actin interaction, we have examined the effects of culture cell caldesmon and tropomyosin (TM) on actin binding activity of human fascin. Caldesmon alone or TM alone has little or no effect on the actin binding of fascin. However, caldesmon together with TM completely inhibits actin binding of human fascin. When calmodulin is added, the inhibition of fascin-actin interaction by caldesmon and TM becomes Ca\(^{2+}\) dependent because Ca\(^{2+}\)/calmodulin blocks actin binding of caldesmon. Furthermore, as phosphorylation of caldesmon by cdc2 kinase inhibits actin binding of caldesmon, phosphorylation can also control actin binding of fascin in the presence of TM. As expected by the inhibition of fascin-actin binding, caldesmon coupled with TM also inhibits actin bundling activity of fascin. Whereas smooth muscle caldesmon alone or TM alone shows no effect, caldesmon together with TM completely inhibits actin bundling activity of fascin. This inhibition is again Ca\(^{2+}\) dependent when calmodulin is added to the system. These results suggest important roles for caldesmon and TM in the regulation of the function of human fascin.

Fascins belong to a unique family of actin-bundling proteins (1, 2), which include sea urchin fascin (3–8), HeLa 55-kDa actin-bundling protein (9, 10), and the gene products of *Drosophila singed* (11–13). All of these proteins make F-actin aggregate side-by-side into bundles (4, 8–10) and are localized in the structures containing actin bundles including filopodia and stress fibers of cultured cells (1, 9, 10, 14), bristles of *Drosophila*, actin bundles of *Drosophila* nurse cells (11, 13), and microspikes and microvilli of sea urchin eggs and coelomocytes (6, 7). Some of these structures such as filopodia and microspikes are known to be dynamic structures responding to various biological signals. For example, the fertilization of sea urchin eggs induces the formation of fascin-containing microvilli on their surfaces (7). Filopodia contain fascin-actin bundles and are actively extending and retracting during cell movement of fibroblasts. Fascin should thus be involved in the assembly and disassembly of actin bundles in such structures. However, the mechanisms for the regulation of actin binding of fascin are not well understood.

One way to regulate fascin-actin interaction is phosphorylation. We have shown that human fascin is phosphorylated at Ser-39 in *vivo* in human neuroblastoma cells upon treatment with 12-O-tetradecanoylphorbol-13-acetate, a tumor promoter (16). The same site is phosphorylated by protein kinase C, which results in the inhibition of actin binding of fascin (16, 17). However, the stoichiometry of fascin phosphorylation by protein kinase C is low, suggesting that kinases other than protein kinase C may be involved. Furthermore, the stoichiometry of *in vivo* phosphorylation is also low in the absence of TPA, which suggests the presence of other mechanisms to control fascin-actin interactions under normal conditions.

Another possibility for the control mechanism of fascin-actin interaction is that other actin binding proteins modulate actin-fascin association. Our previous study indeed showed that skeletal muscle tropomyosin (TM\(^1\)) inhibits actin-fascin binding (18). However, we found that TM isolated from cultured rat cells has only a slight effect on actin binding of human fascin (18). On the other hand, human fascin inhibits actin binding of cultured rat cell TMs, depending on TM isoforms. Rat cultured cells contain at least six isoforms (19–23), which are divided into two groups based on the molecular weights and the strength of their actin binding. The isoforms with high $M_r$ (high $M_r$ TMs) bind to actin more strongly than the isoforms with low $M_r$ (low $M_r$ TMs) (21). Fascin completely inhibits actin binding of low $M_r$ TMs but shows little effect on high $M_r$ TMs. These observations suggest that TM could control the fascin-actin interactions if actin binding of TM is high enough. It is thus possible that a protein that can increase actin affinities of TMs may be able to modulate fascin-actin interactions. Caldesmon is a candidate as caldesmon and TM increase each other’s actin binding affinities (24–27). Caldesmon is an actin- and calmodulin binding protein (28, 29), the actin binding of which is regulated by Ca\(^{2+}\)/calmodulin. Caldesmon is also reported to bind to other proteins including myosin and TM. In *in vitro* reconstituted system, caldesmon is known to inhibit actomyosin ATPase. We showed that caldesmon together with TM inhibits actin-severing activity of gelsolin (30). These results suggest the regulatory roles of caldesmon in actomyosin-based motility and the organization of the actin cytoskeleton.

We have thus examined whether caldesmon has any effects on the interactions between human fascin and TMs. We have found that caldesmon, when coupled with TMs, not only blocks the binding of human fascin to actin but also dissociates fascin from actin. Our results suggest that caldesmon coupled with TM controls the formation of fascin-actin bundles.

MATERIALS AND METHODS

Cell Culture—SV40-transformed rat embryo cells (REF-WT4A) and HeLa cells were cultured in Dulbecco’s modified Eagle’s medium con-
Actin Binding Assay—Actin binding of cultured cell TMs, caldesmon, and human fascin was assayed in the following four conditions. (i) F-actin (final concentration, 12 μM) was first incubated for 1 h with varying concentrations of human fascin (0–3.6 μM) in 100 mM KCl, 0.5 mM dithiothreitol, 20 mM imidazole-HCl buffer (pH 7.0), and then a constant concentration of high M₆ TMs (2.1 μM) or low M₆ TMs (5.2 μM) was added in a final volume of 60 μL. In some experiments, nonmuscle caldesmon (3.3 μM) was also added. (ii) F-actin (final concentration, 12 μM) was first incubated for 1 h with a constant concentration of human fascin (7.1 μM) in the same buffer as described above, and varying concentrations of nonmuscle caldesmon (0–3.3 μM) and a constant concentration of high M₆ TMs (2.1 μM) or low M₆ TMs (5.2 μM) were added. (iii) F-actin (final concentration, 12 μM) was first incubated for 1 h at room temperature with human fascin (2.2 μM) in the same imidazole buffer except that either 0.1 mM CaCl₂ or 1 mM EGTA was included. Nonmuscle caldesmon (3.3 μM) and calmodulin (24 μM) with either low M₆ TMs (5.2 μM) or high M₆ TMs (2.1 μM) were then added. (iv) F-actin (final concentration, 12 μM) was first incubated for 1 h at room temperature with human fascin (2.2 μM) in the same imidazole buffer. Caldesmon, which had been phosphorylated by cdc2 kinase as described previously (32), was then added together with either low M₆ TMs (5.2 μM) or high M₆ TMs (2.1 μM) as a control, without phosphorylated caldesmon (32). In all actin binding experiments described above, the samples were further incubated for 1 h and centrifuged at 140,000 × g (Beckman Airfuge, 28 p.s.i.) for 20 min. Both supernatants and pellets were dissolved in an equivalent volume of SDS sample buffer and analyzed by 12.5% SDS-polyacrylamide gel electrophoresis. Gels were stained with Coomassie Brilliant Blue R-250 and scanned with a Joyce Loebl Chromoscan 3 densitometer (Vickers Instrument Inc., Malden, MA) to quantitate the amount of actin, human fascin, caldesmon, and TMs.

Actin Bundling Activity—Actin bundling activity was measured by low speed centrifugation assay and fluorescence microscopy. In low speed centrifugation assay, recombinant human fascin (0–0.89 μM) was mixed with gizzard TM (2.3 μM), gizzard caldesmon (1.7 μM), calmodulin (12 μM), and F-actin (7.1 μM) in 100 mM KCl. 20 mM dithiothreitol, 20 mM imidazole-HCl buffer (pH 7.0) either in the presence of 1 mM CaCl₂ or 1 mM EGTA. After incubation for 30 min at room temperature, the mixtures were centrifuged at 8,000 × g for 20 min. Both supernatants and pellets were dissolved in an equivalent volume of SDS sample buffer, and the amount of actin was determined by SDS-polyacrylamide gel electrophoresis followed by densitometry. In fluorescence microscopy, F-actin filaments (7.1 μM) containing 10% rhodamine-phalloidin-labeled F-actin (33) were mixed with 0.89 μM recombinant fascin, 2.3 μM gizzard TM, 1.7 μM gizzard caldesmon, and 12 μM calmodulin in 100 mM KCl, 20 mM dithiothreitol, 20 mM imidazole-HCl buffer (pH 7.0) either in the presence of 1 mM CaCl₂ or 1 mM EGTA. After incubation for 30 min at room temperature, the mixture was sandwiched between a coverslip and slide glass, sealed with petroleum jelly, and observed with a fluorescence microscope (Axioplan; Zeiss, Oberkochen, Germany) equipped with a 100× oil lens (Plan-NEOFLAR; Zeiss).

Other Procedures—SDS-polyacrylamide gel electrophoresis was performed as described by Blatter et al. (34) using 12.5% polyacrylamide except that the buffer system of Laemmli et al. (35) was used. Protein concentrations were determined by the Bradford method (36).

RESULTS

Caldesmon, Together with TMs, Can Dissociate Human Fascin from Actin—We have first examined whether caldesmon and/or TMs have any effects on fascin-actin interactions. F-actin was first incubated with varying concentrations of human fascin to form fascin-actin complexes, and then a constant concentration of TMs (2.1 μM) and/or TMs was added. In the absence of TMs, caldesmon is strongly inhibited when caldesmon together with high TMs (●) or low TMs (▲) was added. The experiments were repeated three times, and representative data are shown here.

Actin binding ability of our low M₆ TM preparation; Helfman's group has revealed, using recombinant TM isoforms, that TM-4 shows much weaker actin binding than TM-5 (37). As Fig. 1 shows, caldesmon alone (open squares) or TM alone (open circles, high M₆ TMs; open triangles, low M₆ TMs) has little or no effect on actin-fascin binding (compare with a control, ×). On the contrary, when both caldesmon and TMs (closed circles, high M₆ TMs; closed triangles, low M₆ TMs) were added, fascin-actin binding was greatly abolished. Although 2.2 μM human fascin shows saturated binding to actin in the absence of either TMs or caldesmon, the addition of both TMs and caldesmon causes the dissociation of more than 90% of fascin from actin. It should be noted that caldesmon and TMs were added after the formation of fascin-actin complexes, indicating that caldesmon together with TMs can cause the dissociation of fascin from actin.

In the same experimental conditions as described above, we have determined how actin binding of high M₆ or low M₆ TMs is affected by fascin. Fig. 2A shows actin binding of high M₆ TMs in the presence of varying amounts of fascin. In the absence of caldesmon, human fascin slightly inhibits actin binding of high M₆ TMs by about 20% (open circles), which is consistent with our previous result (18). In the presence of caldesmon, fascin does not inhibit actin binding of high M₆ TMs at a concentration below 2 μM fascin (closed circles). However, above 2 μM fascin, actin binding of high M₆ TMs is decreased to a similar extent as observed in the absence of caldesmon. Fig. 2B shows the effects of fascin on actin binding of low M₆ TMs. In the absence of caldesmon, human fascin greatly inhibits actin binding of low M₆ TMs. Actin binding is decreased from 100 to 40% at 5.2 μM low M₆ TMs (open squares) and is decreased from 28 to less than 5% at 2.1 μM low M₆ TMs (open
Disassembly of Fascin-Actin Bundles by Caldesmon and TM

that an increase in actin-TM interactions by caldesmon allows TMs to compete with fascin in actin binding.

We have also examined whether fascin affects the actin binding of caldesmon in the same conditions described for Fig. 3. As Fig. 4 shows, in the absence of TM, fascin reduces caldesmon-actin binding to a 50% level (open circles), indicating that fascin and caldesmon compete in actin binding and that actin binding of fascin is stronger than actin binding of caldesmon. In the presence of either high (open squares) or low M₉ TM (open triangles), however, fascin has little effect on the actin binding of caldesmon (compare with the control, closed circles).

Regulation of Fascin-Actin Binding by Ca²⁺-Caldesmon-Calmodulin—The binding of caldesmon is regulated by calmodulin in a calcium-dependent way. In the absence of Ca²⁺, calmodulin does not bind to caldesmon, and caldesmon binds to actin. On the other hand, in the presence of Ca²⁺, calmodulin binds to caldesmon, and a Ca²⁺-calmodulin-caldesmon complex does not bind to actin (28, 29). We have thus examined whether actin binding of fascin can be regulated in a calcium-dependent way by controlling actin binding of caldesmon with Ca²⁺/calmodulin. As Fig. 5A shows, when calcium is absent, calmodulin has no effect on actin binding of caldesmon, and thus, caldesmon coupled with high M₉ TM inhibits actin-fascin binding almost completely. In the presence of calcium, caldesmon binding to actin is inhibited by calmodulin; the molar ratio of fascin to actin is increased from 0.19 to 0.07. At the same time, the molar ratio of fascin to actin is increased from 0.007 to 0.07. Fascin does not bind to calmodulin, and actin binding of fascin is not regulated by Ca²⁺/calmodulin (data not shown). Thus, the regulation of fascin-actin binding is through the regulation of caldesmon-actin binding. Similar calcium-dependent regulation of fascin-actin binding is observed when low M₉ TM was used instead of high M₉ TM (Fig. 5B). The molar ratio of fascin to actin is increased from 0.04 to 0.12 when calcium is added.

Regulation of Fascin-Actin Binding through Phosphorylation of Caldesmon—Phosphorylation of caldesmon by cdc2 kinase causes the dissociation of caldesmon from actin (32, 38). We have thus examined effects of caldesmon phosphorylation on
actin binding of fascin and found that phosphorylation of caldesmon by cdc2 kinase also controls fascin-actin binding. As Fig. 6 shows, most (more than 80%) of phosphorylated caldesmon does not bind to F-actin even in the presence of high M<sub>f</sub> TMs (lane 3, precipitate; lane 4, supernatant) or low M<sub>f</sub> TMs (lane 7, precipitate; lane 8, supernatant). As a result, fascin-actin binding is not inhibited; about 60% of fascin are bound to F-actin in this condition (lanes 3 and 7). On the other hand, unphosphorylated caldesmon together with high M<sub>f</sub> TMs (lane 1, precipitate; lane 2, supernatant) or low M<sub>f</sub> TMs (lane 5, precipitate; lane 6, supernatant) greatly inhibits actin binding of fascin; only 20–30% of fascin binds to F-actin (lanes 1 and 5).

**Regulation of Actin Bundling Activity of Fascin by Caldesmon and TM**—The inhibition of fascin-actin binding by caldesmon coupled with TM indicates that actin bundling activity of fascin is also blocked by caldesmon together with TM. We have confirmed this by two independent assays, i.e. low speed centrifugation, in which only bundles of actin filaments are precipitated, and fluorescent microscopy to directly observe actin bundles. Instead of cultured cell caldesmon and TM, we used smooth muscle caldesmon and TM for this purpose because the properties of smooth muscle caldesmon and those of TM are very similar to those of nonmuscle caldesmon and TM.

Fig. 7A and Table I show the effect of TM and caldesmon on actin bundling activity of fascin examined by the low speed centrifugation assay. When 7.1 μM actin is incubated with 0.86 μM fascin, about 75% of actin is precipitated (lanes 3 and 4). The addition of either caldesmon alone (lanes 5 and 6) or TM alone (lanes 7 and 8) to the fascin-actin mixture does not alter the amount of actin pelleted by low speed centrifugation. On the other hand, when both caldesmon and TM are added (lanes 9 and 10), the amount of pelletable actin is reduced to 20%, a level of which is similar to that found in the control without fascin (lanes 1 and 2). Fig. 7B shows the actin bundling activity of fascin with or without addition of caldesmon and TM when fascin concentrations are changed. In the presence of both caldesmon and TM, fascin cannot precipitate F-actin in low speed centrifugation even at high fascin concentrations (closed circles). In the absence of caldesmon and TM (control, open circles), on the other hand, about 75% of F-actin is precipitated by 0.86 μM fascin. These results indicate that the bundling activity of fascin is almost completely blocked by the addition of both caldesmon and TM just as actin binding activity of fascin is inhibited by caldesmon coupled with TM.

To further confirm the inhibition, we have directly observed the fluorescently labeled actin-fascin bundles (Fig. 8). In the absence of fascin, no actin bundles are observed (Fig. 8A). When fascin is mixed with actin filaments, very thick F-actin bundles are formed (Fig. 8C). Similar bundles are observed when caldesmon alone (Fig. 8D) or TM alone (Fig. 8E) is added to fascin-actin mixtures. On the contrary, when fascin is mixed with F-actin in the presence of both caldesmon and TM, no such bundles but very thin filaments are observed (Fig. 8F). These thin filaments seem to represent bundles consisting of a few

![Graph](image-url)

**Fig. 4. Effects of fascin on caldesmon-actin binding.** Instead of fascin-actin binding, caldesmon-actin binding was determined under the same conditions as described for Fig. 3. In the absence of TM (○), fascin inhibits actin binding of caldesmon by 50% when compared with a control (●, no addition of TM or fascin). In the presence of either high M<sub>f</sub> TMs (□) or low M<sub>f</sub> TMs (△), fascin shows little or no effects on actin binding of caldesmon.

**Fig. 5. Ca<sup>2+</sup> regulation of fascin-actin binding by a caldesmon-calmodulin complex.** Fascin (2.2 μM) was first complexed with 12 μM F-actin, and then 3.3 μM caldesmon and 24 μM calmodulin either with 2.1 μM high M<sub>f</sub> TMs (A, indicated by TM 1 and TM 2) or 5.2 μM low M<sub>f</sub> TMs (B, indicated by TM 4.5) were added in the presence of 1 mM Ca<sup>2+</sup> or 1 mM EGTA. Lane 1, molecular mass markers; lane 2, supernatant in the presence of Ca<sup>2+</sup>; lane 3, precipitate in the presence of Ca<sup>2+</sup>; lane 4, supernatant in the presence of EGTA; lane 5, precipitate in the presence of EGTA; lane 6, supernatant in the presence of Ca<sup>2+</sup>, but calmodulin was omitted from the reaction mixture; lane 7, precipitate in the presence of Ca<sup>2+</sup>, but calmodulin was omitted from the reaction mixture. Whereas fascin-actin binding is greatly inhibited in the absence of Ca<sup>2+</sup> or calmodulin, fascin can bind to F-actin in the presence of Ca<sup>2+</sup>/calmodulin/caldesmon. Note that actin binding of caldesmon is also inhibited by Ca<sup>2+</sup>/calmodulin. The experiments were repeated three times, and representative data are shown here.
FIG. 6. Phosphorylation of caldesmon by cdc2 regulates fascin-actin binding. F-actin (final concentration, 12 μM) was first incubated with human fascin (2.2 μM), and then either phosphorylated or unphosphorylated caldesmon was added together with either high M, TMs (2.1 μM) or low M, TMs (5.2 μM). Lanes 1–4, high M, TMs (indicated by TM 1 and TM 2); lanes 5–8, low M, TMs (indicated by TM 4,5). Lane 1, precipitate with unphosphorylated caldesmon; lane 2, supernatant with unphosphorylated caldesmon; lane 3, precipitate with phosphorylated caldesmon; lane 4, supernatant with phosphorylated caldesmon; lane 5, precipitate with phosphorylated caldesmon; lane 6, supernatant with unphosphorylated caldesmon; lane 7, precipitate with phosphorylated caldesmon; lane 8, supernatant with phosphorylated caldesmon. Phosphorylated caldesmon does not bind to F-actin; as a result, fascin can bind to actin, whereas unphosphorylated caldesmon binds to F-actin and inhibits fascin-actin binding in the presence of TM. The experiments were repeated at least three times, and the representative data are shown here.

actin filaments. These observations are consistent with the results obtained by the low speed centrifugation assay described above.

The fluorescent microscopy method has allowed us to examine how quickly caldesmon coupled with TM can disassemble preformed fascin-actin bundles. The disassembly of fascin-actin bundles occurs rather quickly. Within 3 min, roughly 80% of thick fascin-actin bundles disappear; in 20 min, no such bundles are observed.

Ca"/calmodulin can regulate the actin binding activity of fascin when both TM and caldesmon are present. It is thus expected that Ca"/calmodulin also controls the actin binding activity of fascin in the presence of caldesmon and TM. As Fig. 9A and Table I show, fascin precipitates only 16% of actin filaments when caldesmon, TM, and calmodulin are present in the presence of 1 mM EGTA (lanes 1 and 2). This level of actin precipitation is comparable with that in the absence of fascin, indicating that caldesmon coupled with TM and calmodulin inhibits the actin bundling activity in the absence of Ca".

Again, very thin filaments were observed by fluorescent microscopy (Fig. 9B), which is similar to those shown in the absence of calmodulin (see Fig. 8F). When EGTA is replaced with CaCl₂, the amount of precipitated actin increases to 54% (lanes 3 and 4). Fluorescent microscopy has revealed the appearance of thick actin bundles (Fig. 9C). These results confirm that calmodulin together with caldesmon and TM can confer Ca" sensitivity to the actin bundling activity of fascin.

**DISCUSSION**

**Regulation of the Actin Bundling of Fascin by Caldesmon and TM**—We have revealed that caldesmon coupled with cultured cell TM inhibits actin binding and bundling activities of fascin. Our results suggest a possible mechanism that caldesmon together with TM regulates the assembly and disassembly of actin bundles present in filopodia, membrane ruffles, and stress fibers. In fact, all three proteins are, at least to some

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**FIG. 7. Inhibition of actin bundling activity of fascin by TM and caldesmon.** Fascin at various concentrations (0.86 μM in panel A and 0–0.86 μM in panel B) was mixed with 7.1 μM actin filaments in the absence or presence of 1.7 μM smooth muscle caldesmon and 2.3 μM smooth muscle TM (a mixture of alpha and beta forms). Actin bundling activities were measured by centrifugation at 8,000 × g for 20 min. A, lanes in odd numbers and those in even numbers represent supernatants and precipitates, respectively. Lanes 1 and 2, actin only; lanes 3 and 4, plus fascin; lanes 5 and 6, plus caldesmon/fascin; lanes 7 and 8, plus TM/fascin; lanes 9 and 10; plus caldesmon/TM/fascin; lanes 11 and 12, plus caldesmon. Lanes 13 and 14, plus TM. Note that actin bundling activity of fascin is inhibited by the addition of both caldesmon and TM (see lanes 9 and 10), whereas caldesmon alone (lanes 5 and 6) or TM alone (lanes 7 and 8) does not inhibit fascin’s actin bundling. B, the amounts of actin precipitated by low speed centrifugation were plotted against the concentration of fascin added. ○, fascin only; □, in the presence of TM and caldesmon.

**TABLE I**

| Proteins | Precipitated actin | S.D. (n = 3) |
|----------|--------------------|-------------|
| Actin    | 14.3               | 2.4         |
| Actin + tropomyosin | 15.0 | 1.4         |
| Actin + caldesmon | 22.8 | 4.1         |
| Actin + fascin | 75.0 | 6.2         |
| Actin + fascin, tropomyosin | 74.3 | 0.5         |
| Actin + fascin, caldesmon | 76.0 | 10.6        |
| Actin + fascin, tropomyosin, caldesmon | 20.0 | 5.0         |
| Actin + fascin, tropomyosin, caldesmon, calmodulin (1 mM CaCl₂) | 54.3 | 6.6         |
| Actin + fascin, tropomyosin, caldesmon, calmodulin (1 mM EGTA) | 16.0 | 2.4         |

* Protein concentrations were as follows: actin, 7.1 μM; fascin, 0.89 μM; tropomyosin, 2.3 μM; caldesmon, 1.7 μM; calmodulin, 12 μM; in 100 mM NaCl, 20 mM dithiothreitol, and 20 mM imidazole-Cl (pH 7.0).

* Solutions were centrifuged at 8,000 × g for 20 min at room temperature.
Dissociation of actin bundling sites of fascin, caldesmon, and TMs results in actin bundle disassembly. This process is regulated in a Ca²⁺-dependent manner.

Competition in Actin Binding between Fascin and TM—Our results indicate that fascin competes with TM and caldesmon for actin binding and that the binding affinity of each protein for actin seems to be a major factor to determine which protein is dissociated from actin. For example, fascin (Kₐ = 5–7 × 10⁶ M⁻¹, Ref. 16) greatly inhibits actin binding of low Mₚ TMs (Kₐ = 3–4 × 10⁵ M⁻¹, Ref. 15) but decreases actin binding of high Mₚ TMs (Kₐ = 5–7 × 10⁶ M⁻¹, Ref. 15) by only 20%. These TM isoforms cannot dissociate fascin from actin. On the other hand, we have previously shown that skeletal muscle TM (Kₐ = 0.5–1 × 10⁷ M⁻¹, see Ref. 43) can dissociate fascin from actin, whereas fascin cannot dissociate skeletal muscle TM from actin (18). These results correlate well with the fact that low Mₚ TMs show the lowest affinity, whereas skeletal muscle TM has the highest among these TM isoforms. Furthermore, the result that caldesmon coupled with TM can dissociate fascin-actin complexes is explained by the fact that caldesmon and TM increases each other’s actin binding. The apparent binding constants become one order higher (24–27).

Recent structural study by Lehman et al. (44) has demonstrated that the binding site of caldesmon is shared with many actin binding proteins. Indeed, two of the major binding sites of gelsolin are shared with the binding sites of caldesmon and TM, which explains our previous results that caldesmon together with TM inhibits severing action of gelsolin (30, 45). Perhaps, fascin also shares the binding sites on actin with caldesmon and TM. However, it is worthy of note that high Mₚ TMs or caldesmon can still bind to actin, which is almost saturated with fascin, suggesting that the binding site of fascin on actin is not entirely the same as those of caldesmon and TM. The partial overlap of actin binding sites may explain why the dissociation of fascin-actin bundles is rapid. If the actin binding sites of these proteins completely overlap, then the binding of the second protein to actin will only occur after the dissociation of the first protein from actin. It is also possible that the simultaneous binding of TM and caldesmon may shift the binding sites of TM and caldesmon in such a way that TM and caldesmon can compete better with fascin for actin binding. Indeed, the three-dimensional reconstruction study (44) has suggested that caldesmon seems to induce movement of TM.
of fascin seem to contrast with those of caldesmon. There is an increasing body of evidence showing that caldesmon stabilizes microfilaments in nonmuscle cells (28), probably by increasing actin binding of TM (24–27). In vitro, caldesmon and TM combined inhibit both the actin-severing and actin-capping activities of gelsolin, even in the presence of Ca\(^{2+}\) (30, 45). Increased expression of caldesmon in vivo leads to the stabilization of microfilaments (46–48). Conversely, inhibition of caldesmon via microinjection of specific caldesmon antibody results in the disruption of microfilaments (49). On the other hand, fascin inhibits actin binding of cultured cell TM, when caldesmon is absent. Fascin also inhibits actin binding of caldesmon when TM is absent. Thus, fascin is likely to contribute to destabilization of microfilaments by counteracting the actions of caldesmon and TM.

It should be noted that the expression of caldesmon is down-regulated in cell transformation (39, 50, 51) whereas that of fascin is up-regulated (52, 53). In addition, the levels of high M\(_{r}\) TMs are decreased in many transformed cells (54–58). These changes may contribute to microfilament reorganization upon cell transformation. For example, more fascin coupled with less caldesmon and less TM would result in less binding of TM to actin, thereby causing the destabilization of microfilaments. Thus, fascin may express itself to induce more bundling of actin filaments, leading to uncoordinated formation of filopodia and membrane ruffles. In fact, we have recently shown that overexpression of fascin induces membrane protrusions including microspikes and lamellipodia (52). Furthermore, many transformed cells show increased levels of calmodulin (59). Thus, it is also possible that an increased level of calmodulin could decrease the binding of caldesmon, again resulting in the decreased stability of microfilaments and increased formation of actin bundles in filopodia and membrane ruffles.

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REFERENCES

1. Edwards, R. A., and Bryan, J. (1995) Cell Motil. Cytoskeleton 32, 1–9
2. Otto, J. J. (1994) Curr. Opin. Cell Biol. 6, 105–109
3. Kane, R. E. (1975) J. Cell Biol. 66, 305–315
4. Bryan, J. (1986) Methods Enzymol. 134, 13–23
5. Bryan, J., Edwards, R., Matsuura, P., Otto, J., and Wolkehke, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9115–9119
6. Otto, J. J., Kane, R. E., and Bryan, J. (1979) Cell 17, 285–293
7. Otto, J. J., Kane, R. E., and Bryan, J. (1980) Cell Motil. 1, 31–49
8. DeRosier, D. J., and Edds, K. T. (1980) Exp. Cell Res. 120, 490–494
9. Yamashiro-Matsumura, S., and Matsumura, F. (1985) J. Biol. Chem. 260, 5087–5097
10. Yamashiro-Matsumura, S., and Matsumura, F. (1986) J. Cell Biol. 103, 631–640
11. Cant, K., Knowles, B. A., Mooseker, M. S., and Cooley, L. (1994) J. Cell Biol. 125, 369–380
12. Cant, K., and Cooley, L. (1996) Genetics 143, 249–258
13. Tilney, L. G., Tinley, M. S., and Guild, G. M. (1995) J. Cell Biol. 130, 629–638
14. Sasaki, Y., Hayashi, K., Shirao, T., Ishikawa, R., and Kohama, K. (1996) J. Neurochem. 66, 980–986
15. Yamashiro-Matsumura, S., Ishikawa, R., and Yamashiro, S. (1988) Prototplasma 2, 1–9
16. Yamashiro-Matsumura, S., Ishikawa, R., and Yamashiro, S. (1986) J. Biol. Chem. 261, 15230–15238
17. Ono, S., Yamakita, Y., Yamashiro, S., Matsuda, P. T., Gnarra, J. R., Obinata, T., and Matsumura, F. (1997) J. Biol. Chem. 272, 2527–2533
18. Matsumura, F., and Yamashiro-Matsumura, S. (1986) J. Biol. Chem. 261, 4655–4659
19. Matsumura, F., Yamashiro-Matsumura, S., and Lin, J. J.-C. (1983) J. Biol. Chem. 258, 6636–6644
20. Kan, R., and Garrels, J. I. (1984) in Cancer Cells (Levine, A., Vande Woude, G., Topp, W., and Watson, J. D., eds) pp. 137–146, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Matsumura, F., and Yamashiro, M. S. (1985) J. Biol. Chem. 260, 13851–13859
22. Lees-Miller, J. P., and Helfman, D. M. (1991) J. Virol. 65, 147–155
23. Laemmli, U. K. (1970) Nature 227, 685–688
24. Bradford, M. (1976) Anal. Biochem. 72, 248–254
25. Tsien-Rooney, C., Gu, W., and Helfman, D. M. (1996) Cell Motil. Cytoskeleton 33, 225–240
26. Yamashiro, S., Yamakita, Y., Ishikawa, R., and Matsumura, F. (1990) Nature 344, 673–678
27. Kojio-Ohawa, M., Hukura, A., Iida, K., Yahara, I., Sobue, K., and Kacziu, S. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3313–3317
28. Noguera, A., and Lynch, W. (1985) J. Cell Biol. 100, 1656–1663
29. Lin, J. J., Hegmann, T. R., and Lin, J. J. (1988) J. Cell Biol. 107, 563–572
30. Steinfort, R., Zucker, R., and Schatten, G. (1977) Dev. Biol. 58, 185–186
31. Urbanickova, M., and Hitchcock-DeGregori, S. E. (1994) J. Biol. Chem. 269, 24310–24315
32. Lehman, W., Vier, P., and Craig, E. (1997) J. Biol. Chem. 272, 310–317
33. Ishikawa, R., Yamashiro, S., and Matsumura, F. (1989) J. Biol. Chem. 264, 16764–16770
34. Warren, K. S., Shutt, D. C., McDermott, J. P., Lin, J. J., Soll, D. R., and Lin, J. J. (1996) Cell Motil. Cytoskeleton 34, 215–229
35. Castellino, P., Ono, S., Matsumura, F., and Louni, A. (1995) J. Cell Biol. 131, 1223–1230
36. Lamb, N. J., Fernandez, A., Mezgueldi, M., Labbé, J. P., Kassas, R., and Fattoum, A. (1996) Eur. J. Cell Biol. 69, 36–44
37. Yamashiro, S., Yoshida, K., Yamakita, Y., and Matsumura, F. (1994) in International Conference on the Biophysics, Biochemistry, and Cell Biology of Actin (Estes, J. E., and Higgins, P. J., eds) pp. 113–112, Plenum Publishing, Troy, NY
38. Lin, J. J., Raj, S. L., and Lin, J. J. (1991) J. Biol. Chem. 266, 16917–16924
39. Yamashiro, S., Yamakita, Y., Ono, S., and Matsumura, F. (1998) Mol. Biol. Cell 9, 993–1006
40. Morris, S., Yamashiro, S., Baughman, R. W., Matsuda, P., Varah, L., Matsumura, F., Kieff, E., and Birkenbach, M. (1994) J. Virol. 68, 7320–7328
41. Hendricks, M., and Weintrob, H. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5633–5637
42. Leonard, C. L., Warren, R. H., and Rubin, R. W. (1982) Biochim. Biophys. Acta 720, 154–162
43. Matsumura, F., Lin, J. J., Yamashiro, M. S., Thomas, G. P., and Topp, W. C. (1983) J. Biol. Chem. 258, 13954–13964
44. Lin, J. J., Yamashiro-Matsumura, S., and Matsumura, F. (1984) in Cancer Cells (Levine, A., Vande Woude, G., Topp, W. C., and Watson, J., eds) pp. 57–65, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
45. Cooper, H. L., Feusterin, N., Noda, M., and Bassin, R. H. (1985) Mol. Cell. Biol. 5, 972–983
46. Veigl, M. L., Vanaman, T. C., and Sedwick, W. D. (1984) Biochim. Biophys. Acta 758, 21–48

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