The Identification of a Second Cofilin Binding Site on Actin
Suggests a Novel, Intercalated Arrangement of F-actin Binding*

Celine Renoult‡, Diane Ternent‡, Sutherland K. Maciver§, Abdellatif Fattoum¶,
Catherine Astier‡, Yves Benyamin‡, and Claude Roustan‡†

From the §UMR 5539 (CNRS), Laboratoire de Motilité Cellulaire (EcolePratique des Hautes Etudes), Université de Montpellier 2, Place E. Bataillon, CC107, 34095 Montpellier Cedex 5, France, the ‡Department of Biomedical Sciences, Hugh Robson Building, George Square, Edinburgh EH8 9XD, United Kingdom, and the ¶SUPR 1086 (CNRS), Centre de Recherches de Biochimie Macromoléculaire, 1919 Rte. de Mendes, 34293 Montpellier Cedex 5, France

The cofilins are members of a protein family that binds monomeric and filamentous actin, severs actin filaments, and increases monomer off-rate from the pointed end. Here, we characterize the cofilin-actin interface. We confirm earlier work suggesting the importance of the lower region of subdomain 1 encompassing the N and C termini (site 1) in cofilin binding. In addition, we report the discovery of a new cofilin binding site (site 2) from residues 112–125 that form a helix toward the upper, rear surface of subdomain 1 in the standard actin orientation (Kabsch, W., Mannherz, H. G., Suck, D., Pai, E. F., and Holmes, K. C. (1990) Nature 347, 37–44). We propose that cofilin binds “behind” one monomer and “in front” of the other longitudinally associated monomer, accounting for the fact that cofilin alters the twist in the actin (McGough, A., Pope, B., Chiu, W., and Weeds, A. (1997) J. Cell Biol. 138, 771–781). The characterization of the cofilin-actin interface will facilitate an understanding of how cofilin sever and depolymerizes filaments and may shed light on the mechanism of the gelsolin family because they share a similar fold with the cofilins (Hatanaka, H., Ogura, K., Moriyama, K., Ichikawa, S., Yahara, I., and Inagaki, F. (1996) Cell 85, 1047–1055).

Many motile processes in cells require cyclic polymerization and depolymerization of actin filaments. In cell locomotion for example, actin is polymerized at the leading edge of the cell and is recycled by depolymerizing toward the cell center. The rate constants of pure actin have been established (1), and it is clear that a discrepancy exists between these known rates and those calculated from filament turnover in cells (2). A host of actin-binding proteins are known that dramatically alter the behavior of actin; gelsolin (27, 28) is similar to that of the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs (reviewed in Ref. 26). The mechanism by which cofilin depolymerizes actin filament has been contentious. Soon after the discovery of the first member of the family (10), several authors suggested that depolymerization occurred through severing (9, 18). Evidence for a severing mechanism later came from videomicroscopy (19, 20), but this was later challenged (6) as it was shown (6, 21) that cofilins increased the off-rate from the pointed end of the filament. However, the two opinions are not necessarily exclusive (22–24), and a similar mechanism has been proposed for both events (23). We report the identification of a cofilin-actin interface that is compatible with the observation that cofilin increases the twist in the actin filament (25) but does not fit the model presented by these authors. We propose that some of the density attributed to cofilin (25) is actually the bulk of subdomain 2 of actin pushed forward by cofilin. Cofilin lies behind subdomain 2 of one monomer and in front of subdomain 1 of the longitudinally associated monomer, immediately toward the pointed end of the filament.

Our model may also be applicable to the many other ABPs that contain regions homologous to cofilins that bind to actin (reviewed in Ref. 26) and to the gelsolin-villin family of ABPs because the gelsolin fold (27, 28) is similar to that of the ADF-cofilin fold (29).

EXPERIMENTAL PROCEDURES

Proteins and Peptides—Rabbit skeletal muscle actin was isolated from acetone powder (30). Actin was selectively cleaved by S. aureus V8 protease, and the obtained fragments were isolated as described previously (31). Human cofilin was produced in Escherichia coli (BL21.(DE3)), transfected with a T7-based vector (pMW172) carrying a human cofilin-encoding cDNA fragment, and purified as described previously (20, 32). Biotinylation of cofilin was performed according to Bayer and Wüchek (33) via lysine residues. Antibodies directed toward cofilin (32) or actin sequences 75–105 and 105–113 were elicited in rabbits (34, 35). The antibodies directed toward the actin sequences were selectively purified by affinity chromatography (36). Anti-IgG antibodies labeled with alkaline phosphatase were purchased from Sigma.

Synthetic peptides derived from actin sequences were prepared on solid phase support using a 9050 Milligen PepSynthesizer (Millipore, Herts, UK) according to the Fmoc (N-(9-fluorenyl)methoxycarbonyl)/butyl system. The crude peptides were deprotected and thoroughly purified by preparative reverse-phase high pressure liquid chromatog-

* This work was supported by grants from the Association Française contre les Myopathies, the Institut National de la Recherche Agronomique, and the Institut Français de Recherche pour l’Exploitation de la Mer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: UMR 5539, UM2 CC107, Place E. Bataillon, 34095 Montpellier Cedex 5, France. Fax: 33-0467144927; E-mail: roustan@crit.univ-montp2.fr.

1 The abbreviations used are: ADF, actin depolymerizing factor; TFE, trifluoroethanol; 1,1'-AEDANS, N,N'-dioctyl-N,N'-di-sulfopropyl-ethylene diamine; ELISA, enzyme-linked immunosorbent assay; ABP, actin-binding protein; G-actin, monomeric actin; F-actin, filamentous actin; G1, gelsolin segment G.
Identification of a Cofilin Binding Site on Actin

The purified peptides were shown to be homogeneous by analytical high pressure liquid chromatography. Electrospray mass spectra, carried out in the positive ion mode using a Trio 2000 VG Biotech mass spectrometer (Altrincham, UK), were in line with the expected structures.

Peptides were labeled at the cysteine residue with 1,5-AEDANS (37). Excess reagent was eliminated by sieving through a Bio-Gel P-2 column equilibrated with 0.05 mM NaHCO₃ buffer, pH 8.0, and the peptide was then lyophilized. Actin fragments were obtained after S. aureus V₈ protease cleavage was labeled with fluorescein isothiocyanate as described elsewhere (38). Excess reagent was eliminated by chromatography on a PD10 column (Amersham Pharmacia Biotech) in NaHCO₃ buffer, pH 8.6.

Affinity Chromatography—Synthetic peptides were coupled to Sepharose 4B by the cyanogen bromide procedure as described previously (39, 40). The affinity columns were equilibrated with 50 mM NaCl 10 mM Tris, pH 7.5, washing with the same buffer supplemented with 0.15 mM NaCl was then performed to eliminate possible nonspecific interactions. The retained material was finally eluted by 10 mM phosphate, 10% dioxane, pH 12.

Immunological Techniques—ELISA (41), which was described previously in detail (42), was used to monitor interaction between coated peptides or actin and cofilin. Actin (0.5 pg/ml) or peptides (5 pg/ml) in 50 mM NaHCO₃/Na₂CO₃, pH 9.5, were immobilized on plastic microtiter wells. The plate was then saturated with 0.5% gelatin/0.1% gelatin hydrolysate in 140 mM NaCl, 50 mM Tris buffer, pH 7.5. Experiments with coated peptides were performed in 0.15 mM NaCl, 10 mM phosphate, pH 7.5. Binding was monitored at 405 nm using alkaline phosphatase-labeled anti-IgG antibodies (dilution 1/1000) or alkaline phosphatase-labeled streptavidin (dilution 1/1000). Control assays were carried out in wells saturated with gelatin and gelatin hydrolysate used alone. Each assay was conducted in triplicate, and the mean value was plotted after subtraction of nonspecific absorption. Additional details on the different experimental conditions are given in the figure legends.

Fluorescence Measurements—Fluorescence experiments were conducted using an LS 50 Perkin-Elmer luminescence spectrometer. Intrinsic fluorescence spectra were obtained for cofilin in 10 mM Tris, pH 7.5, at 22 °C. The excitation wavelength was set at 280 nm, and the emission spectrum recorded was between 310 and 400 nm. Spectra for dansylated peptides were obtained in the same conditions, with the excitation wavelength set at 340 nm. Fluorescence changes were deduced from the area of the emission spectra.

The fluorescence polarization of 1,5-I-AEDANS peptides in the presence of cofilin was also determined, with the excitation and emission wavelengths being set at 340 and 500 nm, respectively.

Collisional quenching of a fluorophore such as tryptophan in our study is described by the Stern-Volmer equation, \( F/F_0 = 1 + \frac{K_q}{[Q]} \) where \( F \) and \( F_0 \) are the fluorescence intensities in the presence and absence of the quencher, \( Q \), respectively, and \( K_q \) is the Stern-Volmer constant (43). The constant, \( K_q \), depends upon the lifetime of fluorescence without quencher and the bimolecular rate constant for the quencher.

Circular Dichroism Measurements—CD spectra were obtained using a Jobin Yvon Mark V dichrograph and 0.1-cm path length quartz cells. Experiments were performed for a peptide concentration of 0.1 mg/ml in 5 mM phosphate buffer, pH 7.5. Data were collected within the 190–260 nm wavelength range. Four scans of each sample were accumulated.

Analytical Methods—Protein concentrations were determined by UV absorbance using a Varian MS 100 spectrophotometer. SDS-polyacrylamide gel electrophoresis was performed on 15% gels as described by Laemmli (44) and stained with Coomassie Blue.

RESULTS

Involvement of Actin Subdomain 1 in the Interaction with Cofilin—Structural models (45, 46) of F-actin show that the actin N-terminal sequence moiety. Therefore, we searched for a possible involvement of these subdomains in cofilin interaction.

Proteolytic cleavage of actin by S. aureus V₈ protease provides two major fragments (sequences I–226 and 227–375) and a minor one (sequence 5–167) (31). This last fragment covers subdomain 2 and a large part of subdomain 1.

The conformational properties of the 5–167 fragment were checked by fluorometry and CD. This actin sequence included two tropomyosins at positions 79 and 86. The corresponding tropomyosan fluorescence emission spectrum of the isolated fragment is characterized by a peak centered at 340 nm showing that the tropomyosans are located in a relative hydrophobic medium (data not shown). In addition, quenching experiments were performed to test the accessibility of these chromophores. The results obtained (Fig. 1A) show that the two tropomyosans of the 5–167 fragment are somewhat shielded from the solvent because the apparent \( K_q \) (0.5 M⁻¹) is less than for a small tropomyosan peptide (21 amino acids) used as model (\( K_q = 10 \) M⁻¹). In this latter case, the small curvature in the Stern-Volmer plot would probably be caused by the occurrence of multiple conformations in solution. Furthermore, the influence of aspartic residues in close proximity to tropomyosan 79 within the 5–167 actin fragment must also be taken into account (47). The CD spectrum shown in Fig. 1B demonstrated that the 5–167 fragment presented some structure in solution. It is characterized by a negative peak located near 210 nm suggesting the occurrence of essentially strand conformation.

The actin fragment was then labeled with fluorescein isothiocyanate at lysine residues (about 0.1 mol/mol of fragment was modified). As the fragment presented a definite conformation, reactivity of the seven lysines should depend upon their specific environments. To locate some of the modified residues, antibodies directed toward a central region (sequence 75–105) were allowed to react with the labeled fragment. Their interactions induced a decrease in the emission spectrum of the fluorescein chromophore suggesting some possible proximity of the epitope with modified lysines (Fig. 1C, inset).

In an initial experiment, we tested the fluorescein-labeled 5–167 fragment for its ability to interact with cofilin. Fluorescence measurements of the interaction showed that the binding of increasing amounts of cofilin to a fixed quantity of labeled actin fragment resulted in the quenching of the fluorescence by up to about 40%, taking place in a saturable manner (Fig. 1C). Therefore, this result shows the occurrence of an interface for cofilin in the N-terminal part of actin.

To more precisely define the location of a cofilin binding region in this fragment, we tested the effect of specific antibodies directed toward sequence 75–105 of actin upon cofilin binding. This region, in subdomain 1, is implicated in the binding of numerous actin-binding proteins such as profilin (48), cross-linking proteins, myosin head, or gelsolin (27, 40, 49–51). In this context, competitive experiments were conducted in solid phase assays. We observed that the interaction between cofilin and coated actin was significantly decreased (up to 40%) in the presence of increasing antibody concentrations. Similarly, cofilin, although presenting a lower affinity for actin than the antibodies, induces a partial release of the 75–105 antibodies (Fig. 2, A, B, and inset). All these results suggest that an actin-cofilin interface would be located at some spatial proximity from the 75–105 segment in the subdomain 1 of actin.

Determination of an Actin-Cofilin Interface—Two peptides encompassing residues 105–120 and 119–132 were synthesized. Cofilin interaction with the coated peptides was revealed using specific anti-cofilin antibodies. The results shown in Fig. 3A indicate that both peptides 105–120 and 119–132 interacted with cofilin. However, cofilin binding to the 119–132 peptide was of higher affinity (apparent \( K_q = 12 \) \( \mu M \)) compared with the 105–120 peptide (apparent \( K_q > 50 \) \( \mu M \)). The cofilin interaction with G-actin was also reported in Fig. 3A for comparison. The results obtained in the heterogeneous phase (by ELISA) were confirmed in solution using fluorescence polarization measurements. To perform such experiments, peptides synthesized with an extra cysteine at the N-terminal extremity
were fluorescently labeled using 1,5-I-AEDANS. As shown in Fig. 3B, the binding of cofilin enhanced the basal polarization of both peptides. Analysis of the saturation curves showed binding parameters that strongly agree with those obtained by ELISA. Consequently, a new characterized interface can be located within the 105–132 actin sequence.

Identification of the Actin Amino Acid Sequence Implicated in This Interface—To restrict the interface, we have performed competition experiments with specific anti-peptide antibodies (apparent $K_d$ of about 0.3 $\mu$M) directed toward actin sequence 105–113 by ELISA. B, binding of biotinylated cofilin (1.6 $\mu$M) in 2 mM Tris, 0.1 mM CaCl$_2$, and 0.1 mM ATP buffer, pH 7.5, supplemented with 0.5% gelatin, 3% gelatin hydrolysate and monitored by ELISA. B, binding of biotinylated cofilin (1.6 $\mu$M) in 2 mM Tris, 0.1 mM CaCl$_2$, and 0.1 mM ATP buffer, pH 7.5, supplemented with 0.5% gelatin, 3% gelatin hydrolysate to coated actin was performed in the presence of increasing concentrations of antibodies (0–0.2 $\mu$M). Inset, binding of antibodies (15 nM) to coated actin in the presence of increasing cofilin concentrations (0–8 $\mu$M). Interactions were monitored at 405 nm.

were fluorescently labeled using 1,5-I-AEDANS. As shown in Fig. 3B, the binding of cofilin enhanced the basal polarization of both peptides. Analysis of the saturation curves showed binding parameters that strongly agree with those obtained by ELISA. Consequently, a new characterized interface can be located within the 105–132 actin sequence.

Identification of the Actin Amino Acid Sequence Implicated in This Interface—To restrict the interface, we have performed competition experiments with specific anti-peptide antibodies (apparent $K_d$ of about 0.3 $\mu$M) directed toward actin sequence 105–113 by ELISA. B, binding of biotinylated cofilin (1.6 $\mu$M) in 2 mM Tris, 0.1 mM CaCl$_2$, and 0.1 mM ATP buffer, pH 7.5, supplemented with 0.5% gelatin, 3% gelatin hydrolysate and monitored by ELISA. B, binding of biotinylated cofilin (1.6 $\mu$M) in 2 mM Tris, 0.1 mM CaCl$_2$, and 0.1 mM ATP buffer, pH 7.5, supplemented with 0.5% gelatin, 3% gelatin hydrolysate to coated actin was performed in the presence of increasing concentrations of antibodies (0–0.2 $\mu$M). Inset, binding of antibodies (15 nM) to coated actin in the presence of increasing cofilin concentrations (0–8 $\mu$M). Interactions were monitored at 405 nm.

were fluorescently labeled using 1,5-I-AEDANS. As shown in Fig. 3B, the binding of cofilin enhanced the basal polarization of both peptides. Analysis of the saturation curves showed binding parameters that strongly agree with those obtained by ELISA. Consequently, a new characterized interface can be located within the 105–132 actin sequence.
Peptides 105–120 and 119–132, 1,5-I-AEDANS-labeled at their N-terminal cysteine, were incubated with increasing concentrations of cofilin, and the emission fluorescence was recorded. We observed that only peptide 119–132 induced a fluorescence increase (Fig. 4) although both peptides interact with cofilin (Fig. 3, A and B). Thus, the N-terminal sequence of the synthetic peptide 105–120 would be excluded from the interface. In contrast, the dansyl coupled to the 119–132 peptide would be located near the binding site according to the observed fluorescence change. We tested the ability of peptides to bind cofilin in 150 mM NaCl buffer (Fig. 3 C).

Based on these data, a synthetic peptide (sequence 112–125) overlapping the previous peptides was tested for cofilin binding. Sequence 112–125 corresponded to the complete helical region of the actin sequence 105–132 (52). According to the CD spectrum (Fig. 5) that is characterized by a negative band near 200 nm, it was deduced that the 112–125 peptide possesses a random-coil arrangement in aqueous solution. In the presence of the alcohol solvent trifluoroethanol (TFE), the CD spectrum presented a negative peak at 220 nm coupled with a large positive peak in the far UV region (Fig. 5), characteristic of a helical conformation. Therefore, the peptide has a high propensity to adopt helical structure in the presence of TFE.

Binding experiments carried out by ELISA (Fig. 6 A) show a saturation curve for increasing concentrations of cofilin. The interaction is characterized by a high affinity compared with the two other peptides (apparent $K_d$ of about 4 $\mu$M), which substantiates the importance of this sequence in the interface.

To confirm the distinct ability binding of sequences 105–120, 112–125, and 119–132 toward cofilin, the cofilin retention onto affinity columns prepared with an immobilized peptide was tested. Using the peptide 112–125 column, we observed that cofilin was eluted by the dioxan buffer, pH 12 (Fig. 7), whereas no material was retained on the two other columns (data not shown). This result is in agreement with the affinities estimated in solid phase binding assay. The binding of cofilin to sequence 112–125 was also confirmed by fluorescence analysis. The interaction of cofilin with 1,5-I-AEDANS peptide induces an increase of both the emission spectrum intensity and the fluorescence polarization (Fig. 6, B and inset).

The influence of peptide 112–125 binding on the cofilin molecule was also checked by fluorescence measurements. Cofilin indeed possesses only one tryptophan residue at position 104.
Its emission fluorescence spectrum is centered near 330 nm, whereas that of the tryptophan chromophore in solution is near 360 nm. Fig. 8 reports the spectra of cofilin and of a small synthetic peptide (sequence 355–375 of actin) used as a model. In addition, the fluorescence emission spectrum of cofilin treated by 6 M urea is enhanced and shifted to 360 nm. Therefore, the tryptophan fluorescence in cofilin would be quenched, and the residue would be located in a hydrophobic environment. Its accessibility was tested by quenching experiments with iodine. Stern-Volmer plots (Fig. 1A) show that in cofilin, tryptophan is not accessible to iodine and is buried accordingly with tridimensional models (69). Binding of peptide 112–125 induced an enhancement of the fluorescence of the buried tryptophan in cofilin without significant change in the maximum wavelength of emission (Fig. 8). This last result is in favor of an environment modification of the tryptophan in cofilin by local conformational changes upon the 112–125 peptide binding.

**DISCUSSION**

ADF-cofilins are remarkable in that they alter the filament twist of F-actin (25). Nishida et al. (9) had commented that the structure of cofilin-bound filaments was possibly different from that of F-actin alone and noted that the spectra of cofilin-decorated, pyrene-labeled F-actin was similar to that of G-actin. Our model (Fig. 9) explains this. The presence of cofilin pushes the DNase 1 loop from one actin monomer away from the C terminus of the monomer below thus removing that environment afforded by the DNase 1 loop that causes the large change in pyrene fluorescence normally associated with actin polymerization (53). The model proposes that cofilin makes contact with two longitudinally associated actin monomers within the filament, through two sites on cofilin (site 1 and site 2). Site 1 is centered on the N terminus and Lys112 and Lys114 (human ADF and cofilin) at the start of the third helix (α3) and makes contact with the lower part of actin’s subdomain 1. Site 2 is centered on the last helix of cofilin (α4), which we propose makes contact with the 112–125 helix of actin identified as a cofilin binding site in this study. Whereas the region 112–125 is α-helical in the parent actin molecule (27, 52), we have found that this peptide in solution is in a predominantly non-helical state (Fig. 5). It is known that the helical conformation can be selectively stabilized in the presence of TFE, and we have shown by this agent that 112–125 maintains a propensity to adopt an α-helical conformation in solution. Cofilin binds this peptide, but we do not know if in doing so cofilin stabilizes the α-helical state or induces the α-helical state prior to binding. However, in previous work we demonstrated that the binding of the unfolded peptide, thymosin β4, was enhanced in the presence of TFE (54). This model is quite different from that suggested by McGough et al. (25), who placed cofilin “in front” of subdomain 2 of actin in the standard orientation. It is difficult to imagine how such an orientation could result in the change in twist of the actin filament observed, rather this orientation may be expected to alter the twist in the opposite direction. Our model, in which cofilin opens up the interface between the longitudinally associated actin subunits by intercalating between them (Fig. 9), produces the change in filament twist by increasing the angle of rotation between each longitudinally associated actin. The high degree of cooperation evident in the binding of ADF-cofilins to F-actin (20, 21, 25) has been explained by the changed twist they induce in the filament (25). We propose that in opening up a space between two subunits at one side of the filament, this also places a strain on the opposite
longitudinally associated pair of actin subunits across (and along) the filament, allowing another cofilin to bind along the axis of the filament. McGough et al. (25) have suggested that histidines 40, 87, 88, and 101 on the actin surface may contribute to the pH sensitivity of the ADF-cofilins. Our model also suggests that these histidines are likely to be close to the interface between ADF-cofilin and the actin subunits. However, the actin structure in part of this region is disordered (27) or constricted (52) in the available actin structures, making predictions premature.

The cofilin-actin interface has been investigated by a number of methods including chemical cross-linking (19, 55–58), competition with other ABPs (24, 59–63), structural prediction analysis (63), and mutagenesis (64, 65). The consensus of these studies is that subdomain 1 of actin is the principal actin-binding interface that binds the long helix of the ADF-cofilin. However, this remains a contentious area, especially on this second point.

Chemical cross-linking studies indicate that the N and C termini of actin interact with depactin (55) and that the N terminus of depactin contains an actin binding site (56). Subdomain 1 of actin was also implicated in the binding of Acanthamoeba actophorin to G-actin as actophorin competes for cross-linking (19) and binding (24) with profilin. Profilin is known to be cross-linked to glutamic acid 364 of actin (48). Cofilin can be cross-linked to residues 1–12 on actin (58), and ADF can be cross-linked to cysteine 374 on actin (58). Also many have found that various fluorescent labels on cysteine 374 are quenched by ADF-cofilin binding (6, 9, 15, 21, 24, 66).

The position of the actin binding site of ADF-cofilins on actin gained by competition with other ABPs and reagents has produced some contrary evidence. It is known that ADF-cofilin binding is inhibited by phalloidin (6, 19, 60). Tropomyosin is known to compete with ADF for F-actin binding (9, 60). It is tempting to speculate therefore that the tropomyosin and ADF binding sites overlap to some extent; however, it is known that the tropomyosin site is some distance from those of ADF-cofilins (reviewed in Ref. 67). Tropomyosin increases the regularity of the helical twist in actin (68) but does not vary it as ADF-cofilin does (25). It is likely that the twist induced by ADF is not compatible with binding by tropomyosin at its distant site, explaining the apparent competition for binding. The same explanation is very likely for phalloidin. Phalloidin binds F-actin extremely tightly, yet ADF-cofilin competes for binding (60). Myosin, which binds to the subdomain 1, including fragment 96–132 on actin (50), competes for actin binding with ADF-cofilin (9), in agreement with our model. The so called “headpiece” of villin also competes with ADF for binding to actin (61), but only partial and weak competition was evident between α-actinin and cofilin (60).

**Fig. 7.** Binding of cofilin to the actin peptide of sequence 112–125 coupled to Sepharose 4B. Cofilin (300 μg) was passed through a column (1.4 × 2.4 cm) of Sepharose 4B-linked peptide. The column was washed with 50 mM Tris buffer, pH 7.5, supplemented with 0.15 M NaCl. The bound material was eluted with 10 mM phosphate buffer, pH 12, supplemented with 10% dioxan. The eluted fraction was quantified from a UV absorption spectrum (in a typical experiment 25 μg of cofilin were eluted) and analyzed by SDS-polyacrylamide gel electrophoresis. *Inset,* lane 0, molecular mass markers (14.4, 21.5, 31.0, 45.0, 66.2, and 97.4 kDa); lane 1, cofilin sample; and lane 2, eluted material.

**Fig. 8.** Environment of tryptophan 104 in cofilin. Fluorescence emission spectrum of cofilin (1.6 μM) was recorded in the following conditions: cofilin alone in 10 mM Tris buffer, pH 7.5 (—), and cofilin (1.6 μM) in the presence of peptide 112–125 (5 μM) (—). Emission spectrum of a small peptide derived from actin sequence 355–375 was added as a control (—). Excitation wavelength was fixed at 280 nm.

**Fig. 9.** Model for cofilin-actin interaction. *Front view,* two longitudinally associated actin monomers are shown. The lower monomer is in the “standard” orientation with subdomain 1 at the bottom right containing actin's N and C termini. The upper monomer is rotated with respect to the lower monomer in accordance with the finding of McGough et al. (25). A cofilin molecule (dotted) is sandwiched between the two monomers. *Side view,* rotating the filament reveals that site 1 of cofilin associates with the lower region of subdomain 1 of one actin whereas cofilin's site 2 associates with the upper region (actin 112–125) of subdomain 1 of the other actin.
The structures of three cofilins, ADF (29), yeast cofilin (69), and actophorin (70), have been determined. Interestingly, cofilins have an overall fold similar to the gelsolin segment 1 (G1) (27), leading some others (29, 63) to suggest that ADF-cofilin binds actin in a similar manner as G1. Gelsolin is a calcium-sensitive actin-binding, severs, and nucleating protein that is composed of six similar domains (G1–G6) (71). Although the six domains are similar in both sequence and structure (28), it is known that whereas G1 and G4 bind a similar site on actin (72, 73), G2 binds F-actin alone. G2 is thought to bind actin in the region of the outer surfaces of subdomains 1 and 2 (62). In support of this contention, G2–3 competes with cofilin for actin binding (62), however it is also known that G1 also competes with cofilin for actin binding (63). These apparently disparate findings are compatible with our model (Fig. 9). Stable F-actin binding can only take place at low pH values where cofilin binds actin monomers within the filament via site 1 and site 2. These sites are also shared by G1 and G2, respectively.

A very comprehensive mutagenesis study (65) in which the actin interface was systematically explored concluded that cofilin did not bind in a similar manner to G1 but that there were two actin binding sites. One site comprised Arg96 and Lys98, Asp123 and Glu126, and M1 to G5, whereas the other comprised Arg80 and Lys82 and Glu134 to Arg138. These authors suggested the “long helix” (that one of these sites bound one actin monomer within a discussion.

Macromoleculaire, Montpellier, France) for proofreading and valuable

burgh, UK) and Dr. Ridda Kassab (Centre de Recherches en Biochimie complexes.

interpretation of any further atomic structure of their additional firm experimental evidence for the two-sited inter-

high-resolution structures for the actin filament with and with-

task, because the interaction between the two proteins is not structural data, either from NMR or more likely, because of the

G-actin.

Two actin binding sites. One site comprised Arg96 and Lys98, actin interface was systematically explored concluded that co-

findings are compatible with our model (Fig. 9). Stable F-actin

with cofilin for actin binding (63). These apparently disparate

binding (62), however it is also known that G1 also competes

Domains are similar in both sequence and structure (28), it is

composed of six similar domains (G1–6) (71). Although the six

(27), leading some others (29, 63) to suggest that ADF-cofilin

J. Cell Biol. 106, 1263–1259

(1983) J. Cell Biol. 97, 1612–1621

Lopez, I. R., Bonaccorsi, S., Williams, E., Verni, F., Gatti, M., and Goldberg, M. L. (1995) J. Cell Biol. 97, 1263–1259

16. Iida, K., Moriyama, K., Matsumoto, S., Kawasaki, H., Nishida, E., and Yahara, I. (1998) Nature 397, 680–685

23. Maciver, S. K. (1998) FEBS Lett. 421–435

22. Theriot, J. A. (1997) J. Cell Biol. 136, 115–120

8. Moon, A., and Drubin, D. G. (1995) Mol. Biol. Cell 6, 421–435

136, 680–685

124, 115–120

121, 1612–1621

127–132

573–568

109, 619–626

57–62

160, 573–568

1951–1959

222, 127–132

162, 9985–9993

391, 115–120

1201–1217

411–417

5520–5530

124–130

103, 1612–1621

124, 115–120

121, 1612–1621

573–568

127–132

162, 9985–9993

124–130

103, 1612–1621

573–568

127–132

162, 9985–9993

124–130

103, 1612–1621

573–568

127–132

162, 9985–9993

124–130

103, 1612–1621

573–568

127–132

162, 9985–9993

124–130

103, 1612–1621

573–568

127–132

162, 9985–9993

124–130

103, 1612–1621

573–568

127–132

162, 9985–9993

124–130

103, 1612–1621

573–568

127–132

162, 9985–9993

124–130

103, 1612–1621

573–568

127–132

162, 9985–9993

124–130

103, 1612–1621

573–568