UNC-87 Is an Actin-bundling Protein*

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The Caenorhabditis elegans unc-87 gene product is essential for the maintenance of the nematode body wall muscle where it is found colocalized with actin in the I band. The molecular domain structure of the protein reveals similarity to the C-terminal repeat region of the smooth muscle actin-binding protein calponin. In this study we investigated the in vitro function of UNC-87 using both the full-length recombinant molecule and several truncated mutants. According to analytical ultracentrifugation UNC-87 occurs as a monomer in solution. UNC-87 cosedimented with both smooth and skeletal muscle F-actin, but not with monomeric G-actin, and exhibited potent actin filament bundling activity. Actin binding was independent of the presence of tropomyosin and the actin cross-linking proteins filamin and α-actinin. Consistent with its actin bundling activity in vitro, UNC-87 tagged with green fluorescent protein associated with and promoted the formation of actin stress fiber bundles in living cells. These data identify UNC-87 as an actin-bundling protein and highlight the calponin-like repeats as a novel actin-binding module.

The interaction of actin and myosin to produce force is an essential prerequisite for a variety of cellular processes including muscle contraction (1), cell motility, and anchorage (2). The organization of contractile and motile systems based on actin relies on a large family of actin-associated proteins that regulate and define the assembly of actin into filaments and then into filament arrays (3, 4). To date, more than 60 different proteins directly interacting with actin have been identified, but the majority of F-actin-binding proteins populate partially overlapping regions on the filament (5, 6). Despite the large number of actin-binding proteins, functional diversity is reflected by a limited number of basic structural modules (7). Most actin cross-linking proteins exhibit two independent actin-binding domains, each individual actin-binding domain commonly composed of a tandem arrangement of the calponin homology domain module (8) and other modular elements defining the distance between and the relative orientation of the two actin-binding domains, often involving parallel or antiparallel dimerization (7).

We have shown recently that a unique sequence motif found in the C-terminal third of the calponin (CaP)1 molecule and other members of the CaP family of actin-associated proteins (9), namely a 23-amino acid residue repeat, which we will refer to from now on as the CLIK-23 repeat, forms an independent actin-binding site (10). This finding was corroborated by Mino et al. (11) who demonstrated the direct interaction of a peptide corresponding to the first CaP repeat with actin in vitro. A survey of the available data bases identified other proteins with CLIK-23 repeats, in particular the Caenorhabditis elegans body wall muscle protein UNC-87 that exhibits seven tandem CLIK-23 repeats (12). A protein with a similar molecular structure has also been described by Irvine et al. (13) in the filarial worm Onchocerca volvulus. Although the UNC-87 protein was identified as a key molecule for maintenance of the structural integrity of the myoskeletal apparatus in the nematode (12, 14), no further information on its putative biological function or on the mode of interaction with actin was obtained.

In this study we show that UNC-87 is an actin-bundling protein in vitro and in vivo and present evidence for a binding site of the CLIK motif on the actin filament different from that of other actin cross-linking proteins.

EXPERIMENTAL PROCEDURES

Construction of Plasmids—The expression plasmids UNC-87-pEGFP-C1 (for expression of a GFP-tagged full-length UNC-87 protein in mammalian cells) and UNC-87-pMW172 (for bacterial expression of untagged full-length UNC-87) were constructed from the unc-87 full-length cDNA clone pSG3 (accession number U04711; see Ref. 14). This cDNA corresponds to the predominantly transcribed smaller splice variant (UNC-87b) and contains exons A, C, D, E, F, and G (14). Polymerase chain reaction on pSG3 was performed using a forward primer that starts amplification at the first of two potential start sites and introduces a BglII site immediately 5′ to the start codon. The reverse primer introduces an EcoRI site immediately 3′ of the original unc-87 stop codon. The polymerase chain reaction product was digested with BglII and EcoRI and cloned into the corresponding sites of pEGFP-C1 and the BamHI and EcoRI site of pMW172 (15), respectively. All sequences were confirmed by dyeo sequencing using a LI-Cor model 400 automated sequencer (MWG Biotech AG, Ebersberg, Germany).

The deletion mutants containing repeats 2–7 (amino acids 69–374), 3–7 (117–374), 4–7 (170–374), and 5–7 (216–374) were made by polymerase chain reaction essentially the same way as described for the full-length constructs and cloned into pEGFP-C1 and pMW172, respectively.

For construction of the deletion mutant containing repeats 1–3 (amino acids 1–169) full-length UNC-87-pEGFP-C1 was digested with AcI followed by a complete fill-in of the overhang by Klenow fragment. After digestion with BglII the UNC-87 fragment was cloned into the BglII and SmaI site of pEGFP-C1. For bacterial expression this construct was again digested with BglII and BclI and ligated into the BamHI site of pMW172. This orientation was confirmed by sequencing.

Expression and Purification of UNC-87 Proteins—The plasmid UNC-87-pMW172 and the UNC-87 deletion mutants in pMW172, respectively, were transformed into Escherichia coli BL21 DE3. Ampicillin-resistant colonies were scraped off the plate and suspended in 500 ml of LB with ampicillin. The cultures were grown to an A600 of 0.6–0.8 and fibroblasts.

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induced with 1 mM isopropyl-1-thio-β-D-galactosidase. 3–4 h after induction the culture was centrifuged at 1250 × g for 15 min, and the pellet was resuspended in Buffer E (5 mM KH2PO4, 5 mM K2HPO4, 10 mM NaCl, 1 mM EDTA, 1 mM EGTA). The bacteria were lysed in a French® pressure cell press (Spectronic Unicam, Cambridge, United Kingdom) and centrifuged at 25,000 × g for 15 min. The supernatant was applied immediately onto a 5-ml HiTrap SP cation exchange column (Amersham Pharmacia Biotech) equilibrated in Buffer E. Proteins were eluted with a linear gradient, ranging from 0–350 mM NaCl in Buffer E. Peak fractions were pooled and further purified on a Sephacryl S100HR (500 ml) equilibrated in Buffer E. Fractions containing ~98% pure UNC-87 were concentrated on a 1-ml HiTrap SP and eluted again with a gradient of 0–350 mM NaCl.

**Proteins**—Rabbit skeletal muscle actin or turkey gizzard smooth muscle actin was prepared from acetone powder according to Spudich and Watt (16) and Strzelecka-Golaszewska et al. (17), respectively. Recombinant h1 and h2 CaP were expressed and purified as described (19). Tropomyosin and α-actinin were purified from turkey gizzard smooth muscle as described (18, 19). Transfection, Immunoprecipitation, and Immunofluorescence—Mouse melanoma cells (B16F1) or rat embryo fibroblasts (REF 52) grown in Dulbecco’s modified Eagle’s medium + 10% fetal calf serum (PAA Laboratories, Linz, Austria) to 75% confluence were transfected for 24 h using 2 μg of total DNA per construct per 60-mm dish using 8.5 μl of Lipofectamine (Qiagen, Hilden, Germany) and prepared for immunofluorescence as described elsewhere (10). GFP-tagged UNC-87 was visualized by direct fluorescence using the excitation wavelength of fluorescein. F-actin was visualized by incubation with Alexa 568 Phalloidin (Molecular Probes, Leiden, The Netherlands). Fluorescent images were photographed on a Zeiss Axioshot with a ×63 oil immersion lens and Eastman Kodak Co. P400 Tmax film. Immunoprecipitations using a polyclonal antibody to recombinant EGFP were performed essentially as described earlier (20) with minor modifications in the IP buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 300 mM KCl, 5% (v/v) glycerol, 0.5% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3 EDTA, 1 mM EGTA). Proteins were visualized by Western blotting using monoclonal antibodies to EGFP (CLONTECH, Palo Alto, CA) and β-actin (Sigma), respectively.

**Actin Binding Assays**—Codestimation assays with smooth or skeletal muscle F-actin were performed in F-actin buffer (20 mM imidazole, pH 7.0, 2 mM MgCl2, 50 mM NaCl, 100 KCl) or G-actin buffer (20 mM imidazole, pH 7.0, 0.2 mM CaCl2, 0.5 mM ATP, 50 mM NaCl). Proteins were incubated at 25 °C for 30 min and pelleted either at 100,000 × g for 30 min (high speed) using an air-driven ultracentrifuge (Beckman model 5417 R centrifuge (Eppendorf-Netheler-Hinz GmbH, Vienna, Austria). Pellets were resuspended in the same buffer in the starting volume.

**Electrophoresis and Western Blotting**—Analytical SDS gel electrophoresis on 8–22% gradient polyacrylamide mini-slab gels and Western blotting using a polyclonal antibody to recombinant EGFP were performed essentially as described earlier (20) with minor modifications in the IP buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 300 mM KCl, 5% (v/v) glycerol, 0.5% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3 EDTA, 1 mM EGTA). Proteins were visualized by Western blotting using monoclonal antibodies to EGFP (CLONTECH, Palo Alto, CA) and β-actin (Sigma), respectively.

**Protein Extraction**—Transfected cells in 60-mm Petri dishes were washed twice in ice-cold phosphate-buffered saline and subsequently extracted in 250 μl of IP buffer (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1% Triton X-100) supplemented with 200, 400, 600, or 800 mM KCl for 10 min. Extracts were collected with a rubber policeman and centrifuged at 20,000 × g for 15 min. Extracts and Triton X-100-insoluble pellets were brought to equal volumes and prepared for SDS gel electrophoresis and Western blotting as described above.

**Analytical Ultracentrifugation**—Sedimentation velocity profiles of purified proteins (1 mg/ml in 140 mM KCl, 1 mM EDTA, 1 mM EGTA, 5 mM KH2PO4, 5 mM K2HPO4, pH 7.0) were collected in an analytical ultracentrifuge (Optima XL-A, Beckman Instruments, Palo Alto, CA) at 60,000 rpm at 20 °C. The data were analyzed using the program Ultrascan 4.1 (The University of Texas Health Center at San Antonio; see Ref. 211 by using the van Holde-Weischat method (22).

**RESULTS**

**Expression and Purification of Recombinant UNC-87**—The domain structure of UNC-87 is similar to that of the C-terminal third of the CaP molecule (Fig. 1A). The UNC-87 sequence comprises 7 copies of a highly basic repeat of 23–26 amino acid residues, tentatively termed 23-residue calponin-like repeat or CLIK-23 repeat, interspersed by highly acidic “intervening” or linker sequences. Whereas the repeats in UNC-87 show a striking sequence similarity to those present in CaP, the intervening regions are unique in sequence and variable in length but similar to the CaP intervening sequences in their calculated isolectric points. We have cloned the coding region of the major 41-kDa UNC-87 isoform (UNC-87b) and the truncation mutants depicted in Fig. 1B into the prokaryotic expression vector pMW172 for the expression of recombinant, nonfusion proteins. The proteins were purified under native conditions from the bacterial cytosol by alternating ion exchange and gel filtration chromatography (see “Experimental Procedures”). We routinely obtained between 15 and 25 mg of purified UNC-87 protein from a 1-liter bacterial culture in less than 48 h. The final protein was more than 96% pure (Fig. 1C) and was stable in solution at 4 °C for more than 4 weeks.

**UNC-87 Cross-links F-actin in Vitro**—High-speed cosedimentation assays using purified smooth (Fig. 2A-C) or skeletal (not shown) muscle F-actin showed that full-length UNC-87 bound tenaciously to actin with a saturation of binding of UNC-87 to actin at a molar ratio of 1:6–1:4 (Fig. 2A). No UNC-87 was pelleted in the absence of F-actin at 100,000 × g.
Addition of smooth muscle TM to UNC-87-saturated F-actin filaments had no influence on the binding of UNC-87 to actin, and binding of TM was likewise unaffected in the reverse experiment using TM-saturated smooth muscle actin filaments (Fig. 2, B and C).

It has been reported that CaP at high concentrations can induce bundles of smooth muscle F-actin (23). When smooth or skeletal muscle F-actin were incubated together with UNC-87 and centrifuged for 20 min at 18,000 \( \times g \), actin and UNC-87 were found together in the low speed pellet, indicating the induction of actin bundles by UNC-87 (Fig. 2D). This cross-linking effect was concentration-dependent and ceased abruptly when the molar ratio of actin:UNC-87 dropped below 6:1 (Fig. 2D). Thus, UNC-87 functions as an actin cross-linking protein in vitro.

To define the structure of the cross-linked actin assemblies in more detail we analyzed them by electron microscopy. As seen in Fig. 3, A and B, UNC-87 caused the formation of dense, parallel actin bundles. The tight packing of the individual filaments increased the stiffness of the bundles, resulting in abrupt fractures (Fig. 3C). Identical results were obtained with smooth muscle actin (not shown).

Because CaP has been shown to induce actin polymerization at low ionic strength (24) we tested whether UNC-87 shared this ability. As seen in Fig. 4, the UNC-87 protein failed to induce the polymerization of G-actin as judged from both low speed (Fig. 4C) or high speed (not shown) sedimentation. In contrast, A1 CaP efficiently cosedimented with actin at low speed under both F-actin (Fig. 4A) and G-actin conditions (Fig. 4B), indicating the induction of actin polymerization and bundling of the formed filaments. Binding of UNC-87 with actin was unaffected by the presence of the actin-binding proteins \( \alpha \)-actinin (Fig. 5) and filamin (not shown) indicating that \( \alpha \)-actinin and UNC-87 occupy different sites on the actin filament.

**UNC-87 Is a Monomer in Solution**—A prerequisite for the cross-linking activity of actin-binding proteins is the presence of two independent actin-binding sites, either on the single protein subunit or as a result of a dimerization or oligomerization process. Secondary structure predictions failed to identify two potential regions capable of forming actin-binding sites in UNC-87.
The van Holde-Weischet plot from a sedimentation boundary positions correspond to a sedimentation coefficient of 2.0 S (Fig. 6). The deletion mutants of UNC-87 containing the CLIK-23 modules 1–3, 3–7, or 5–7, respectively, all had sedimentation coefficients \( \approx 1.6 \) S (not shown). Thus, the multiple CLIK-23 modules may serve to form at least two independent actin binding interfaces.

**GFP-tagged UNC-87 Induces Stress Fiber Bundling in Cultured Cells**—The cosedimentation data demonstrated that UNC-87 is capable of bundling actin in vitro. For studies of the bundling activity in living cells we cloned the UNC-87 cDNA into the pEGFP C1 vector to generate a mutant protein fused to GFP at its amino terminus. Various cell lines were transfected with this construct, and the cellular localization was determined by fluorescence microscopy. In REF 52 fibroblasts UNC-87 localized to the actin stress fibers, and the ectopic expression of the UNC-87 protein caused a significant increase in stress fiber bundling (Fig. 7, A and B). Moreover, stress fiber formation was significantly enhanced in the mouse melanoma cell line B16F1 (Fig. 7, C and D), consistent with the strong bundling effects observed in our in vitro experiments.

To assay for the strength of association with the actin cytoskeleton in vitro we analyzed the extractability of the UNC-87 protein. The amount of soluble protein was analyzed by Western blotting using a monoclonal antibody to GFP. For comparison, we used extracts of cells transfected with GFP-tagged \( \alpha \)-actinin, extracted in the same way. As demonstrated in Fig. 8, UNC-87 was strongly retained in the Triton X-100-insoluble cytoskeletal fraction even at 800 mM KCl, an ionic strength at which \( \alpha \)-actinin is almost quantitatively extracted under these conditions.

### Table I

| Construct | Actin binding | Actin bundling |
|-----------|---------------|---------------|
| Full-length | +++ | +++ |
| 2–7 | ++ | + |
| 3–7 | ++ | + |
| 4–7 | – | – |
| 5–7 | – | – |
| 1–3 | +/− | +/− |

**Deletion Mutants Pinpoint the First Three Repeats as the Region Essential for Actin Binding**—To further delineate the region(s) involved in the interaction with the actin filament we performed high and low speed cosedimentation assays as above but using the truncated, purified proteins shown in Fig. 1C. As summarized in Table I, deletion of more than the first three repeats (mutant 4–7) completely abolished both binding and bundling activities in vitro, whereas a mutant comprising the first three repeats (mutant 1–3) retained a weak actin association and actin bundling activity. A similar effect was observed in REF 52 cells transiently transfected with the mutant

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**Fig. 5.** UNC-87 and \( \alpha \)-actinin bind simultaneously to F-actin. Low speed cosedimentation assay under the same conditions as in Fig. 2 using decreasing amounts of \( \alpha \)-actinin and UNC-87, respectively, is shown. Note that the actin filaments can be simultaneously saturated with both proteins.

**Fig. 6.** Analytical ultracentrifugation reveals that UNC-87 is a monomer in solution. The van Holde-Weischet plot from a sedimentation velocity experiment shows that the extrapolated sedimentation speed from various sedimentation boundary positions correspond to a sedimentation coefficient of 2.0 S.

**Fig. 7.** Localization of GFP-tagged UNC-87 in transiently transfected REF 52 (A and B) and B16F1 cells (C and D). UNC-87 associates strongly with the actin stress fibers in REF 52 fibroblasts and causes the formation of prominent, thick bundles (A and B). Transfection into B16F1 melanoma cells induces the formation of stress fibers (C and D). Note the absence of actin stress fibers in the surrounding nontransfected cells in C, A and C, F-actin visualized by incubation with Alexa 568 Phalloidin; B and D: GFP fluorescence. Bar is 10 \( \mu \)m.

**Fig. 8.** Western blot probed with a monoclonal anti-GFP antibody. Extracts and insoluble cytoskeletal residues of REF 52 cells transiently transfected with GFP UNC-87 or GFP \( \alpha \)-actinin and extracted at the indicated KCl concentrations in the presence of 1% Triton X-100 are shown. UNC-87 remains associated with the insoluble cytoskeletal fraction at 800 mM KCl, whereas \( \alpha \)-actinin is almost quantitatively extracted under these conditions.
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FIG. 9. Subcellular localization of GFP-tagged UNC-87 mutants in transiently transfected REF 52 fibroblasts. Note the gradual decrease in stress fiber association with progressing deletion of repeats. F-actin was visualized by incubation with Alexa 568 Phalloidin. Bar is 10 μm.

FIG. 10. Coimmunoprecipitation of cytoplasmic β-actin by the bundling-competent mutants of UNC-87. Western blot of precipitated proteins is shown. Antibodies used for precipitation and Western blotting are indicated.

UNC-87 constructs fused to GFP at their amino terminus (Fig. 9). The progressive deletion of repeat sequences was mirrored by a reduction in stress fiber localization. Weak stress fiber localization was still observed for the amino-terminal mutant 1–3.

Finally, we confirmed the loss of binding and bundling activity for the respective UNC-87 mutants by coimmunoprecipitation from cell lysates of transiently transfected REF 52 cells using a polyclonal anti-GFP antibody (Fig. 10). Only those constructs displaying bundling activity in vitro (compare with Table I) coprecipitated actin in this assay.

DISCUSSION

Our findings identify the UNC-87 protein as an actin-bundling molecule, which uses seven copies of an archetypal protein module (the CLIK-23 repeat) to bind to and cross-link F-actin. The overall molecular structure of UNC-87 is similar to that of the C-terminal third of CaP, featuring alternating basic and acidic amino acid stretches (Fig. 1A). The CaP family of actin-binding proteins exhibiting CLIK-23 repeat motifs likely evolved from an ancestral molecule by gene duplication and subsequent diversification. Three copies of the CLIK-23 repeat are found in all calponins flanking the strong actin-binding site and the gradual decrease in actin ATPase inhibitory region responsible for the inhibition of the actin-activated myosin ATPase activity (25, 26). Thus, the CLIK-23 repeats, which constitute the second autonomous actin-binding site (ABS2) in CaP, may serve to attach this molecule to a second site on the filament not directly involved in the regulation of actomyosin interactions.

The sharp breaks seen in the filament bundles suggest that UNC-87 confers rigidity to the actin filaments. A similar observation has been reported earlier for the actin cross-linking protein fimbrin (27). Thus, UNC-87 may act as a structural component of the nematode muscle by cross-linking actin filaments into stable bundles. This hypothesis is consistent with the previous observations that UNC-87 knock-out animals display distorted myofilaments (12, 14). A structural role has also been postulated for CaP (10, 28), which bundles filaments at low ionic strength (29). Thus, we hypothesize that the cytoskeleton-stabilizing function of CaP is contributed by the C-terminal repeats.

In their original work, Goetinck and Waterston (12) mapped UNC-87 to the myofilament system of the invertebrate body wall muscle, and immunocytochemical analyses pointed toward an association with the actin-containing thin filaments. Here we have demonstrated that UNC-87 binds directly to F-actin in vitro and that the protein possesses potent bundling activity both in vitro and in living cells. This result is concordant with the documented importance of the molecule for the maintenance of muscle integrity. The localization of UNC-87 in C. elegans was unaffected in the TM null-mutants, suggesting that the two proteins are capable of coassociating with the thin filament (12), which we now confirmed by in vitro analyses. UNC-87 binding to actin was also unaffected by saturating concentrations of either α-actinin or filamin, indicating the occupation of nonoverlapping sites on the actin filament. In support of this Chalovich and colleagues (28) reported that α-actinin, filamin, and calponin show only little displacement and are capable of binding the actin filament simultaneously in almost stoichiometrical amounts. Thus, the second actin-binding site of CaP (ABS2) and that of UNC-87 are likely to interact with a similar region along the filament. Studies specifically addressing the question of how and where UNC-87 and CaP bind to actin using three-dimensional helical image reconstructions of cryoelectron micrographs are currently underway.

Our deletion studies demonstrate that the individual repeats contribute differently to the overall actin binding activity of UNC-87. Whereas the three N-terminal repeats still show detectable binding activity in vitro and in transfected cells, the C-terminal four repeats are not sufficient for actin binding. Thus, despite the sequence similarity of the CLIK-23 modules, the acidic intervening sequences may also be relevant for the overall structural integrity of the molecule and in particular the actin binding interface(s). Notably, all CLIK-23 modules identified thus far are interspersed by these acidic linker regions and are found exclusively in odd numbers of copies (1, 3,
5, or 7). Detailed structural analyses of the UNC-87 molecule will shed more light on this question in the future.

The exclusive localization of GFP-UNC-87 (and also that of Myc-tagged UNC-87; data not shown) along actin stress fibers is similar to the subcellular localization seen with HA- or GFP-tagged smooth muscle CaP and the C-terminally truncated versions of all three CaP isoforms (30). GFP-tagged UNC-87 caused the condensation of actin stress fibers into thick bundles in transfected cultured REF 52 cells and induced the formation of prominent stress fibers in the mouse melanoma cell line B16F1. Together with our data from the in vitro binding assays and the coimmunoprecipitations this illustrates that UNC-87, like CaP, is capable of interacting with both muscle- and nonmuscle-type vertebrate actins. The interaction of UNC-87 with filamentous actin was essentially insensitive to ionic strength. Actin cosedimentation under both low and high speed conditions was unchanged at salt concentrations ranging from 50–300 mM KCl (data not shown). More significantly, the majority of the UNC-87 protein remained associated with the Triton X-100-insoluble cytoskeletal fraction even at 800 mM KCl, whereas α-actinin was readily extracted from the cells under these conditions. Thus, electrostatic interactions may play a subordinate role in the binding of UNC-87 to actin. Taken together, the binding and extraction data point toward a high affinity interaction of UNC-87 with actin.

We conclude that the basic CLIK-23 repeats and not the acidic intervening sequences are responsible for contacting the actin filament. First, the intervening sequences of CaP and UNC-87 share no apparent similarities other than the predicted acidic pl (see also Fig. 1A), but the isolated repeat region of CaP colocalized with actin stress fibers in transfected cells (10).2 Secondly, Mino et al. (11) have shown the direct interaction of a peptide corresponding to the first repeat of CaP with actin. Emerging structural information suggests that nature operates with a limited number of actin binding motifs (31). The CLIK-23 repeats share no apparent similarity with any of the currently known actin binding sequences. An actin-binding protein containing six copies of the Kelch motif and consisting predominantly of β-sheet structures is found in the Limulus sperm protein scrin (5), which also bundles actin in vitro (32).

Computer-assisted secondary structure analysis (data not shown) of UNC-87 or the corresponding repeat region in CaP predict for both sequences a high content (70%) of unstructured β-sheet folds, clearly different from scrin. Thus, the actin-binding site(s) formed by the multiple CLIK-23 repeats in CaP and UNC-87 appears to constitute a novel structural actin binding motif, which awaits further analysis.

The UNC-87 protein appears as a monomer in solution according to the data from the analytical ultracentrifugation. Similarly, the deletion mutants 3–7, 5–7, and 1–3 also sedimented at values indicating a monomeric molecule corresponding to the calculated molecular mass. Chemical cross-linking, using the zero length cross-linker 1-ethyl-3-[3(dimethyl amino) propyl] carbodiimide (EDC), and also [bis sulfosuccinimidyl] suberate (BS3) or m-Meleimidoobenzoyl-N-hydroxysuccinimide ester (MBS), confirmed these results (data not shown). Thus, UNC-87 is likely to function as a monomer in vivo. Because cross-linking activities depend on the presence of at least two binding interfaces these results indicate that the seven CLIK 23 repeats form a minimum of two actin-binding sites. The fact that the binding and bundling capabilities were lost simultaneously in the deletion mutants argues for a structural requirement that involves both N- and C-terminal repeat regions. Notably, under the same buffer conditions used for the cross-linking and sedimentation assays both full-length UNC-87 and the deletion mutants migrated significantly faster on analytical gel filtration columns, and the proteins eluted at positions corresponding to 1.5–2.3 times the calculated molecular mass, indicating that the UNC-87 molecule may form an extended, rigid rod structure.3

The expression of CaP isoforms is not restricted to muscle tissue, and different members of the CaP family have been identified in a wide range of vertebrate cells. CaP variants have thus been implicated in the regulation of a variety of actomyosin-based processes, including neurite outgrowth (33) and the organization of the nonmuscle cell cytoskeleton (34). The CLIK-23 module may have acquired a specialized function in the course of evolution from invertebrates to vertebrates and the concomitant specialization of different muscle types (see also Ref. 35). It is, however, worth noting that the C. elegans genome contains proteins closely related to the SM22-like members of the CaP family (consisting of a single copy of both a CLIK-23 repeat and a single N-terminal calponin homology domain) but also Vav and IQGAP-like proteins harboring a single calponin homology domain (8, 9) and no CLIK-23 module. Thus, UNC-87 may be a CaP ortholog specialized for actin filament assembly processes in invertebrate obliquely striated muscle.

Conclusion—In summary, we have demonstrated that UNC-87 interacts directly with F-, but not G-actin, and causes the formation of rigid actin bundles. We suggest a physiological role for this protein as a “rectifying” component of the actomyosin system in nematodes and propose a similar structural role, performed by the three homologous CLIK-23 repeats in the smooth muscle protein calponin in vertebrate muscle.

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