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Two Mouse Cofilin Isoforms, Muscle-Type (MCF) and Non–Muscle Type (NMCF), Interact with F-Actin with Different Efficiencies

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Two cofilin isoforms, a muscle-type (MCF) and a non–muscle-type (NMCF), are co-expressed in developing mammalian skeletal and cardiac muscles. To clarify how they are involved in the actin filament dynamics during myofibrillogenesis, we examined their localization in muscle tissues and cultured muscle cells using immunocytochemical methods, and their interaction with F-actin *in vitro*. NMCF was mostly detected in a diffuse pattern in the cytoplasm but MCF was partly localized to the striated structures in myofibrils. The location of chicken cofilin, a homologue of MCF, in the I-bands of myofibrils was determined by an immunocytochemical method. It is suggested that MCF could be associated with actin filaments in muscle cells more efficiently than NMCF. Using purified recombinant MCF and NMCF, their interaction with F-actin was examined *in vitro* by a cosedimentation assay method. We observed that MCF was precipitated with F-actin more effectively than NMCF. When MCF and NMCF were simultaneously incubated with F-actin, MCF was preferentially associated with F-actin. MCF and NMCF inhibited the interaction of F-actin with tropomyosin, but the former suppressed the actin-tropomyosin interaction more strongly than the latter. These results suggest that MCF interacts with F-actin with higher affinity than NMCF, and although both of them are involved in the regulation of actin assembly in developing myotubes, the two proteins may play somewhat different roles.

Key words: actin, actin filament dynamics, cofilin, muscle development, myofibrillogenesis.

Abbreviations: CF, cofilin; MCF, muscle-type cofilin; MCF1, a monoclonal antibody to muscle-type cofilin; NMCF, non–muscle-type cofilin; PBS, phosphate-buffered saline.

The ADF/cofilin family proteins are widely distributed in eukaryotic organisms and play a critical role in the actin filament dynamics in a variety of cell types (*1–3*). They bind to G-actin as well as actin monomeric units in F-actin in a stoichiometry of 1 to 1, and enhance the turnover of the monomeric units within F-actin filaments by regulating the rate constant of depolymerization and polymerization at the filament ends (*4*), severing the filaments (*5*, *6*), and changing the twists of the filaments (*7*). In mammals, there are three different ADF/cofilin proteins, namely ADF (also called destrin) (*8*) and two cofilin isoforms, while in chicken, there is only one isoform for each protein.

Although both ADF and cofilin are present in developing muscle (*9–11*), ADF expression in muscle ceases at an early developmental stage, while cofilin expression persists throughout muscle development although the level decreases somewhat (*11*, *12*) and increases markedly under muscle degeneration conditions (*12–14*). Therefore, cofilin may be more important as a regulator as to actin dynamics than ADF during muscle morphogenesis and muscle degeneration.

Two cofilin (CF) isoforms, a nonmuscle-type (NMCF) or cofilin-1 and a muscle-type (MCF) or cofilin-2, are present in mammals (*15–17*). MCF is highly homologous to chicken cofilin (*18*), the only cofilin isoform present in chicken. Mouse MCF and chicken cofilin exhibit 96% identity in amino acid sequence, while MCF and NMCF show only 81% identity (*15*, *18*). According to the results of Northern blot and in situ hybridization analyses (*15*, *17*, *19*), NMCF, which was originally discovered in porcine brain (*5*), is expressed in a variety of tissues other than mature skeletal muscle. In contrast, MCF is predominantly expressed in muscle tissues including skeletal, cardiac and smooth muscles (*15*). However, both are expressed in developing skeletal muscle at early fetal stages through to a young postnatal stage (*20*). During postnatal development, NMCF expression in muscle is down-regulated and MCF becomes the only isoform in mature skeletal muscle (*15*, *19*, *20*). Both are also present in cultured muscle cells, namely myotubes, but the onset of MCF expression is coupled with terminal differentiation of muscle cells (*20*). NMCF, in addition to MCF, exists in mature cardiac muscle, although the expression level is low (*20*). The differential expression of the cofilin isoforms in particular tissues suggests their specialized roles in the regulation of certain types of actin

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cytoskeleton. It is of particular interest as to whether MCF plays any specific role during muscle morphogenesis, especially during myofibrillogenesis. In the case of the nematode *Caenorhabditis elegans*, two ADF/cofilin proteins are generated from a single gene by alternative splicing (*21*). They are functionally distinct and one of them is specifically required for the proper assembly of actin into myofibrils (*22–24*). With regards to mammalian ADF/cofilin family proteins, it has been demonstrated that they quantitatively differ in their activities, although they share basic common properties (*17*, *25*, *26*). As judged from their functional properties, NMCF is more like ADF than MCF (*17*, *26*). Generally ADF/cofilin family proteins show higher affinity with ADP-bound actin than ATP-bound actin. ADF in chicken, however, can bind to ATP-G-actin with higher affinity than chicken cofilin and may function as an ATP-G-actin sequestering protein (*26*), and, in addition, it depolymerizes F-actin more effectively in a pH-dependent manner (*19*, *26*). MCF is less efficient in turning over actin filaments and promoting actin disassembly than NMCF (*17*). Chicken cofilin and maybe MCF as well have weaker actin depolymerizing activity and promote actin assembly (*17*, *26*). These unique properties of MCF may be required for the assembly and maintenance of myofibrils (*17*).

In this study, we aimed to clarify whether the two mammalian cofilin isoforms are involved differently in the regulation of actin assembly during myofibrillogenesis in the cytoplasm, where both of them are present. We first examined the localization of the two cofilins in developing myotubes in culture and cardiac muscle cells, which contain both cofilin isoforms. Then, their interaction with F-actin *in vitro* was examined either individually or in combination. The results show that MCF preferentially binds to actin filaments in muscle.

MATERIALS AND METHODS

Preparation of Proteins—Recombinant mouse muscle-type cofilin (MCF) and mouse non-muscle-type cofilin (NMCF) were prepared using an *E. coli* expression system as previously described (*27*) with cDNAs encoding the respective proteins (*15*). Skeletal muscle actin was prepared from acetone-dried powder of rabbit skeletal muscle by the method of Spudich and Watt (*28*) and purified by gel filtration on a Sephadex G-100 column. Non-muscle actin was obtained from porcine brain by the method of Maekawa *et al*. (*29*). Tropomyosin was obtained from rabbit skeletal muscle by the method of Bailey (*30*).

Cosedimentation Assay—The binding of cofilin to F-actin was examined by ultracentrifugation as described previously (*31*). Cofilin (0–6 μM) and F-actin (4 μM) were incubated in 0.1 M KCl, 2 mM $MgCl_2$, 20 mM HEPES-KOH (pH 7.2), 0.01% NaN_3, and 0.1 mM DTT for 2 h at 20°C. When the effects of tropomyosin on cofilin-actin interaction were examined, tropomyosin (final concentration, 1 μM) was preincubated with F-actin before the addition of cofilin. The mixtures were then centrifuged at 100,000 × *g* for 20 min, and the resultant supernatants and pellets were examined by SDS-PAGE. In each assay, measurements were carried out 5 to 10 times, and the average values and the standard error of each value were determined.

Gel Electrophoresis and Immunoblotting—SDS-PAGE was carried out using a 13.5% polyacrylamide gel and a discontinuous Tris-glycine buffer system according to Laemmli (*32*). For immunoblotting, the proteins were transferred electrophoretically from SDS–polyacrylamide gels to a nitrocellulose membrane in a solution comprising 25% ethanol, 20 mM Tris-HCl, and 150 mM glycine, pH 8.3, for 1 h according to Towbin *et al*. (*33*). The nitrocellulose membrane was treated with 5% skim milk and then incubated with antibodies to cofilin for 1 h at room temperature, followed by treatment with alkaline phosphatase (AP)–labeled 2nd antibodies for 1 h at room temperature. After the immunoreaction, the membrane was washed with 0.1 M NaCl, 5 mM $MgCl_2$, and 0.1 M Tris-HCl, pH 9.5. The AP-labeled antibodies bound to the membrane were detected by incubation with a mixture of 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt (BCIP) and nitroblue tetrazolium chloride (NBT).

Antibodies—A monoclonal antibody (MCF1) specific for MCF was prepared by immunizing a rat with a synthetic peptide, with the partial MCF sequence WQVNGLDDIKDRS (residues 135–147 of MCF sequence), as an immunogen (*20*). Rabbit anti-serum to NMCF (anti-NMCF) was prepared with synthetic peptides KKNIILEEGKE and LQANCYEEVK, residues 44–54 and 135–144 of the NMCF sequence, respectively, as immunogens. The specificity of the antibodies has been established by immunoblotting combined with SDS-PAGE (*20*). A monoclonal antibody to cofilin (MAB22) that recognizes both isoforms was prepared as described (*11*). A monoclonal antibody to sarcomeric actin (SkA-06) (*34*) and the polyclonal antibody to slow myosin light chain (*35*) were described previously. Alkaline phosphatase (AP)–labeled goat anti-mouse IgG (GAM) and AP-labeled goat anti-rabbit IgG (GARb) were purchased from Bio-Rad (Richmond, CA). AP-labeled goat anti-rat IgG (GARt) was from Chemicon (Temecula, CA). Fluorescein (FITC)-labeled GARt, FITC-labeled GAM, tetramethylrhodamine (TRITC)-labeled donkey anti-rabbit IgG (DARb), and TRITC-labeled GARb were from Tago (Burlingame, California).

Cell Culture—Mouse mononucleated myogenic cells were dissociated from the leg (soleus) muscles of adult mice by treatment with 2 mg/ml of collagenase for 1 h at 37°C and then cultivated in 100-mm culture dishes. The culture medium consisted of Dulbecco's Modified Eagle's medium (DMEM, Nissui Co. Tokyo) supplemented with 20% fetal bovine serum and 50 ng/ml of bFGF. At 7 days culture, cells were collected from the dishes by trypsinization, and fibroblasts in the cell mixtures were removed by means of differential adhesion to culture plates. Myogenic cells were then plated on glass slides coated with collagen in 35-mm culture dishes at a density of 1×10^5 cells per dish. Three days later, the medium was replaced with DMEM containing 5% horse serum to promote muscle cell differentiation. The cultures were maintained under a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Fluorescence Microscopy—Cultured cells were fixed with 4% paraformaldehyde in PBS (0.15 M NaCl and 10 mM sodium phosphate, pH 7.0) for 20 min at room temperature, and further fixed and permeabilized with 100% methanol for an additional 5 min at –20°C. Cryosections of adult mouse muscle were prepared as described previously (*13*) except that methanol was used for fixation in place of

acetone. The specimens were then exposed to anti-cofilin antibodies, followed by staining with FITC-labeled GARt or TRITC-labeled DARb. Pieces of anterior latissimus dorsi (ALD) muscle from a 11-day-old chicken were stretched, tied to a stick at both ends, and then quick-frozen in isopentane cooled with liquid N_2. They were then fixed with 100% acetone at –20°C and 4% paraformaldehyde at 20°C, infused with 2.3 M sucrose, and then re-frozen in liquid N_2. Semithin frozen sections (about 0.5 μm thickness) were prepared at –60°C as described by Tokuyasu *et al*. (*36*). They were stained dually with MAB-22 and anti–slow myosin light chain antibodies followed by staining with FITC-labeled GAM and TRITC-labeled GARb. The specimens treated with antibodies were washed thoroughly with PBS, and then mounted with 90% glycerol containing 1 mg/ml *p*-phenylenediamine and PBS. They were examined under an epifluorescence microscope, Axioscope (Carl Zeiss: Jena, Germany), with a cooled charge-coupled device camera (CoolSNAP, Nippon ROPER, Chiba, Japan).

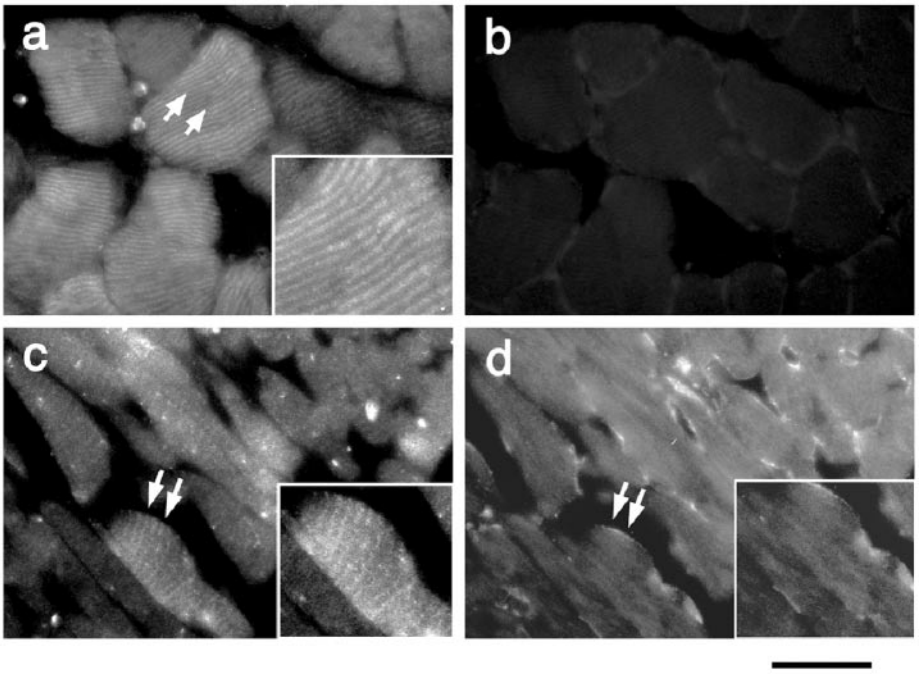

Fig. 1. **Detection of muscle-type (MCF) and non-muscle type (NMCF) cofilins in mouse striated muscles**. Cryo-sections of mouse leg (soleus) muscle (a, b) and ventricular muscle (c, d) were stained doubly with a monoclonal antibody specific for MCF (MCF1) (a, c) and a polyclonal antibody specific for NMCF (b, d), followed by treatment with FITC-GARt and TRITC-DARb. The skeletal muscle section was positively stained with MCF1 but not with anti-NMCF, while the cardiac muscle section was stained with both antibodies. MCF was detected in a striated pattern in myofibrils, both in skeletal and cardiac muscles, as visualized in the insets (a, c) at a higher magnification, but NMCF was scarcely detected in a striated pattern (see the inset in d). See the patterns in the regions indicated by double arrows in the insets. Bar: 50 μm.

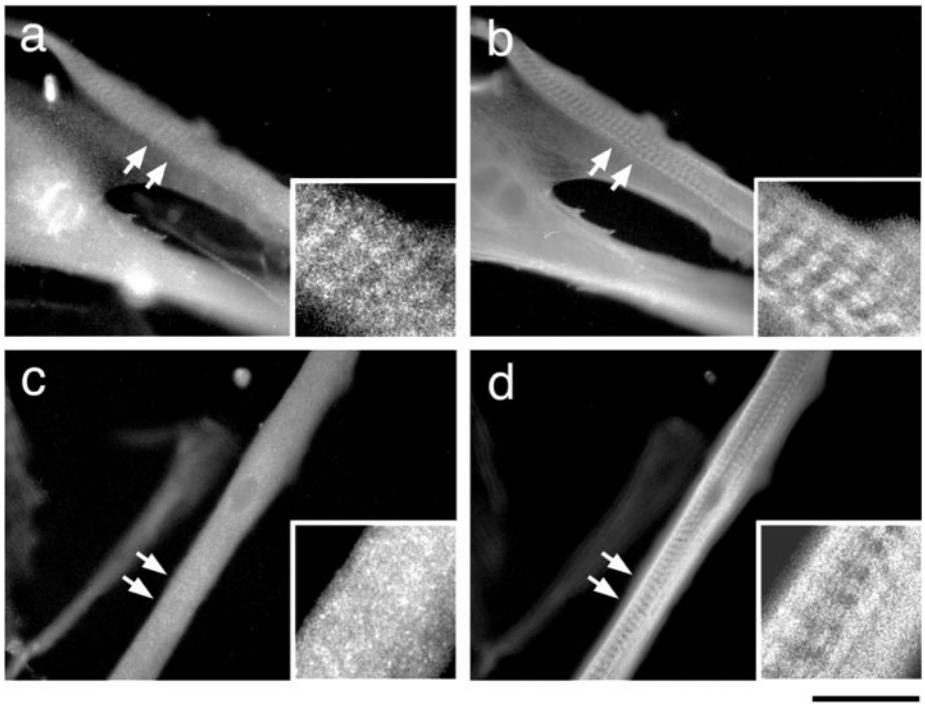

Fig. 2. **Detection of muscle-type (MCF) and non-muscle type (NMCF) cofilins in the cultured myotubes of mouse skeletal muscles**. Primary cultures of mouse myotubes were stained doubly with MCF1 and anti-actin (a, b) or anti-NMCF and anti-actin (c, d), followed by treatment with FITC- or TRITC-labeled secondary antibodies. The myotubes were positively stained with the anti-MCF (a) and anti-NMCF(c) antibodies. MCF was localized to the sarcomeric structures in the myotubes, as visualized at a higher magnification (see the inset in a), but NMCF was scarcely detected in a striated pattern (see the inset in c). The localization patterns of actin are demonstrated in b and d. See the patterns in the regions indicated by double arrows in the insets. Bar: 50 μm.

RESULTS

Differential Localization of Muscle-Type Cofilin (MCF) and Non–Muscle-Type Cofilin (NMCF) in Muscle Cells—When cryosections of adult mouse skeletal (soleus) and cardiac muscles were treated with the antibodies specific for MCF or NMCF, anti-MCF stained both skeletal and cardiac muscles (Fig. 1, a and c), while anti-NMCF stained only cardiac muscle (Fig. 1, b and d). These observations indicate that in mature skeletal muscle, only MCF is expressed, but that in cardiac muscle, both are expressed even in adult mice, which is consistent with the previous report (*20*). The localization patterns of MCF and NMCF in the muscle sections were different. MCF was localized in a striated pattern in the sections of both skeletal and cardiac muscles (Fig. 1, a and c), suggesting that MCF is partly associated with sarcomeric actin filaments. In contrast, NMCF was only detected in a diffuse pattern in sections of cardiac muscle (Fig. 1d). The location of MCF and NMCF in muscle cells was further examined in cultured myotubes by means of immunocytochemical methods. Both antibodies positively stained multinucleated myotubes, which were formed in a differentiation medium (Fig. 2, a and c). Staining with anti-MCF, MCF1, yielded striated patterns that were similar to the staining patterns with anti-actin, SkA-06 (Fig. 2, a and b). However, the striated pattern was not observed on staining with the anti-NMCF (Fig. 2c), indicating that NMCF is mostly distributed diffusely in the cytoplasm. These results suggest that MCF could be associated with actin filaments in myofibrils more efficiently than NMCF.

In order to clarify the location of cofilin in myofibrils more precisely, we performed further examination with chicken slow anterior latissimus dorsi (ALD) muscle. This muscle was used since it expresses a considerable amount of cofilin (*14*) and is easy to manipulate for thin cryosections. In addition, chickens have only one cofilin isoform that is highly homologous to MCF (*15*) and is specifically recognized by an anti–pan-cofilin antibody (MAB-22) (*11*). The ALD muscle of neonatal chicken was quick-frozen, and fixed with a combination of acetone and paraformaldehyde, and then cryosections of the muscle were prepared at about 0.5 μm thickness. They were dually stained with MAB-22 and anti-myosin light chain. As shown in Fig. 3a, both antibodies stained the myofibrils in striated patterns, but the regions stained by two antibodies were obviously different. The MAB-22-positive striated regions were regarded as actin-containing I-bands, as judged on comparison of them with those stained with the anti-myosin light chain (Fig. 3, b and c). The staining with MAB-22 appears a little fuzzy in some regions. This may be due to the presence of cofilin that is free from myofibrils and distributed diffusely in the cytoplasm.

Interaction of Recombinant MCF and NMCF with F-Actin In Vitro—Using purified recombinant MCF and NMCF that were produced in an *E. coli* expression system, we compared the interaction of MCF and NMCF with muscle F-actin *in vitro* by means of a cosedimentation assay. Under the current experimental conditions, actin without cofilin was only detected in the precipitates, while both cofilins were only detected in the supernatants in the absence of actin (Fig. 4a). When cofilin was mixed with F-actin, small but significant amounts of actin became detectable in the supernatants because of cofilin-induced depolymerization and/or fragmentation of F-actin, and a considerable amount of MCF or NMCF was precipitated together with F-actin (Fig. 4b). The amounts of cofilin in the precipitates increased as the cofilin concentrations

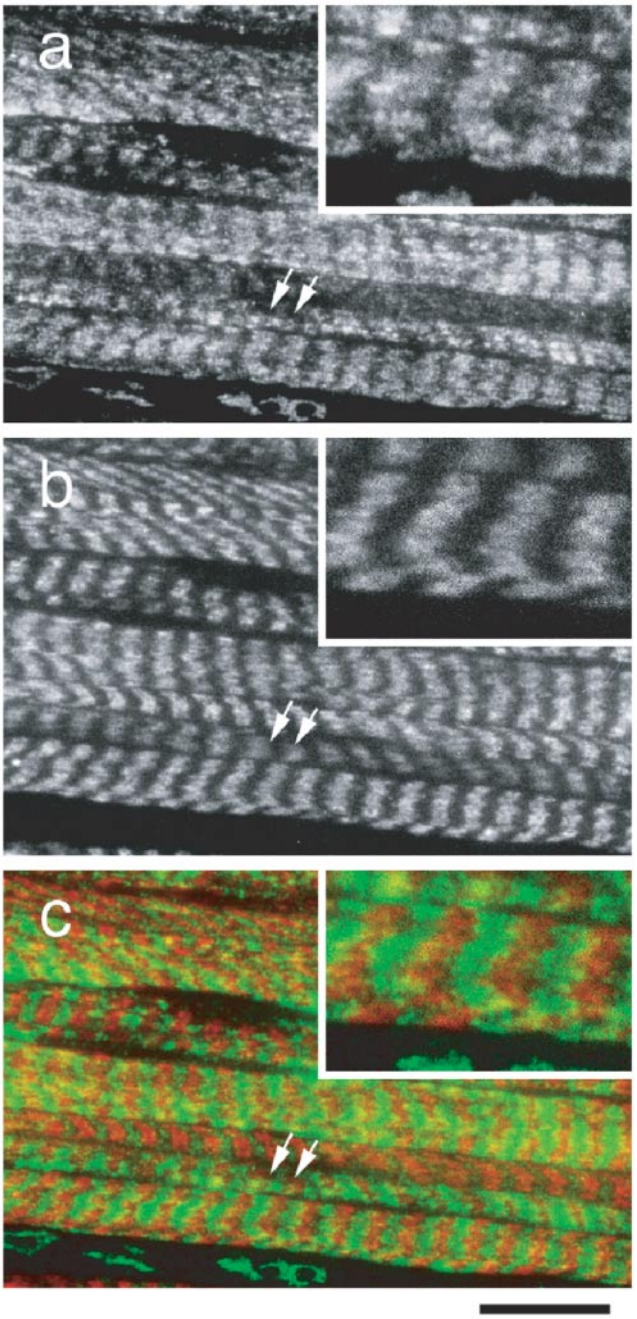

Fig. 3. **Location of cofilin in slow skeletal muscle.** Semithin frozen sections of 11-day-old chicken ALD muscle (about 0.5 μm thickness) were stained dually with a monoclonal anti-cofilin antibody (MAB-22) (a) and the antibody to slow myosin light chain (b), followed by treatment with FITC- or TRITC-labeled secondary antibodies. Merged images of double staining of cofilin (green) and myosin light chain (red) are shown in (c). Cofilin was localized mostly in the regions in which myosin was absent. The insets show enlarged images of the region including the structure indicated by double arrows. Bar: 10 μm.

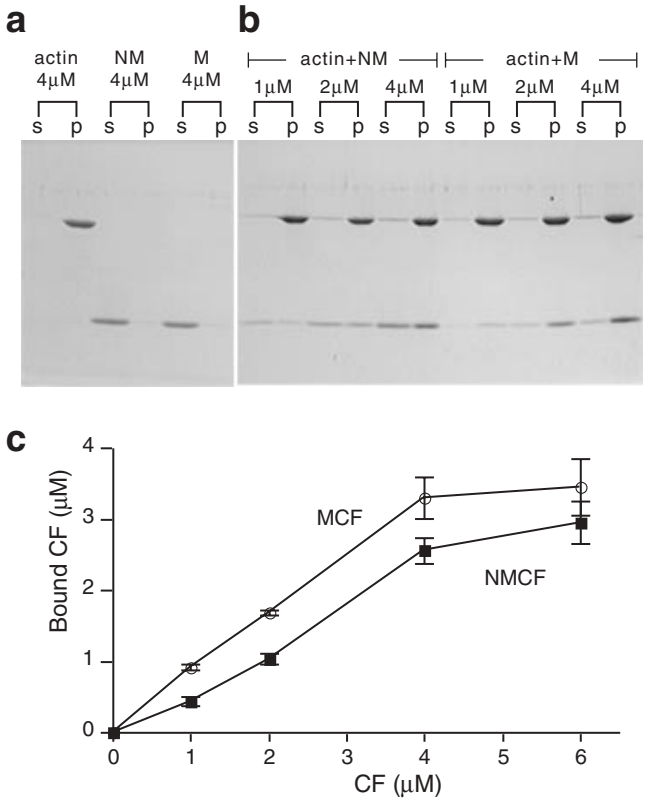

Fig. 4. **Interaction of MCF and NMCF with skeletal muscle F-actin**. Different concentrations (final concentrations, 0–6 μM) of the purified recombinant MCF or NMCF were added to F-actin (final concentration, 4 μM) in a buffer solution comprising 0.1 M KCl, 2 mM $MgCl_2$, 0.1 mM DTT, 0.01% NaN_3, and 20 mM HEPES-KOH, pH 7.2. After incubation of the protein mixtures for 2 h at 20°C, the mixtures were centrifuged at 100,000 × *g* for 20 min, and the pellets (p) and supernatants (s) was subjected to SDS-PAGE (panel b). A solutions containing cofilin alone or actin alone were similarly treated and subjected to SDS-PAGE (panel a). The amounts of cofilin precipitated as a complex with F-actin were determined by densitometry (panel c).

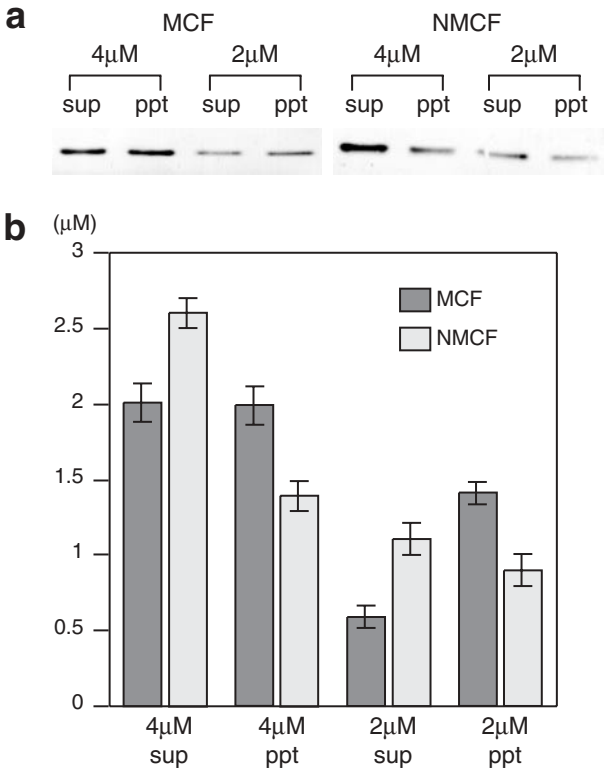

Fig. 5. **Competition of MCF with NMCF in their interaction with skeletal muscle F-actin**. A mixture of MCF and NMCF was added to F-actin (final concentration, 4 μM) in a buffer solution comprising 0.1 M KCl, 2 mM $MgCl_2$, 0.1 mM DTT, 0.01% NaN_3, and 20 mM HEPES-KOH, pH 7.2. The concentrations of MCF and NMCF were equally adjusted to either 2 μM or 4 μM. After incubating the protein mixtures for 2 h at 20°C, samples were centrifuged at 100,000 × *g* for 20 min, and the pellets (ppt) and supernatants (sup) were subjected to SDS-PAGE. The proteins in the gel were transferred to a nitrocellulose membrane, and the MCF and NMCF bands were detected (panel a) and quantified by quantitative immunoblot assay as described (*12*) (panel b) using antibodies specific for the respective cofilin isoforms.

increased up to roughly 6 μM in the presence of 4 μM actin (Fig. 4c). Importantly, with all cofilin concentrations tested, greater amounts of MCF were co-precipitated with actin than those of NMCF (Fig. 4c). These results suggest that MCF binds to F-actin with higher affinity than NMCF.

In order to directly compare the affinities of the two cofilin isoforms with F-actin under the same conditions, the same concentrations of MCF and NMCF were mixed and incubated with F-actin. Then, their binding to F-actin was again examined by means of a cosedimentation assay. Namely, the mixture of actin, MCF and NMCF was spun at high speed, and then the amounts of MCF and NMCF in the precipitates as well as in the supernatants were determined by a quantitative immunoblot assay method (*12*) with antibodies that distinguish the two cofilin isoforms. As shown in Fig. 5, more MCF was precipitated with F-actin than NMCF at two different cofilin concentrations (2 and 4 μM), indicating that MCF binds to F-actin more efficiently than NMCF. We further examined the interaction of the two cofilin isoforms with F-actin using non-muscle actin. A mixture of MCF and NMCF at the same concentration was incubated with non-muscle F-actin, and then their binding to F-actin was examined by means of a cosedimentation assay in combination with an immunoblot assay method. As shown in Fig. 6, MCF also bound to non-muscle actin more effectively than NMCF.

Cofilin is known to perturb the binding of tropomyosin to F-actin (*5*). We examined which cofilin variant, MCF or NMCF, disturbs the binding of tropomyosin to F-actin more efficiently. Cofilin was added to a mixture of tropomyosin and F-actin in a physiological salt solution, pH 7.0. and then the cosedimentation of tropomyosin as well as cofilin with F-actin was examined by high-speed centrifugation. Both cofilins inhibited the interaction of F-actin with tropomyosin. By increasing the concentration of cofilin added, either MCF or NMCF, the amount of tropomyosin precipitated, namely tropomyosin bound to F-actin, proportionally decreased (Fig. 7). It should be noted that MCF suppressed the actin–tropomyosin interaction more strongly than NMCF. This is consistent with our data showing that MCF can be preferentially associated with F-actin.

DISCUSSION

Two cofilin isoforms, namely NMCF (cofilin-1) and MCF (cofilin-2), are co-expressed in developing skeletal and cardiac muscles of mammals, although MCF is solely expressed in mature skeletal muscle while both are present in mature cardiac muscle (*20*). It is poorly understood how the two isoforms are involved in the actin filament dynamics and whether one of them plays a particularly important role during myofibril organization.

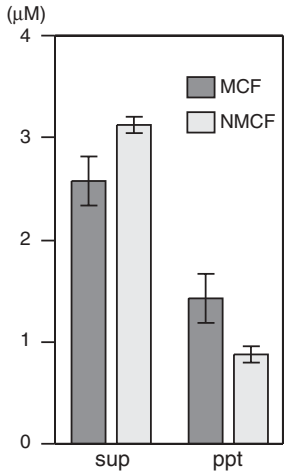

Fig. 6. **Competition between MCF and NMCF in their interaction with non-muscle F-actin**. A mixture of MCF and NMCF, 4 μM, respectively, was incubated with non-muscle F-actin (final concentration, 4 μM) in the same buffer solution as in the legend to Fig. 5, and then the protein mixtures were incubated for 2 h at 20°C. The amounts of MCF and NMCF bound to F-actin were estimated by means of a cosedimentation assay, followed by quantitative immunoblot analysis as described in the legend to Fig. 5.

Previous studies demonstrated that NMCF and MCF interact with actin in different manners *in vitro* (*17*, *25*). Then, it became a matter of interest as to whether the two cofilin isoforms interact with actin differently during myofibrillogenesis. In order to visualize how MCF and NMCF are localized in muscle cells and whether they are differentially associated with myofibrillar actin filaments in muscle cells, we prepared specific antibodies for the respective cofilin isoforms. Since the two antibodies were generated in different animals, one as a monoclonal antibody in rat and the other as a polyclonal antibody in rabbit, we were able to selectively detect each cofilin isoform even in the cytoplasm of muscle cells that contain both cofilin isoforms. Using these antibodies, we compared the locations of the two cofilin isoforms with that of myofibrillar actin in muscle cells. In slow (soleus) muscle, which contains more MCF than fast skeletal muscle, but lacks NMCF, MCF was detected in association with sarcomeric structures. The location of cofilin in the I-band regions of myofibrils was clearly observed in semithin frozen sections (*36*) of chicken slow ALD muscle. Since chicken cofilin is highly homologous to mammalian muscle-type cofilin (MCF) from structural and functional viewpoints (*15*, *17*, *26*), this observation strongly suggests that MCF is also localized in the actin-containing I-bands of myofibrils in mammalian slow muscle. In heart cells and cultured skeletal muscle cells, which contain both MCF and NMCF, MCF was detected in a striated pattern, while NMCF was detected in a diffuse pattern in the cytoplasm. It is likely that MCF tends to bind to actin filaments in premyofibrils or myofibrils with higher affinity than NMCF. ADF is known to bind to ATP-G-actin, a major monomeric actin form in the cytoplasm, with higher affinity to sequester monomeric actin (*26*). Since NMCF is more like ADF (*17*, *26*), NMCF might be associated with monomeric G-actin and thereby be distributed diffusely in the cytoplasm.

To further clarify the differential association of NMCF and MCF with actin filaments in a physiological salt

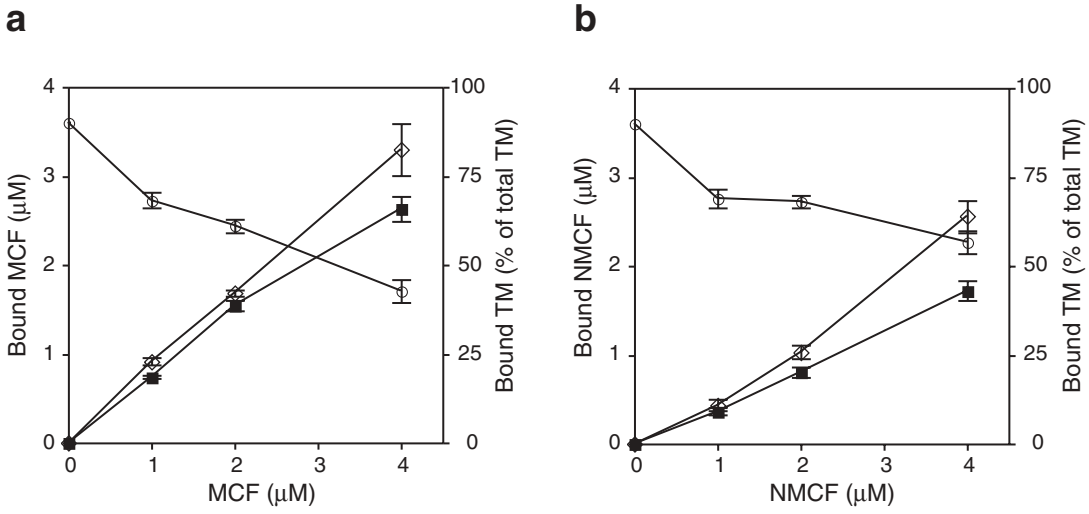

Fig. 7. **Competition of cofilin isoforms, MCF and NMCF, with tropomyosin in their interaction with F-actin**. The interaction of 0–4 μM MCF (a) and 0–4 μM NMCF (b) with F-actin (final concentration, 4 μM) in the absence (open diamonds) or presence (solid squares) of tropomyosin (final concentration, 1 μM) was examined by means of a cosedimentation assay as described in the legend to Fig. 4. The concentrations of precipitated cofilin (open diamonds, solid squares) as well as tropomyosin (open circles) are plotted as a function of the total concentration of cofilin.

solution, binding of recombinant NMCF and MCF with purified F-actin was examined either individually or in combination *in vitro*. When NMCF and MCF were examined separately, MCF was more effectively precipitated with F-actin than NMCF, as described previously (*17*), but the difference was greater than that observed in the previous study. To compare their ability to bind to F-actin more precisely, NMCF and MCF were mixed and then incubated with F-actin, followed by cosedimentation assaying. Each cofilin in the precipitates and supernatants was selectively detected and quantified using specific antibodies. Thus, their differential binding to F-actin was examined under exactly the same conditions. The results showed definitely that MCF preferentially binds to F-actin *in vitro* as well. Because of the higher affinity of MCF for F-actin, MCF inhibited the binding of tropomyosin to F-actin more efficiently than NMCF.

The results of this investigation show that MCF rather than NMCF preferentially binds to F-actin *in vitro* as well as to myofibrillar actin filaments in muscle cells. Since NMCF has high actin-depolymerizing activity just like ADF (*17*, *26*), NMCF may be involved in the disassembly of pre-existing actin filaments in pre-myofibrils and/or in the cortical regions of myogenic cells, while MCF may contribute by enhancing nucleation for actin polymerzation and promoting actin assembly. The combination of NMCF and MCF seems to facilitate dynamic reorganization of actin filaments in the early process of myofibrillogenesis. The specific role of MCF may become more important at later stages when myofibril formation is progressing rapidly, since *de novo* assembly of actin is particularly needed for the growth of myofibrils in developing muscle cells.

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