Actin filaments function as a tension sensor by tension-dependent binding of cofilin to the filament

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ntracellular and extracellular mechanical forces affect the structure and dynamics of the actin cytoskeleton. However, the underlying molecular and biophysical mechanisms, including how mechanical forces are sensed, are largely unknown. Actin-depolymerizing factor/cofilin proteins are actin-modulating proteins that are ubiquitously distributed in eukaryotes, and they are the most likely candidate as proteins to drive stress fiber disassembly in response to changes in tension in the fiber. In this study, we propose a novel hypothesis that tension in an actin filament prevents the filament from being severed by cofilin. To test this, we placed single actin filaments under tension using optical tweezers. When a fiber was tensed, it was severed after the application of cofilin with a significantly larger delay in comparison with control filaments suspended in solution. The binding rate of cofilin to an actin bundle decreased when the bundle was tensed. These results suggest that tension in an actin filament reduces the cofilin binding, resulting in a decrease in its effective severing activity.

Results and discussion

Single actin filaments function as a mechanosensor

An in vitro reconstituted system comprised of only actin filaments and recombinant (dephosphorylated) cofilin was used to directly test the aforementioned hypothesis. We prepared...
and direct kinetic measurements (Andrianantoandro and Pollard, 2006), showing that severing activity of cofilin is slower than cofilin binding. In contrast, when tension (≥30 pN) was generated in a filament, it was not severed or was severed by cofilin with a significantly larger (P < 0.01) delay (43.2 ± 5.1 s; n = 15; Fig. 1 E and Video 1). After the cessation of optical trapping, the filament was severed by cofilin within 15.1 ± 3.7 s (n = 10). These results demonstrate directly that tension in the actin filament prevents, or delays, the filament severing by cofilin (Fig. 1, F and G).

Magnetic micromanipulation of micrometer-sized magnetic particles provides a means to probe force-dependent molecular interactions (Fig. 1 H; Wang et al., 1993). Tension-dependent severing of actin filaments by cofilin was examined by using magnetic beads (~1 µm in diameter) conjugated with phalloidin and that were attached to the actin filaments tethered on the glass surface by NEM-myosin. Individual beads were pulled...
cofilin to the actin filaments. Quantitative analysis showed that the short-lived fluorescence was not seen in the absence of actin filaments, indicating that it takes place only during transient binding of cofilin already bound to the filament. The effect of tension on the cofilin binding was examined using actin filaments under different mechanical conditions. Actin filaments were tethered at multiple sites on the coverslip by NEM-myosin, and 50 nM 5-iodoacetamide fluorescein (IAF)–labeled cofilin was applied; brief fluorescence emission from IAF-cofilin was observed under total internal reflection fluorescence (TIRF) microscopy. The fluorescence was not seen in the absence of actin filaments, indicating that it takes place only during transient binding of cofilin to the actin filaments. Quantitative analysis showed that the cofilin-binding rate to actin filament meshwork was low (0.025 ± 0.006 events/s in 1 µm² of meshwork; n = 3), and the mean duration of fluorescence was short (41 ± 26 ms; n = 147). However, the rate of binding and fluorescence duration increased (to 0.21 ± 0.12 events/s µm² [n = 3] and 91 ± 102 ms [n = 415], respectively) when the meshwork of actin filaments was severed by scratching the mesh with a pipette tip (Fig. 2 A). Analysis was performed in a narrow (2-µm width) area facing the scratched region, and examples of the data analyzed are shown in Fig. 2 (B–E) and Video 3. Although most of the binding was brief, slow binding (1–3 s) was sometimes detected (a few percentages of binding events). The area free of F-actin gradually expanded during 60 s of observation, suggesting that cofilin had severed the actin filaments. The tethering of actin filaments at multiple sites on the glass surface decreases actin filament flexibility (Pavlov et al., 2007), whereas severing results in a freeing of the ends of the actin filaments, allowing their relaxation and tension reduction compared with the tethered filaments. These findings suggest that cofilin tends to bind to flexible, not tensed, actin filaments and severs them, as hypothesized (Michelot et al., 2007; Pavlov et al., 2007).

The dissociation time constant of cofilin from actin filaments was reported to be 0.18 s⁻¹ (Cao et al., 2006). The long duration (1–3 s) of cofilin binding to the actin filaments detected in this study roughly agrees with the aforementioned estimation (i.e., the dissociation time constant 0.18 s⁻¹ corresponds to a 5.6-s duration of binding).

Sliding a fine pipette along the surface, actin meshwork often formed a bundle of actin filaments from the actin meshwork, which consisted of F-actin and NEM-myosin; NEM-myosin binds to actin filaments and facilitates bundle formation by the zipperpering together of the actin filaments. The cofilin-binding rate to the bundle was 6.5 ± 4.5 events/s per 1 µm of bundles (n = 7; Fig. 2 [F and G] and Video 4). The rate decreased to 2.9 ± 2.7 events/s when the bundle was stretched 20–30% by
To examine the tension-dependent cofilin binding to the actin stress fibers in living cells, a GFP-cofilin expression construct was introduced into HUVEC cells. In control cells, GFP-cofilin was distributed uniformly in the cytosol and in the lamellipodia (Fig. 4 A), as reported previously (Obinata et al., 1997). When the prestretched elastic substratum was relaxed (20%), GFP-cofilin was translocated to actin stress fibers within 1 min (Figs. 4 B and S2), suggesting that the binding of cofilin to stress fibers also depends on tension in the fiber in living cells. These stress fibers were disassembled within 30 min when the tension in the stress fibers was decreased; similar observations were described earlier (Ono et al., 1996; Katoh et al., 2001). These results account for the compressive stress-induced severing of actin bundles (Medeiros et al., 2006) and disassembly of stress fibers (Ono et al., 1996) in intact cells.

How does tension prevent cofilin binding to actin filaments?

Detailed examination by EM (McGough et al., 1997; Galkin et al., 2001) revealed a unique property of cofilin; it induces an ~25% reduction in the pitch of the actin helix while keeping the original length of the filament (i.e., the binding of cofilin increases the degree of filament twisting). Such a conformational change in the filament is postulated to induce cooperative binding of cofilin to the filament (McGough et al., 1997; Galkin et al., 2001).

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Tension-dependent cofilin binding to stress fibers

Tension-dependent cofilin binding to actin stress fibers was also examined in semi-intact human umbilical vein endothelial cells (HUVECs). Semi-intact cells on a prestretched (20%) elastic substrate were relaxed in ATP-free buffer (pH 6.5) with 500 nM purified cofilin, chemically fixed within 1 min, and stained with an anticofilin antibody. The experiment showed that cofilin distributed along the actin stress fibers in parallel to the axis of relaxation (Fig. 3, D–F). However, this characteristic staining pattern was not observed when the cells were 10% stretched (Fig. 3, A–C), demonstrating that tension prevents the cofilin binding to the tensed stress fibers in semi-intact cells. Quantitative fluorescence image analyses confirmed the tension-dependent cofilin distribution along the actin stress fibers (Fig. 3, G–K). Furthermore, in ATP-free DK buffer (Mackay et al., 1997), which reduces tension in stress fibers by attenuating actomyosin activity, the stress fibers in semi-intact cells were disassembled by 250 nM cofilin in relaxed cells (Fig. 3 M) but not in stretched cells (Fig. 3 L).
would twist actin filaments to a degree comparable with the twist when filaments are decorated with cofilin (McCough et al., 1997). These results suggest that spontaneous structural fluctuations of actin filaments enable cofilin binding to the filaments and are consistent with the slow-association kinetics (Cao et al., 2006). Conceivably, cofilin prefers to associate with the twisted actin filament, which would induce twisting of the neighboring region of the filament, resulting in a cooperative form of cofilin binding.

Actin filaments behave like a twin strand of beads that can be easily twisted (Huxley and Brown, 1967). Precise x-ray diffraction studies indicate that stretching the actin filament is associated with changes in its helical structure (Huxley and Brown, 1967; Wakabayashi et al., 1994). Based on geometrical considerations of the helical structure of the actin filament, it can be postulated that stretching the filament causes untwisting of its right-handed genetic helix. Thus, when the filament is stretched, the amplitude of torsional fluctuations of the filament will be reduced, resulting in an inhibition of the cofilin binding to actin filaments. A tension-dependent reduction in the torsional fluctuations in the single actin filament was demonstrated very recently by molecular dynamics simulations (Matsushita et al., 2011). Besides, an evaluation of the effect of long axis tension on phalloidin fluorescence (Shimozawa and Ishiwata, 2009) suggests that external forces distort the filament structure. In this study, a direct measurement of the torsional fluctuations of a single actin filament under different stresses was performed by using a method of Tsuda et al. (1996). Experiments demonstrated that the SD of the fluctuations was reduced by 55–72% (P < 0.05) by an applied force of ∼5 pN (n = 5; Fig. S3). Cofilin binding increases the bending (McCullough et al., 2008; Pfaendtner et al., 2010) and twisting (Prochniewicz et al., 2005) elasticity of actin filaments, suggesting that any changes in elasticity will affect cofilin binding and severing. Therefore, reduction in the torsional fluctuation could be a potential molecular mechanism by which actin filament tension prevents cofilin binding.

This study demonstrates that a low level of tension (>2 pN) prevents (or delays) the severing of actin filaments by cofilin. This effect accounts for the frequent observations in cells that actin filaments with less tension, i.e., filaments not in use, are severed easily, whereas those in use, and thus generating larger tension, are not severed. The regulation of cofilin binding to the actin filament by tension may be behind these observations, at least in part, as schematically illustrated in Fig. 4 in addition to biochemical regulation of cofilin activity (Yang et al., 1998; Bamburg, 1999). This study raises the intriguing possibility that actin cytoskeletons are endowed with an ability to sense and respond to mechanical stress, as in the case of focal contact-associated proteins, e.g., p130Cas (Sawada et al., 2006).

Materials and methods

Direct observation and manipulation of single actin filaments

Rhodamine-labeled cytoplasmic β-actin (Cytoskeleton) was polymerized in F-buffer at a 1-mg/ml concentration for 12 h at 4°C. The F-actin solution was diluted at a final concentration of 1 µg/ml in F-buffer with beads (4.3 µm in diameter) coated with NEM-treated skeletal muscle myosin and placed on an observation chamber (0.5 × 2 × 18 mm). Phalloidin was not added to the solution because it inhibits the binding of cofilin to F-actin. During the experiment, an actin filament with one end attached to a 4–10-µm bead was selected, whereas the other end of the filament was trapped at a 0.3-µm bead that was manipulated by optical tweezers. The actin filaments were tensed by the optical tweezers. 500 nM mouse recombinant muscle cofilin (a gift from T. Obinata, Teikyo University, Tokyo, Japan) was applied by perfusion, allowing the measurement of the delay between the cofilin

Figure 4. Cofilin binding to actin stress fibers in intact cells. (A) GFP-cofilin was distributed uniformly in the perinuclear cytosol but was often condensed in the ruffling membrane; GFP-cofilin did not associate with the stress fibers in control cells. (B) When the substratum was relaxed 20%, GFP-cofilin was translocated to actin stress fibers (shown by the arrows: see also Fig. S2) within 1 min. (C) Intensity profiles of the GFP-cofilin along the top lines are shown (the top profile for control cell and the bottom profile for relaxed cell). The broad peak (a) in the top profile corresponds to the cofilin condensed in the ruffling membrane (corresponding with letters in A). The narrow peaks (a, b, and c) in the bottom profile correspond to the stress fibers (corresponding with letters in B). (D) Percentage of cells positive for GFP-cofilin translocated to actin stress fibers increases from 0/17 to 8/17, with relaxing of the substrate [the number of cells examined was 17 from seven independent experiments]. Translocation of GFP-cofilin to actin stress fibers (SFs) was not detected in control cells in the same period of observations (five independent experiments). *(P < 0.05, using Fisher’s exact test. (E–G) Schematic drawings of the mechanosensing by the actin stress fibers and the regulation of cofilin binding to actin stress fibers in cells. (E) Actin stress fibers generate contractile force in adherent cells, resulting in generation of tension in stress fibers, which prevents the binding of cofilin to the fibers. (F) When the tension declines (e.g., by relaxing the cell substratum or by decreasing the contractile force in the actin filaments), cofilin binds to and disassembles the fibers (G).
application and the severing of the actin filament, although 500 nM of cofilin severs actin filaments less effectively than 10 nM (Andrianantoandro and Pollard, 2006). Severing of actin filaments by mouse cofilin at the concentration 500, 250, 100, or 50 nM was examined. Rapid severing of the filaments was observed with 500 and 250 nM cofilin, and it was slower with 100 nM (or 50 nM) cofilin. We chose 500 nM in optical trapping experiments and 250 nM for magnetic force experiments to examine the effect of tension to the severing activity of cofilin. The severing of the actin filaments by cofilin was observed with an epitfluorescence microscope (TE2000-U; Nikon) equipped with a high-NA lens (Plan Apo TIRF, 60×, 1.45 NA; Nikon) and a charge-coupled device camera (Cascade512B; Roper Scientific) or another epitfluorescence microscope with image intensifiers (IIs; CB600; Hamamatsu Photonics) and charge-coupled device cameras (CCD-900; ULTIMATE; Watec Co., Ltd.). No severing was seen in the control perfusate (F-buffer with 10 mM of dithiothreitol, pH 8.0) without cofilin in both tense or not tensed actin filament up to 120 s in our experimental condition. The speed of solution flow was ~100 µm/s, which was not different between control and cofilin-containing solutions. Therefore, the filament fluctuations were not changed obviously during perfusion. All imaging experiments were performed at room temperature (25°C–27°C).

Magnetic force application to beads attached to the actin filament while mechanically stretching the filament

A streptavidin-conjugated polystyrene bead (2 µm in diameter) was attached to a biotin-labeled actin (Cytoskeleton) containing actin filament (~1 µm) that was tethered to a gelsolin molecule on the glass surface. The rotational angular fluctuation of a bead was observed by a particle-tracking method of MicroMorph image-analyzing software. A schematic drawing of the experimental setup is shown in Fig. S2. An equal volume of 100 nM IAF-labeled cofilin was optically trapped, and the actin filament was stretched by moving the trapping point in the downward direction (~5 pN).

Online supplemental material

Fig. S1 shows the interaction of IAF-cofilin with actin. Fig. S2 shows the colocalization of GFP-cofilin with actin stress fibers in cells upon relaxation. Fig. S3 shows a decrease in the rotational angular fluctuation of a bead attached to an actin filament by mechanical stretching of the filament. Video 1 shows that tension in the actin filament prevents the filament from being severed by cofilin. Video 2 shows time-lapse images of an actin filament severed by cofilin. Video 3 shows the binding of cofilin to a severed actin meshwork. Video 4 shows the binding of IAF-cofilin to the relaxed actin bundle. Video 5 shows the binding of IAF-cofilin to the tensed actin bundle. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201102039/DC1.

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