Biochemical and Biophysical Research Communications 438 (2013) 728–731

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Competitive displacement of cofilin can promote actin filament severing

W. Austin Elam, Hyeran Kang, Enrique M. De La Cruz *

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 12 July 2013
Available online 2 August 2013

Keywords:
Actin binding protein
Competition
Cytoskeleton
Myosin
Phalloidin

ABSTRACT

Cofilin is an essential actin filament severing protein that functions in the dynamic remodeling of the actin cytoskeleton. Filament severing activity is most efficient at sub-stoichiometric cofilin binding densities (i.e. <1 cofilin per actin filament subunit), and peaks when the number density of boundaries (i.e. junctions) between bare and cofilin-decorated segments is maximal. A model in which local topological and mechanical discontinuities lead to preferential fragmentation at boundaries accounts for available experimental data, including direct visualization of cofilin and actin during real-time severing events. The boundary-severing model predicts that ligands (e.g. other actin-binding proteins) that compete with cofilin for actin filament binding and modulate cofilin occupancy on filaments will alter the bare-decorated segment boundary density, and thus, the filament severing activity of cofilin. Here, we directly test this model prediction by evaluating the effects of phalloidin and myosin, two ligands that compete with cofilin for filament binding, on the actin filament binding and severing activities of cofilin. Our experiments demonstrate that competitive displacement of cofilin lowers cofilin occupancy and promotes severing when initial cofilin occupancy is high (i.e. >50%). Even in the presence of competitive ligands, maximum severing activity occurs when cofilin-decorated boundary density is highest, consistent with preferential fragmentation at boundaries. We propose a general "severodyne" framework for the modulation of cofilin-mediated actin filament severing by small molecule or actin-binding protein ligands that compete with cofilin for actin filament binding.

1. Introduction

Remodeling of the actin cytoskeletal network is vital for many fundamental cellular processes including cell division, growth, and motility [1–3]. Cofilin is an essential actin regulatory protein that binds and severs actin filaments [4–7]. Human cofilin binds vertebrate actin filaments cooperatively [8–10], and a one-dimensional lattice model with nearest neighbor cooperative binding interactions reliably describes the equilibrium [11] and kinetics [12,13] of binding.

Filaments partially decorated with cofilin sever more readily than bare or saturated filaments [5,7,11,14–20]. Severing activity is maximal at half-stoichiometric cofilin occupancy (i.e. binding density, $v_{cof} = 0.5$ cofilin per actin filament subunit) [5,18,20], where the number of boundaries between bare and cofilin-decorated filament regions is highest [5,7,18]. Direct real-time visualization of cofilin-mediated severing events indicates that severing occurs preferentially at or near boundaries [18,20]. Preferential severing at boundaries is attributed to mechanical (e.g. filament bending and twisting compliance) and topological (e.g. filament twist and subunit tilt) discontinuities between bare and cofilin-decorated segments [17,18].

Central to understanding cofilin-mediated filament severing *in vivo* is knowledge of how other actin regulatory proteins modulate cofilin binding and severing activity. Actin regulatory proteins that compete with cofilin for filament binding can modulate cofilin binding density along filaments and, therefore, severing activity. The boundary-severing mechanism specifically predicts that displacement of bound cofilin will either increase or decrease filament severing, depending on the initial cofilin occupancy. Because severing activity peaks at a cofilin binding density of ~0.5, competitive displacement of bound cofilin is predicted to increase the boundary density and promote severing when initial cofilin occupancy is high ($v_{cof} > 0.5$) but lower the boundary density and reduce severing when initial occupancy is low ($v_{cof} < 0.5$).

In this study, we directly test this model prediction through evaluation of the effects of phalloidin and myosin on cofilin binding and severing of actin filaments. At high cofilin occupancy ($v_{cof} = 0.9$), inclusion of either phalloidin or myosin displaces cofilin, which increases the boundary density and promotes filament severing. Filaments partially decorated with cofilin and competing ligands sever maximally when the cofilin binding density is

Abbreviations: L_{avg}, average filament length; v_{cof}, cofilin binding density; NEM, N-ethylmaleimide; S1, subfragment 1; XAip1, *Xenopus* Actin-interacting protein 1.

* Corresponding author. Address: 260 Whitney Avenue, J.W. Gibbs Building, New Haven, CT 06520-8114, USA.
E-mail address: enrique.delacruz@yale.edu (E.M. De La Cruz).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.07.109

approximately half-saturating ($v_{cof} \sim 0.5$), consistent with preferential severing at boundaries ofcofilin-decorated segments.

2. Materials and methods

2.1. Protein expression, purification, and labeling

All reagents were the highest purity commercially available and purchased from Sigma–Aldrich, unless otherwise noted. Vertebrate skeletal muscle actin was purified from rabbit back and leg muscle, labeled (efficiency > 0.85) with pyrenyl-iodoacetamide, Alexa 488-, or Alexa 594-succimidyl ester (Molecular Probes), and filtered at 4 °C over Sephacryl S-300 (equilibrated in 0.2 mM ATP, 0.1 mM $CaCl_2$, 0.5 mM DTT, 1 mM NaN_3, 2 mM Tris–HCl, pH 8.0) as described [17,18,21]. Ca^{2+}-actin was converted to Mg^{2+}-actin on ice with 0.2 mM EGTA and 20–80 μM $MgCl_2$ before polymerizing with 0.1 vols of 10× polymerizing buffer (500 mM KCl, 20 mM $MgCl_2$, 200 mM imidazole, pH 6.8) and freshly dissolved DTT, yielding a solution of Mg^{2+}-actin filaments in $KMI_{6.8}$ buffer (50 mM KCl, 2 mM $MgCl_2$, 2 mM DTT, ~0.2 mM ATP, ~1 mM NaN_3, 20 mM imidazole, pH 6.8).

Recombinant human nonmuscle cofilin-1 was expressed and purified as described [11,17]. Cofilin concentration was determined spectroscopically by the Edelhoch method [22]. Purified rabbit skeletal muscle myosin S1 was provided as a generous gift from David D. Thomas (University of Minnesota), and labeled with N-ethylmaleimide (Thermo Fisher Scientific) as described [23]. Phalloidin stock (1 mM) was prepared in methanol.

2.2. Equilibrium binding assays

Cofilin binding to pyrene-labeled actin filaments was assayed by fluorescence and cosedimentation [11,12]. Equilibrium binding of human cofilin to 2.5 μM pyrene-labeled actin filaments (in the presence and absence of the indicated phalloidin or NEM myosin S1 concentrations) was monitored by fluorescence (λ_{ex} 366 nm, λ_{em} 407 nm) with a SpectraMax Gemini XPS plate reading fluorimeter (Molecular Devices). Measurements were made at 22 °C in $KMI_{6.8}$ buffer. Observed intensities were converted to cofilin and competitor binding densities as described [11,12]. Cofilinbinding to Alexa-labeled actin filaments used in microscopy assays was validated by cosedimentation [11,12]. No detectable depolymerization occurred in the presence of cofilin under our conditions, as reported [16].

2.3. Fluorescence microscopy

Alexa 488 or Alexa 594-labeled actin filaments equilibrated with cofilin and/or competitors (myosin or phalloidin) were diluted in $KMI_{6.8}$ buffer supplemented with 20 μg/mL catalase, 100 μg/mL glucose oxidase, and 10 mM glucose and free cofilin (and competitor, where appropriate, at corresponding concentrations to ensure initial occupancy did not change [17,18]) and immobilized on poly-L-lysine treated slides. Filaments were imaged at room temperature (~22 °C) using a Till iMic digital microscope system equipped with a 100× objective (Olympus), cooled Andor iXon897 EMCCD camera, and LiveAcquisition software. Digital images were processed using ImageJ software (NIH). Single actin filaments longer than 6 pixels (0.07 μm/pixel) were detected and reconstructed using a custom Matlab script, with manual user verification to exclude bundles [17,18,21]. The average contour lengths and bending persistence lengths of actin filaments at varying cofilin, phalloidin, and myosin occupancies were determined from analysis of 100 to 500 individual filaments (~20 images) in each data set, as described in detail [17,18,21].

3. Results

3.1. Phalloidin and myosin compete with cofilin for actin filament binding

Phalloidin and myosin compete with cofilin for actin filament binding [9,24–27]. Myosin and cofilin binding sites are overlapping (comparison of Refs. [28,29]). Phalloidin and cofilin binding sites are distinct, but linked allosterically [9,27,30,31]. These two ligands therefore provide distinct classes of chemical linkage for competing cofilin from actin filaments.

We performed competitive equilibrium binding assays to determine conditions under which myosin or phalloidin displace cofilin from actin to yield filaments with a range of cofilin and myosin or phalloidin binding densities. Competitive displacement of cofilin from actin filaments at high initial cofilin occupancy ($v_{cof} > 0.9$) was measured by pyrene fluorescence enhancement as either phalloidin or myosin was titrated over a broad concentration range (Fig. 1A and B). Because phalloidin and NEM-treated myosin bind actin filaments more tightly than cofilin [11,12,26,32,33], they effectively displace bound cofilin and allow us to identify experimental conditions for evaluation of severing activity in which the cofilin binding density can be precisely controlled through competition.

3.2. Phalloidin and myosin promote filament severing by displacement of cofilin at high initial occupancy

Severing lowers the average actin filament length (L_{avg}) at steady state and provides a quantitative metric of filament severing activity [18,34,35] As previously reported [18,20], filaments half-saturated with cofilin ($v_{cof} = 0.5$) possess the maximum allowable boundary density [5,13], sever with the greatest frequency [18,20], and thus display the shortest observed L_{avg} (Fig. 2A and C–E). Filaments with low (i.e. $v_{cof} \to 0$) or high (i.e. $v_{cof} \to 1.0$) cofilin binding densities contain a low bare-decorated boundary

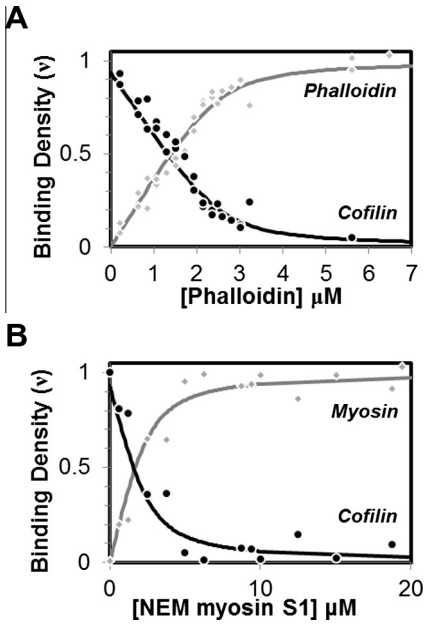

Fig. 1. Phalloidin and myosin dissociate cofilin from actin filaments. (A) Cofilin (black) and phalloidin (gray) binding densities derived from equilibrium fluorescence titrations. (B) Cofilin (black) and NEM-myosin S1 (gray) binding densities derived from fluorescence data. Solid lines through the data represent the best fits of the data to a two ligand, linked equilibrium competitive binding model [46], where phalloidin and myosin have no binding cooperativity.

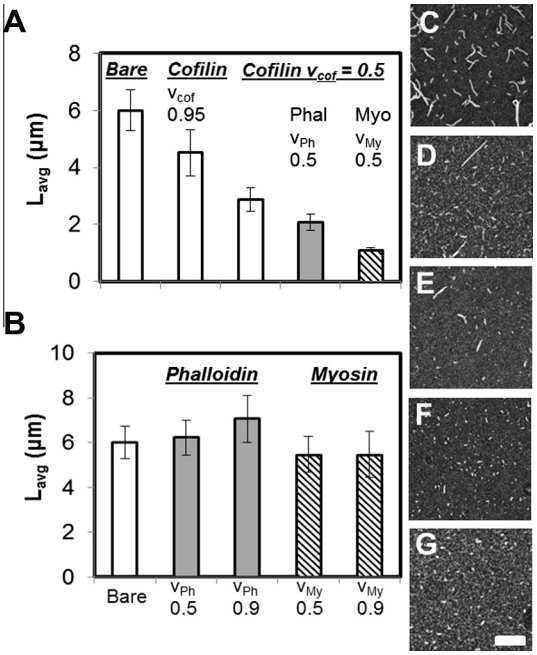

Fig. 2. Phalloidin and myosin competition enhances cofilin-mediated filament severing. (A) L_{avg} of bare actin and filaments decorated with either cofilin or both cofilin and competitor (i.e. phalloidin or myosin) at various occupancies. Uncertainty bars represent the standard deviation of the mean filament contour lengths. Note that filaments decorated with cofilin and phalloidin or myosin have shorter average lengths, consistent with the mechanical gradient and topological discontinuities at boundaries contributing to severing efficiency [5,7,17,18,20]. (B) L_{avg} of bare filaments and filaments decorated with phalloidin or myosin S1 at binding densities (v) of 0.5 or 0.9. Uncertainty bars represent the standard deviation of the mean filament contour lengths. (C–G) Representative images of Alexa-labeled actin filaments: (C) Bare actin. (D) Cofilin v = 0.95. (E) Cofilin v = 0.5. (F) Cofilin v = 0.5, phalloidin v = 0.5. (G) Cofilin v = 0.5, NEM myosin S1 v = 0.5. Scale bar is 5 μm.

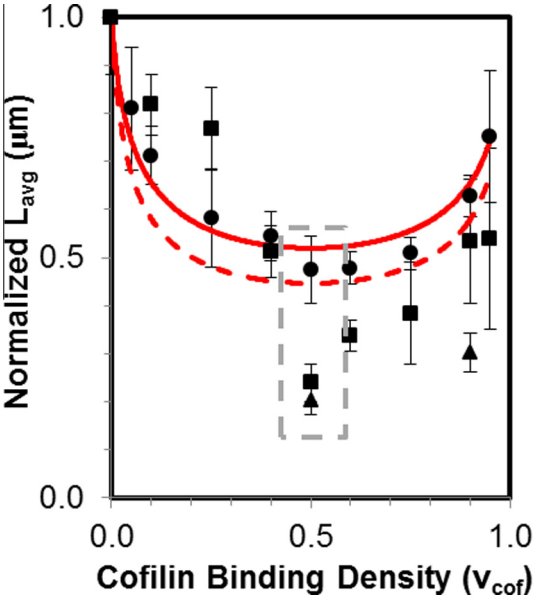

Fig. 3. Cofilin-mediated severing activity is maximal when filaments are partially decorated. Cofilin binding density dependence of L_{avg} in the presence of cofilin alone (circles), cofilin and phalloidin (squares), or cofilin and myosin (triangles). The lines (solid, cofilactin; dashed, cofilin–phalloidin) through the data represents the best fits of the data to an equilibrium severing model [18]. The gray box highlights the regime of maximal severing (shortest L_{avg}). Average lengths were normalized to bare actin (cofilin alone) or to filaments saturated with the appropriate competitor.

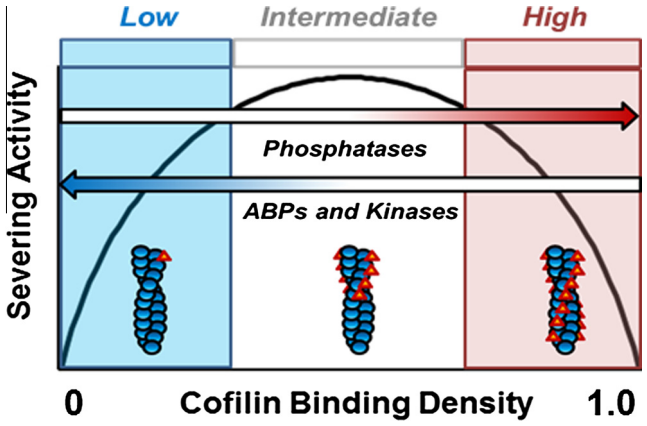

Fig. 4. Severodyne framework for the modulation of cofilin severing activity. Depending on the initial cofilin occupancy of actin filaments, competing ligands (e.g. small molecule or other actin-binding proteins) and regulatory kinases have the capacity to differentially modulate cofilin activity. Severing activity is depicted by the solid black line. Cofilin occupancy is shaded blue, white, or red to indicate low, intermediate, or high binding densities. The severodyne scheme is drawn symmetrically for presentation, but cofilactin filaments are predicted to sever more readily than bare filaments [18].

density so they sever less readily and display a longer L_{avg} than partially decorated filaments.

Addition of competing ligands (phalloidin or myosin) to filaments with high initial cofilin occupancies (v_{cof} = 0.9), dissociates bound cofilin (Fig.1) and lowers L_{avg} until approximately half-saturation of cofilin is achieved (i.e. v_{cof} = 0.5; Figs. 2A, F, G, and 3), indicating that competitive displacement of near-saturating cofilin (i.e. $v_{cof} \rightarrow 1.0$) promotes filament severing. Neither phalloidin nor myosin severs filaments in the absence of cofilin, as indicated from L_{avg} (Fig. 2B), so severing must result from cofilactin interactions [5,7,17,18,20]. Displacement of bound cofilin below half-saturation ($v_{cof} < 0.5$) reduces severing (Fig. 3). Maximum severing of filaments occupied with bound cofilin and competitive ligands occurs at a cofilin binding density of ~0.5 – when the cofilin-decorated boundary density is maximal, consistent with preferential fragmentation at boundaries of cofilin-decorated and either bare or competitor-decorated segments.

4. Discussion

The data presented in this study demonstrate that both phalloidin and myosin can displace cofilin from actin filaments and promote severing of filaments densely decorated with cofilin. Severing results from competitive displacement of bound cofilin, which increases the number density of boundaries where severing occurs. This behavior is thermodynamic in origin (i.e. chemical linkage and mass action), and thus expected to be general. That is, any ligand (small molecule or actin-binding protein) that competes with cofilin for actin filament binding could potentially promote severing of cofilactin.

Competition with the large number of actin regulatory proteins could amplify cofilin-dependent filament severing *in vivo*, which may contribute to differences between cofilin severing *in vivo* and *in vitro* [36,37]. For example, Okada et al. reported that *Xenopus* Actin-interacting protein 1 (XAip1) enhances cofilin-mediated filament severing [38]. The authors attribute this behavior to inhibition of depolymerization and re-annealing of severed filaments resulting from filament end capping [38]. However, XAip1 also binds filament sides and could promote severing at high cofilin occupancies through competitive displacement of bound cofilin and/or changes in cofilin binding mode [39], provided the distinct binding modes [13] differentially affect filament mechanics and/or

structure. Similarly, cofilin severing of fascin bundled filaments [40] may be enhanced by competition and formation of boundaries.

We propose a "severodyne" framework (from "heterodyne" in radio signal processing, Fig. 4) as a general mechanism for regulation of cofilin activity through alteration of cofilin occupancy of actin filaments. Ligands (proteins and small molecules, e.g. [41–43]) or post-translational modifications [44,45] that modulate cofilin occupancy can either inhibit or promote severing, depending on the initial cofilin binding density. Ligands or modifications that dissociate cofilin from filaments through competition or changes in binding affinity will promote severing when initial cofilin occupancy is high($v_{cof} > 0.5$) and inhibit severing when cofilin occupancy is low ($v_{cof} < 0.5$). In contrast, ligands or modifications that recruit cofilin or increase binding affinity will enhance severing when initial cofilin occupancy is low ($v_{cof} < 0.5$) and inhibit severing when cofilin occupancy is high ($v_{cof} > 0.5$). When cofilin is half-saturating ($v_{cof} = 0.5$), ligands that dissociate or recruit cofilin will lower the cofilin boundary density and inhibit filament severing.

Acknowledgments

This work was supported by National Institutes of Health Grant RO1-GM097348 and American Heart Association Established Investigator Award 0655849T awarded to E.M.D.L.C. We thank Anthony Schram for preparation of actin, David D. Thomas (University of Minnesota) for the gift of the myosin S1, and Jiin-Yu Chen for comments on an earlier draft of the manuscript. The authors declare no conflicts of interest.

References

[1] T.D. Pollard, G.G. Borisy, Cellular motility driven by assembly and disassembly of actin filaments, Cell 112 (2003) 453–465.
[2] J. Fass, S. Gehler, P. Sarmiere, et al., Regulating filopodial dynamics through actin-depolymerizing factor/cofilin, Anat. Sci. Int. 79 (2004) 173–183.
[3] Q. Chen, T.D. Pollard, Actin filament severing by cofilin is more important for assembly than constriction of the cytokinetic contractile ring, J. Cell Biol. 195 (2011) 485–498.
[4] J.R. Bamburg, Proteins of the ADF/cofilin family: essential regulators of actin dynamics, Annu. Rev. Cell. Dev. Biol. 15 (1999) 185–230.
[5] E.M. De La Cruz, How cofilin severs an actin filament, Biophys. Rev. 1 (2009) 51–59.
[6] B.W. Bernstein, J.R. Bamburg, ADF/cofilin: a functional node in cell biology, Trends Cell Biol. 20 (2010) 187–195.
[7] W.A. Elam, H. Kang, E.M. De La Cruz, Biophysics of actin filament severing by cofilin, FEBS Lett. 587 (2013) 1215–1219.
[8] M. Hawkins, B. Pope, S.K. Maciver, et al., Human actin depolymerizing factor mediates a pH-sensitive destruction of actin filaments, Biochemistry 32 (1993) 9985–9993.
[9] A. McGough, B. Pope, A.G. Weeds, Cofilin changes the twist of F-actin: implications for actin filament dynamics and cellular function, J.Cell Biol. 138 (1997) 771–781.
[10] F. Ressad, D. Didry, G.X. Xia, et al., Kinetic analysis of the interaction of actin-depolymerizing factor (ADF)/cofilin with G- and F-actins: comparison of plant and human ADFs and effect of phosphorylation, J. Biol. Chem. 273 (1998) 20894–20902.
[11] E.M. De La Cruz, Cofilin binding to muscle and non-muscle actin filaments: isoform-dependent cooperative interactions, J. Mol. Biol. 346 (2005) 557–564.
[12] W. Cao, J.P. Goodarzi, E.M. De La Cruz, Energetics and kinetics of cooperative cofilin–actin filament interactions, J. Mol. Biol. 361 (2006) 257–267.
[13] E.M. De La Cruz, D. Sept, The kinetics of cooperative cofilin binding reveals two states of the cofilin–actin filament, Biophys. J. 98 (2010) 1893–1901.
[14] B.J. Pope, S.M. Gonsior, S. Yeoh, et al., Uncoupling actin filament fragmentation by cofilin from increased subunit turnover, J. Mol. Biol. 298 (2000) 649–661.
[15] S. Yeoh, B. Pope, H.G. Mannherz, et al., Determining the differences in actin binding by human ADF and cofilin, J. Mol. Biol. 315 (2002) 911–925.
[16] E. Andrianantoandro, T.D. Pollard, Mechanism of actin filament turnover by severing and nucleation at different concentrations of ADF/cofilin, Mol. Cell 24 (2006) 13–23.
[17] B.R. McCullough, L. Blanchion, J.L. Martiel, et al., Cofilin increases the bending flexibility of actin filaments: implications for severing and cell mechanics, J. Mol. Biol. 381 (2008) 550–558.
[18] B.R. McCullough, E.E. Grintsevich, C.K. Chen, et al., Cofilin-linked changes in actin filament flexibility promote severing, Biophys. J. 101 (2011) 151–159.
[19] D.A. Pavlov, A. Muhlrad, J. Cooper, et al., Actin filament severing by cofilin, J. Mol. Biol. 365 (2007) 1350–1358.
[20] C. Suarez, J. Roland, R. Boujemaa-Paterski, et al., Cofilin tunes the nucleotide state of actin filaments and severs at bare and decorated segment boundaries, Curr. Biol. 21 (2011) 862–868.
[21] H. Kang, M.J. Bradley, B.R. McCullough, et al., Identification of cation-binding sites on actin that drive polymerization and modulate bending stiffness, Proc. Natl. Acad. Sci. USA 109 (2012) 16923–16927.
[22] H. Edelhoch, Spectroscopic determination of tryptophan and tyrosine in proteins, Biochemistry 6 (1967) 1948–1954.
[23] D.M. Warshaw, J.M. Desrosiers, S.S. Work, et al., Smooth muscle myosin cross-bridge interaction modulate actin filament sliding velocity in vitro, J. Cell Biol. 111 (1990) 453–463.
[24] E. Nishida, K. Iida, N. Yonezawa, et al., Cofilin is a component of intracellular and cytoplasmic actin rods induced in cultured cells, Proc. Natl. Acad. Sci. USA 84 (1987) 5262–5266.
[25] N. Yonezawa, E. Nishida, S. Maekawa, et al., Studies on the interaction between actin and cofilin purified by a new method, Biochem. J. 251 (1988) 121–127.
[26] E.M. De La Cruz, T.D. Pollard, Transient kinetic analysis of rhodamine phalloidin binding to actin filaments, Biochemistry 33 (1994) 14387–14392.
[27] E. Prochniewicz, N. Janson, D.D. Thomas, et al., Cofilin increases the torsional flexibility and dynamics of actin filaments, J. Mol. Biol. 353 (2005) 990–1000.
[28] K. Holmes, I. Angert, F.J. Kull, et al., Electron cryo-microscopy shows how strong binding of myosin to actin releases nucleotide, Nature 425 (2003) 423–427.
[29] V.E. Galkin, A. Orlova, D.S. Kudryashov, et al., Remodeling of actin filaments by ADF/cofilin proteins, PNAS 108 (2011) 20568–20572.
[30] A. Muhlrad, I. Ringel, D. Pavlov, et al., Antagonistic effects of cofilin, beryllium fluoride complex, and phalloiding on subdomain 2 and nucleotide-binding cleft in F-actin, Biophys. J. 91 (2006) 4490–4499.
[31] D. Scoville, J.D. Stamm, C. Altenbach, et al., Effects of binding factors on structural elements in F-actin, Biochemistry 48 (2009) 370–378.
[32] H. Nagashima, S. Asakura, Studies on co-operative properties of tropomyosin–actin and tropomyosin–troponin–actin complexes by the use of N-ethylmaleimide-treated and untreated species of myosin subfragment 1, J. Mol. Biol. 155 (1982) 409–428.
[33] E.M. De La Cruz, T.D. Pollard, Kinetics and thermodynamics of phalloidin binding to actin filaments from three divergent species, Biochemistry 35 (1996) 14054–14061.
[34] D. Sept, J. Xu, J.A. McCammon, Annealing accounts for the length of actin filaments formed by spontaneous polymerization, Biophys. J. 77 (1999) 2911–2919.
[35] A.E. Carlsson, Stimulation of actin polymerization by filament severing, Biophys. J. 90 (2006) 413–422.
[36] S.H. Zigmond, Recent quantitative studies of actin filament turnover during cell locomotion, Cell Motil. Cytoskeleton 25 (1993) 309–316.
[37] H. Chen, B.W. Bernstein, J. Bamburg, Regulating actin-filament dynamics in vivo, Trends Biochem. Sci. 25 (2000) 19–23.
[38] K. Okada, L. Blanchoin, H. Abe, et al., *Xenopus* actin-interacting protein 1 (XAip1) enhances cofilin fragmentation of filaments by capping filament ends, J. Biol. Chem. 277 (2002) 43011–43016.
[39] M.G. Clark, J. Teply, B.K. Haarer, et al., A genetic dissection of Aip1p's interactions leads to a model for Aip1p-cofilin cooperative activities, Molecular Biology of the Cell 17 (2006) 1971–1984.
[40] D. Breitsprecher, S.A. Koestler, I. Chizhov, et al., Cofilin cooperates with fascin to disassemble filopodial actin filaments, J. Cell Sci. 124 (2011) 3305–3318.
[41] V.Y. Gorbatyuk, N.J. Nosworthy, S.A. Robson, et al., Mapping the phosphoinositide-binding site on chick cofilin explains how PIP2 regulates the cofilin–actin interaction, Mol. Cell 24 (2006) 511–522.
[42] N. Yonezawa, Y. Homma, I. Yahara, et al., A short sequence responsible for both phosphoinositide binding and actin binding activities of cofilin, J. Biol. Chem. 266 (1991) 17218–17221.
[43] H. Zhao, M. Hakala, P. Lappalainen, ADF/cofilin binds phosphoinositides in a multivalent manner to act as a PIP2-density sensor, Biophys. J. 98 (2010) 2327–2336.
[44] S. Arber, F.A. Barbayannis, H. Hanser, et al., Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase, Nature 393 (1998) 805–809.
[45] M. Sakuma, Y. Shirai, K. Yoshino, et al., Novel PKCa-mediated phosphorylation site(s) on cofilin and their potential role in terminating histamine release, Mol. Biol. Cell 23 (2012) 3707–3721.
[46] Z.X. Wang, An exact mathematical expression for describing competitive binding of two different ligands to a protein molecule, FEBS Lett. 360 (1995) 111–114.