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Modulation of Actin Mechanics by Caldesmon and Tropomyosin

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Abstract

The ability of cells to sense and respond to physiological forces relies on the actin cytoskeleton, a dynamic structure that can directly convert forces into biochemical signals. Because of the association of muscle actin-binding proteins (ABPs) may affect F-actin and hence cytoskeleton mechanics, we investigated the effects of several ABPs on the mechanical properties of the actin filaments. The structural interactions between ABPs and helical actin filaments can vary between interstrand interactions that bridge azimuthally adjacent actin monomers between filament strands (i.e. by molecular stapling as proposed for caldesmon) or, intrastrand interactions that reinforce axially adjacent actin monomers along strands (i.e. as in the interaction of tropomyosin with actin). Here, we analyzed thermally driven fluctuations in actin's shape to measure the flexural rigidity of actin filaments with different ABPs bound. We show that the binding of phalloidin increases the persistence length of actin by 1.9-fold. Similarly, the intrastrand reinforcement by smooth and skeletal muscle tropomyosins increases the persistence length 1.5- and 2- fold respectively. We also show that the interstrand crosslinking by the C-terminal actin-binding fragment of caldesmon, H32K, increases persistence length by 1.6-fold. While still remaining bound to actin, phosphorylation of H32K by ERK abolishes the molecular staple (Foster et al. 2004. J Biol Chem 279;53387–53394) and reduces filament rigidity to that of actin with no ABPs bound. Lastly, we show that the effect of binding both smooth muscle tropomyosin and H32K is not additive. The combination of structural and mechanical studies on ABP-actin interactions will help provide information about the biophysical mechanism of force transduction in cells.

Keywords

actin-binding proteins; persistence length; flexural rigidity; cytoskeleton; filament mechanics

INTRODUCTION

Cells are dynamic structures that are subjected to a variety of mechanical stimuli and necessarily must respond to both intra- and extracellular forces [Janmey, 1998]. For example, various mechanical stimuli play a role in normal physiological development in the heart [Kendrick-Jones et al., 1971], vasculature [Skalak and Price, 1996], and bone [Turner et al., 1995]. Furthermore, exposure to abnormal mechanical forces often results in pathologic conditions such as cardiac hypertrophy, carpal tunnel syndrome, atherosclerosis and vascular smooth muscle apoptosis [Goldschmidt et al., 2001; Katsumi et al., 2004].

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A characterization of the transmission of force from the external environment into the cell is crucial to our understanding the response of cells to forces. Specialized sensory cells, including skin mechanoreceptor cells and cochlear hair cells, display unique adaptations that sense their mechanical environment. Here, mechanical stimuli are directly coupled to ion channel opening and electrical signaling [Corey and Hudspeth, 1983]. In other instances, distension may decrease the lateral spacing between cells thereby increasing extracellular ligand concentrations and activating signaling pathways [Tschumperlin et al., 2002]. In some cases, a direct effect of stretch on signal transduction has been observed when the plasma membrane and its associated receptors have been removed [Sawada and Sheetz, 2002]. Thus, direct stretching of the cytoskeleton can trigger mechanotransduction.

The cytoskeleton is a dynamic structure comprised of many proteins including actin. Actin filaments can be linked together via actin-binding proteins (ABP) to form a mesh-like network, which acts as the cytoskeleton scaffolding. Integrins, which attach to the actin matrix via additional scaffolding proteins such as talin and vinculin, often serve as force transducers between intracellular actin filaments and the extracellular matrix [Wang et al. 1993]. External forces applied to the cell will deform the cytoskeleton by differing amounts depending on the viscous and elastic properties of the mesh and the intrinsic mechanical properties of the actin filaments and their associated ABPs.

ABPs can affect the mechanical properties of the cytoskeleton by promoting filament assembly and disassembly or by structural reinforcement of the filament network. For example, mechanical stress on human umbilical cells promotes the expression of the cytoskeletal proteins caldesmon, calponin, and tropomyosin [Cevallos et al., 2006], leading to filament elongation and stability. Similarly, repeated stress to smooth muscle tissue can cause dense body formation, expression of Erk1/2, and cytoskeletal remodeling [Kim and Hai, 2005]. Formin binding can also affect actin polymerization directly by inducing torsional strain [Shemesh and Kozlov, 2007; Shemesh et al., 2005]. All of these effects come from the dynamic response of the cytoskeleton to applied mechanical stress, which is modulated by ABPs.

The ability of the cytoskeleton to sense and respond to forces also depends on the mechanical properties of the actin filaments themselves. ABPs can buttress the structural organization of actin filaments by reinforcing the interstrand interactions between opposite strands of the actin helix, the intrastrand interactions between successive monomers along filaments, or both. Furthermore, ABP binding could affect actin-actin interactions via an allosteric mechanism. These varied interactions can affect the torsional, flexural, and tensile properties of an actin filament differently. As a first step toward understanding the effects of ABP binding, we have undertaken a study to correlate structural information about actin-binding protein interactions with the mechanical effects of ABP binding on flexural rigidity.

We measured the persistence length, a parameter that is directly related to flexural rigidity by a simple linear transform (See Eq. 3 in the “Materials and Methods” section), of actin-ABP complexes by observing actin filaments vibrating under thermal fluctuations using fluorescence microscopy. We found that phalloidin increases the persistence length of actin by 1.9-fold. Consistent with kinetic [Lehrer and Morris, 1984] and structural differences [Lehman et al., 2000], the intrastrand reinforcement of tropomyosin increases persistence length, with differences between smooth and skeletal muscle isoforms, being 1.5- and 2-fold respectively. We also show that the interstrand crosslink of the C-terminal actin-binding fragment of caldesmon, H32K, increases persistence length by 1.6-fold while, consistent with the structure of phosphorylated H32K-actin complexes [Foster et al., 2004], phosphorylation by ERK kinase reverses this effect. Lastly, we show that the effect of binding both smooth muscle tropomyosin and H32K is not additive.

MATERIALS AND METHODS

Actin

Unlabeled actin was prepared from chicken pectoralis muscle acetone powder using the method of Straub [1942] with the modification of Drabikowski and Gergely et al. [1964]. The actin was suspended in actin buffer (25 mM KCl, 1 mM EGTA, 10 mM DTT, 25 mM imidazole, 4 mM MgCl₂).

Rhodamine-labeled rabbit skeletal muscle actin was purchased from Cytoskeleton (Denver, CO). Twenty micrograms of lyophilized actin was prepared in 50 μ L of 0.2 mM CaCl₂, 0.2 mM ATP, 5 mM Tris-HCl, 10 mM DTT pH 8.0. Actin was polymerized by increasing KCl to 50 mM, MgCl₂ to 20 mM, ATP to 10 mM, and DTT to 10 mM followed by incubation at room temperature for 1 h. To increase filament length polymerized actin was diluted 500-fold in the presence of a 10-fold molar excess of unpolymerized actin, vortexed, and incubated at 4°C overnight.

Actin-Binding Proteins

Smooth muscle tropomyosin was purified by the method of Cohen and Cohen [1972] with the modifications of Lehman et al. [1989]. Skeletal muscle tropomyosin was a gift from L.S. Tobacman. Chicken gizzard “H32K” representing caldesmon residues Met⁵⁶³–Pro⁷⁷¹ was prepared as described by Huang et al. [2003] in *E. coli* BL21-DE3 cells and purified with a DE52 and CaM sepharose columns.

Sample Preparation

Slides and coverslips were pretreated twice with 1 mM BSA for 1 min to prevent nonspecific binding of actin and ABPs. Rhodamine-labeled actin, diluted to 10 nM, was mixed with 2 μ M unlabeled actin in degassed actin buffer containing oxygen scavengers (2 mM dextrose, 160 U glucose oxidase, 2 μ M catalase; Sigma, St. Louis, MO). In experiments with caldesmon, 10 μ M H32K was incubated overnight in 500 mM DTT to prevent aggregation [Haeblerle et al., 1992; Huang et al., 2003]. Samples of phosphorylated H32K were prepared by incubating H32K with Erk2 (New England Biolabs, Ipswich, MA) according to Foster et al. [2004]. For all experiments with H32K, a final concentration of 5 μ M was used while for all experiments using tropomyosin, a final concentration of 2 μ M was used. Then, a final mixture of 3 nM labeled actin, 600 nM unlabeled actin, 15 mM BSA, and appropriate ABPs was prepared, which resulted in an ~8 and ~23-fold excess of ABPs to actin target sites for H32K and tropomyosin, respectively. Three microliters of this sample was gently compressed (to avoid shear) between the coverslip and the slide to give a narrow flow cell (~1- to 1.5- μ m thick), which was held together with nail polish. Filament lengths ranged from 4 to 25 μ m.

Microscopy

Rhodamine-labeled actin filaments were observed on a Nikon Eclipse TE2000-U microscope (Nikon, Melville, NY) with standard epifluorescence illumination. The images were recorded using video microscopy and captured to a Scion frame grabber (Model AG-5). Using Scion Image (Scion, Frederick, MD), images were captured at 1 second intervals to ensure that filament shapes were uncorrelated [Gittes et al., 1993].

Image Processing and Analysis

The flexural rigidity of actin filaments was measured using the method of Gittes et al. [1993], however, a minor modification was required to correct for filament motion constrained to two dimensions. Modal analysis is a useful method for measuring the flexural rigidity because every mode analyzed will give an independent measurement of the flexural rigidity

of actin, providing an internal validation of consistency. Filament images were processed with a 9 pixel Gaussian filter, thresholded and then skeletonized using ImageJ. Rarely, ImageJ would export the point coordinates out of sequence, giving filament lengths much longer than the true filament length and these points were dropped. Also, if the filament drifted out of the plane of focus, a much shorter filament length would be measured and thus these points were dropped. The (x, y) coordinates of the skeletonized image were recorded and converted to coordinates (s, θ) where s is the length along the filament and θ is the tangent angle of the skeletonized point with respect to the x -axis. This data was then converted to a Fourier series:

$$\theta(s) = \sqrt{\frac{2}{L}} \sum_{n=0}^{\infty} a_n \cos\left(\frac{n\pi s}{L}\right) \quad (1)$$

where as in Gittes et al. [1993], the variance in the modes is given by:

$$\text{var}(a_n) = \frac{kT}{EI} \left(\frac{L}{n\pi}\right)^2 \quad (2)$$

where a_n is the mode amplitude, n is the mode number, kT is the thermal energy, and EI is the flexural rigidity. For N -dimensional space, the persistence length, L_p , is defined as [Wiggins et al., 1998]:

$$L_p = \frac{2}{(N-1)} \frac{EI}{kT} \quad (3)$$

Since actin is a semiflexible polymer and the thickness of the flow cell is less than the length of the filament, the filament could be considered to be oscillating in two dimensions [Hendricks et al., 1995]. Then substituting Eq. 3 into Eq. 2, we find:

$$L_p = \frac{L^2}{2n^2\pi^2 \text{var}(a_n)} \quad (4)$$

Although absent in Gittes et al., [1993] the factor of 2 correction in the denominator of Eq. 4 is necessary to get the true value of the persistence length for the two-dimensional motion observed in this type of experiment. The Fourier decomposition was performed with Mathematica (Wolfram, Champaign, IL) and the mode amplitudes and persistence lengths were extracted for each frame of the 60 frame movie. Sixty frames appeared sufficient for mode convergence to a stable value (see results, Fig. 1a).

Statistics

A Welch's t -test was used to examine the significance of the addition of ABPs with respect to undecorated rhodamine-labeled actin and to test the differences between tropomyosin isoforms. The P value was calculated from the Student's t -test distribution. The t -tests were corrected for multiple comparisons against a single control using the Bonferroni t -test.

RESULTS

Actin-ABP complexes were introduced into a narrow flow chamber that restricted motion to two dimensions (see "Materials and Methods" section) and epifluorescence microscopy was used to observe rhodamine-labeled actin filaments fluctuating under Brownian motion. Under

these conditions, the degree of thermally driven curvature will depend on the intrinsic material properties of the polymer, temperature, and viscosity of the medium and thus can be used to determine actin persistence length. The image of the filament was recorded by video microscopy and its shape was determined via digitization and a skeletonization algorithm. The normal modes were extracted from the filament coordinates and, as the shape fluctuated over time due to thermal fluctuations, the amplitude of each of the modes also fluctuated. The variance in the amplitude of the modes in turn provided a measure of persistence length (Eq. 4).

The measured variance of the modes contains components due to Brownian motion and potentially, experimental error. Previous measurements using this method did not investigate the convergence of the modes as a function of data processed [Gittes et al., 1993]. To ensure that experimental error was negligible and that the measured variance provided a true measure of the Brownian motion induced variance rather than the variance due to experimental error, the persistence length as a function of the number of frames processed was calculated (Fig. 1a). Here, we show experimentally that since each mode gives an independent measurement of the persistence length, as more frames were processed, the value for the persistence length for each mode converged to a stable value that was consistent across the modes by 60 frames (Fig. 1a). The only exception was mode 1, which did not converge within 60 frames presumably because it is dominated by convective currents [Gittes et al., 1993]. In all of the data, modes 3–4 were analyzed for 60 frames to ensure that the convergence had occurred.

Persistence length is proportional to the length of the filament squared (Eq. 4); thus care must be taken to ensure the proper definition of filament shape. Under-sampling the data will cause measurement of a shorter persistence length with a larger standard deviation in the value measured across the modes. Although not observed in our experiments, it is expected that over sampling the data would result in an artificially longer persistence length and a larger standard deviation [Isambert et al., 1995; Ott et al., 1993]. Previous application of the Fourier decomposition method relied on defining filament shape by hand with a limited number of data points and therefore could result in an incorrect determination of the persistence length. The skeletonization method used here defines filament shape based on the filament intensity and the maximum pixel density of the imaging system. To optimize the number of data points needed to clearly define the filament shape, points were systematically removed from the full data set obtained from the skeletonization procedure. The average and standard deviation in the persistence length of actin are plotted as a function of percent data included (Fig. 1b). When 45–80% of the data is included, a plateau region is observed where the standard deviation across the modes is minimized and the average persistence length measured is stable. Thus, all experiments were performed in the plateau region to avoid sampling artifacts.

Using modal analysis, the persistence length of actin was measured by itself and in the presence of several ligands (Fig. 2). The persistence length measured for rhodamine-labeled actin is $9.1 \pm 0.5 \mu\text{m}$. The addition of the phalloidin increased persistence length 1.9-fold ($P < 0.0001$ compared to undecorated rhodamine-labeled actin) to $17.7 \pm 1.5 \mu\text{m}$, agreeing well with previously determined values for phalloidin decorated actin [Yanagida et al. 1984; Gittes et al., 1993; Ott et al., 1993; Isambert et al., 1995]. Similarly intrastrand reinforcing ABPs, smooth and skeletal muscle tropomyosin, increase persistence length 1.5 ($P < 0.0001$ compared to undecorated rhodamine-labeled actin) and 2-fold ($P < 0.0001$ compared to undecorated rhodamine-labeled actin), respectively.

In the case of the H32K fragment of caldesmon that forms a “molecular staple” ligating the long-pitch helical strands of actin [Foster et al., 2004], we expected that H32K would also stabilize F-actin and rigidify filaments. Consistent with this idea, Fig. 2 shows that H32K binding to actin also causes an increase in the persistence length of actin by 1.6-fold ($P < 0.0001$

compared to undecorated rhodamine-labeled actin) (Fig. 2). Structural [Foster et al., 2004] and fluorescence [Huang et al., 2003] studies of actin-phosphorylated H32K complexes show that part of the H32K bridge between adjacent long-pitch helical strands of actin breaks after phosphorylation. Therefore, to ascertain the specific effects of this bridge, we also tested the effects of Erk2 phosphorylated H32K on filament mechanical properties. Indeed, phosphorylated H32K, while remaining bound to actin, relieves the mechanical effects imposed by interstrand reinforcement of F-actin by returning persistence length to a similar value as actin lacking any bound ABPs ($P = 0.15$), thus showing that ABP binding does not a priori increase filament rigidity. We also found that the mechanical consequences of binding both tropomyosin and H32K are not additive, increasing the persistence length to $16 \pm 1 \mu\text{m}$ ($P < 0.0001$ compared to undecorated rhodamine-labeled actin) (Fig. 2).

DISCUSSION

Mechanotransduction, namely the ability of cells to respond biochemically to their mechanical environment, is poorly understood. While many cells are sensitive to mechanical stimulation, the molecular basis for the force dependent cellular responses is unclear. The cytoskeleton, a complex network of protein filaments, plays a key role in the force response of eukaryotic cells. An understanding of the generation and transmission of forces in cells relies on a basic understanding of the mechanical properties of the cytoskeletal filament complexes and the effects of associated binding proteins. Here we have modified and extended the Fourier modal analysis of Gittes et al. [1993], to study the rigidifying effect of tropomyosin, phalloidin, and caldesmon binding to actin. Furthermore, we show that physiologically relevant phosphorylation of the C-terminal fragment of caldesmon can reverse the filament stiffening thus providing an example whereby cytoskeletal properties could be altered rapidly in vivo.

Phalloidin

When actin is decorated with phalloidin, a bicyclic peptide that stabilizes filamentous actin, the persistence length is measured to be $\sim 17 \mu\text{m}$. Several groups that have also reported a similar persistence length for actin using fluorescently labeled phalloidin derivatives to visualize the filament [Yanagida et al., 1984; Gittes et al., 1993; Ott et al., 1993; Isambert et al., 1995]. However, here we show, consistent with Isambert et al. [1995], that actin labeled directly, without the use of phalloidin, has a much shorter persistence length of $\sim 8 \mu\text{m}$ thus showing that the phalloidin itself stabilizes actin structure and increases filament rigidity.

Compared to native actin filaments, phalloidin stabilized actin filaments have been shown to exhibit an extra mass between the two long-pitch helical strands [Steinmetz et al., 1997]. This extra mass reinforces the intersubunit contacts both along and between the long-pitch helix thus suggesting a mechanism for the increase in filament rigidity. Phalloidin based increases in persistence length are consistent with phalloidin's role as a toxin that prevents depolymerization of actin. It is possible that structural stabilization makes actin less susceptible to depolymerization and therefore less easily broken by smaller forces such as Brownian motion [Dancker et al., 1975].

Tropomyosin

Tropomyosin molecules bind in an end-to-end fashion to form a cable that lies in parallel with the actin strands, reinforcing the monomer-monomer intrastrand interactions within an actin filament (Fig. 3). These strands are known to undergo regulatory movement laterally over the surface of the actin filament. In the so-called "blocked" position, tropomyosin lies over successive actin subunits on their outer domains while in the "closed" position, tropomyosin lies on the actin inner domain near the junction of the inner and outer domains [Lehman et al., 2000]. When smooth muscle tropomyosin is bound to actin, it increases the persistence length

by a factor of 1.5 while skeletal muscle tropomyosin increases the persistence length by a factor of 2. The differences between the flexural rigidities of the two isoforms is statistically significant ($P < 0.0001$). EM reconstructions [Lehman et al., 2000] show that on average smooth muscle tropomyosin associates in the blocked position whereas skeletal muscle tropomyosin lies in the closed state (Fig. 3). Also, biochemical studies indicate that the energy barrier between blocked and closed states is lower for smooth muscle tropomyosin than for skeletal muscle tropomyosin [Lehrer and Morris, 1984], suggesting that the interactions between actin and tropomyosin depend on tropomyosin isoform. Therefore, the observed differences in actin filament mechanics between smooth muscle and skeletal muscle tropomyosins could result from differences in tropomyosin position or actin-tropomyosin interactions.

Caldesmon C-Terminal Fragment (H32K)

Caldesmon consists of three functional domains: (1) an N-terminal myosin binding domain, (2) a central α -helical spacer, absent in the nonmuscle isoform, and (3) a C-terminal domain that binds to actin and may inhibit myosin ATPase. There are two primary sites of interaction between the C-terminal fragment (H32K) and actin. One site connects subdomains 1 and 3 of sequential actin monomers along the long pitch helix of F-actin. The second actin-binding site acts as an intrastrand “molecular staple” that bridges the gap between neighboring actin monomers on separate strands of the F-actin long-pitch helix [Foster et al., 2004] (Fig. 3). Interstrand crosslinking is not unique to H32K, but may also be induced by the troponin I [Pirani et al., 2006] and the acrosomal protein scruin [Owen and DeRosier, 1993]. Consistent with the fortification of F-actin structure by the H32K fragment, our results show that H32K increases persistence length of actin 1.6-fold.

Although H32K binds obliquely along the actin filament axis there is little overlap between the positions of H32K and tropomyosin [Lehman et al. 2000; Foster et al., 2004] and thus the binding of one would not be expected to influence the other. However, contrary to this expectation, the effect of H32K and smooth muscle tropomyosin do not appear additive because the persistence length was only increased by 1.8-fold when both are bound (Fig. 2). This unexpected result could be due to several different, nonexclusive factors. One possibility is that the nonadditivity stems from caldesmon-induced repositioning of tropomyosin [Hodgkinson et al., 1997]. It is also possible that the mechanical stiffening of the actin occurs as a result of ABP induced allosteric changes in the structure of the actin filament [Prochniewicz et al., 1996] where both H32K and smooth muscle tropomyosin induce similar conformational changes in actin. Observing the effects of subsaturating amounts of ABPs on flexural rigidity may shed light on this possibility. Lastly, it is possible that the effects of inter- and intra-strand binding proteins are interdependent. To probe further the relationship between the interstrand and intrastrand interactions requires information about the torsional rigidity of the filament and the degree of anisotropy within the actin rod. It is interesting to note that although the flexural rigidity of actin is increased in the presence of phalloidin, Yoshimura et al. [1984] reported that phalloidin does not affect the torsional rigidity. This result would be consistent with different modes of actin binding affecting the mechanical properties of actin differently.

Phosphorylation has been proposed to regulate caldesmon binding to actin. Phosphorylation *in vitro* can be achieved via PKC, CamKII, PKA, casein kinase, Erk2, p34^{cdc2}, and Pak, although *in vivo* evidence supports a role for the proline-directed MAP kinases, such as cdc2 kinase and Erk2 [Yamashiro et al., 1991; Adam et al., 1992; Childs and Mak, 1993; D’Angelo et al., 1999; Hedges et al., 2000]. Structural [Foster et al., 2004] and spectroscopic [Huang et al., 2003] data suggest that phosphorylation of H32K causes one side of the “molecular staple” to detach from the actin while the other side remains bound (Fig. 3). Our results show that phosphorylation of H32K by Erk kinase reverses the rigidifying effect of H32K binding and

reduces persistence length to a value comparable to that of bare F-actin. Since the molecular staple that reinforces intrastrand connections between helical strands of actin is disrupted by phosphorylation [Foster et al., 2004] the rigidifying effects of H32K can be attributed to this specific interaction and not simply the effect of H32K binding.

The mechanical reinforcement of actin by caldesmon binding has several potential physiological consequences. Caldesmon has been linked to cytoskeletal remodeling dependent processes such as exocytosis [Burgoyne et al., 1986], receptor capping [Mizushima et al., 1987; Walker et al., 1989], and oncogenic transformation of stress fibers (42). Similarly, phosphorylation of caldesmon has been shown to precede cell remodeling associated with cytokinesis presumably by promoting the recruitment of actin from stress fibers to the contractile ring at the cleavage furrow [Hosoya et al., 1993]. Similar to phalloidin [Dancker et al., 1975], caldesmon stabilizes thin filaments to prevent severing and depolymerization [Ishikawa et al., 1989a,b; Kordowska et al., 2006]. A phosphorylation induced decrease in the interaction of caldesmon with actin filaments could expose actin filaments to fragmentation, thus facilitating the severing process, which would precede cytoskeletal remodeling [Yamashiro et al., 1991; Pawlak and Helfman, 2001; Cuomo et al., 2005].

Integrin clustering activates the MAPK pathways when cells are subjected to force. Erk2 dependent caldesmon phosphorylation would relieve the mechanical reinforcement of caldesmon thus destabilizing the cytoskeleton and promoting cytoskeletal reorganization. It is plausible that partial dissociation of H32K upon phosphorylation might be kinetically advantageous, whereby phosphorylation by Erk can quickly exert its regulatory effects without the expense of caldesmon detachment and rebinding. This dynamic process of phosphorylation in response to an external force allows for rapid cytoskeletal reorganization.

Extension to Other Actin-Binding Proteins

Here, we showed the effects of several ABPs on actin's mechanical properties. One might have thought that binding any protein to actin would increase the flexural rigidity however, phosphorylated H32K, while still reinforcing the long-pitch strand of actin, has the same persistence length as undecorated actin, thus indicating that the mechanical effects depend on the specific ABP. Although all of the binding proteins shown here either increase or have no effect on flexural rigidity, it was shown that formin binding to actin decreases the flexural rigidity of actin [Bugyi et al., 2006]. Similarly, it has been shown using time-resolved phosphorescence anisotropy that cofilin [Prochniewicz et al., 2005] and gelsolin [Prochniewicz et al., 1996] increase the torsional flexibility of actin, whereas myosin decreases the torsional flexibility [Prochniewicz and Thomas, 1997] and phalloidin has no effect at all [Yoshimura et al., 1984]. Furthermore, EM data shows that cortactin binding to actin causes an increase in the variance in the angle of twist of actin [Pant et al., 2006] and thus an increase in the torsional flexibility [Yoshimura et al., 1984]. Thus it appears that different ABPs can affect the torsional and flexural properties of actin differently. These differences could stem from alternative modes of ABP binding to actin, ABP induced structural changes in the actin filament, or the mechanical properties of the ABPs themselves.

CONCLUSIONS

In summary, we have shown that several ABPs decrease the flexural rigidity of actin filaments, while the degree of rigidification was shown to depend on ABP isoform and post-translational modifications. Understanding the regulation of ABP binding will help to provide an understanding of how cells respond to mechanical force and how alterations in actin mechanics affect cytoskeletal reorganization. The cytoskeleton acts as a molecular force transducer, and the manner of transduction can be changed by alterations in the mechanical properties of the cytoskeleton. For example, Janmey [1998] suggested that stiffening the cytoskeleton allows

more efficient transmission of force over a larger distance. Modulation of the stiffness by ABPs will affect the way that the cytoskeleton is able to transduce and respond to force. In addition, changes in the stiffness may permit more rapid actin filament assembly and disassembly, allowing for dynamic modulation of cytoskeletal mechanics. The use of ABPs to modulate cell stiffness rather than using a cellular strategy to thicken actin bundles may be energetically favorable for rapid responses to applied forces.

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Fig. 1.

(a) The value of the persistence length was calculated as a running average based on the number of frames processed. Frames were taken 1 second apart. Convergence of the modes is observed at around 60 frames. Mode 1 is not plotted because it is subject to more noise due to convective currents. (b) The persistence length of actin was calculated based on the skeletonization procedure described in the “Materials and Methods” section. To find the optimal number of points to sample and avoid artifacts due to over or under sampling, data was systematically removed and the persistence length of the filament was calculated as a function of data points processed. The average and standard deviation of the value given for the persistence length

was calculated by examining several modes for convergence. Dropping 20 % of the data appeared sufficient to negate any sampling artifacts.

Fig. 2.

Measured persistence length of actin with different ABPs. Shown is the mean $\pm$ the standard deviation with significance $P < 0.0001$ (compared to rhodamine-labeled actin) indicated by an asterisk. The number of filaments analyzed for each ABP is shown inside the bar however, multiple modes for each filament contributed to the average and standard deviation. Shown are rhodamine-labeled actin (actin), phalloidin decorated rhodamine-labeled actin (phalloidin), H32K decorated rhodamine-labeled actin (H32K), rhodamine-labeled actin decorated with phosphorylated H32K (H32K Phos), rhodamine-labeled actin decorated with skeletal muscle tropomyosin (Sk Tm), rhodamine-labeled actin decorated with smooth muscle tropomyosin (Sm Tm), and rhodamine-labeled actin decorated with both H32K and smooth muscle tropomyosin (Sm Tm + H32K).

Fig. 3. Graphical depiction of actin complexed with ABPs. **(a)** Actin filaments can be reinforced mechanically by interstrand (blue) or intrastrand (red) binding proteins. **(b)** Tropomyosin, an intrastrand binding protein, binds along the actin filament in the blocked (red) and open (orange) positions. **(c)** Caldesmon C-terminal fragment H32K is an interstrand binding protein that acts as a molecular staple between actin strands. Under the conditions employed here H32K binding will saturate actin filament binding sites (not shown for image clarity). Phosphorylation (P) of H32K causes one side of the staple to release from the actin, while still remaining bound to actin via the other side of the staple [Foster et al., 2004].