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Mechanotransduction by the Actin Cytoskeleton: Converting Mechanical Stimuli into Biochemical Signals

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Abstract

Force transmission through the actin cytoskeleton plays a central role in cell movements, shape change, and internal organization. Dynamic reorganization of actin filaments by an array of specialized binding proteins creates biochemically and architecturally distinct structures, many of which are finely tuned to exert or resist mechanical loads. The molecular complexity of the actin cytoskeleton continues to be revealed by detailed biochemical assays, and the architectural diversity and dynamics of actin structures are being uncovered by advances in super-resolution fluorescence microscopy and electron microscopy. However, our understanding of how mechanical forces feed back on cytoskeletal architecture and actin-binding protein organization is comparatively limited. In this review, we discuss recent work investigating how mechanical forces applied to cytoskeletal proteins are transduced into biochemical signals. We explore multiple mechanisms for mechanical signal transduction, including the mechanosensitive behavior of actin-binding proteins, the effect of mechanical force on actin filament dynamics, and the influence of mechanical forces on the structure of single actin filaments. The emerging picture is one in which the actin cytoskeleton is defined not only by the set of proteins that constitute a network but also by the constant interplay of mechanical forces and biochemistry.

Contents

ORGANIZING THE ACTIN CYTOSKELETON

Perhaps the most commonly discussed protein in descriptions of cell shape change, movement, and force generation is actin. For decades, researchers have imaged the actin cytoskeleton using fluorescent labeling, typically fluorescently labelled phalloidin (66) or fluorescent fusions to actin-binding proteins (11, 79), to observe and classify a range of subcellular architectures. With the advent of super-resolution optical microscopy and improvements in electron microscopy techniques, the field is beginning to uncover just how broad a range of beautifully complex and distinct actin structures exist (3, 12, 29, 96). So striking are the architectural differences between parts of the actin cytoskeleton that architecture has become dogma for descriptions of the actin cytoskeleton and what it does in cells—stress fibers that contract, filopodial bundles that poke, and branched (or dendritic) networks that push. Adding to the underlying complexity belied by these simple descriptions are their inherent dynamics and ability to rapidly reorganize in response to external stimuli (69).

A perplexing and persistent question is why actin structures that all share the same common cytoplasm and pool of subunits are so distinct. One answer is that actin filaments interact with different sets of ancillary nucleation-promoting and actin-binding proteins that endow the structures with their unique architectural plasticity. In this framework, it becomes easy to view actin as a passive structural element interacting with different crosslinking, bundling, and branching proteins to assemble different structures in an almost Lego brick–type manner. This, of course, is a dramatic oversimplification given the role of actin networks in cell shape change and migration, where they must sense, transduce, and generate mechanical forces.

One intriguing possibility is that actin filaments are active sensors of mechanical tension rather than passive building blocks. In 2011, Hayakawa et al. (39) used optical tweezers to apply mechanical tension across single actin filaments and reported that the actin-severing protein cofilin binds to and severs actin filaments in a tension-dependent manner. In 2012, a review by Galkin et al. (32) elegantly discussed the concept of actin filaments as tension sensors and speculated on possible mechanisms for this process. Since this work, however, there has been relatively little further direct evidence of actin filaments sensing mechanical tension and feeding back on protein binding, beyond a few examples, including the actin-binding protein cofilin (38, 39). In addition, it has been difficult to connect the existence of polymorphic structural states of F-actin with variations in mechanical load, primarily because of the challenge of combining high-resolution electron microscopy imaging with precise control of the mechanical load on an actin filament (30, 32, 33). Several studies have indicated that the conformational state of actin is polymorphic and

Table 1 Estimation of filament-level forces in different actin structures

	Actin structure			
	Lamellipodium	**Stress fibers**	**Cortex**	**Filopodia**
Characteristic actin-binding proteins	Arp2/3, capping protein, cofilin	Myosin, α-actinin	Myosin, Arp2/3, formins, α-actinin, filamin	Formins, fascin
Force action on actin	Compression	Tension	Tension	Compression
Force magnitude	5 nN (77)	1–10 nN (57)	400 pN (89)	10 pN (53)
Filament density estimate	50–250 per micron	30 per fiber	100-nm mesh size	10–30 per filopodia
Estimated filament level load (pN)	0.2–10	~10–100	20–40	0.3–5

feeds back on binding protein affinity, but the role of mechanical forces on actin in this context remains elusive (45, 49, 50, 80, 82).

In this review, we discuss the broader concept of mechanical regulation of the actin cytoskeleton and the multiple modes of action of this process. To elucidate whether forces at the level of a single actin filament could be relevant for larger-scale cytoskeletal regulation, we first collect estimates of the mechanical load on actin filaments in different cytoskeletal structures from recent literature.

MECHANICAL LOADING OF THE ACTIN CYTOSKELETON

Different actin structures appear to be used for defined mechanical tasks, such as the generation of protrusion forces that push against obstacles or contractile forces that pull on a substrate. To explore the potential role of mechanical forces as regulators of protein organization, we first consider the actin filament organization and mechanical loads felt by individual filaments within these structures in this section.

Most of the insights that we have into the structure of polymerized actin filaments comes from electron microscopy image data (30, 33), which show that filaments are double-stranded, right-handed helices with an average pitch of 36 nm (30). In a cellular context, filaments assemble into higher-order structures, including dendritic networks, branched networks, bundles, and meshes through their interaction with a vast set of actin-binding proteins (6). Because different actin structures all share a common pool of biochemical constituents, competition among actin structures balances and regulates the assembly of different structures (2, 15, 34, 59, 69). Several recent reviews have discussed actin biochemistry, its filament-forming kinetics, and the biochemical composition of different actin networks (6, 76), and we summarize some of the key molecular and architectural components of the cytoskeleton in **Figure 1** and estimate single-filament forces in these structures in **Table 1**.

Single-Filament Forces in Compressed Actin Structures

At the leading edge of migrating cells, the lamellipodium is a thin (50–150 nm), protrusive actin structure (98) (**Figure 1**). Actin filaments within the lamellipodium are nucleated in close proximity to the plasma membrane by the Arp2/3 complex and its associated activating factors (62). When imaged using electron microscopy, lamellipodial actin networks appear as interweaving dense filament networks pressed with filament barbed ends at steep angles to the membrane plane. Since the plasma membrane itself is under tension (22), addition of actin monomers to filament ends in contact with the membrane exerts a force that displaces the membrane and compresses the network (75). Several studies have aimed to quantify the capability of branched actin networks to push against an external barrier and drive the cell forward in cell culture (72, 77) and in reconstituted systems (5, 62a, 74a). Considering a membrane with a line tension of ~100 pN/μm (71), a filament

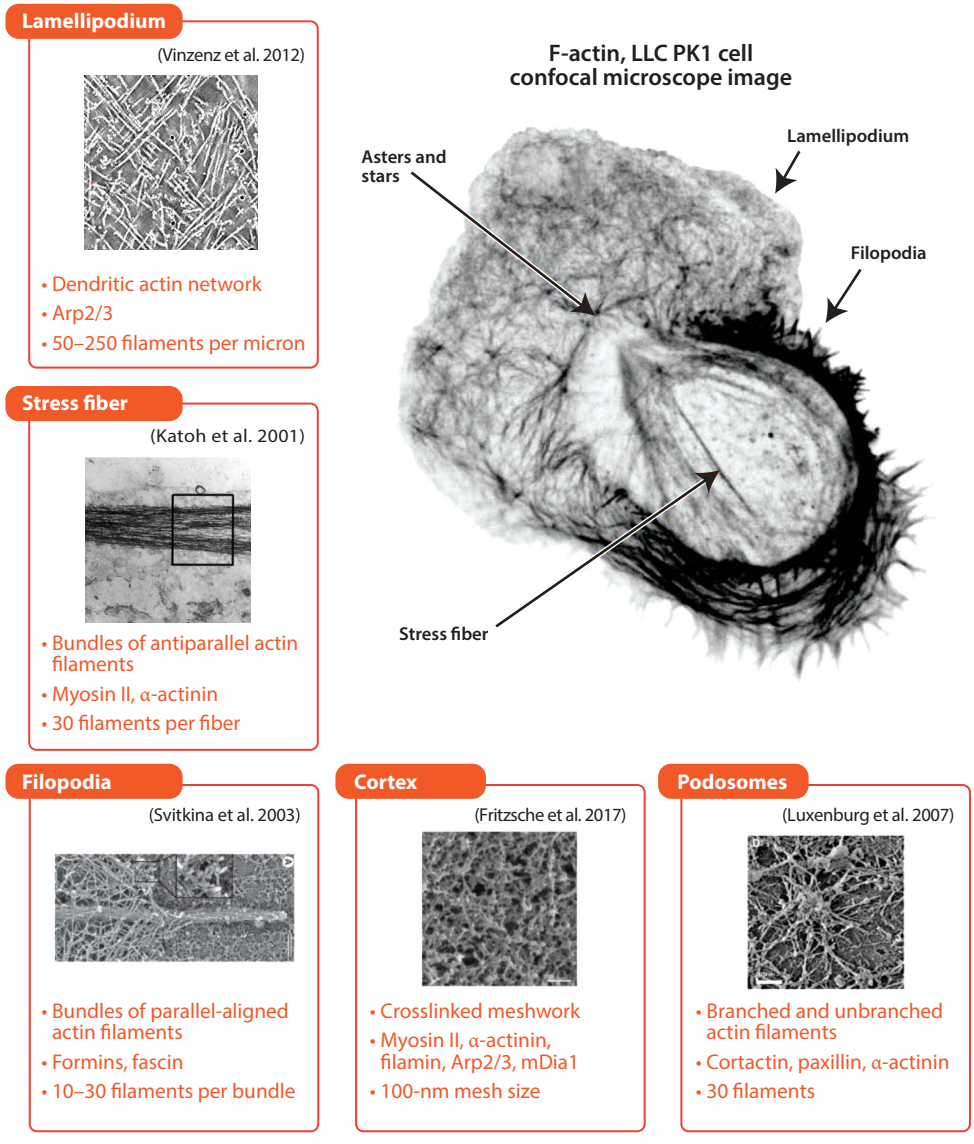

Figure 1

The actin cytoskeleton defined by architecture. The actin cytoskeleton consists of several architecturally distinct regions that can be observed with optical microscopy (*right*) and at the ultrastructural level with electron microscopy (*boxes*). Different actin architectures can be observed within a common cytoplasm and have distinct biochemical compositions. We have highlighted a few examples of proteins contained within these different structures and the density and organization of actin filaments within them.

network density of a hundred filaments per micrometer (considering the lamellipodium to be a largely two-dimensional structure) (35), and pure orthogonal compression, we estimate the average compressive force per filament to be on the order of 1 pN within this structure.

In some cell types, the lamellipodium is interceded by periodic bundles of actin filaments between the dendritic regions that form into filopodial protrusions (87) (**Figure 1**). Filopodia

contain aligned actin filaments that are nucleated by various formins, including Daam1 (47), and crosslinked by the actin-binding protein fascin (1). Filopodia are highly dynamic structures and have been reported to produce both protrusive and contractile forces (7). Forces on filopodia have been measured using optical traps and found to be on the order of 1–10 pN (53). For a filament density of 10–30 filaments per bundle, the compressive force on an actin filament is on the order of ~0.5 pN. While the average protrusive forces generated by growing actin filaments in the lamellipodium and filopodia are relatively small, larger forces have been reported for actin protrusions such as podosomes. Recently, Labernadie et al. (56) used a combination of cells grown on a thin, deformable Formvar sheet and atomic-force microscopy to measure the protrusive forces generated by podosomes. The authors measured the indentations generated by podosomes in the Formvar sheet that had a calibrated stiffness, and calculated the average force generated by a podosome to be 94 nN. This is significantly larger than the protrusive forces reported for lamellipodia and filopodia, perhaps reflecting the complex architecture of actin filaments (8, 61) and composition of this structure (**Figure 1**).

Single-Filament Forces in Tensed Actin Structures

Stress fibers are perhaps the clearest example of actin structures under tension. Directed motion of the myosin motor heads within these aligned bundles of actin generates a tensile load within the fiber (2, 44, 51, 52). The tensile force in stress fibers is clearly apparent upon laser dissection, where large scale recoiling of the fiber can be observed (51, 55). Since a single myosin exerts roughly 3–4 pN of tensile force (27), we expect the tensile force applied to a stress fiber bundle to be in the range of 100 pN to 1 nN, depending on the number of myosin motors acting on the fiber and the fiber width. Consistent with this estimate, the native tension in contractile stress fibers of cells plated on raised micropatterns was measured to be several nanonewtons (57). Considering a filament density of roughly 30 filaments per fiber, the force per filament is on the order of ~10–100 pN.

Most of the actin structures that have been discussed thus far are prominent in either highly migratory or strongly adherent cells. Cortical actin networks are present in most cell types and play a particularly important role during cell division. Rounded mitotic cells have a thin layer of short crosslinked actin filaments just beneath the plasma membrane (83). This shell of actin is approximately 200 nm to 1 μm thick, with a mesh size between filaments on the order of 100 nm and a rich biochemical composition of crosslinkers, motor proteins, and actin nucleators (9, 17, 70, 83) (**Figure 1**). The actin cortex has a central role in generating the contractile forces that are required to separate two daughter cells during cytokinesis. Here, activation of the small G-protein Rho activates myosin contractility to generate tensile forces at the cleavage furrow (86, 95). Actin crosslinking proteins couple the forces generated by myosin motors on individual filaments into network-level deformations. Thus the connectivity between filaments and maintenance of cortical architecture plays an important role in the generation of cortical tension (16, 17, 83, 89). Values for tensile load in the cortex are on the order of several hundred piconewtons per micron (89). An estimate of the number of filaments on this length scale can be made from the mesh size in the cortex, yielding a filament-level tension in the tens of piconewtons range.

Toward Direct Measurements of Single-Filament Forces

The estimates of mechanical loading of actin structures presented here is confounded by the assumptions of equal load sharing by actin filaments and homogeneous architecture. The measurement of forces on the cytoskeleton with atomic-force microscopy (16, 77), micropipette

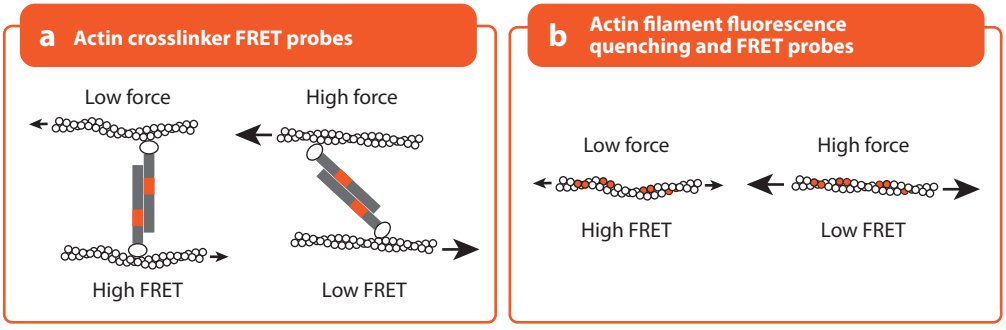

Figure 2

Methods to measure intracellular forces based on optical microscopy. Few methods exist to measure intracellular cytoskeletal forces. Because of the small length scale needed to probe forces on the cytoskeleton, a few optical approaches have been developed. Most of these are based on Förster resonance energy transfer (FRET) pairs (*a*) inserted into actin crosslinking proteins or (*b*) attached to actin monomers within filaments. Tension within the cytoskeleton results in changes in distance and orientation between FRET pairs or quenched dyes, causing changes to fluorescent output that can be measured directly.

aspiration (16, 84), and micropillars (88) provides valuable information on the bulk forces on each of these structures but averages out the finer details of forces on filaments within that structure. In fact, there are presently no direct measurements of forces on individual actin filaments within cells at the microstructural level. This is primarily due to the lack of appropriate experimental measurement techniques.

Interestingly, super-resolution microscopy has been used to reveal a rich landscape of actin microstructures (29, 96). Actin asters are structures found in the cell cortex but are not necessary for maintaining cell mechanical properties, indicating that distribution of mechanical load is not necessarily equal among all filaments. To address this problem, attempts have been made to develop fluorescent probes that report on the forces on the actin cytoskeleton that would report an ultrastructural-level force (36, 67, 68, 85) (**Figure 2**). Most of these examples are based on Förster resonance energy transfer (FRET) pairs that decrease in FRET as fluorophores become spaced apart (28). FRET modules have been inserted into actin crosslinkers such as filamin and α-actinin so that tension across these actin crosslinkers results in a change in FRET efficiency (**Figure 2*a***). In the context of forces across single actin filaments, FRET measurements are more difficult. The small size of changes that occur to actin filament structure means that more sensitive techniques are needed, such as polarization FRET or FRET using chemical dyes with high fluorescence output (68) (**Figure 2*b***). While technical limitations have prevented their widespread use in cells, these or related strategies would help provide a more detailed understanding of the mechanical forces at the single-filament level in different actin networks.

MECHANICAL REGULATION OF THE ACTIN CYTOSKELETON

In the previous section, we provided estimates of the mechanical load per filament in a variety of different actin structures. There is a clear link between actin network architecture and mechanical loading of filaments, consistent with a specific mechanical role existing for different networks in cell physiology. In this section, we highlight what the downstream effects of these mechanical-loading conditions are on cytoskeletal regulation, including actin filament conformation, polymerization kinetics, and protein binding.

Actin Filament Conformation and Force Feedback

The textbook picture of an actin filament is that it is a double-helical polymer with an angular twist of ~167°, ~9-nm width, and ~27.6-Å rise/subunit (32, 33). However, it is unclear whether these values are fixed and depict the static state of an actin filament or they are a snapshot of an ensemble of conformations (30, 32). A high-resolution actin structure by Galkin et al. (33) supports the notion of filament structural polymorphism and shows that flexibility in subdomain 2 of actin monomers could be at the origin of the various structural states. Binding of regulatory proteins to actin filaments has been shown to drive changes in the filament conformation, further supporting the idea that actin filaments are not structurally static (65). Proteins containing calponin homology domains have been shown to modulate filament conformation by rearranging monomeric subdomain positions. Fimbrin's actin-binding domain 2 (ABD2) stabilizes actin filaments and reduces their structural variability (31, 37). Similarly, calponin has been shown to drive rearrangement of subdomain 1 toward subdomain 3 when bound to tropomyosin-decorated filaments (43).

The actin-severing protein cofilin has been extensively studied for its effect in increasing filament flexibility. In fact, cofilin has been demonstrated to change the filament crossover length from 365 Å to 270 Å by changing the subunit angular twist (65). This increase in twist is further manifested in anisotropy measurements that revealed a decrease in torsional rigidity of cofilin-decorated filaments (78). Flexural rigidity has also been shown to decrease by more than 75%, corresponding to a reduction in filament persistence length from 9.8 μm down to 2.2 μm (63). These flexural rigidity changes induced by cofilin binding are believed to be central to its severing mechanism (25, 63, 64). Given the cooperative nature of cofilin binding to filaments, local structural changes to the filament could propagate outside of the cofilin–actin binding interface. Umeki and colleagues (92) and Ngo and colleagues (73) showed that a cofilin–actin fusion protein allosterically induces conformational changes to the actin filament that propagated over several subunits. The conformational changes to the actin filament then were shown to feed back on the binding affinity of both cofilin and myosin. Changes in conformations of an actin filament have been observed not only with side-binding proteins but also with actin nucleators. Formin-nucleated filaments are thought to exhibit an increase in flexibility that is reduced under the stabilizing effects of tropomyosin and myosin binding (74, 90, 91).

Do actin filaments change conformational state under mechanical tension (**Figure 3**)? Shimozawa & Ishiwata (85) provided one piece of evidence supporting structural rearrangement of stabilized filaments under mechanical load. Using labeled actin as a probe, they showed that the fluorescence intensity can decrease by 6%, independent of photobleaching effects, when the filament is tensed beyond the minimal straightening force (~5 pN). A second piece of evidence comes from Hayakawa et al. (38, 39), who showed that tensile forces of up to 30 pN applied to a single filament in vitro decreased cofilin severing activity. Scratching assays of a tethered actin mesh further demonstrated a bias in cofilin binding to relaxed filaments. A third piece of evidence comes from the finding that force on a filament increases the affinity of myosin II motor domain to the filament (93). Preferential binding to stretched filaments was observed under physiological changes to contractility and when external perturbations were applied. Uyeda et al. (93) proposed a positive feedback loop model where mechanical perturbations and myosin II binding increase tension in actin filaments, locking them in a stable structural state to which myosin II favorably binds. In contrast, the myosin I motor domain did not show an increase in localization (93). Bias in protein binding to a mechanical state of actin structures has also been observed with actin nucleators. Risca et al. (80) found that curvature biases branch formation through Arp2/3-mediated nucleation through a shift in the bending fluctuation spectrum resulting from applied tension.

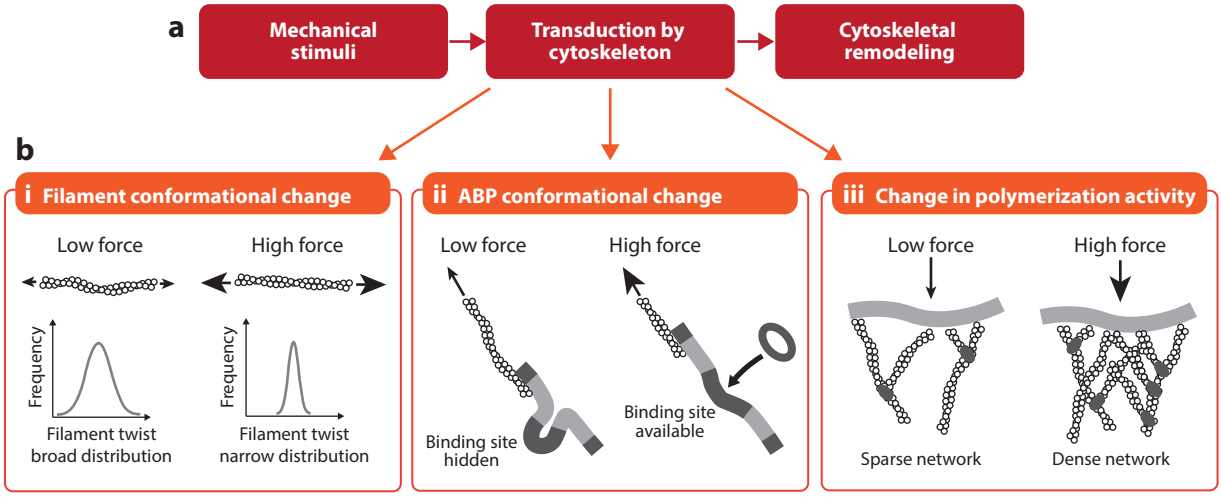

Figure 3

Mechanical force transduction by the actin cytoskeleton. (*a*) Mechanical forces on cells are transduced by the actin cytoskeleton into biochemical signals. These often culminate in cytoskeletal remodeling as the cell responds by changing shape. (*b*) We highlight three main modes for mechanical force transduction by the actin cytoskeleton. (*b*, *i*) Firstly, actin filaments themselves can be susceptible to mechanical forces, changing conformational state under mechanical load. (*b*, *ii*) Secondly, actin-binding proteins can change conformation under mechanical load, exposing binding sites for other proteins that were previously unavailable. (*b*, *iii*) Finally, the polymerization kinetics of actin-binding proteins can be influenced by the mechanical load upon them, thus changing the network density and growth rate. Abbreviation: ABP, actin-binding protein.

Mechanical Changes to the Conformational States of Actin-Binding Proteins

Perhaps the most well-studied examples of mechanosensitive proteins are those that link the actin cytoskeleton to other interfaces, such as adhesions to the cell substrate or those between cells. In this framework, mechanical tension is generated across a protein that is anchored at one end to a surface (i.e., the plasma membrane), and at the other end to the cytoskeleton. This tension conformationally unfolds the protein, revealing previously unavailable binding sites for other proteins (**Figure 3*b***, summarized in **Table 2**). One example of this is the focal adhesion–associated protein talin and the actin-binding protein vinculin (21). A similar mechanism has been shown to exist for the adherens junction protein α-catenin. Remarkably, this complex requires force to form a high-affinity interaction with F-actin (10). This interaction was shown with optical tweezers to depend on the unfolding of α-catenin to reveal binding sites for vinculin.

Interestingly, a similar mechanism exists for actin crosslinking proteins. The actin-binding protein filamin displays mechanosensitive behavior in reconstituted actin gels in vitro (24) and in vivo (46). Filamin A forms a homodimer at its C-terminus and binds to actin through its N-terminal calponin homology domain. Mechanical force across filamin A homodimers causes changes to the conformation of the rod2 domain that impact its affinity for filGAP, a GTPase involved in the regulation of the signaling protein Rac (24). In addition, myosin, α-actinin 4, and filamin B can crosslink actin filaments and show preferential accumulation in response to mechanical load (60, 84). The changes in protein localization in response to stress were attributed to changes in binding-protein turnover, as measured using fluorescence recovery after photobleaching. This observation corresponded to increases in binding density during cytokinesis and in response to micropipette

Table 2 Mechanisms of mechanical regulation of the actin cytoskeleton

	Mechanosensitive protein	Force response (pN)	Effect	Measurement method	Sample reference
Conformational change of actin-binding proteins	Myosin	4	Myosin step	Optical tweezers	27
	α-Catenin	5	Unveils vinculin binding site	Magnetic tweezers	101
	Talin	5	Unveils vinculin binding site	Magnetic tweezers	21
	Cadherin–catenin	10	Increased dissociation	Optical tweezers	10
	Filamin	15	Release of autoinhibition	Optical tweezers	81
	Vinculin	30	Dissociates from catenin	Magnetic tweezers	100
Change in polymerization kinetics	Formin Bni1p	0.3	Decrease in polymerization rate	Microfluidic flow	20
	Formin mDia1	3	Increase in polymerization rate	Microfluidic flow	48
	Dendritic actin	–	Change in network density	Atomic-force microscopy	5
Filament-level conformational change	Cofilin	30	Doubled delay in severing	Magnetic tweezers	39
	Myosin II	–	Increased affinity for stretched actin	Optical microscopy	93
	Arp2/3	–	Branching bias on convex curvature	Single-filament branching assay	80

aspiration of the cell cortex. In particular, the mechanosensitive response of myosin was shown to be dependent on the lever arm length of the myosin molecule (60).

Force Regulation of Polymerization Kinetics

One of the most important properties of actin filaments is their ability to convert biochemical energy into mechanical work through directed polymerization. Polymerization of actin filaments has been found to be modulated by force in two different ways (**Figure 3**).

Firstly, the force on different actin nucleators can regulate their polymerization activity. In particular, the polymerizing activity of some formin homology proteins has been shown to be force sensitive. In vivo, cells overexpressing formin have a larger pool of F-actin upon release of tension, suggesting a mechanical mechanism is regulating formin-mediated polymerization of actin. This mechanosensitive response is independent of Ca^{2+}, Rho, and kinase signaling but depends on profilin activity (20, 42). More directly, in vitro reconstitution assays of FH1-anchored formins show that, in the absence of profilin, application of tensile forces slows down actin polymerization. Meanwhile, filament elongation rates increase under tension at optimal profilin concentrations (20, 54). Microfluidic assays have also been used to apply forces to growing actin filaments by fluid shear (48). In this assay, the FH2 dimer conformation was sensitive to force and impacted the elongation rate of actin filaments by this nucleation factor.

Secondly, actin polymerization occurring against an obstacle can affect architectural and dynamic properties of network structure (5, 19, 72). When growth is opposed by large forces, polymerization can stall. Prior to stall, the network growth rate and mechanical properties are altered by force due to changes in the assembly rates of network components. Multiple models have been developed to describe the force dependence of branched actin network assembly, including the autocatalytic model (13) and several Brownian ratchet-based models (71, 71a, 71b, 75). While each captures experimentally observed features of branched actin network assembly—tethering of the network to the nucleation surface, reduction of monomer on-rate with force, increasing network density with force—none fully describe the complex mechanical response of branched networks to applied loads. For example, the force velocity characteristics for branched networks can be directly measured in vitro but are not well described by a single curve owing to history-dependent effects arising from changing network density with load (5, 72, 74a). Indeed, force must be considered as a factor in all aspects of actin network assembly under load, as the alternative of a molecular assembly process insensitive to forces actin on its constituent components is difficult to imagine.

OUTLOOK

Mechanotransduction by the actin cytoskeleton is accomplished through specialized actin structures that are built for the mechanical environment in which they function. Estimates of the forces felt by single filaments indicate they carry on average ~1 pN of compressive load in the lamellipodia and ~10–100 pN of tensile load in stress fibers, though the actual distribution of filament loads within a network is expected to be highly variable. In addition to being organized by sets of specialized binding proteins, actin networks appear to be regulated by mechanical forces. In this review, we have highlighted three key mechanisms by which mechanical forces are transduced into biochemical changes through the actin cytoskeleton. Firstly, actin filament structure can be altered by mechanical force in ways that alter the affinity of actin binding and regulatory proteins. Secondly, actin-binding proteins can conformationally unfold under mechanical force, revealing binding sites for other regulatory proteins. Finally, mechanical forces can directly influence the density and organization of actin filament networks through their interplay with actin polymerization kinetics. Taken together, these mechanisms paint a picture wherein the actin cytoskeleton actively responds to mechanical load exerted on it by shaping its composition, organization, and function, enabling cells to sense and rapidly adapt to forces within their environment.

The implications of better understanding how actin networks use forces to regulate their architecture and behavior extend well beyond fundamental biophysical and cell biology of the cytoskeleton. Indeed, a range of diseases arise from mutant forms of actin-binding proteins that may be linked to the cell's compromised ability to properly transduce mechanical forces. Two key examples of this are associated with actin crosslinking proteins (97). Mutated forms of α-actinin and filamin are associated with diseases, including focal segmental glomerulosclerosis and skeletal disorders (atelosteogenesis, skeletal dysplasia) (18, 23, 41, 58, 99). The expression of mutant α-actinin in podocytes causes truncation of cellular processes and an inability of these cells to sustain mechanical forces (26). Mutations to the actin-binding domain of dystrophin are associated with muscular dystrophy and protein instability (40). During development, mechanosensitive processes associated with the actin cytoskeleton direct tissue morphogenesis (14).

Continued development of fluorescence microscopy and electron microscopy techniques—and their extension to quantify forces in actin networks—has the potential to significantly advance our understanding of mechanotransduction by the actin cytoskeleton in cell physiology, embryonic development, and different pathologies. In addition, new experimental techniques, both in live cells and in vitro, will be needed to reveal the molecular mechanisms responsible for mechanical

regulation of actin networks and provide a more complete understanding of the often unseen, but always felt, influence of force.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Aramaki S, Mayanagi K, Jin M, Aoyama K, Yasunaga T. 2016. Filopodia formation by crosslinking of F-actin with fascin in two different binding manners. *Cytoskeleton* 73(7):365–74
2. Beach JR, Bruun KS, Shao L, Li D, Swider Z, et al. 2017. Actin dynamics and competition for myosin monomer govern the sequential amplification of myosin filaments. *Nat. Cell Biol.* 19(2):85–93
3. Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, et al. 2006. Imaging intracellular fluorescent proteins at nanometer resolution. *Science* 313(5793):1642–45
4. Deleted in proof
5. Bieling P, Weichsel J, McGorty R, Jreij P, Huang B, et al. 2016. Force feedback controls motor activity and mechanical properties of self-assembling branched actin networks. *Cell* 164(1):115–27
6. Blanchoin L, Boujemaa-Paterski R, Sykes C, Plastino J. 2014. Actin dynamics, architecture, and mechanics in cell motility. *Physiol. Rev.* 94(1):235–63
7. Bornschlögl T. 2013. How filopodia pull: what we know about the mechanics and dynamics of filopodia. *Cytoskeleton* 70(10):590–603
8. Bouissou A, Proag A, Bourg N, Pingris K, Cabriel C, et al. 2017. Podosome force generation machinery: a local balance between protrusion at the core and traction at the ring. *ACS Nano* 11(4):4028–40
9. Bovellan M, Romeo Y, Biro M, Boden A, Chugh P, et al. 2014. Cellular control of cortical actin nucleation. *Curr. Biol.* 24(14):1628–35
10. Buckley CD, Tan J, Anderson KL, Hanein D, Volkmann N, et al. 2014. The minimal cadherin-catenin complex binds to actin filaments under force. *Science* 346(6209):1254211
11. Burkel BM, von Dassow G, Bement WM. 2007. Versatile fluorescent probes for actin filaments based on the actin-binding domain of utrophin. *Cytoskeleton* 64(11):822–32
12. Burnette DT, Manley S, Sengupta P, Sougrat R, Davidson MW, et al. 2011. A role for actin arcs in the leading-edge advance of migrating cells. *Nat. Cell Biol.* 13(4):371–82
13. Carlsson AE. 2003. Growth velocities of branched actin networks. *Biophys. J.* 84(5):2907–18
14. Chanet S, Miller CJ, Vaishnav ED, Ermentrout B, Davidson LA, Martin AC. 2017. Actomyosin meshwork mechanosensing enables tissue shape to orient cell force. *Nat. Commun.* 8:15014
15. Christensen JR, Hocky GM, Homa KE, Morganthaler AN, Hitchcock-DeGregori SE, et al. 2017. Competition between Tropomyosin, Fimbrin, and ADF/Cofilin drives their sorting to distinct actin filament networks. *eLife* 6:e23152
16. Chugh P, Clark AG, Smith MB, Cassani DA, Dierkes K, et al. 2017. Actin cortex architecture regulates cell surface tension. *Nat. Cell Biol.* 19(6):689–97
17. Clark AG, Dierkes K, Paluch EK. 2013. Monitoring actin cortex thickness in live cells. *Biophys. J.* 105(3):570–80

18. Clark AR, Sawyer GM, Robertson SP, Sutherland-Smith AJ. 2009. Skeletal dysplasias due to filamin A mutations result from a gain-of-function mechanism distinct from allelic neurological disorders. *Hum. Mol. Genet.* 18(24):4791–4800
19. Cojoc D, Difato F, Ferrari E, Shahapure RB, Laishram J, et al. 2007. Properties of the force exerted by filopodia and lamellipodia and the involvement of cytoskeletal components. *PLOS ONE* 2(10):e1072
20. Courtemanche N, Lee JY, Pollard TD, Greene EC. 2013. Tension modulates actin filament polymerization mediated by formin and profilin. *PNAS* 110(24):9752–57
21. del Rio A, Perez-Jimenez R, Liu R, Roca-Cusachs P, Fernandez JM, Sheetz MP. 2009. Stretching single talin rod molecules activates vinculin binding. *Science* 323(5914):638–41
22. Diz-Muñoz A, Thurley K, Chintamen S, Altschuler SJ, Wu LF, et al. 2016. Membrane tension acts through PLD2 and mTORC2 to limit actin network assembly during neutrophil migration. *PLOS Biol.* 14(6):e1002474
23. Ehrlicher AJ, Krishnan R, Guo M, Bidan CM, Weitz DA, Pollak MR. 2015. Alpha-actinin binding kinetics modulate cellular dynamics and force generation. *PNAS* 112(21):6619–24
24. Ehrlicher AJ, Nakamura F, Hartwig JH, Weitz DA, Stossel TP. 2011. Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. *Nature* 478(7368):260
25. Elam WA, Kang H, De La Cruz EM. 2013. Biophysics of actin filament severing by cofilin. *FEBS Lett.* 587(8):1215–19
26. Endlich K, Kliewe F, Endlich N. 2017. Stressed podocytes—mechanical forces, sensors, signaling and response. *Pflüg. Arch. Eur. J. Physiol.* 469(7–8):937–49
27. Finer JT, Mehta AD, Spudich JA. 1995. Characterization of single actin-myosin interactions. *Biophys. J.* 68(Suppl. 4):291S
28. Freikamp A, Mehlich A, Klingner C, Grashoff C. 2017. Investigating piconewton forces in cells by FRET-based molecular force microscopy. *J. Struct. Biol.* 197(1):37–42
29. Fritzsche M, Li D, Colin-York H, Chang VT, Moeendarbary E, et al. 2017. Self-organizing actin patterns shape membrane architecture but not cell mechanics. *Nat. Commun.* 8:14347
30. Fujii T, Iwane AH, Yanagida T, Namba K. 2010. Direct visualization of secondary structures of F-actin by electron cryomicroscopy. *Nature* 467(7316):724–28
31. Galkin VE, Orlova A, Cherepanova O, Lebart M-C, Egelman EH. 2008. High-resolution cryo-EM structure of the F-actin-fimbrin/plastin ABD2 complex. *PNAS* 105(5):1494–98
32. Galkin VE, Orlova A, Egelman EH. 2012. Actin filaments as tension sensors. *Curr. Biol.* 22(3):R96–101
33. Galkin VE, Orlova A, Vos MR, Schröder GF, Egelman EH. 2015. Near-atomic resolution for one state of F-actin. *Structure* 23(1):173–82
34. Gateva G, Kremneva E, Reindl T, Kotila T, Kogan K, et al. 2017. Tropomyosin isoforms specify functionally distinct actin filament populations in vitro. *Curr. Biol.* 27(5):705–13
35. Grimm HP, Verkhovsky AB, Mogilner A, Meister J-J. 2003. Analysis of actin dynamics at the leading edge of crawling cells: implications for the shape of keratocyte lamellipodia. *Eur. Biophys. J.* 32(6):563–77
36. Guo J, Sachs F, Meng F. 2014. Fluorescence-based force/tension sensors: a novel tool to visualize mechanical forces in structural proteins in live cells. *Antioxid. Redox Signal.* 20(6):986–99
37. Hanein D, Matsudaira P, DeRosier DJ. 1997. Evidence for a conformational change in actin induced by fimbrin (N375) binding. *J. Cell Biol.* 139(2):387–96
38. Hayakawa K, Sakakibara S, Sokabe M, Tatsumi H. 2014. Single-molecule imaging and kinetic analysis of cooperative cofilin–actin filament interactions. *PNAS* 111(27):9810–15
39. Hayakawa K, Tatsumi H, Sokabe M. 2011. Actin filaments function as a tension sensor by tension-dependent binding of cofilin to the filament. *J. Cell Biol.* 195(5):721–27
40. Henderson DM, Lee A, Ervasti JM. 2010. Disease-causing missense mutations in actin binding domain 1 of dystrophin induce thermodynamic instability and protein aggregation. *PNAS* 107(21):9632–37
41. Henderson JM, Alexander MP, Pollak MR. 2009. Patients with ACTN4 mutations demonstrate distinctive features of glomerular injury. *J. Am. Soc. Nephrol.* 20(5):961–68
42. Higashida C, Kiuchi T, Akiba Y, Mizuno H, Maruoka M, et al. 2013. F- and G-actin homeostasis regulates mechanosensitive actin nucleation by formins. *Nat. Cell Biol.* 15(4):395–405

43. Hodgkinson JL, El-Mezgueldi M, Craig R, Vibert P, Marston SB, Lehman W. 1997. 3-D image reconstruction of reconstituted smooth muscle thin filaments containing calponin: visualization of interactions between F-actin and calponin. *J. Mol. Biol.* 273(1):150–59
44. Hu S, Dasbiswas K, Guo Z, Tee Y-H, Thiagarajan V, et al. 2017. Long-range self-organization of cytoskeletal myosin II filament stacks. *Nat. Cell Biol.* 19(2):133–41
45. Huang DL, Bax NA, Buckley CD, Weis WI, Dunn AR. 2017. Vinculin forms a directionally asymmetric catch bond with F-actin. *Science* 357(6352):703–6
46. Huelsmann S, Rintanen N, Sethi R, Brown NH, Ylänne J. 2016. Evidence for the mechanosensor function of filamin in tissue development. *Sci. Rep.* 6:32798
47. Jaiswal R, Breitsprecher D, Collins A, Corrêa IR, Xu M-Q, Goode BL. 2013. The formin Daam1 and fascin directly collaborate to promote filopodia formation. *Curr. Biol.* 23(14):1373–79
48. Jégou A, Carlier M-F, Romet-Lemonne G. 2013. Formin mDia1 senses and generates mechanical forces on actin filaments. *Nat. Commun.* 4:1883
49. Jégou A, Romet-Lemonne G. 2016. Single filaments to reveal the multiple flavors of actin. *Biophys. J.* 110(10):2138–46
50. Jensen MH, Morris EJ, Huang R, Rebowski G, Dominguez R, et al. 2012. The conformational state of actin filaments regulates branching by actin-related protein 2/3 (Arp2/3) complex. *J. Biol. Chem.* 287(37):31447–53
51. Kassianidou E, Brand CA, Schwarz US, Kumar S. 2017. Geometry and network connectivity govern the mechanics of stress fibers. *PNAS* 114:2622–27
52. Katoh K, Kano Y, Amano M, Kaibuchi K, Fujiwara K. 2001. Stress fiber organization regulated by MLCK and Rho-kinase in cultured human fibroblasts. *Am. J. Physiol. Cell Physiol.* 280(6):C1669–79
53. Kress H, Stelzer EH, Holzer D, Buss F, Griffiths G, Rohrbach A. 2007. Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity. *PNAS* 104(28):11633–38
54. Kubota H, Miyazaki M, Ogawa T, Shimozawa T, Kinosita K Jr., Ishiwata S. 2017. Biphasic effect of profilin impacts the formin mDia1 force-sensing mechanism in actin polymerization. *Biophys. J.* 113(2):461–71
55. Kumar S, Maxwell IZ, Heisterkamp A, Polte TR, Lele TP, et al. 2006. Viscoelastic retraction of single living stress fibers and its impact on cell shape, cytoskeletal organization, and extracellular matrix mechanics. *Biophys. J.* 90(10):3762–73
56. Labernadie A, Bouissou A, Delobelle P, Balor S, Voituriez R, et al. 2014. Protrusion force microscopy reveals oscillatory force generation and mechanosensing activity of human macrophage podosomes. *Nat. Commun.* 5:5343
57. Labouesse C, Gabella C, Meister J-J, Vianay B, Verkhovsky AB. 2016. Microsurgery-aided in-situ force probing reveals extensibility and viscoelastic properties of individual stress fibers. *Sci. Rep.* 6:23722
58. Lee SH, Weins A, Hayes DB, Pollak MR, Dominguez R. 2008. Crystal structure of the actin-binding domain of α-actinin-4 Lys255Glu mutant implicated in focal segmental glomerulosclerosis. *J. Mol. Biol.* 376(2):317–24
59. Lomakin AJ, Lee K-C, Han SJ, Bui DA, Davidson M, et al. 2015. Competition of two distinct actin networks for actin defines a bistable switch for cell polarization. *Nat. Cell Biol.* 17(11):1435
60. Luo T, Mohan K, Iglesias PA, Robinson DN. 2013. Molecular mechanisms of cellular mechanosensing. *Nat. Mater.* 12(11):1064–71
61. Luxenburg C, Geblinger D, Klein E, Anderson K, Hanein D, et al. 2007. The architecture of the adhesive apparatus of cultured osteoclasts: from podosome formation to sealing zone assembly. *PLOS ONE* 2(1):e179
62. Machesky LM, Mullins RD, Higgs HN, Kaiser DA, Blanchoin L, et al. 1999. Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. *PNAS* 96(7):3739–44
62a. Marcy Y, Prost J, Carlier MF, Sykes C. 2004. Forces generated during actin-based propulsion: a direct measurement by micromanipulation. *PNAS* 101(16):5992–97
63. McCullough BR, Blanchoin L, Martiel J-L, Enrique M. 2008. Cofilin increases the bending flexibility of actin filaments: implications for severing and cell mechanics. *J. Mol. Biol.* 381(3):550–58
64. McCullough BR, Grintsevich EE, Chen CK, Kang H, Hutchison AL, et al. 2011. Cofilin-linked changes in actin filament flexibility promote severing. *Biophys. J.* 101(1):151–59

65. McGough A, Pope B, Chiu W, Weeds A. 1997. Cofilin changes the twist of F-actin: implications for actin filament dynamics and cellular function. *J. Cell Biol.* 138(4):771–81
66. Melak M, Plessner M, Grosse R. 2017. Actin visualization at a glance. *J. Cell Sci.* 130(3):525–30
67. Meng F, Sachs F. 2011. Visualizing dynamic cytoplasmic forces with a compliance-matched FRET sensor. *J. Cell Sci.* 124(2):261–69
68. Meng F, Sachs F. 2012. Orientation-based FRET sensor for real-time imaging of cellular forces. *J. Cell Sci.* 125(3):743–50
69. Michelot A, Drubin DG. 2011. Building distinct actin filament networks in a common cytoplasm. *Curr. Biol.* 21(14):R560–69
70. Moeendarbary E, Valon L, Fritzsche M, Harris AR, Moulding DA, et al. 2013. The cytoplasm of living cells behaves as a poroelastic material. *Nat. Mater.* 12(3):253
71. Mogilner A, Edelstein-Keshet L. 2002. Regulation of actin dynamics in rapidly moving cells: a quantitative analysis. *Biophys. J.* 83(3):1237–58

71a. Mogliner A, Oster G. 1996. Cell motility driven by actin polymerization. *Biophys. J.* 71(6):3030–45

71b. Mogliner A, Oster G. 2003. Force generation by actin polymerization II: the elastic ratchet and tethered filaments. *Biophys. J.* 84(3):1591–605

72. Mueller J, Szep G, Nemethova M, de Vries I, Lieber AD, et al. 2017. Load adaptation of lamellipodial actin networks. *Cell* 1:188–200.e16
73. Ngo KX, Umeki N, Kijima ST, Kodera N, Ueno H, et al. 2016. Allosteric regulation by cooperative conformational changes of actin filaments drives mutually exclusive binding with cofilin and myosin. *Sci. Rep.* 6:35449
74. Papp G, Bugyi B, Ujfalusi Z, Barkó S, Hild G, et al. 2006. Conformational changes in actin filaments induced by formin binding to the barbed end. *Biophys. J.* 91(7):2564–72

74a. Parekh SH, Chaudhuri O, Theriot JA, Fletcher DA. 2005. Loading history determines the velocity of actin-network growth. *Nat. Cell Biol.* 7:1219–23

75. Peskin CS, Odell GM, Oster GF. 1993. Cellular motions and thermal fluctuations: the Brownian ratchet. *Biophys. J.* 65(1):316–24
76. Pollard TD. 2016. Actin and actin-binding proteins. *Cold Spring Harb. Perspect. Biol.* 8:a018226
77. Prass M, Jacobson K, Mogilner A, Radmacher M. 2006. Direct measurement of the lamellipodial protrusive force in a migrating cell. *J. Cell Biol.* 174(6):767–72
78. Prochniewicz E, Janson N, Thomas DD, Enrique M. 2005. Cofilin increases the torsional flexibility and dynamics of actin filaments. *J. Mol. Biol.* 353(5):990–1000
79. Riedl J, Crevenna AH, Kessenbrock K, Yu JH, Neukirchen D, et al. 2008. Lifeact: a versatile marker to visualize F-actin. *Nat. Methods* 5(7):605–7
80. Risca VI, Wang EB, Chaudhuri O, Chia JJ, Geissler PL, Fletcher DA. 2012. Actin filament curvature biases branching direction. *PNAS* 109(8):2913–18
81. Rognoni L, Stigler J, Pelz B, Ylänne J, Rief M. 2012. Dynamic force sensing of filamin revealed in single-molecule experiments. *PNAS* 109(48):19679–84
82. Romet-Lemonne G, Jégou A. 2013. Mechanotransduction down to individual actin filaments. *Eur. J. Cell Biol.* 92(10):333–38
83. Salbreux G, Charras G, Paluch E. 2012. Actin cortex mechanics and cellular morphogenesis. *Trends Cell Biol.* 22(10):536–45
84. Schiffhauer ES, Luo T, Mohan K, Srivastava V, Qian X, et al. 2016. Mechanoaccumulative elements of the mammalian actin cytoskeleton. *Curr. Biol.* 26:1473–79
85. Shimozawa T, Ishiwata S. 2009. Mechanical distortion of single actin filaments induced by external force: detection by fluorescence imaging. *Biophys. J.* 96(3):1036–44
86. Srivastava V, Robinson DN. 2015. Mechanical stress and network structure drive protein dynamics during cytokinesis. *Curr. Biol.* 25(5):663–70
87. Svitkina TM, Bulanova EA, Chaga OY, Vignjevic DM, Kojima S, et al. 2003. Mechanism of filopodia initiation by reorganization of a dendritic network. *J. Cell Biol.* 160(3):409–21
88. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. 2003. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *PNAS* 100(4):1484–89

89. Tinevez J-Y, Schulze U, Salbreux G, Roensch J, Joanny J-F, Paluch E. 2009. Role of cortical tension in bleb growth. *PNAS* 106(44):18581–86
90. Ujfalusi Z, Kovács M, Nagy NT, Barkó S, Hild G, et al. 2012. Myosin and tropomyosin stabilize the conformation of formin-nucleated actin filaments. *J. Biol. Chem.* 287(38):31894–904
91. Ujfalusi Z, Vig A, Hild G, Nyitrai M. 2009. Effect of tropomyosin on formin-bound actin filaments. *Biophys. J.* 96(1):162–68
92. Umeki N, Hirose K, Uyeda TQ. 2016. Cofilin-induced cooperative conformational changes of actin subunits revealed using cofilin-actin fusion protein. *Sci. Rep.* 6:20406
93. Uyeda TQ, Iwadate Y, Umeki N, Nagasaki A, Yumura S. 2011. Stretching actin filaments within cells enhances their affinity for the myosin II motor domain. *PLOS ONE* 6(10):e26200
94. Vinzenz M, Nemethova M, Schur F, Mueller J, Narita A, et al. 2012. Actin branching in the initiation and maintenance of lamellipodia. *J. Cell Sci.* 125:2775–85
95. Wagner E, Glotzer M. 2016. Local RhoA activation induces cytokinetic furrows independent of spindle position and cell cycle stage. *J. Cell Biol.* 213(6):641–49
96. Wang Y, Kanchanawong P. 2016. Three-dimensional super resolution microscopy of F-actin filaments by Interferometric PhotoActivated Localization Microscopy (iPALM). *JoVE* 118:e54774
97. Weins A, Schlondorff JS, Nakamura F, Denker BM, Hartwig JH, et al. 2007. Disease-associated mutant α-actinin-4 reveals a mechanism for regulating its F-actin-binding affinity. *PNAS* 104(41):16080–85
98. Wu C, Asokan SB, Berginski ME, Haynes EM, Sharpless NE, et al. 2012. Arp2/3 is critical for lamellipodia and response to extracellular matrix cues but is dispensable for chemotaxis. *Cell* 148(5):973–87
99. Wyss HM, Henderson JM, Byfield FJ, Bruggeman LA, Ding Y, et al. 2011. Biophysical properties of normal and diseased renal glomeruli. *Am. J. Physiol. Cell Physiol.* 300(3):C397–405
100. Yao M, Goult BT, Chen H, Cong P, Sheetz MP, Yan J. 2014. Mechanical activation of vinculin binding to talin locks talin in an unfolded conformation. *Sci. Rep.* 4:4610
101. Yao M, Qiu W, Liu R, Efremov AK, Cong P, Seddiki R. 2014. Force-dependent conformational switch of α-catenin controls vinculin binding. *Nat. Commun.* 5:4525