Actin Mechanics and Fragmentation

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Cell physiological processes require the regulation and coordination of both mechanical and dynamical properties of the actin cytoskeleton. Here we review recent advances in understanding the mechanical properties and stability of actin filaments and how these properties are manifested at larger (network) length scales. We discuss how forces can influence local biochemical interactions, resulting in the formation of mechanically sensitive dynamic steady states. Understanding the regulation of such force-activated chemistries and dynamic steady states reflects an important challenge for future work that will provide valuable insights as to how the actin cytoskeleton engenders mechanoresponsiveness of living cells.

Actin Cytoskeleton

The non-covalent polymerization of the cytoskeleton protein actin (Fig. 1) into linear filaments powers a variety of non-muscle cell movements underlying their migration, division, and assembly into multicellular tissue (1). Cross-linking proteins arrange filaments into higher-order assemblies such as parallel bundles of filopodia and microvilli, isotropic networks of the cortex, and contractile bundles in the lamella that provide cells with mechanical integrity, sensing, and shape (2–4). Contractile myosin motor proteins generate force and work output (i.e. motility and transport) along filament tracks, in both muscle and non-muscle cells (5).

Actin filaments grow and shrink from their ends through subunit addition and loss, respectively. Accordingly, the overall assembly dynamics and turnover of the actin cytoskeleton in cells is influenced by factors that modulate the filament end concentration. Nucleating proteins and complexes generate new filament ends, often branching from existing filament templates (1). Capping proteins bind filament ends and slow or inhibit subunit addition (1). Severing and contractile proteins fragment actin filaments and accelerate actin turnover in cells and in reconstituted biomimetic systems (6–12).

Actin Structure

Actins are single polypeptide chain proteins (~375 amino acids with an ~42-kDa molecular mass) folded into a U-shaped structure containing four subdomains (SD1–4) with a bound adenine nucleotide, either ATP, ADP-Pi, or ADP, and an associated divalent cation, Mg2+ in cells, bound within the cleft separating subdomains 1–2 and 3–4 (Fig. 2A). Actin filaments are commonly described as either a one-start, left-handed helix of subunits with a 2.77-nm rise per subunit (13) or a two-start, right-handed helix with half-staggered filament strands displaying an ~72-nm repeat (14). However, filaments in solution adopt multiple distinct structural states (referred to as “structural polymorphism”(15)) with variable twist and subunit tilt distributions that are influenced by regulatory protein occupancy and external forces. Thus, one actually refers to a subset of populated states when describing actin structure.

Polymerization is associated with a conformational change in actin, such that incorporated filament subunits appear “flattened” when compared with free monomers (16, 17). Extensive intersubunit contacts stabilize filaments (16–18), and an individual subunit contacts four neighboring actin molecules (Fig. 2A). Longitudinal contacts within a strand are thought to be stronger than lateral contacts across the strands (19). The DNase I binding loop (residues 36–52 of SD2; Fig. 2A) adopts a variety of conformations (20), some of which make important long-axis, intersubunit contacts, and plays a central role in regulating actin filament structure and mechanical properties (21, 22).

Actin Filament Mechanical Properties

Actin filaments are polymers with lengths ranging up to ~10 μm in solution (23) (Fig. 2B). The local asymmetry arising from the ribbon structure averages out to a diameter of ~6 nm on the length scales associated with analysis of individual filament and network mechanics. How filaments deform in response to force is governed by their mechanical elastic properties. Recent biochemical, biophysical, and computational studies have revealed the molecular and geometric origins of actin filament mechanical properties and how solution conditions, particularly cations, influence them.

Bending, torsional, and twist-bend coupling elasticities dominate individual actin filament mechanical properties. The flexural, or bending, rigidity as well as the extent of deformation determine the energy required to bend a filament segment of a certain length. Likewise, the energy required to twist a filament is determined by the twisting rigidity and the torsional angle that overwinds or unwinds the filament. Twist-bend coupling represents an obligatory coupling between bending and twisting motions, such that filament bending introduces twist and vice versa (24, 25) (Fig. 2C). Actin filaments are rather resistant to stretching (26). However, stretching forces dampen bending

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and twisting motions (27), and can have significant effects on filament structural dynamics.

Filament mechanical properties are determined by the strength and distribution of intersubunit contacts (24). Subunit compliance and compressibility (28, 29) presumably play a role in overall filament mechanics. However, mathematical and computational models that treat filament subunits as incompressible entities capture the reported mechanical parameters with reasonable accuracy (24, 25). Filaments twist more easily than they bend, which manifests itself as a larger bending than torsional rigidity.

Solution cations bind and stiffen some, but not all (e.g. Saccharomyces cerevisiae), actin filaments (20, 30). Substitution of a single amino acid residue positioned between adjacent, long-axis filament subunits confers S. cerevisiae actin filaments with salt-dependent bending stiffness (30). The filament bending stiffness (i.e. flexural rigidity, \(\kappa\)) is equal to the product of the shape-independent stiffness of the protein material (i.e. apparent Young’s modulus, \(E_{\text{app}}\)) and the shape-dependent second moment of area (\(I\)), which is determined by the strength and distribution of intersubunit contacts. Filaments possessing this “stiffness cation site” display salt-dependent structure and intersubunit contacts, suggesting that stiffness cations may exert their effects on filament mechanics by binding to discrete sites at subunit interfaces and altering the radial distribution (\(I\)) and/or the strength (\(E_{\text{app}}\)) of the intersubunit contacts (18). A mechanism in which cations bind at distal sites and allosterically alter intersubunit contacts is also plausible (31). The DNase I binding loop of SD2 participates in longitudinal intersubunit contacts, plays a central role in modulating the filament bending stiffness and displays salt-dependent conformations, making it an attractive structural element for linking salt-dependent filament structure and mechanics. Changes in filament stiffness can also arise from side binding proteins. For example, tropomyosin stiffens actin filaments by increasing its geometrical movement (32).

Mechanically strained filaments (e.g. deformed in bending and/or twisting) store elastic free energy in their shape. The
stored energy density depends on the deformation amplitude and the bending rigidity; larger deformations store more energy than small ones, and stiff filaments store more energy than compliant ones at identical deformations. Relaxation back to the straight conformation can potentially be used to generate work and/or force. Alternatively, the deformation amplitude and stored energy can reach the point of irreversibility, causing the filament to fragment.

Thermal energy ($k_B T$, where $k_B$ is Boltzmann’s constant and $T$ is temperature in Kelvin) can be sufficiently large to induce actin filament shape fluctuations. The filament length at which the deformation energy compares to thermal energy defines the persistence length, which can be defined for bending ($L_B$), twisting ($L_T$), and twist-bend coupling ($L_{TB}$) deformations (Fig. 2D). Filaments behave as rigid rods at lengths much shorter than the persistence length, as flexible polymers much larger than the persistence length, and as semi-flexible polymers at lengths comparable with the persistence length (33). The bending fluctuations reduce the filament end-to-end length, and the force required to extend and “straighten” the filament is determined by the energy required to reduce the bending fluctuation amplitude. This entropic spring constant is both highly sensitive to the length of filament segment and becomes highly non-linear as the end-to-end length approaches the full polymer contour length (34) (Fig. 3).

Compressive forces tend to excite the longest wavelength (softest) bending mode, which is comparable with $k_B T$ for micrometer length filaments. At a critical compressive force, referred to as critical or Euler force, buckling will occur. The critical force scales linearly with filament bending rigidity and inversely with the square of the filament length (e.g. it takes four times less force to buckle a 2-$\mu$m filament than a 1-$\mu$m one with identical bending rigidity) (35). This different response to extension and compression gives filaments a highly asymmetric force extension curve (Fig. 3) that can be approximated in coarse-grained models as cables (36) and is a powerful mechanism by which actin networks can sense (and respond) to different types of stresses (9).

Cross-linking proteins organize filaments into parallel (or antiparallel) bundles. The mechanical parameters (twist, bend, and extension) of bundles depend on the width of the bundle and length of the filaments comprising them, as well as the
density, mechanics, affinity, and exchange kinetics of the cross-links. For instance, filaments in bundles formed with a compliant cross-linker (e.g. plasmin) can readily slide past each and are weakly coupled such that the bundle stiffness increases linearly with the number of actin filaments (37). By contrast, when filaments become tightly coupled to each other by use of crowding agents or high density of compact cross-linker (e.g. fascin), the bundles act as single unit that displays a bending persistence length that scales quadratically with the number of filaments in the bundle (23, 37). In some cases, the cross-link density can impact the degree of coupling and the observed scaling of bundle stiffness (37, 38). Because actin cross-linking proteins are dynamic, the bundle mechanics are also frequency-dependent and depend on the cross-link dynamics as well (37, 39).

Filament Fragmentation

Fragmentation by severing and contractile motor proteins accelerates actin network turnover and assembly dynamics by increasing the concentration of filament ends where subunits can add and dissociate. As described above, compressive stresses buckle filaments. However, filaments under compressive loads do not deform indefinitely. Rather, they buckle and bend until they reach a deformation curvature where the stored elastic energy exceeds that holding the subunits together and it becomes energetically more favorable for the filament to fragment than remain intact (40). Estimating this stored energy and force requires that all elastic contributions be considered (e.g. bending, twisting, and coupling) because they all contribute to the local energy density that eventually causes filament subunit interface “bonds” to rupture (40).

Several classes of regulatory filament-severing proteins have been identified (14). Gelsolin and ADF3/cofilin family members have been characterized most extensively at the biochemical and biophysical level with purified components (14). Gelsolin severs filaments by inserting one of its structural domains between long axis filament subunits, compromising stabilizing intersubunit interactions and promoting fragmentation. Formin INF2 may sever filaments by an analogous insertion, or “wedging,” mechanism (41).

The filament-severing mechanism of ADF/cofilin (heretofore referred to as cofilin) appears to be distinct from that of gelsolin and INF2. Cofilin isoforms within and across organisms and species are not identical, and often the observed biochemical activities depend on the isoform and conspecific nature of the actin (42, 43), indicating that the chemical and physical properties of actin itself can influence cofilin function.

Cofilins bind weakly to “young” ATP- and ADP-Pi actin filaments and bind orders of magnitude more strongly to “old” ADP-actin filaments (42), which allows for spatially targeted disassembly (44). Vertebrate cofilin binding is positively cooperative (43). Association is far below the diffusion limit for encounter (45) and limited by filament shape fluctuations (45, 46), suggesting opportunistic binding to compliant filament segments (45). Severing occurs preferentially at or near boundaries (e.g. junctions) between bare and cofilin-decorated segments (47), explaining why the severing activity peaks when filaments are partially decorated (31, 43, 47–51).

Multiple factors likely influence the severing activity of ADF/cofilin, and the contributions of some have yet to be fully resolved. Cofilin alters filament twist (52) and subunit tilt (53), renders filaments more compliant in bending (54) and twisting (55, 56) by dissociating filament-associated stiffness cations (31), and binds filaments in at least two distinct binding modes that may be differentially associated with severing and/or depolymerization activities (44). Thermally driven severing (e.g. in the absence of applied external loads) is weak and presumably dominated by thermodynamic effects originating from structural phase discontinuities at boundaries between bare and decorated segments. Boundaries fragment at smaller deformations than either parent filament (50), suggesting that boundaries are “bad joints” that are susceptible to fragmentation, analogous to the interfacial fracture of some non-protein materials (12). Srv2/CAP (cyclase-associated protein) may enhance severing by cofilin by inducing further conformational changes at boundaries (57). It is also conceivable that cofilin at the end of a bound cluster (43, 44) preferentially adopts a distinct binding mode (e.g. “severing”) from cofilin within a cluster (e.g. “non-severing”). However, a correlation between filament dynamics and thermally driven fragmentation has also been observed (31, 49, 50, 58), suggesting that filament mechanics and the structural dynamics at boundaries can potentially play a role, even in the absence of external load. An attractive model (53) implicates allosteric propagation of cofilin-linked conformational changes (47, 55, 59) to bare, undecorated segments where local, stabilizing cofilin-actin interactions are absent, thus leading to fragmentation within the bare side of boundaries.

Actin-interacting protein 1 (Aip1 (60)) accelerates the filament-severing activity of cofilin. One model of enhanced severing (61) implicates further structural changes in cofilin-actin induced by Aip1, possibly a wedge-like mechanism where cofilin is inserted between adjacent long-axis neighbors. Consistent with a functional ternary Aip1-cofilin-actin complex, Aip1 does not dissociate cofilin and enhances severing at all cofilin occupancies (62). However, a different study (63) favors an alternate mechanism (64) in which Aip1 simply competes with cofilin, dissociating it from actin and introducing additional boundaries where severing can occur.

The various factors contributing to filament severing may play different roles under cellular conditions. For example, active forces generated by contractile proteins may play a more dominant role (8, 11, 12). The mechanical properties and response of filaments are central to understanding fragmentation in this context because the mechanical heterogeneity and discontinuities introduced by cofilin will lead to unique behaviors that deviate from mechanically homogenous, bare actin filaments (40). Similarly, confinement within the elastic matrix of the cytoplasm (65) will influence the deformation and presumably fragmentation.

Control over Actin Network and Bundle Architecture

Actin cross-linking and bundling proteins assemble filaments into larger scale networks and bundles. Cross-linking

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3 The abbreviations used are: ADF, actin depolymerizing factor; Aip1, actin-interacting protein 1; pN, piconewtons.
proteins are monomeric or homodimeric proteins that contain two filament binding sites. Cross-linking proteins can vary considerably in their affinity, compliance, and distance between actin binding sites. Smaller cross-linkers (e.g. fascin and fimbrin) form compact bundles, whereas larger, more compliant cross-linkers (e.g. filamin and α-actinin) form loose bundles or “mesh-like” networks. Most cross-linkers exhibit both behaviors with the tendency to form at high cross-linker concentrations. When a stress is applied to an actin network or bundle, the deformations and dynamics of filaments and actin cross-linking proteins determine the mechanical response. In general, this mechanical response will, in some circumstances, behave like an elastic solid, and in others, it will behave like a viscous fluid. Thus, actin networks can be considered viscoelastic in their mechanical response.

The architecture of cross-linked actin networks is determined both by filament assembly dynamics (rate of nucleation, growth, and severing) and by factors controlling cross-linking (cross-link kinetics and concentration) and actin filament concentration and length (66, 67). In the absence of cross-linkers, assembled filaments display a homogeneous distribution with a relatively uniform average distance between filaments (termed the “mesh size”). Filaments longer than the mesh size are sterically entangled in the network. Steric entanglements constrain filament motions and, consequently, give rise to viscoelastic behavior (68, 69). Steric entanglements also prevent realignment of filaments into bundles (66). Thus, the architecture of cross-linked networks is determined by competing timescales of bundle formation and the arrested mobility that occurs with entanglement. For a given cross-link type and density, fast growing filaments form fine meshworks, whereas slowly elongating filaments form networks of dense bundles (70). Thus, architecture of cross-linked actin network is kinetically determined, reflecting an arrested or kinetically “trapped” state rather than the lowest energy state defined by true thermodynamic equilibrium (66, 71).

As a result, the actin cytoskeletal architectures found within living cells will exist in a dynamic steady state determined by a balance of filament assembly and disassembly dynamics and regulatory protein interactions. These microscopic rates, as discussed above, may also be force-regulated.

**Actin Network Mechanics**

The mechanical properties of the actin networks will determine the extent and pattern of deformation in response to mechanical stress. The type of response will depend on the spatial scale, direction, duration, and magnitude of the applied stress. For example, at the scale of a single bundle or filament, the mechanics can be dominated by the local compressional, stretching, or bending rigidity of the filament/bundle depending on the direction of the applied force. At longer length scales, the mechanical response is determined both by the mechanics of individual elements as well as by how the filaments/bundles are connected together. Thus, actin network architecture determines the mechanical properties of the elements as well as which types of microscopic deformations occur in response to a macroscopic stress. Below, we describe two regimes that dominate actin network mechanics.

In filament networks or bundles that are densely cross-linked, the deformations are self-similar, or affine, from microscopic to macroscopic length scales. For shear or extensional stress, this results in a mechanical response that is dominated by stretching of filaments or bundles (Fig. 4A). The relationship between the stress (force per unit area) and strain (relative deformation) provides a measure of elastic modulus. The elastic modulus has units of energy per volume and can be thought of as the amount of energy required to deform the medium. The elastic modulus of filament networks is determined both by the actin filament/bundle concentration and the stretching and/or by the bending modulus of the filament/bundle itself. Thus, factors that affect the stretching/bending modulus of the filament or bundle (e.g. time scales and applied stress) will directly impact the macroscopic mechanical response. Indeed, the nonlinearity of the extensional spring constant of actin filaments can directly manifest into a nonlinear stiffening of cross-linked networks in response to stresses applied externally (72, 73) or generated internally (e.g. by myosin motors) (74). Strain stiffening in networks composed of linearly elastic filaments/bundles can also arise from geometric effects (75, 76).

As the cross-link or filament density is reduced, the sparse connectivity within the network results in deformations that are not self-similar (non-affine) from microscopic to macroscopic length scales. For instance, shear deformation at the macroscopic length scale generates filament bending, with a small fraction of filaments/bundles bearing the majority of the stress. Here the bending-dominated response at the microscopic scale results in softening of the network at macroscopic scale.
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The mechanical response of filament networks is nearly always dependent on the measurement frequency. Energy dissipation arises over a broad range of time scales, ranging from filament fluctuations at high frequencies to cross-link unbinding/unfolding and filament turnover at long time scales. Energy dissipation results in the networks behaving like a fluid, whereby applied stress results in a constant rate of deformation (strain rate) and the ratio determines the viscosity. Experiments of stabilized filaments with dynamic cross-links yield viscoelasticity over a large range of time scales, with approximately equal contributions of elasticity and viscosity to the mechanical response. However, it is still uncertain whether networks formed with dynamic filaments could be well approximated as a fluid. Recent simulations suggest that this may be the case (77) as models of actin as a viscous fluid appear to perform reasonably well in recapitulating observed flows in cells (78).

Mechanochemical Feedback in Dynamic Steady States

The response of actin filaments and associated binding proteins to applied stress provides a natural mechanism to modify the large-scale network architecture in response to externally applied or internally generated stresses. For actin networks that are in a dynamic steady state with polymer disassembly/assembly, forces that alter the local rate of filament stability (disassembly), assembly, or binding of actin-binding proteins will induce a transition to a new dynamic steady state. Although the experimental characterization of such mechanochromically responsive actin networks is still in its nascent stages, recent data suggest that distinct modules may differentiate branched versus contractile networks in response to stress.

The filament binding affinity of the Arp2/3 complex depends on the filament curvature (79), raising the possibility that a branched network exhibiting a compressive load could respond both by modulating the branch density and possibly by coflin-mediated severing and filament subunit exchange. Together, this will increase the branch density and decrease average filament length, resulting in increased force generation network stiffness as a result of the applied load.

In contractile networks containing actin and myosin II filaments, network contraction arises from filament bending and buckling (9). This results in a preferential severing of compressed, buckled filaments, thereby altering the local filament end density. In the presence of barbed end capping proteins, this could result in local disassembly from the pointed end, whereby in the presence of barbed end elongation factors, this would result in enhanced assembly. Unraveling how these mechanochromical feedbacks can be used to achieve the dynamic steady states observed in cells and enable cell mechanoensing will be an exciting area of future study.

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