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Journal
Current Opinion in Cell Biology, 25(1)

ISSN
0955-0674

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Publication Date
2013-02-01

DOI
10.1016/j.ceb.2012.11.007

Peer reviewed

Published in final edited form as:
Curr Opin Cell Biol. 2013 February ; 25(1): 6–13. doi:10.1016/j.ceb.2012.11.007.

In vitro studies of actin filament and network dynamics

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Abstract

Now that many genomes have been sequenced, a central concern of cell biology is to understand how the proteins they encode work together to create living matter. In vitro studies form an essential part of this program because understanding cellular functions of biological molecules often requires isolating them and reconstituting their activities. In particular, many elements of the actin cytoskeleton were first discovered by biochemical methods and their cellular functions deduced from in vitro experiments. We highlight recent advances that have come from in vitro studies, beginning with studies of actin filaments, and ending with multi-component reconstitutions of complex actin-based processes, including force-generation and cell spreading. We describe both scientific results and the technical innovations that made them possible.

Actin filament architecture

The actin cytoskeleton plays a role in many different cellular processes, including maintenance of cell shape and mechanics, membrane trafficking, cell division, and locomotion. Assembly of functional actin networks (Figure 1) requires collaboration between a variety of regulators—filament crosslinkers, nucleation and elongation factors, and motor proteins—but the basic unit of these networks is the actin filament.

Recent work has significantly deepened our understanding of actin filament architecture and the ways in which regulatory proteins modulate it. The atomic structure of monomeric actin was solved in 1980 but definitive high-resolution structures of filamentous actin have proven harder to obtain. The best filament models derive from electron microscopy and image reconstruction. New methods of sample preparation and data collection have now pushed the resolution of actin filament models well below 10 Å and provide mechanistic explanations for key features of filament assembly.

Namba and co-workers [1••], for example, made use of energy filtering, optimal ice thickness, and liquid helium cooling to obtain high-contrast images of frozen, hydrated actin filaments. From these they computed a filament model with 6.6 Å resolution, one which clearly resolves protein secondary structures. In their filament, the four subdomains of actin are rotated to flatten the molecule against the filament axis. Longitudinal interactions between protomers along the two-start helices turn out to be very strong, while interactions between the two strands of the filament are weaker than expected. The ‘hydrophobic plug’, for example, which was proposed to connect the two strands, does indeed make cross-strand connections but they turn out to be hydrophilic in nature.

In a similar study, Wakabayashi and colleagues [2••] stabilized actin filaments with high concentrations of phosphate and obtained a reconstruction with 5 Å resolution. These authors identified both Mg^{++} and phosphate ions in their filament and proposed a

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convincing mechanism by which ATP hydrolysis is stimulated by filament formation. They also identified a 6 Å × 18 Å cylindrical 'tunnel' in the heart of the filament, through which phosphates, cleaved from ATP, escape. At the upper end of this 'phosphate escape tunnel' the authors identified a phosphate binding site (Figure 2) that stabilizes the interaction of three adjacent protomers in the filament, providing a molecular explanation for both the slow kinetics of phosphate release and the stability of ADP-Pi actin filaments.

Some cytoskeletal regulators have evolved to make use of the conformational flexibility of actin filaments. When the actin depolymerization factor cofilin, for example, binds a filament, it alters the helical pitch [3] and this alteration propagates for some distance down the filament. The spread of this conformational change enables cofilin to communicate its presence to other nearby actin binding proteins. This promotes the binding of additional cofilin molecules while greatly accelerating dissociation of the Arp2/3 complex, a filament nucleating and branching factor [4]. This effect has been estimated to propagate over a distance of seven monomers, almost 20 nm. It is appealing to imagine actin filaments as conduits for communication between actin binding proteins. Such a mechanism could explain the simultaneous construction of distinct actin networks (*e.g.* 'lamellipodial' and 'lamellar' actin networks near the leading edges of migrating cells), with different architectures and different cadres of binding proteins, in more-or-less the same space.

Actin filament dynamics

Early studies of actin dynamics relied on interpreting bulk properties of actin-containing solutions, such a flow birefringence, light scattering, or the fluorescence of environmentally sensitive dyes. Assays like these remain important—partly because they are easy—but they provide less information than methods based on imaging individual filaments. The first single-filament studies of actin relied on electron microscopy but, around twenty years ago, improvements in (1) light microscope optics; (2) sensitivity of CCD cameras; and (3) coupling of fluorescent dyes made it relatively easy to image single, dynamic actin filaments. More recent technical innovations have further expanded the power and scope of single filament studies. These innovations include: (1) the use of total internal reflection fluorescence (TIRF) microscopy, (2) improved chemical passivation of glass surfaces, (3) improved methods for immobilizing proteins, and (4) the use of microfluidics to rapidly switch buffer conditions and protein concentrations.

Single-filament TIRF studies were critical in demonstrating that some actin regulators are insertional polymerases, surfing on the ends of growing filaments and accelerating their elongation. Formin-family proteins were the first to be shown to have this activity. Their interaction with actin filaments is remarkably processive, with individual formin molecules remaining attached to a growing end for more than 1000 s [5]. Similar experiments demonstrated that Ena/VASP proteins also accelerate filament elongation, but interact with growing filaments by a different mechanism and fall off much more quickly than formins, after only 1–2 s [6,7•,8•]. Importantly, these in vitro experiments resolved outstanding questions about the activity of Ena/VASP proteins. From in vivo experiments, these proteins were known to promote polymerization [9,10] but the molecular mechanism was, for several years, unclear.

New dyes and more sensitive cameras have expanded the color range of fluorescence microscopy beyond the traditional reds and greens. A recent, and beautiful, three-color TIRF experiment, for example, uncovered a remarkable collaboration between two actin regulators: a filament nucleation factor, adenomatous polyposis coli (APC), and a formin-family polymerase, mammalian Diaphanous 1 (mDia1) [11••]. These molecules interact to form a complex which both nucleates new filaments and promotes their rapid growth. The

nucleation factor, APC, remains associated with the filament at the site of nucleation while the polymerase, mDia1, dissociates and rides away on the growing barbed end. This has been described as a 'rocket-launcher' mechanism and may explain interactions observed between other actin nucleators and polymerases (*e.g.* the spire-family nucleators and cappuccino-family formins [12]).

In addition to facilitating new discoveries, new experimental techniques can also be used to revisit long-standing questions. Arguably the most sophisticated system for studying single filament dynamics was developed by Jégou *et al.* [13••] to study disassembly of actin filaments and kinetics of phosphate release following ATP hydrolysis. These authors combined TIRF microscopy with microfluidics to enable them to both image single filaments and to rapidly change protein concentrations. They minimized interaction between proteins and glass surfaces by using spectrin–actin complexes to attach their filaments to coverslips only at one end. To measure lengths they used fluid flow to align their filaments and stretch them out parallel to the coverslip. To study depolymerization, they first grew long filaments from attached seeds then initiated disassembly by rapidly diluting the monomeric actin. By carefully monitoring the rate of filament disassembly and correlating it with the nucleotide state of the filament, Jégou and co-workers showed convincingly that phosphate dissociation from actin protomers is a random process that is not influenced by neighboring molecules. This is true for all but the terminal subunits in the filament, which appear to lose their phosphate groups more rapidly than subunits deeper in the filament.

Initiating actin filament assembly

Several factors are now known to nucleate new actin filaments, including the Arp2/3 complex, formin-family proteins, and spire-like proteins. At first glance, the spire-family proteins would seem the easiest to understand: they bind multiple actin monomers and stabilize their interaction [14]. Newly solved atomic structures of spire-actin complexes, however, reveal that spire does not position monomers in the orientation found in an intact filament [15,16•]. This discovery might provide a structural explanation for recent biochemical studies demonstrating that spire can profoundly destabilize preformed filaments [17,16•]. Based on these results, it might be best to think of spire-family proteins as constructing transitional structures, intermediate between monomers and filaments. Like an enzyme stabilizing a transition state, spire might facilitate both forward and reverse reactions: promoting filament assembly in some cases and disassembly in others.

The mechanism of nucleation by formins is still a mystery. In fact, not all formins are capable of nucleating new filaments in vitro and, even for the nucleation-competent formins, it is unclear whether their primary role in cells is to create new filaments or enhance growth of filaments nucleated by other mechanisms [11••]. Formin mutations that uncouple nucleation from processive polymerase activity would be extremely useful in sorting out this question.

The Arp2/3 complex contains what appears to be a cryptic filament barbed end, constructed from the actin-related proteins, Arp2 and Arp3. It binds preexisting filaments and creates new filaments that grow as branches off the older ones. The activity of the complex is controlled by upstream regulators, called nucleation promoting factors (NPFs). Rather than being a simple switch, however, the complex is more like a Chinese Puzzle Box that requires binding of two nucleation promoting factors [18], and a handoff of actin monomers from the activators to the complex [19,20•], that induces a conformational change [21,19]. The two NPF-binding sites on the complex have significantly different affinities and each appears to execute a distinct step in the nucleation reaction [22,23] but the details of the activation mechanism are still under study.

The activity of nucleation promoting factors is regulated, in turn, by upstream signaling molecules, including small G-proteins. N-WASP was the first NPF whose regulation was understood at the molecular level [24]. The regulation of WAVE, which helps generate actin filaments near the leading edges of motile cells, has proven more complicated. WAVE proteins exist as part of a five-subunit complex (the WAVE Regulatory Complex or WRC). Recent work using recombinant WRC is now beginning to fill some of the gaps in our understanding. Like N-WASP, the WRC is auto-inhibited and integrates multiple upstream inputs, including Rac1 and Arf1 GTPases [25••], PI(3,4,5)P_3, and IRSp53 [26,27,28••]. Phospohorylation of the native WAVE2 complex is also required for Rac2-GTP and PIP_3 to function as activators [27].

Assembling complex, biomimetic structures in vitro: protrusive networks and spreading cells

Over a decade ago the Carlier laboratory succeeded in reconstituting assembly of motile, dendritic actin networks from purified components [29]. They constructed branched filament networks with the Arp2/3 complex, activated by immobilized NPFs, and modulated by accessory proteins, including capping protein. Since that time, the field has developed increasingly sophisticated reconstitutions and used them to study many different aspects of actin network assembly and function. One important difference between reconstitution systems is the method by which they control the localization of active nucleation promoting factors. Substrates used to localize nucleation promoting factors include (Figure 3): (1) rigid particles, (2) lipid-coated particles, (3) lipid vesicles with external actin networks, (4) lipid vesicles encapsulating internal actin networks, and (5) encapsulating vesicles interacting with solid surfaces. Each localization method has advantages and disadvantages and the choice of method is generally dictated by the question being asked.

Rigid particles, uniformly coated with covalently attached nucleation promoting factors are excellent analogs for pathogens that hijack the actin cytoskeleton to move from cell to cell. Such particles are relatively simple to produce and have been used to study many aspects of network formation, including mechanical properties of Arp2/3-generated actin networks. Kawska *et al.* [30] recently followed the growth of small, dendritic subnetworks on the surfaces of polystyrene microspheres and watched them merge into a single, entangled structure. Interestingly, the final, unified network appears to retain weak spots at the boundaries between the original subnetworks that can fracture under mechanical loading.

Nucleation promoting factors covalently attached to rigid surfaces can activate the Arp2/3 complex but, because they are not free to move, their own dynamics cannot be studied. Coating a solid surface with a lipid bilayer, enables membrane-anchored nucleation promoting factors to diffuse and rearrange in response to network growth. Lipid-coated microspheres are also amenable to reconstitution of more complicated upstream signaling events that control actin assembly and in studying the role of lipids in the regulation of actin assembly. In a beautiful recent study, Koronakis *et al.* [25••] mapped the signaling landscape for actin networks assembled from lipid bilayers of different compositions. By constructing and isolating actin networks from lipid-coated microspheres in brain extracts, they discovered that two small G proteins, Rac1 and Arf1, synergize to activate the WAVE regulatory complex. Consistent with previous reports, they also found that N-WASP- and WAVE-dependent actin assembly are promoted by different phospholipids: PIP_2 and PIP_3, respectively. PIP_3 has been specifically implicated in regulating WAVE complex activity but it remains unclear whether this effect is owing to localization or allosteric activation.

In vivo, the assembly of actin filaments around unsupported vesicle occurs during many processes, including late stages of endocytosis. Unilamellar vesicles are more difficult to

produce in vitro, but, in addition to mimicking physiological processes, the release of mechanical constraints makes them useful for answering basic questions that cannot be addressed using lipid-coated particles. For example, actin-dependent deformation of vesicles in vitro has been used to calculate mechanical forces generated by growing actin networks [31]. Also, by growing actin networks on the surfaces of giant unilamellar vesicles, the Fletcher laboratory discovered that actin assembly can drive large-scale phase separation of lipids [32]. This group also used giant unilamellar vesicles to reconstitute formation of filopodia that project into the vesicle lumen [33].

To fully reconstitute the actin-membrane interactions that control cell shape, mechanics, and motility requires reproducing the geometry of the cell itself. That is, we must encapsulate actin and its regulatory factors inside closed, membrane-bound compartments. Controlled encapsulation of soluble and membrane-associated proteins inside well defined, unilamellar vesicles remains difficult but several new technologies now make this process easier. Reverse emulsion techniques can produce vesicles with well defined contents, surrounded by lipid bilayers of a specified composition [34]. Encapsulation techniques that utilize microfluidic jetting are even more technically challenging but have many important advantages [35,36,37•]: (a) they produce vesicles of a highly uniform size and (b) they can mix multiple solutions at the moment of encapsulation to initiate a biochemical reaction inside the vesicle. Several groups have encapsulated actin and observed shape changes in vesicles upon polymerization. Recently, however, the Sykes group used reverse emulsion techniques to encapsulate membrane-associated nucleation promoting factors along with actin and other factors required to generate dynamic, Arp2/3-nucleated actin networks [38]. They used these internal networks as a model for the cell cortex and they characterized the physical and geometrical constraints controlling the thickness of the actin network and the rate at which filaments in the network turn over. This is an important first step toward reconstituting more dramatic cell-like behaviors, including: (a) localized control of mechanical properties; (b) regulated shape change and; ultimately, (c) directed migration. One requirement to reconstitute, or at least mimic, migration on a two-dimensional surface is adhesion. In a recent, and very clever, study the Sykes group encapsulated a minimal system for reconstituting Arp2/3-dependent actin assembly inside lipid vesicles, made with nickel-conjugated lipids [39•]. After allowing time for the actin inside the vesicles to polymerize, the authors dropped them onto glass surfaces coated with poly-histidine. Tight interactions between nickel and poly-histidine drove spreading of the vesicles across the glass, enabling the Sykes group to study how the mechanics of the underlying actin network affects spreading dynamics. A next, obvious step along this line will be to use actin assembly itself to generate forces that drive spreading.

In addition to regulated polymerization and network assembly, several groups are now reconstituting and studying the behavior of motor-powered, contractile actin networks. In one set of studies [40•,41], actin filaments were tethered, with random orientations, to rigid microspheres, which were, in turn, attached to an elastic, poly-acrylamide substrate (Figure 4a). Actin filaments attached to adjacent particles often overlapped and, in the presence of myosin and ATP, these overlapping filaments became crosslinked into mixed-polarity bundles. Motor activity of the myosin molecules caused the bundles to contract and contractile forces were measured by displacement of the actin-associated particles. Remarkably, the myosin motors also self-organized into discrete clusters, similar to those observed in vivo. In a recent and very elegant study, Reymann *et al.* [42••] patterned nucleation promoting factors on a glass surface and used them to create Arp2/3-generated actin networks (Figure 4b) in which different fractions of filaments overlapped in parallel or anti-parallel orientations. When these authors added myosin motors, the networks became contractile but their behavior depended strongly on local network architecture. When they altered the pattern to increase the number of antiparallel filaments they observed stronger

contraction and pronounced filament disassembly. This represents the most satisfying reconstitution to date of a type of myosin-dependent actin network disassembly thought to occur in vivo.

This is an exciting time in the field. Complex reconstitutions are now enabling us to answer important, and previously inaccessible, questions in basic cell biology. Soon they may enable us to engineer new biomimetic devices that may find applications in nanotechnology and/or medicine.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1••. Fujii T, Iwane AH, Yanagida T, Namba K. Direct visualization of secondary structures of F-actin by electron cryomicroscopy. Nature. 2010; 467:724–728. This paper presents a high resolution (6.6 Å) structure of filamentous actin, derived from cryo-electron microscopy and image averaging. The structure reveals new details about lateral and longitudinal interactions that hold the filament together. The 'hydrophobic' plug, for example, which has been proposed to connect the two strands of the filament, turns out to be a 'hydrophilic' plug. [PubMed: 20844487]

2••. Murakami K, Yasunaga T, Noguchi TQ, Gomibuchi Y, Ngo KX, Uyeda TQ, Wakabayashi T. Structural basis for actin assembly, activation of ATP hydrolysis, and delayed phosphate release. Cell. 2010; 143:275–287. Similar to [1••], this paper presents a 5 Å resolution structure of phosphate-stabilized actin filaments. These authors focused on understanding the structural basis of ATP hydrolysis and phosphate release. They propose that a specific, polymerization-linked conformational positions a catalytic water in proximity to the gamma phosphate of bound ATP. They also identified bound Mg^{++} and phosphate ions and proposed a mechanism by which bound phosphate stabilizes actin filaments. [PubMed: 20946985]

3. McGough A, Pope B, Chiu W, Weeds A. Cofilin changes the twist of F-actin: implications for actin filament dynamics and cellular function. J Cell Biol. 1997; 138:771–781. [PubMed: 9265645]

4. Chan C, Beltzner CC, Pollard TD. Cofilin dissociates Arp2/3 complex and branches from actin filaments. Curr Biol. 2009; 19:537–545. [PubMed: 19362000]

5. Kovar DR, Pollard TD. Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces. Proc Natl Acad Sci USA. 2004; 101:14725–14730. [PubMed: 15377785]

6. Breitsprecher D, Kiesewetter AK, Linkner J, Urbanke C, Resch GP, Small JV, Faix J. Clustering of VASP actively drives processive, WH2 domain mediated actin filament elongation. EMBO J. 2008; 27:2943–2954. [PubMed: 18923426]

7•. Hansen SD, Mullins RD. VASP is a processive actin polymerase that requires monomeric actin for barbed end association. J Cell Biol. 2010; 191:571–584. This paper demonstrates that VASP is a weakly processive polymerase that accelerates barbed end filament elongation. Using single-molecule imaging technique the authors track individual VASP tetramers as they diffuse along the sides of actin filaments and deliver monomers to growing barbed ends. [PubMed: 21041447]

8•. Breitsprecher D, Kiesewetter AK, Linkner J, Vinzenz M, Stradal TE, Small JV, Curth U, Dickinson RB, Faix J. Molecular mechanism of Ena/VASP-mediated actin-filament elongation. EMBO J. 2011; 30:456–467. This paper demonstrates that enhanced actin elongation, driven by Ena/VASP proteins, depends critically on properties of the (WH2-like) G-actin binding domain of VASP. They identify a direct correlation between affinity for monomeric actin and enhancement of elongation and propose a mathematical model to describe the effect. [PubMed: 21217643]

9. Bear JE, Svitkina TM, Krause M, Schafer DA, Loureiro JJ, Strasser GA, Maly IV, Chaga OY, Cooper JA, Borisy GG. Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. Cell. 2002; 109:509–521. [PubMed: 12086607]

10. Svitkina TM, Bulanova EA, Chaga OY, Vignjevic DM, Kojima S, Vasiliev JM, Borisy GG. Mechanism of filopodia initiation by reorganization of a dendritic network. J Cell Biol. 2003; 160:409–421. [PubMed: 12566431]

11••. Breitsprecher D, Jaiswal R, Bombardier JP, Gould CJ, Gelles J, Goode BL. Rocket launcher mechanism of collaborative actin assembly defined by single-molecule imaging. Science. 2012; 336:1164–1168. This paper provides experimental evidence for a new mechanism of collaboration between actin nucleators and polymerases. The authors demonstrate that an actin nucleation factor, adenomatous polyposis coli, and a filament elongation factor, a diaphanous-family formin, form a complex that produces a new actin filament. Remarkably, the generation of the new filament disrupts the complex and drives the two components apart. [PubMed: 22654058]

12. Quinlan ME, Hilgert S, Bedrossian A, Mullins RD, Kerkhoff E. Regulatory interactions between two actin nucleators, Spire and Cappuccino. J Cell Biol. 2007; 179:117–128. [PubMed: 17923532]

13•. Jégou A, Niedermayer T, Orbán J, Didry D, Lipowsky R, Carlier MF, Romet-Lemonne G. Individual actin filaments in a microfluidic flow reveal the mechanism of ATP hydrolysis and give insight into the properties of profilin. PLoS Biol. 2011; 9:e1001161–02022. This paper describes a sophisticated system for studying the dynamics of single actin filaments. It combines: (1) microfluidic switching of protein concentrations and buffer conditions; (2) flow orientation of end-tethered filaments; and (3) total internal reflection fluorescence microscopy. [PubMed: 21980262]

14. Quinlan ME, Heuser JE, Kerkhoff E, Mullins RD. Drosophila Spire is an actin nucleation factor. Nature. 2005; 433:382–388. [PubMed: 15674283]

15. Ducka AM, Joel P, Popowicz GM, Trybus KM, Schleicher M, Noegel AA, Huber R, Holak TA, Sitar T. Structures of actin-bound Wiskott-Aldrich syndrome protein homology 2 domains of Spire and the implication for filament nucleation. Proc Natl Acad Sci USA. 2010; 107:11757–11762. [PubMed: 20538977]

16•. Chen CK, Sawaya MR, Phillips ML, Reisler E, Quinlan ME. Multiple forms of Spire-actin complexes and their functional consequences. J Biol Chem. 2012; 287:10684–10692. This paper describes multiple spire-actin complexes and demonstrates that, in some conditions, spire can promote filament formation while, under other conditions, it can promote filament disassembly. [PubMed: 22334675]

17. Husson C, Renault L, Didry D, Pantaloni D, Carlier MF. Cordon-Bleu uses WH2 domains as Multifunctional organizers if actin filament assembly. Mol Cell. 2011; 43:464–477. [PubMed: 21816349]

18. Padrick SB, Cheng HC, Ismail AM, Panchal SC, Doolittle LK, Kim S, Skehan BM, Umetani J, Brautigam CA, Leong JM, et al. Hierarchical regulation of WASP/WAVE proteins. Mol Cell. 2008; 32:426–438. [PubMed: 18995840]

19. Dayel MJ, Mullins RD. Activation of Arp2/3 complex: addition of the first subunit of the new filament by the WASP protein triggers rapid ATP hydrolysis on Arp2. PLoS. 2004; 2:0476–0485.

20•. Padrick SB, Doolittle LK, Brautigam CA, King DS, Rosen MK. Arp2/3 complex is bound and activated by two WASP proteins. Proc Natl Acad Sci USA. 2011; 108:E472–E479. This paper was the first to identify a second binding site for nucleation promoting factors on the Arp2/3 complex and to demonstrate that maximal nucleation activity requires engagement of both sites. [PubMed: 21676863]

21. Rouiller I, Xu XP, Amann KJ, Egile C, Nickell S, Nicastro D, Li R, Pollard TD, Volkmann N, Hanein D. The structural basis of actin filament branching by the Arp2/3 complex. J Cell Biol. 2008; 180:887–895. [PubMed: 18316411]

22. Xu X, Rouiller I, Slaughter BD, Egile C, Kim E, Unruh JR, Fan X, Pollard TD, Li R, Hanein D, et al. Three-dimensional reconstructions of Arp2/3 complex with bound nucleation promoting factors. EMBO J. 2011; 31:236–247. [PubMed: 21934650]

23. Ti S, Jurgenson CT, Nolan BJ, Pollard TD. Structural and biochemical characterization of two binding sites for nucleation-promoting factor WASp-VCA on Arp2/3 complex. Proc Natl Acad Sci USA. 2011; 108:E463–E471. [PubMed: 21676862]

24. Rohatgi R, Nollau P, Ho HY, Kirschner MW, Mayer BJ. Nck and phosphatidylinositol 4,5-bisphosphate synergistically activate actin polymerization through the N-WASP-Arp2/3 pathway. J Biol Chem. 2001; 276:26448–26452. [PubMed: 11340081]

25••. Koronakis V, Hume PJ, Humphreys D, Lui T, Horning O, Jensen ON, McGhie EJ. WAVE regulatory complex activation by cooperating GTPases Arf and Rac1. Proc Natl Acad Sci USA. 2011; 108:14449–14454. These authors use lipid-coated microspheres and bovine brain extracts to demonstrate that small GTPases, Rac1 and Arf1, act synergistically to stimulate the nucleation promoting activity of the WAVE complex. [PubMed: 21844371]

26. Ismail AM, Padrick SB, Chen B, Umetani J, Rosen MK. The WAVE regulatory complex is inhibited. Nat Struct Mol Biol. 2009; 16:561–563. [PubMed: 19363480]

27. Lebensohn AM, Kirschner MW. Activation of the WAVE complex by coincident signals controls actin assembly. Mol Cell. 2009; 36:512–524. [PubMed: 19917258]

28••. Chen Z, Borek D, Padrick SB, Gomez TS, Metlagel Z, Ismail AM, Umetani J, Billadeau DD, Otwinowski Z, Rosen MK. Structure and control of the actin regulatory WAVE complex. Nature. 2010; 468:533–538. This paper describes the first atomic-resolution structure of the five-subunit WAVE regulatory complex and provides insight into the mechanisms of WAVE's autoinhibition and activation. [PubMed: 21107423]

29. Loisel TP, Boujemaa R, Pantaloni D, Carlier MF. Reconstitution of actin-based motility of Listeria and Shigella using pure proteins. Nature. 1999; 401:613–616. [PubMed: 10524632]

30. Kawska A, Carvalho K, Manzi J, Boujemaa-Paterski R, Blanchoin L, Martiel JL, Sykes C. How actin network dynamics control the onset of actin-based motility. Proc Natl Acad Sci USA. 2012; 109:14440–14445. [PubMed: 22908255]

31. Giardini PA, Fletcher DA, Theriot JA. Compression forces generated by actin comet tails on lipid vesicles. Proc Natl Acad Sci USA. 2003; 100:6493–6498. [PubMed: 12738883]

32. Liu AP, Fletcher DA. Actin polymerization serves as a membrane domain switch in model lipid bilayers. Biophys J. 2006; 91:4064–4070. [PubMed: 16963509]

33. Liu AP, Richmond DL, Maibaum L, Pronk S, Geissler PL, Fletcher DA. Membrane-induced bundling of actin filaments. Nat Phys. 2008; 4:789–793. [PubMed: 19746192]

34. Pautot S, Frisken BJ, Weitz DA. Engineering asymmetric vesicles. Proc Natl Acad Sci USA. 2003; 100:10718–10721. [PubMed: 12963816]

35. Richmond DL, Schmid EM, Martens S, Stachowiak JC, Liska N, Fletcher DA. Forming giant vesicles with controlled membrane composition, asymmetry, and contents. Proc Natl Acad Sci USA. 2011; 108:9431–9436. [PubMed: 21593410]

36. Stachowiak JC, Richmond DL, Li TH, Brochard-Wyart F, Fletcher DA. Inkjet formation of unilamellar lipid vesicles for cell-like encapsulation. Lab Chip. 2009; 9:2003–2009. [PubMed: 19568667]

37•. Stachowiak JC, Richmond DL, Li TH, Liu AP, Parekh SH, Fletcher DA. Unilamellar vesicle formation and encapsulation by microfluidic jetting. Proc Natl Acad Sci USA. 2008; 105:4697–4702. This paper describes a method for encapsulating defined, protein-containing solutions inside lipid vesicles by using microfluidic jetting. One significant advantage of this approach is the ability to mix two solutions together at the moment of encapsulation. [PubMed: 18353990]

38. Pontani LL, van der Gucht J, Salbreux G, Heuvingh J, Joanny JF, Sykes C. Reconstitution of an actin cortex inside a liposome. Biophys J. 2009; 96:192–198. [PubMed: 19134475]

39•. Murrell M, Pontani LL, Guevorkian K, Cuvelier D, Nassoy P, Sykes C. Spreading dynamics of biomimetic actin cortices. Biophys J. 2011; 100:1400–1409. This paper describes the closest biomimetic model produced to date of an adherent, spreading cell. The authors encapsulate actin and some of its assembly regulators inside lipid vesicles that contain a small amount of Ni^{+}-conjugated lipids. When dropped onto poly-histidine-coated cover-slips, the vesicles undergo adhesion-driven spreading, whose kinetics are determined by the state of the actin cytoskeleton inside the vesicle. [PubMed: 21402021]

40•. Thoresen T, Lenz M, Gardel ML. Reconstitution of contractile actomyosin bundles. Biophys J. 2011; 100:2698–2705. These authors develop a system for generating contractile actin bundles and measuring the forces they generate. [PubMed: 21641315]

41. Stachowiak MR, McCall PM, Thoresen T, Balcioglu HE, Kasiewicz L, Gardel ML, O'Shaughnessy B. Self-organization of myosin II in reconstituted actomyosin bundles. Biophys J. 2012; 103:1265–1274. [PubMed: 22995499]

42••. Reymann AC, Boujemaa-Paterski R, Martiel JL, Guerin C, Cao W, Chin HF, De La Cruz EM, Thery M, Blanchoin L. Actin network architecture can determine myosin motor activity. Science. 2012; 336:1310–1314. This paper describes a set of experiments that reconstitute motor-dependent depolymerization of actin networks. The authors use micro-patterned glass surfaces to generate overlapping, anti-parallel actin filament arrays. In the presence of processive myosin motors these arrays contract. Motor-driven contraction also promotes disassembly of the network, a process that resembles motor-dependent network disassembly observed in vivo. [PubMed: 22679097]

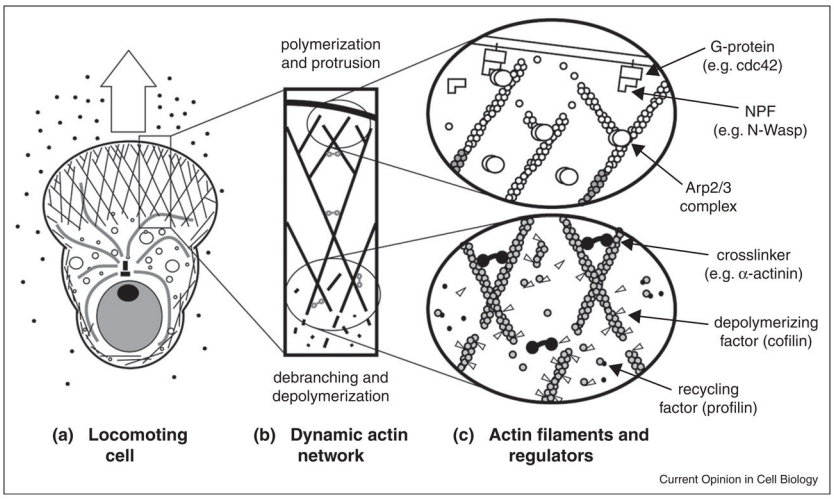

Figure 1.

Levels of complexity in the study of the actin cytoskeleton. **(A)** Eukaryotic cells rely on the actin cytoskeleton to maintain their shape, to control their mechanical properties, to shuffle internal membranes, and to move. **(B)** Dynamic actin networks, such as the lamellipodial actin network under the leading edge of an advancing cell must be assembled and disassembled on a rapid time scale. **(C)** The cellular functions of many actin filament binding proteins have been inferred from in vitro biochemical and biophysical studies. Specific activities that have been reconstituted in vitro include actin filament nucleation, capping, crosslinking, depolymerization, and monomer recycling. One frontier of modern in vitro studies is the reconstitution of complex network behaviors from combinations of actin and its associated regulators.

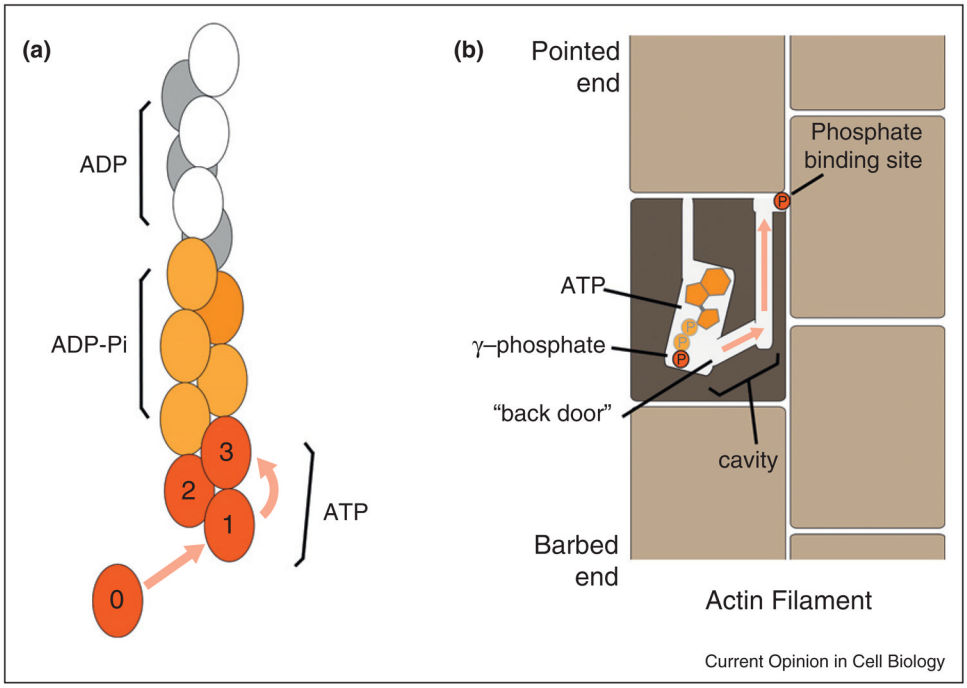

Figure 2.

Model for polymerization-dependent ATP hydrolysis in an actin filament and the subsequent dissociation of the cleaved phosphate. **(A)** Based on high-resolution filament models derived from electron microscopy Wakabayashi and co-workers [2••] proposed that the three terminal subunits at the fast-growing barbed end of the filament are not in the proper conformation to hydrolyze ATP. Binding of an additional subunit (labeled 0) to the end of the filament alters the position of the terminal monomer (labeled 1), shifting a helix from subdomain 2 of monomer 1 into a pocket between subdomains 1 and 3 of the protomer above it (labeled 3). According to the model, this brings a catalytic water into close proximity with the gamma phosphate in monomer 3 and stimulates ATP hydrolysis. **(B)** Release of cleaved phosphate from the actin filament. Wakabayashi and colleagues [2••] identified a 'cavity' in near the center of the actin filament through which they propose cleaved gamma phosphate groups could exit the filament. In their high-resolution filament model they discovered a phosphate bound to a site near the exit of this cavity. The bound phosphate makes contact with three adjacent protomers in the filament is proposed to stabilize the filament structure.

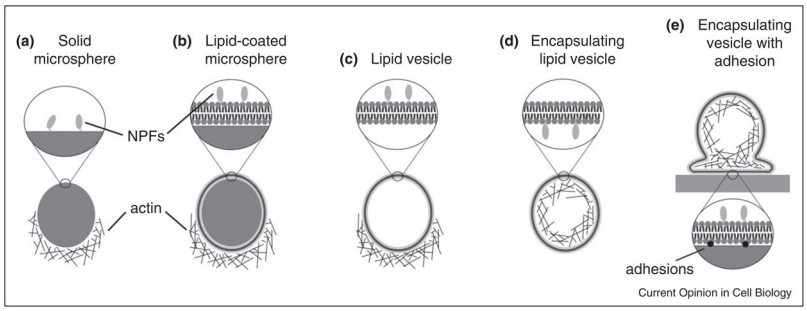

Figure 3.

Substrates and attachment geometries used for in vitro reconstitution of dynamic actin networks. **(a)** Nucleation promoting factors attached to solid particles, including glass beads and polystyrene microspheres [30]. This system mimics certain pathogens that have evolved to exploit the host cell cytoskeleton [29]. **(b)** Lipid-coated particles, including glass microspheres, are useful to maintain mobility of nucleation promoting factors and to study the contribution of lipids to the control of actin assembly [25••]. **(c)** Lipid vesicles have been used to assemble external actin networks. These systems can be used to probe forces actin on the surface and to mimic actin-dependent movement of endomembranes [31,32,33]. **(d)** Lipid vesicles encapsulating internal actin networks are more difficult to make and control but they mimic the geometry of actin networks that form the cell cortex [38]. **(e)** A lipid vesicle encapsulating a dynamic actin network and adhering tightly to a glass substrate mimics some of the features of a spreading cell [39•].

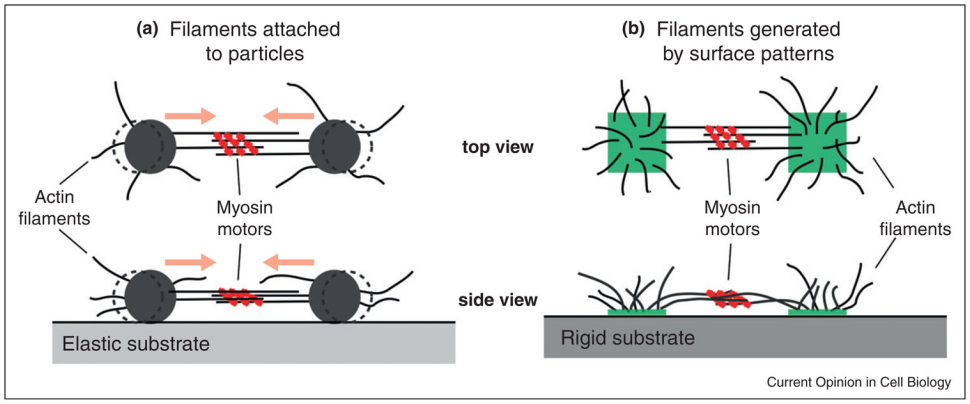

Figure 4.

Reconstitution of contractile actin networks. **(a)** Thoresen *et al.* [40•] attached actin filaments to rigid microspheres using biotin-neutravidin linkages. The microspheres were attached to a flexible, poly-acrylamide substrate. When they added myosin II thick filaments (red particles) at sufficiently high concentrations they bundled overlapping filaments from adjacent particles. In the presence ATP, the bundles contracted, displacing the microspheres from their original positions (dashed circles). Knowing the stiffness of the substrate, Thorensen *et al.* were able to calculate force-velocity curves for their contractile bundles. **(b)** Reymann *et al.* [42••] generated actin filaments by immobilizing nucleation promoting factors to microscale patterns on a glass surface and then using the Arp2/3 complex to nucleate filament assembly from the pattern. When two NPF patches of were placed in proximity, they produced a zone of anti-parallel filament overlap. When the authors added the processive, minus end-directed motor protein, Myosin VI, it crosslinked the bundles and produced strong contractile forces that eventually detached the network from the micropatterned surface and caused it to disassemble.