Comparison of Filamin A-induced Cross-linking and Arp2/3 Complex-mediated Branching on the Mechanics of Actin Filaments* [S]

Received for publication, November 27, 2001
Published, JBC Papers in Press, January 10, 2002, DOI 10.1074/jbc.M111297200

Fumihiko Nakamura, Eric Osborn, Paul A. Janmey, and Thomas P. Stossel‡

From the Hematology Division, Brigham and Women’s Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115

We compared the effects of human filamin A (FLNa) and the activated human Arp2/3 complex on mechanical properties of actin filaments. As little as 1 FLNa to 800 polymerizing actin monomers induces a sharp concentration-dependent increase in the apparent viscosity of 24 μm actin, a parameter classically defined as a gel point. The activated Arp2/3 complex, at concentrations up to 1:25 actins had no detectable actin gelation activity, even in the presence of phalloidin, to stabilize actin filaments against debranching. Increasing the activated Arp2/3 complex to actin ratio raises the FLNa concentration required to induce actin gelation, an effect ascribable to Arp2/3-mediated actin nucleation resulting in actin filament length diminution. Time lapse video microscopy of microparticles attached to actin filaments or photoactivation of fluorescence revealed actin microscopy of microparticles attached to actin filaments in contrast to diffusion of Arp2/3-branched actin filaments. The experimental results support theories predicting that polymer branching absent cross-linking does not lead to polymer gelation and are consistent with the observation that cells deficient in actin filament cross-linking activity have unstable surfaces. They suggest complementary roles for actin branching and cross-linking in cellular actin mechanics in vivo.

The major ingredient of living cells is water, but unlike water, cells exhibit elastic as well as viscous properties. The elasticity of cells, historically designated as gel-like, results from the interactions of intracellular solute, principally cytoskeletal polymers. One of these polymers, actin, dominates the periphery of motile non-muscle cells and accounts for their susceptibility to gelation by imposition of cross-linking of these filaments is necessary to provide sufficient coherence to account for the protrusive activity and deformation resistance of cells. Cells contain many actin filament-cross-linking proteins to accomplish this task.

A phase transition that marks the cross-linking of actin filaments into a cohesive structure is an easily discernable abrupt consistency increase inducible by imposing a critical number of actin filament-cross-linking molecules. This transition point, the system converts from predominantly overlapping linear chains into a giant continuously linked molecular structure, operationally defined as a gel. With other polymers, the length of actin filaments is directly proportional to their susceptibility to gelation by imposition of cross-links.

The first actin filament cross-linking protein identified in non-muscle cells was filamin A (FLNa), previously named actin-binding protein or ABP-280 (17, 18). Filamin A increases the consistency of filamentous actin abruptly at concentrations close to the cross-link value predicted from statistical first principles (1), implying that each FLNa molecule recruits an actin filament into the three-dimensional network. In direct comparisons with FLNa, higher concentrations of other actin filament-cross-linking proteins such as myosin or α-actinin are necessary to bring the same actin preparation to its gel point (12, 20). Because all of these proteins bind two actin filaments with similar apparent affinities (21), the functional differences in actin filament cross-linking by different agents are attributable to variations in the geometry of actin filament overlap angles they impose. FLNa promotes high angle branching of actin filaments. This branching, documented in electron micrographs (22–24), prevents redundant cross-linking of closely aligned filaments. The orthogonal branching morphology of actin cytoskeletal networks observable in de-membranated cells spreading on surfaces strikingly resembles that of reconstituted actin-FLNa gels constituted in vitro, and specific anti-FLNa antibodies selectively label actin filament crossover points in such cytoskeletons. The antibodies label actin filament T-shaped and Y-shaped junctions (branches) as well as X-shaped overlaps (cross-links) (24–28).

† Mammalian cells that constitutively do not express FLNa

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental videos that relate to Fig. 3.

* This research was supported by the United States Public Health Service, National Institutes of Health Grant HL19429 and HL54145, by the Edwin S. Webster Foundation, and by the American Cancer Society. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: FLNa, filamin A; N-WASp, neural Wiskott-Aldrich syndrome protein; V, verproline-like region; C, central region; A, acidic region; GST, glutathione S-transferase; PIPES, 1,4-piperazinediethanesulfonic acid; PAF, photoactivation of fluorescence; CR-actin, caged-resorufin iodacetamide-labeled actin; G′, dynamic modulus.
reveal the importance of FLNa-mediated actin filament cross-linking in vivo. These cells have a reduced cytoplasmic viscoelasticity, unstable membranes that convulsively bleb, and they are unable to undergo translational locomotion. These cells can transiently extend flat surface protrusions, but compared with FLNa-expressing cells, the extensions contain increased filamentous actin concentrations organized in a much less orthogonal architecture. Restoration of FLNa expression to these cells increases their rigidity, counteracts membrane blebbing, and restores their high angle actin filament branching as well as their capacity to crawl (28–30).

Recently the Arp2/3 complex, a tightly associated group of seven polypeptides, has been proposed to be sufficient for actin filament cohesion at the leading edge (note reviews by Borisy and Switkina (31) and by Higgs and Pollard (32)). The Arp2/3 complex promotes actin filament nucleation, leading to an array of actin filaments branching at near precisely 70° angles in vitro (33–37). An underlying assumption behind the Arp2/3 complex influencing leading edge mechanics is that actin filament branching resulting in a dendritic structure has the same or a similar coherence-producing effect on actin filaments as actin filament cross-linking in orthogonal networks, but no information exists regarding the mechanical effects of actin filament branching induced by the Arp2/3 complex. Therefore, we compared the actin gelation activity of FLNa and of the Arp2/3 complex in vitro.

**EXPERIMENTAL PROCEDURES**

Expression of Recombinant FLNa in a Baculovirus Expression System—A FLNa cDNA was excised from a pREP4 construct (Invitrogen) FLNa (38) by NotI/BssHI digestion and cloned into BssHI/NotI sites of the expression vector pFASBAC (Invitrogen). A virus was generated with the BAC-TO-BAC system (Invitrogen). Sf9 cells were cultured in SF-900 II SFM (Invitrogen) supplemented with 50 units/ml penicillin and 50 μg/ml streptomycin and used for virus amplification and protein expression. Protein extracts were prepared 48 h after infection by suspending the Sf9 cells in a solution containing 10 mM Tris-HCl, pH 8.5, 100 mM NaCl, 1% Triton X-100, 10 mM EDTA, 1 mM EGTA, 5 mM 2-mercaptoethanol, and a mixture of protease inhibitors (10 μg/ml each chymostatin, leupeptin, and pepstatin and 1 mM phenylmethylsulfonyl fluoride) and disrupting them in a Dounce homogenizer for 20 min. The lysates were rapidly frozen in liquid nitrogen and stored at −80 °C.

Protein Purification—Recombinant FLNa was purified from Sf9 cell lysates by several chromatographic steps performed at 4 °C. During each step, fractions containing recombinant FLNa protein were identified by coelectrophoresis with polyclonal antibodies against the hinge region of FLNa (28) or by Coomassie Brilliant Blue staining. Either His6- or His6/NotI/BssHI digested FLNa was expressed in insect cells and purified on glutathione-Sepharose 4B beads (Amersham Biosciences, Inc.) and stored at −80 °C described by Blanchoin et al. (30). GST-CA was covalently immobilized to glutathione-Sepharose 4B with glutaraldehyde as follows. Three ml of glutathione-Sepharose 4B, which fully retains GST-CA, was incubated in 12 ml of 1.0% glutaraldehyde in phosphate-buffered saline for 10 min at room temperature. Reducible cross-links and the remaining aldehydes were quenched with 1% NaBH4 in phosphate-buffered saline for 30 min and 100 mM Tris-HCl, pH 7.4, for 1 h with three washes with phosphate-buffered saline between each reaction. The resin was packed in a column, washed with buffer CA (20 mM Tris-HCl, pH 7.4, 25 mM KCl, 1 mM 2-mercaptoethanol, 1 mM MgCl2, 0.5 mM EDTA, 0.1 mM ATP) containing 0.2 mM MgCl2, and then equilibrated with buffer CA. After loading the sample from HiTrap, the GST-CA column was washed with 50 mM of buffer CA containing 10% glycerol, 100 mM NaCl, 100 μM ATP, 0.2 mM CaCl2, pH 7.4, kept overnight on ice, and then centrifuged at 300,000 × g for 40 min at 4 °C. The supernatant fluid was further diluted in buffer G in final concentration of 50 μM and stored in the depolymerized form at 4 °C.

Measurements of Apparent Viscosity and Gelation—Gelation was determined using a miniature falling ball viscometer (42). Actin polymerization was initiated by the addition of 24 μM unpolymerized actin in the presence or absence of different concentrations of FLNa, the Arp2/3 complex, GST-CA, free VCA, or 24 μM phallloidin alone or in combinations in buffer B (20 mM Tris-HCl, 100 mM KCl, 2 mM MgCl2, 0.5 mM EDTA, 0.5 mM ATP). The mixtures were immediately drawn into vertically positioned 100-μl capillary tubes (Fisher) and incubated at 25 °C for 80 min unless indicated otherwise. The time required for a 0.7-mm diameter stainless steel ball to fall 5 cm was measured.

**Actin Polymerization Assays**—Actin polymerization was measured as a function of time by monitoring the fluorescent intensity of pyrene-actin. The reaction was started by adding 2 μM pyrene-actin to the preincubated protein mix in buffer B, and the fluorescence change was recorded at 386 nm with excitation at 340 nm using a luminescence spectrometer (LS50B, PerkinElmer Life Sciences). Actin filaments assembled in the presence of the activated Arp2/3 complex or of FLNa were visualized by tetrathiomolybdate isoiooctyanate-phalloidin as described by Blanchon et al. (43).

**Particle Tracking**—One-micron diameter carboxylated polystyrene microspheres (Polysciences) were coated with polysyline as described by McGrath et al. (44). Samples of 24 μM unpolymerized actin alone or with...


FIG. 1. Purified FLNa and the Arp2/3 complex proteins used for comparison studies on actin. A, Coomassie Blue-stained polyacrylamide gels of purified recombinant human FLNa expressed in SF9 cells or of purified Arp2/3 complex from human platelets after electrophoresis in SDS. B, effect of purified FLNa, Arp2/3 complex plus or minus GST-VCA, and FLNa plus the Arp2/3 complex on actin polymerization. Measurement of pyrene fluorescence was used to monitor the polymerization rates of 2.0 μM G-actin in the presence of indicated concentration of added proteins.

Various additives in 50 μl volumes were supplemented with 0.01% (w/v) beads before polymerization. Polymerizing actin was immediately loaded in glass microcapillary tubes (Friedrich & Dimmock, 0.05 × 0.5 mm, inner diameter, 50 mm), and the sides were sealed with Seal-Ease (Becton Dickinson). The capillaries were attached on glass slides with Scotch tape and maintained at room temperature for 60 min. Particle tracking was performed with a Zeiss inverted microscope with bright-field optics and a 100 × oil immersion objective. The microscope was focused on the middle of the sample. Images were obtained using a Hamamatsu charged coupled device camera, and frames were captured at 1-s intervals. Movies were generated using ImageJ 1.22d software (rsb.info.nih.gov/ij/plugins/multitracker.html), and tracking was processed by MultiTracker plugin (rsb.info.nih.gov/ij/plugins/multitracker.html).

Photobleaching of Actin Fluorescence (PAF)—Caged-resorufin iodacetamide-labeled actin (CR-actin) was synthesized (45), and PAF experiments were conducted as previously described (46). Briefly, 2 μM purified CR-actin monomer was polymerized in the presence of equimolar amounts of phallacidin in 1 mg/ml bovine serum albumin-coated microcapillary tubes alone or after the addition of 1.5 VCA and 1.50 Arp2/3/2/3 in the presence or absence of 1:100 FLNa. One hour after polymerization, a 30-μm-wide photoactivated band was illuminated across one dimension of the microcapillary tube. After uncaged monomer diffusion was complete, the change in photoactivated bandwidth and centerline fluorescence decay was monitored over time to infer the mobility and lifetime of fluorescent actin filaments from the characteristic fluorescent decay time constant (τ) and the fraction of total actin polymerized (PF). Parallel sample preparations were negatively stained, and at least 100 actin filaments were measured with electron microscopy to determine the actin filament length distribution and number-average actin filament length (Ln).

Rheometry—Measurements of the dynamic shear storage modulus (G′) of actin filament solutions were made with a plate and plate rheometer (Rheometrics Fluid Spectrometer II, Rheometrics Inc., NJ) (48).

RESULTS

Purity and Function of the FLNa and Arp2/3 Reagents—Human FLNa protein expressed in SF9 cells constituted ~7% of the total cell protein, as estimated by densitometric analysis of Coomassie blue-stained 9% polyacrylamide gel after electrophoresis. SF9-derived FLNa protein eluted in the same fractions on Superose 6 gel filtration columns as dimeric FLNa protein purified from human platelets and is homogeneous (Fig. 1A). The Arp2/3 complex purified from human platelets by the three-step method described under “Experimental Procedures” has the seven polypeptide subunits characteristic for the complex (Fig. 1A) and expresses actin nucleation activity after activation by GST-VCA, as reported in many recent publications (Fig. 1B). FLNa had no detectable actin nucleation activity and did not measurably influence actin nucleation induced by the Arp2/3 complex and GST-VCA (Fig. 1B).

Liquid-to-gel Transition of Actin by FLNa or GST-VCA but Not Arp2/3 or Arp2/3-GST-VCA—Fig. 2A shows the apparent viscosity of 24 μM actin polymerized with various concentrations of FLNa, the Arp2/3 complex, GST-VCA, or free VCA. B, apparent viscosity of 24 μM actin polymerized in the presence of FLNa or the Arp2/3 complex activated by GST-VCA. The inset shows that GST-VCA or free VCA activate the Arp2/3 complex to form branched actin filaments stained with tetramethylrhodamine isothiocyanate-phalloidin. The actin concentration was 24 μM, and the preparation was diluted 1000-fold for microscopy.

FIG. 2. FLNa, but not the activated Arp2/3 complex, induces actin filament gelation. A, apparent viscosity of 24 μM actin polymerized in the presence or absence of various concentrations of purified FLNa, the Arp2/3 complex, GST-VCA, or free VCA. B, apparent viscosity of 24 μM actin polymerized in the presence of FLNa or the Arp2/3 complex activated by GST-VCA. The inset shows that GST-VCA or free VCA activate the Arp2/3 complex to form branched actin filaments stained with tetramethylrhodamine isothiocyanate-phalloidin. The actin concentration was 24 μM, and the preparation was diluted 1000-fold for microscopy.

2 E. Osborn, J. L. McGrath, S. K., Chalos, M. Schleicher, C. F. Dewey, Jr., and J. H. Hartwig, submitted for publication.
and the Arp2/3 complex with 0.6 μM FLNa polymerized in the presence of various concentrations of FLNa—the 2000-fold excess of free Arp2/3 to 50 actins, the addition of a 5 complex had no actin-gelating effect. At a molar ratio of 1 GST-VCA alone, the combination of GST-VCA and the Arp2/3 complex had no actin-gelating effect. VCA minus the GST moiety has no actin filament-cross-linking activity up to a molar ratio of 1:5 compared with actin (Fig. 2A). As previously reported (51, 52), GST-VCA was more efficient at activating the Arp2/3 complex than free VCA, 8-fold more free VCA than GST-VCA being required for maximal activation of polymerization of 2 μM actin (data not shown).

Fig. 2B compares the apparent viscosities of 24 μM actin polymerized in the presence of various concentrations of FLNa and the Arp2/3 complex with 0.6 μM GST-VCA. In contrast to GST-VCA alone, the combination of GST-VCA and the Arp2/3 complex had no actin-gelating effect. At a molar ratio of 1 Arp2/3 to 50 actins, the addition of a 5–2000-fold excess of free VCA over Arp2/3 also had no actin-gelating effect. Fluorescence micrographs of the actin filaments polymerized with the Arp2/3 complex and VCA demonstrated branched structures (Fig. 2B, inset). The images were taken after the addition of buffer to the sample as previously described (43), and the total fluorescent intensities in different fields in a unit square were similar, indicating that the material is homogeneous after dilution. By contrast, actin and FLNa resisted dilution, which is characteristic of a gel.

In contrast to the cross-links formed by FLNa with actin (53), the branches made by the Arp2/3 complex are unstable and dissociate with a half-time of about 5 min. However, the addition of phallloidin stabilizes Arp2/3-mediated actin filament branches for hours (43). Therefore, we also included phallloidin concentrations equimolar to actin in the same experiments described above. Again, we observed no evidence for actin gelation by Arp2/3. As another approach to addressing transient actin filament cross-linking by the Arp2/3 complex, we measured the time course of changes in apparent viscosity after starting polymerization. The Arp2/3 complex did not promote any detectable increase of the apparent viscosity at any time points measured (Fig. 3). On the other hand, the viscosity increases rapidly in the presence of FLNa, and phallloidin accelerates this effect, presumably by increasing the average actin filament length distribution at early times as compared with actin polymerizing without this stabilizing factor.

Effect of Combining FLNa and Arp2/3 on Actin Gelation—At fixed concentrations of FLNa and phallloidin-treated actin, increasing amounts of the Arp2/3 complex activated by VCA decreased the apparent viscosity of the protein mixtures in a concentration-dependent manner. Increasing the FLNa concentration counteracted this effect (Fig. 4A). Conversely, increasing concentrations of the Arp2/3 complex increased the critical concentration of FLNa required for actin gelation (Fig. 4B).

Effect of FLNa and Arp2/3 on the Brownian Movement of Actin Filaments—Videomicroscopic recordings (see the supplemental videos that relate to Fig. 5) show the motions of polylysine-coated 1-μm-sized particles sampled at a rate of 1 frame/s over 1 min embedded in 24 μM filamentous actin in the presence or absence of FLNa, activated Arp2/3, or FLNa plus activated Arp2/3. Fig. 5 summarizes the particle trajectories. Filamentous actin alone accommodated easily detectable particle movement (supplemental video A and Fig. 5a), whereas the same actin concentration polymerized with FLNa (1:100) essentially immobilized the particles (supplemental video B and Fig. 5b). Actin polymerized with 1 VCA-activated Arp2/3 per 100 actins was as or more mobile than actin alone (supplemental video C and Fig. 5c). Inclusion of 1 FLNa dimer per 100 actins markedly reduced the mobility of the Arp2/3-actin mixture (supplemental video D and Fig. 5d).
Comparison of Cross-linking and Branching on Actin Mechanics

**Fig. 5.** The Brownian motion of a microsphere embedded in actin filaments is restricted by FLNa but not by the Arp2/3 complex. Trajectories of the particles are shown. Supplemental time-lapse videos (available online) show the movement of polylysine-coated microparticles embedded in 24 μM actin (supplemental video A), 24 μM actin plus 0.24 μM FLNa (supplemental video B), 24 μM actin plus 0.48 μM Arp2/3 complex and 4.8 μM VCA (supplemental video C), or 24 μM actin plus 0.24 μM FLNa, 0.48 μM Arp2/3 complex, and 4.8 μM VCA (supplemental video D). The particles were monitored for 1 min at 1 Hz. Supplemental videos A–D correspond to panels a–d in this figure.

**Effect of the Arp2/3 Complex and FLNa on the Diffusion of Actin Filaments—**The ability of FLNa and the activated Arp2/3 complex to hinder the diffusion of actin filaments was compared with PAF 1 h after the initiation of polymerization. Pyrene-actin nucleation assays performed under the same conditions as the PAF experiments, replacing CR-actin with pyrene-labeled actin monomer, reveal comparable levels of actin assembly 1 h after polymerization regardless of the presence of the activated Arp2/3 complex or FLNa. PAF results at this time point are comparable, therefore, even though a true steady state has not yet been achieved (data not shown). After fluorescent monomer diffusion is complete, 30-μm photoactivated bands of purified, polymerized actin at steady state decay simultaneously due to actin filament diffusion and filament turnover. By polymerizing actin in the presence of equimolar phalloidin, newly formed actin filaments are stabilized against actin filament diffusion, with filament cycling contributing to a much lesser extent in the time-dependent centerline fluorescence decay. Thus, increases in the characteristic fluorescence decay time constant, confirmed by visualizing and the elimination of photoactivated band broadening, represent decreased diffusivity of actin filaments in the actin network as a result of increased entanglements or cross-linking.

Thirty-μm-wide photoactivated bands of purified CR-actin broadened similarly regardless of the presence of activated Arp2/3 (data not shown) and exhibited comparable time constants describing centerline fluorescence decay (Table I). The addition of gelling amounts of FLNa eliminated photoactivated band broadening (data not shown) and increased the characteristic fluorescence decay time constant, alterations in the fluorescence dynamics consistent with FLNa, limiting the ability of actin filaments to escape the photoactivated band. The number-average actin filament length measured by electron microscopy decreases in the presence of the activated Arp2/3 complex as compared with the presence of FLNa, consistent with the results observed by tetramethylrhodamine isothiocyanate-phalloidin staining and gel point determinations.

**Table I**

|                  | τ (h) | PF | L (μm) |
|------------------|-------|----|--------|
| 2 μM F-actin + 1:1 phallacidin (n = 7) | 4.4 ± 2.4a | 0.65 ± 0.04a | 2.3 ± 0.3 |
| 2 μM F-actin + 1:1 phallacidin + 1:5 VCA + 1:50 Arp2/3 (n = 7) | 4.9 ± 1.0a | 0.77 ± 0.05a | 1.7 ± 0.2a |
| 2 μM F-actin + 1:1 phallacidin + 1:5 VCA + 1:50 Arp2/3 + FLNa (n = 6) | 12.2 ± 4.3a | 0.71 ± 0.05 | 2.4 ± 0.1b |

* a p < 0.1, b p < 0.05.

**DISCUSSION**

The cortex of cells maintains its coherence in the face of very high shear stresses, with estimated values up to 1000 Pa (55), and regions at the leading edge can transmit stresses >10 kPa to attachment points on the substrate (56). As the principal structural component of peripheral cytoplasm, filamentous actin is responsible for this coherence. As previously reported, cross-linking of actin filaments by FLNa generates a gel, defined as a macroscopic collection of immobilized polymer chains. In contrast, simple branching of actin filaments by the Arp2/3 complex does not gel actin. These findings to our knowledge represent the first specific test of theoretical analyses postulating that polymer branching without cross-linking does not lead to a gel-like structure (57, 58). These theoretical treatments assumed cross-linking or branching of freely flexible polymer chains. Because actin polymers are not random coil polymers, the possibility remained that branching of semiflexible rods might be sufficient to generate an elastic structure. Nevertheless, our experiments suggest that this is not the case.

Gel point determinations showed that although a stoichiometric amount of FLNa immobilizes actin filaments into a coherent structure capable of resisting imposed shear, the Arp2/3 complex does not detectably affect the viscosity of filamentous actin under these conditions. The ineffectiveness of the Arp2/3 complex in increasing the consistency of filamentous actin is not likely to be the result of branch instability, because no viscosity increases were notable early in the course of actin polymerization in the presence of the Arp2/3 complex, which was also without effect in the presence of phalloidin, which stabilizes branching. Controlling for the effects of GST-
VCA, an Arp2/3 complex activator unexpectedly revealed an additional example of a true actin filament cross-linker for comparison. The lower efficiency of what is presumably dimeric GST-VCA compared with FLNa in actin filament gelation is consistent with the fact that it is a relatively small polypeptide compared with the large filamentous FLNa dimer capable of promoting more orthogonal actin filament branching. The Arp2/3 complex completely abrogates the effect of GST-VCA on actin cross-linking, presumably by blocking one of its actin filament-binding sites.

Actin filament branching is similar to annealing and, by increasing the length of individual polymer chains, could reduce the critical cross-link concentration required for incipient gelation. The Arp2/3 complex nucleates actin filament growth, however, increasing actin filament number and offsetting the branching effect. The kinetics of nucleated branching of actin by the Arp2/3 complex favors actin filament number over length because the Arp2/3 complex reduced the gelating ability of FLNa in a concentration-dependent manner.

Results of particle tracking and PAF experiments showed that actin filaments branched by the Arp2/3 complex are freely diffusible on a microscopic scale as well, whereas FLNa restricts filament diffusion, consistent with its effects on macroscopic actin network formation. These findings indicate that the branching imposed by the Arp2/3 complex does not lead to entanglements that importantly impede the diffusive motions of actin filaments and that the Arp2/3 complex does not directly contribute to actin filament coherence on a macroscopic scale.

The Arp2/3 complex is essential for the directed movement of intracellular bacteria such as Listeria monocytogenes and of vesicles and beads (59, 60). It clearly initiates the conversion of actin monomers into filaments at the bacterial, vesicular, or bead surface, but how this conversion mediates the mechanics of movement is unclear. The role of branching is also uncertain. Some have suggested that the branching per se is important for protrusive activity (61), whereas others see it as primarily a way to amplify actin nucleation (35). The fixed 70° branching of actin filaments nucleated by the Arp2/3 complex has suggested that it might exert mechanical effects at the leading edge to cause protrusion there as well. This restricted branching angle has been observed, however, only in actin filaments adherent to surfaces, and the branching angle off of the parental filament only persists for a short distance (33, 34). Actin filaments are sufficiently flexible that they can coil considerably in vitro and in vivo, implying that even a static branch angle cannot confer mechanical stability very far from the branch.

Cells lacking FLNa expression have unstable surfaces and are unable to crawl, although they can transiently protrude flat lamellae (28). Hence, FLNa is required for locomotion and surface stability, but the findings do not rule out a requirement for the Arp2/3 complex in leading edge protrusion. Impairing side binding activity of the Arp2/3 complex by the microinjection of antibodies that recognize the actin filament-binding site of the complex inhibited leading edge extension of MTLn3 mammary carcinoma cells after epidermal growth factor stimulation (62). Even if Arp2/3 complexes do not have direct mechanical effects at the leading edge, by offsetting the entropic and ionic drives promoting parallel alignment of actin filaments (47), they may facilitate the ability of true actin filament cross-linking molecules to stabilize actin filaments in other configurations than tight bundles ranging from different parallel arrays to orthogonal networks. Sorting out the factors behind the evident synergy manifested in branching and cross-linking of leading edge actin filaments is a rich opportunity for cell motility research.
Comparison of Cross-linking and Branching on Actin Mechanics

9154

45. Theriot, J., and Mitchison, T. (1991) Nature 352, 126–131
46. McGrath, J. L., Tardy, Y., Dewey, C. F., Jr., Meister, J. J., and Hartwig, J. H. (1996) Biophys. J. 75, 2070–2078
47. Tang, J., Ito, T., Tao, T., Traub, P., and Janmey, P. (1997) Biochemistry 36, 12600–12607
48. MacKintosh, F., and Janmey, P. (1997) Current Opinion in Solid State and Materials Science 2, 355–357
49. Miki, H., Miura, K., and Takenawa, T. (1996) EMBO J. 15, 5326–5335
50. Lim, K., Ho, J. X., Keeling, K., Gilliland, G. L., Ji, X., Rucker, F., and Carter, D. C. (1994) Protein Sci 3, 2233–2244
51. Higgs, H., and Pollard, T. (2000) J. Cell Biol. 150, 1311–1320
52. Marchand, J., Kaiser, D., Pollard, T., and Higgs, H. (2001) Nat. Cell Biol. 3, 76–82
53. Janmey, P. A., Hvidt, S., Lamb, J., and Stossel, T. P. (1999) Nature 345, 89–92
54. Mullins, R., Kelleher, J., Xu, J., and Pollard, T. (1998) Mol. Biol. Cell 9, 841–852
55. Evans, K., Leung, A., and Zhelev, D. (1993) J. Cell Biol. 122, 1329–1336
56. Dembo, M., and Wang, Y. L. (1998) Biophys. J. 76, 2307–2316
57. Flory, P. (1947) J. Am. Chem. Soc. 69, 2893–2899
58. Tobita, H. (1998) J. Polym. Sci. Part B Polymer Phys. 36, 2015–2018
59. Welch, M., Iwamoto, A., and Mitchison, T. (1997) Nature 385, 265–269
60. Pantaloni, D., Le Clair, C., and Carlier, M.-F. (2001) Science 292, 1502–1506
61. Mogliner, A., and Oster, G. (1996) Biophys. J. 71, 3030–3045
62. Bailly, M., Ichetovkin, I., Grant, W., Zebda, N., Machesky, L., Segall, J., and Condeelis, J. (2001) Curr. Biol. 11, 620–625
Comparison of Filamin A-induced Cross-linking and Arp2/3 Complex-mediated Branching on the Mechanics of Actin Filaments
Fumihiko Nakamura, Eric Osborn, Paul A. Janmey and Thomas P. Stossel

J. Biol. Chem. 2002, 277:9148-9154.
doi: 10.1074/jbc.M111297200 originally published online January 10, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M111297200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2002/03/12/277.11.9148.DC1

This article cites 60 references, 24 of which can be accessed free at http://www.jbc.org/content/277/11/9148.full.html#ref-list-1