

Dendritic Branching and Homogenization of Actin Networks Mediated by Arp2/3 Complex

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The cytoskeleton of motile cells exploits accessory proteins to locally modulate its organization and micromechanics. Here, we demonstrate that the Arp2/3 complex plays the role, unique among other cytoskeleton proteins, of an actin network “homogenizer,” promoting the extremely rapid formation of homogeneous and stiff networks. Nanotracking of microspheres imbedded in F-actin networks reveals that the Arp2/3 complex promotes the formation of networks that are remarkably more homogeneous than control networks, a distinctive feature that coordinates a dramatic burst of elasticity. These results suggest that the Arp2/3 complex possesses a unique function of stabilizing membrane protrusions through the formation of homogeneous and stiff actin cytoskeleton at the leading edge of crawling cells.

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Cell motility is essential for tissue formation, embryo development, immune responses, and cancer metastasis [1]. Cells move by extending a leading edge via the coordinated assembly of actin filaments and organizing these filaments into mechanically resilient networks [1–4]. To prevent rearward movements of the filaments, the actin network needs to be both anchored to the substratum and cross-linked into stiff structures [1]. How filamentous actin could be cross-linked into a sufficiently stiff network to generate propulsive forces at the leading edge is not well understood [5,6]. The Arp2/3 complex [7] seems to be a good candidate for effectively cross-linking actin filaments since it promotes the nucleation of side-branched dendritic actin networks [8]. Moreover, unlike most other known actin–cross-linking proteins, Arp2/3 is localized only at the leading edge of motile cells, from which other cross-linkers such as myosin, α -actinin, and filamin are excluded.

Here, we demonstrate that the Arp2/3 complex plays the role, unique among other cytoskeleton proteins, of an actin network “homogenizer,” promoting the extremely rapid formation of homogeneous and stiff networks. We use real-time tracking of particles imbedded in F-actin networks to show that the Arp2/3 complex promotes the formation of networks that are remarkably more homogeneous than control networks, a distinctive feature that coordinates a dramatic increase in global elasticity. These results suggest that the Arp2/3 complex possesses a unique ultrastructural function of stabilizing membrane protrusions through the formation of a homogeneous and stiff actin cytoskeleton at the leading edge of crawling cells.

Cytoskeletal proteins actin, the Arp2/3 complex, the Wiskott-Aldrich syndrome protein (WASp), fascin, filamin, and α -actinin were purified following published protocols [9–13]. Fascin, filamin, and α -actinin are known F-actin–cross-linking or bundling proteins; the Arp2/3 com-

plex in the presence of its activator WASp [14] promotes the nucleation of F-actin into dendritic networks [12]. Purified actin was stored as Ca^{2+} -actin in continuous dialysis at 4 °C against buffer G (0.2 mM ATP, 0.5 mM DTT, 0.2 mM CaCl_2 , 1 mM sodium azide, and 2 mM Tris-HCl, pH 8.0). Mg^{2+} -actin filaments were generated by adding 0.1 volume of 10-x KMEI (500 mM KCl, 10 mM MgCl_2 , 10 mM EGTA, 100 mM imidazole, pH 7.0), polymerizing salt to 0.9 volume of G-actin in buffer G. Multiple-particle tracking was conducted as described [15]. Briefly, 1- μm diameter fluorescent latex microspheres (Molecular Probes, Eugene, OR) were dispersed in solutions containing 24 μM actin and the reported concentrations in actin-binding proteins. Movies of the fluctuating fluorescent microspheres were recorded by a silicon-intensifier target camera (VE-100 Dage-MTI, Michigan City, IN). This camera was mounted on an inverted epifluorescence microscope (Eclipse TE300, Nikon, Melville, NY) [16] equipped with a 100-X Plan Fluor oil-immersion objective (N.A. 1.3). Movies of fluctuating microspheres were analyzed by a custom multiple-particle-tracking routine incorporated into the software METAMORPH (Universal Imaging Corp., West Chester, PA) as described [17]. The trajectories of the microspheres centroids were simultaneously monitored in the focal plane of the microscope for 20 s at a rate of 30 Hz. Microspheres were tracked with ≈ 5 nm resolution, as determined by immobilizing beads on a glass microslide using a strong adhesive and tracking their apparent displacements [18]. From the coordinates of the particle centroids, $[x(t), y(t)]$ where t is the elapsed time, time-averaged mean-squared displacements (MSD) were calculated, $\langle \Delta r^2(\tau) \rangle = \langle [x(t + \tau) - x(t)]^2 + [y(t + \tau) - y(t)]^2 \rangle$ where τ is the time lag. The distributions of MSDs of ~ 150 particles were statistically characterized for each tested condition as described [19]. Contributions of the 10%, 25%, and 50% highest MSD values at either

0.1 or 1 s time lags were compared to the mean MSD (see also Ref. [15]). Those contributions were verified to be close to 10%, 25%, and 50% for homogeneous liquids like glycerol and close to unity for highly heterogeneous solutions [17]. For more details about the multiple-particle-tracking method and analysis of the MSD distribution, see Ref. [20]. Quantitative rheology of actin solutions undergoing gelation was conducted as described [15]. Briefly, solutions of actin mixed with polymerizing salt were rapidly deposited in the space between a 50-nm diameter cone and plate. Small oscillatory deformations (1% amplitude) were applied every 30 s onto the polymerizing actin solutions and the in-phase component of the stress was measured. That component divided by the strain amplitude is the elastic modulus reported here.

Multiple-particle tracking [15] was used to quantify actin network homogeneity. The Brownian displacements of polystyrene microspheres imbedded in an actin network undergoing gelation were tracked simultaneously [15] [Fig. 1(a)]. Statistical analysis of the distributions of the microspheres' displacements helps quantify the degree of heterogeneity of the network. The distribution of the MSD of the individual microspheres is rapidly narrow [Fig. 1(a)] when actin polymerizes in the presence of the Arp2/3 complex [inset, Fig. 1(a)] compared to an actin solution without the Arp2/3 complex (data not shown; see Ref. [15]). The contributions are 10%, 25%, and 50% in a homogeneous material, but close to 100% in a highly heterogeneous material [15]. Simply, all particles imbedded in homogeneous liquid display MSDs that are very similar to each other; hence, individual MSD profiles contribute similarly to the ensemble-averaged MSD. This is, indeed, what we obtain for glycerol (bottom Fig. 2). The

high degree of network homogeneity in the presence of the F-actin-nucleating factor, the Arp2/3 complex, is remarkable as these parameters reach their "homogeneous" values of 10%, 25%, and 50% within minutes [Fig. 1(b)]. In contrast, the degree of heterogeneity in actin filament networks in the absence of the Arp2/3 complex remains high, even at long times (inset, Fig. 1(b)).

We have shown previously that the global stiffness of an F-actin network undergoing gelation is mostly controlled (and delayed) by the presence of network defects [15]. Accordingly, the rate of gelation of actin networks in the absence of the Arp2/3 complex is slow [Fig. 3(a)]; such a delay between actin polymerization and actin network gelation makes it impossible for F-actin alone (without Arp2/3) to both push the cell membrane and form stiff networks. The presence of Arp2/3 renders the actin networks more solidlike as measured by a rapidly decreasing phase angle [Fig. 3(b)]. Increasing the concentration in the Arp2/3 complex with its activator WASp further enhances the rates of network homogenization (Fig. 2) and network gelation [Fig. 3(a)]. Electron microscopy shows that the Arp2/3-complex-rich actin cytoskeleton at the leading edge of a motile cell is remarkably more homogeneous than regions of the cell farther away from the leading edge, regions where the Arp2/3 complex is absent and other cross-linking proteins such as filamin and α -actinin are present [21]. Recent particle-tracking measurements indeed show quantitatively that the leading edge of a migrating cell is significantly stiffer and more homogeneous than other regions of the cytoplasm [22].

Conventional cytoskeletal cross-linkers, which organize actin filaments into ordered structures in various parts of the cell, including α -actinin, fascin, and filamin, slow the pace of network homogenization (Fig. 2) and network gelation [Fig. 3(a)]. Indeed, we have shown previously

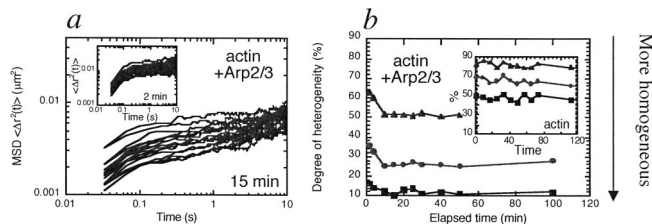


FIG. 1. Multiple-particle tracking in actin solutions containing the Arp2/3 complex. (a) Typical mean-squared displacements (MSD) of 1- μ m latex microspheres imbedded in actin filament networks in the presence of the 0.06 μ M WASp-activated Arp2/3 complex measured 2 min after the onset of polymerization. Inset: Typical MSDs measured 15 min after onset of polymerization. (b) Heterogeneity of polymerizing actin solutions. Time-dependent contributions of the 10%, 25%, and 50% highest MSD values ($n = 150$) to the ensemble-averaged MSD for actin solutions containing the WASp-activated Arp2/3 complex. A low value for these parameters means a relatively more homogeneous solution as indicated by the right arrow. Inset: Same parameters for actin solution in the absence of the Arp2/3 complex. Actin concentration is 24 μ M.

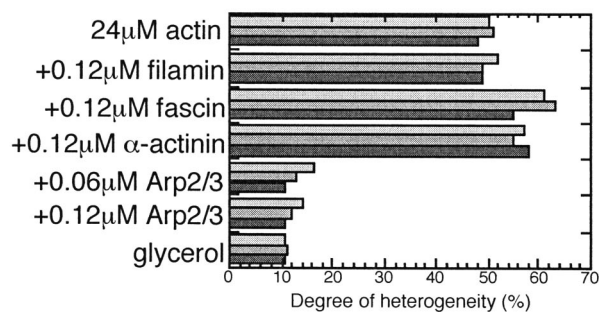


FIG. 2. The Arp2/3 complex accelerates the homogenization of actin filament networks. Degree of actin heterogeneity measured by the contributions of the 10% highest MSD values for glycerol and for actin networks containing a filamin, fascin, α -actinin, or WASp-activated Arp2/3 complex. The upper bar, intermediate bar, and lower bar for each type of cross-linking protein corresponds to 10, 20, and 30 min after onset of actin polymerization. Actin concentration is 24 μ M in all experiments.

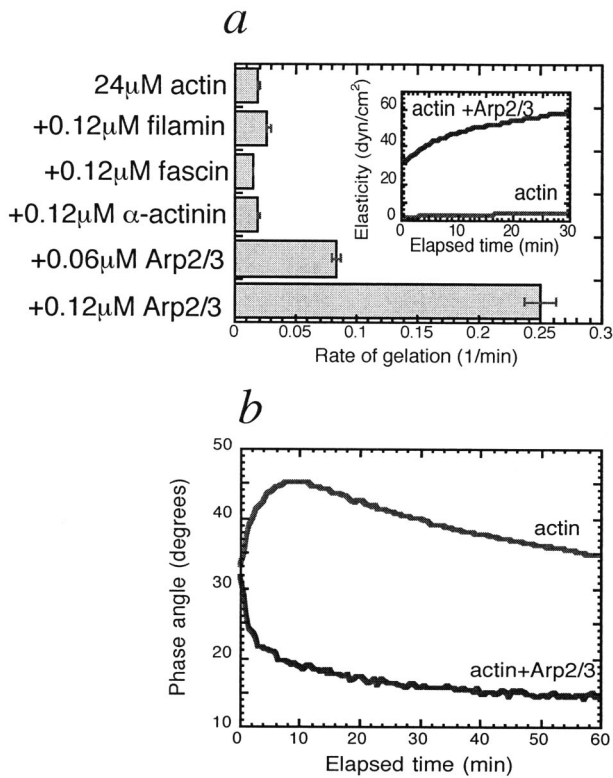


FIG. 3. Rate of actin filament network gelation in the presence of auxiliary proteins. (a) Rates of actin gelation measured by the inverse of the time required to reach 90% of the steady state (or maximum) elasticity during actin polymerization as measured by rheometry. The concentration of filamin, fascin, and α -actinin is $0.12 \mu\text{M}$. Inset: Time course for the elasticity of actin networks in the presence and the absence of the $0.12 \mu\text{M}$ WASp-activated Arp2/3 complex. (b) Time-dependent phase angle, $\delta = \tan^{-1}(G''/G')$, where G' is the elastic modulus and G'' is the viscous modulus of the actin networks in the presence and absence of the $0.12 \mu\text{M}$ WASp-activated Arp2/3 complex. Actin concentration is $24 \mu\text{M}$ in all experiments.

that the rate of gelation of an actin network is set by its degree of spatial homogeneity [15]. Conventional cross-linkers prevent free sliding of actin filaments past one another, and slow down the movement of the actin polymers, thereby slowing down the homogenization of the network. Cross-linkers like α -actinin, fascin, and filamin frustrate the networks into highly heterogeneous structures even at long time scales (Fig. 2).

A possible mechanism for the Arp2/3 complex-mediated network homogenization is as follows: the Arp2/3 complex mediates the localized nucleation and polymerization of filament branches from “mother” filaments [1,6,12]. Because of steric interactions, these new growing branches preferentially fill in the voids in the network, which therefore rapidly homogenizes. In turn, this enhanced network homogenization stiffens [15] the actin network much more effectively than conventional actin filament cross-linkers. This model of network homogenization remains to be tested directly. We conclude that the Arp2/3 complex presents a unique geometric function of network homogenizer among cytoskeleton proteins, which is adapted to the Arp2/3 complex’s peripheral localization *in vivo* [1,2] and presumed mechanical role in cell motility [1].

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