Reconstituted actin filament networks have been used extensively to understand the mechanics of the actin cortex and decipher the role of actin cross-linking proteins in the maintenance and deformation of cell shape. However, studies of the mechanical role of the F-actin cross-linking protein filamin have led to seemingly contradictory conclusions, in part due to the use of ill-defined mechanical assays. Using quantitative rheological methods that avoid the pitfalls of previous studies, we systematically tested the complex mechanical response of reconstituted actin filament networks containing a wide range of filamin concentrations and compared the mechanical function of filamin with that of the cross-linking/bundling proteins α-actinin and fascin. At steady state and within a well defined linear regime of deformation, F-actin solutions behave as highly dynamic networks (actin polymers are still sufficiently mobile to relax the stress) below the cross-linking-to-bundling threshold filamin concentration, and they behave as covalently cross-linked gels above that threshold. Under large deformations, F-actin networks soften at low filamin concentrations and strain-harden at high filamin concentrations. Filamin cross-links F-actin into networks that are more resilient, stiffer, more solid-like, and less dynamic than α-actinin and fascin. These results resolve the controversy by showing that F-actin/filamin networks can adopt diametrically opposed rheological behaviors depending on the concentration in cross-linking proteins.

Monomeric filamin is a high molecular mass (250 kDa), 80-nm actin-binding protein that features an actin-binding domain in its N terminus and forms a V-shaped, flexible dimer (1). Filamin cross-links actin filaments into orthogonal networks below a threshold filamin concentration and bundles them above that threshold in vitro (2). Filamin isoforms are ubiquitously expressed in unicellular and multicellular organisms, eukaryocytes, and prokaryocytes (1). In cultured non-muscle adherent cells such as fibroblasts, filamin localizes to the cortical actin network, the base of cell membrane protrusions, and along stress fibers (3). In dividing cells, filamin is concentrated in the cleavage furrow, where it remains associated at the mid-body region until the completion of cell division (4). Expression of the dysfunctional human filamin-A causes the genetic disorder of ventricular heterotopia, presumably due to reduced neuronal migration to the cortex (5, 6). Filamin-A-null melanoma cells display plasma membrane blebbing (7), reduced migratory speed (8), and increased susceptibility to force-induced membrane leakage (9), all of which are phenotypes attributed to reduced stiffness of cortical actin. These observations, along with filamin’s key role in actin organization, membrane stabilization, and the anchoring of transmembrane cell receptor proteins to the actin cytoskeleton, suggest that filamin has an important mechanical function (2).

The mechanical function of filamin in vitro has been tested extensively but is the subject of much debate. Cell migration requires sol-gel transitions that result from coordinated rearrangements of the cortical actin network (10). It is believed that these transformations can be accounted for by the dynamical behavior of F-actin cross-linking proteins, an argument supported by in vitro studies using reconstituted actin networks in the presence of cross-linking proteins (11). Indeed, actin filament solutions containing the cross-linking protein α-actinin stiffen under high rate deformations and deform readily under slow deformation (11), which was also observed in initial rheological studies of filamin (12). Such a dynamical cross-linking mechanism would be particularly suitable in fast motility events such as membrane ruffling in fast moving cells like keratocytes. However, later studies of filamin’s mechanical function in vitro suggest that cortical actin is rheologically equivalent to a covalently cross-linked gel at all tested rates of deformation (13). Such solid-like behavior would be particularly suitable for maintaining cell shape for long periods of time. To deform, the cytoskeleton would have to rely solely on mechanisms that modulate the actin network stiffness through the regulation of filament length (e.g. by gelsolin) or cross-linking activity (e.g. phosphatidylinositol 4,5 bisphosphate (PIP2) for α-actinin) (13). Resolution of this issue is of physiological relevance, as it will help identify some of the filamin-based structural motifs necessary for actin network organization, which is central to cell shape maintenance and cell locomotion.

These seemingly contradictory results about filamin’s mechanical behavior were initially attributed to the use of rheological methods that broke filaments and reduced artificially the solution’s viscosity in earlier studies of filamin and α-actinin (13, 14). Our paper shows that the apparently contradictory results are also attributable to both the effect of the filamin-to-actin molar ratio on actin network mechanics and the applications of ill-defined strain and frequency sweep assays (see details under “Results”) in the rheological characterization of actin networks containing filamin. Results presented in this

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The Mechanical Function of Filamin Is Bimodal

RESULTS

F-actin Network Gelation in the Presence of Filamin Is Monotonic at Low Filamin Concentrations and Biphasic at High Filamin Concentrations—To characterize the mechanical properties of actin filament networks in the presence of filamin, we measured first the rate and extent of gelation of F-actin solutions containing filamin upon the addition of polymerizing salt by using quantitative rheological methods. Gelation is defined here as the process of formation of a stiff network presumably caused by the onset of topological overlaps among polymerizing actin filaments and filament cross-linking/bundling by filamin, and it is monitored by the network’s elasticity (i.e., stiffness) at a fixed frequency of 1 radian/s and a deformation amplitude of 1% (20). When elasticity reached a steady state value, elastic and viscous modulus measurements were made as a function of frequency and deformation amplitude to probe network dynamics and possible network stiffening under shear deformation, respectively.

Up to 0.12 μM filamin in 24 μM actin ([filamin]:[actin] = 1:200), the elasticity of the network increased monotonically until it reached a steady state value, typically 2–3 h after the addition of the polymerizing salt to the filament/G-actin mixtures (Fig. 1A). For a filamin-to-actin molar ratio $\geq$1:133 (0.18 μM filamin), however, the time course of elasticity featured a distinct maximum that occurred at a time (≈2 min) that increased slightly for increasing filamin concentration (Fig. 1B). The phase angle of F-actin networks containing up to 1:200 filamin decreased steadily during the first 2 h of gelation and remained mostly constant during the rest of the experiment (≈5 h) (Fig. 1C). This means that for long periods of time the increase in viscosity paralleled the increase in elasticity of the network undergoing gelation. In contrast, for filamin-to-actin molar ratios higher than 1:133, the phase angle decreased promptly to extremely low values (≈2–3°), rendering the network mostly solid-like in minutes (Fig. 1D) (21). For comparison, a liquid such as glycerol displays a constant phase angle of 90°, and a Hookean solid displays a phase angle of 0°. The molar ratio at which the transition from monotonic to non-monotonic gelation kinetics occurs corresponds to the threshold filamin concentration at which a transition from orthogonal networks to tight bundles occurs (2).

F-actin Solutions Behave as Highly Dynamic Networks at Low Filamin Concentrations and as Covalently Cross-linked Gels at High Filamin Concentrations—When the elastic modulus reached a steady state value, actin filament networks were subject to small (1%) oscillatory deformations of increased frequency to probe the dynamical behavior of filaments/bundles in solution. In the absence of filamin, the elastic modulus, $G'(\omega)$, of actin filament networks displayed a relatively steep frequency dependence at high frequency (Fig. 2A). At high filamin concentration, $G'(\omega)$ profiles became independent of frequency (Fig. 2A), suggesting that, up to time scales of 2π/ω~600 s, actin polymers showed little mobility. This absence of relaxation behavior was maintained up to time scales of ~600 s as confirmed by stress relaxation measurements (Fig. 4C). Hence, from a rheological standpoint, F-actin solutions behave as relatively dynamic networks at low filamin concentrations and as covalently cross-linked gels at high filamin concentrations.

The concentration-dependent elastic modulus of F-actin solutions (measured at 1 radian/s) increased steadily at low actin-to-filamin molar ratio, tripling in value between 0 and 1:200 (0.12 μM filamin) (Fig. 2B). However, $G'$ increased >8.4-fold for molar ratios between 1:200 (0.12 μM) and 1:100 (0.24 μM) at 1 radian/s (Fig. 2B) and 14-fold at 0.1 radian/s. Similarly, the phase angle underwent a sharp transition at a filamin-to-actin...
molar ratio of 1:133 (0.18 μM), from δ = −30° to δ < 3° (Fig. 2, C and D).

F-actin Networks Soften under Shear at Low Filamin Concentrations and Strain-harden at High Filamin Concentrations—In vivo experiments suggest that filamin plays a central mechanical role during motility events (2), but how filamin may provide the actin cytoskeleton with mechanical resilience remains unclear. The resilience and strain-dependence of the mechanical properties of F-actin/filamin networks have been measured previously using the strain-sweep assay (13), a method that we now show is fundamentally flawed (see also “Discussion”). In the strain-sweep assay, multiple oscillatory
deformations of controlled frequency are applied, and in-phase and out-of-phase components of the oscillatory stress induced within the network are monitored to extract elastic and viscous moduli as a function of deformation amplitude. As illustrated in Fig. 3, this assay is unsuitable because, at large deformation amplitudes, the stress becomes non-periodic and the stress amplitude decreases over time (Fig. 3D, inset) presumably due to filament breakage/alignment during the assay itself (compare Fig. 3, A and B, obtained at deformation amplitudes of $\gamma_0 = 1$ and 35%, respectively). The effect is also detectable in Lissajous figures where the induced time-dependent stress is plotted against the applied time-dependent strain (Fig. 3C and D). Lissajous figures obtained at large deformation amplitudes show a stress that increases dramatically during the first oscillation but is greatly decreased during subsequent oscillations (Fig. 3D). These problems cause the computation of $G'$ and $G''$ (which, at its core, assumes that stress and strain are periodic functions) to become ill-defined, which defeats the purpose of the strain-sweep assay, because its purpose is to probe the network modulus beyond the linear regime (see more under “Discussion”).

Here, the mechanical response of F-actin/filamin networks under increasing shear was rigorously quantified by subjecting the networks to step deformations of increasing shear amplitudes (22). The step-deformation assay avoids the limitations and approximations of the strain-sweep assay. A rapidly imposed step deformation of total amplitude $\gamma_0$ causes stress within the network, which relaxes because of polymer motion in solution (20, 23). The deformation-dependent shear modulus profile is defined as a the ratio of the measured time-dependent stress $\sigma(t)$ and the deformation amplitude, $G(t; \gamma_0) = \sigma(t; \gamma_0)/\gamma_0$, superimposed for deformations up to $\sim 10\%$ (Fig. 4A). This

![Image](image_url)
value of strain amplitude is the yield strain $\gamma_y$, which defines the limit of the linear rheological regime for which deformations are small enough ($\gamma_0 < \gamma_y$) that the stress $\sigma$ induced in the network proportional to the applied deformation amplitude and the network modulus $G$ is independent of $\gamma_0$ (21). For $\gamma_0 > \gamma_y = 10\%$, the modulus decreased steadily over all probed time scales (Fig. 4, A and B), due presumably to filament breakage and/or shear-induced orientation (22). This means that actin filament networks containing low filamin concentrations “soften” when subjected to large deformations.

In startling contrast to the case of low filamin concentrations, filament-to-actin molar ratios $>1:133$ produced actin filament networks that stiffened when subjected to large deformations (Fig. 4C). At high filament concentrations, F-actin networks displayed a modulus independent of amplitude up to a deformation of $\sim3\%$ (22), reducing the extent of the linear regime before undergoing a 60% increase of $G$ for $\gamma_0$ between 3 and 12% (Fig. 4E, inset). Despite the fact that F-actin is bundled for an actin-to-filamin molar ratio of 1:133 (2), this strain-induced hardening phenomenon occurs (less strongly) in F-actin networks cross-linked by $\alpha$-actinin but not the $\alpha$-actinin, two relatively well characterized actin-cross-linking/bundling proteins and fascin (20, 21). The resilience of F-actin networks, defined here as the strain amplitude for which the shear modulus decreases below its baseline value, increased monotonically with increased filamin concentration (Fig. 4F).

Filamin Helps Produce F-actin Networks that Are More Resilient, Stiffer, More Solid-like, and Less Dynamic than the F-actin Cross-linking/Bundling Proteins $\alpha$-Actinin and Fascin—We compared systematically the rheological properties of actin filament networks in the presence of filamin to the properties of networks in the presence of fascin or $\alpha$-actinin, two relatively well characterized actin-cross-linking/bundling proteins (16, 24). The resilience of F-actin networks containing filamin was dramatically higher than that of the two control networks. The resilience of F-actin/filamin networks was 5-fold higher than the resilience of F-actin/$\alpha$-actinin networks and...
Fig. 5. Mechanical behavior of filamin (Fil) compared with α-actinin (α-A) and fascin (F)-response to large deformations. A, graphical definitions of network resilience, maximum modulus, and strain-hardening effect. Actin concentration is 24 μM, and filamin concentration is 0.24 μM. B, resilience of F-actin/filamin, F-actin/α-actinin, and F-actin/fascin networks for concentrations in cross-linking proteins of 0, 0.12, and 0.24 μM. The extent of strain hardening (C) and the maximum modulus (D) obtained while sweeping from low to high strain amplitudes are depicted. Asterisks indicate that no strain hardening occurs. Mechanical properties in panels A–D were evaluated at a time scale of 1 s. Actin concentration is 24 μM is all panels.

10-fold higher than the resilience of F-actin/fascin networks (Fig. 5, A and B). Strain hardening, defined as the relative (maximum) increase of the shear modulus compared with its baseline value at small deformation amplitudes (i.e. in the linear regime) (Fig. 5A) (22), was similar for F-actin/filamin and F-actin/α-actinin networks at low filamin concentrations (Fig. 5C). But strain hardening was much more pronounced in F-actin/filamin networks than in F-actin/α-actinin networks at high cross-linker concentrations (Fig. 5C). In contrast to filamin and α-actinin, fascin did not produce any measurable strain hardening in F-actin networks, even at high fascin concentrations (Fig. 5C). The maximum network elasticity reached by sweeping from low to high shear deformation amplitudes (see definition in Fig. 5A) was similar for F-actin/filamin and F-actin/α-actinin at low concentrations in cross-linking proteins but became much more pronounced at high filamin concentrations than at high α-actinin concentrations (Fig. 5D).

As assessed by the frequency dependence of \(G'(\omega)\), actin filament networks cross-linked/bundled by α-actinin or fascin were typically more dynamic than networks cross-linked/bundled by filamin, at least at high filamin concentrations (Fig. 6A). That difference in network dynamics diminished greatly at low filamin concentrations. The mean elasticity of F-actin networks measured at 1 radian/s was similar to for all tested cross-linkers at low concentrations (Fig. 6B). Here again, differences in stiffness became significant at high concentrations in actin-cross-linking proteins, with F-actin/filamin network by far the stiffest (Fig. 6B) and most solid-like (Fig. 6C) among the three tested types of cross-linked networks.

**DISCUSSION**

The Rheological Behavior of F-actin/Filamin Networks Depends Critically on Filamin Concentration—Our results show that at steady state F-actin/filamin networks behave either as dynamic structures or as covalently cross-linked gels, depending on the local filamin concentration. This transition in rheological behavior occurs at the actin-to-filamin molar ratio for which actin filament organization changes from mostly orthogonal networks to bundles (3). As indicated by the strong frequency dependence of \(G'(\omega)\), F-actin/filamin networks are dynamic at filamin-to-actin molar ratios as high as 1:200. Following classical interpretations of polymer physics (20, 23), such a frequency profile suggests that actin filaments can relax the stress via fast lateral fluctuations between points of polymer overlap at short time scales, creating a steep frequency dependence of \(G'(\omega)\) at high frequency before these filament fluctuations encounter the constraining tube-like region formed by the entanglements of surrounding filaments, creating the beginning of a quasi-plateau at long time scales or low frequencies (Fig. 2A). Increasing filamin concentration eliminates progressively the frequency dependence of \(G'(\omega)\) (Fig. 2A); polymer movements are constrained earlier (at higher frequencies) by cross-linkers, and longitudinal movements of filaments become prohibited (25).

If filamin were a true covalent cross-linker, even low filamin concentrations would stop all polymer motion in solution and render \(G'(\omega)\) independent of frequency. Therefore, actin filament networks adopt a covalently cross-linked behavior at high filamin-to-actin molar ratios >1:200, because the number of cross-linking proteins per filament reaches a threshold beyond which the probability of all (dynamic) cross-linkers being detached from the filament simultaneously becomes zero (26). The fact that we conducted these dynamic measurements at a strain amplitude that was explicitly verified to be within the linear regime eliminates the possibility that the low elasticity of F-actin/filamin at low filamin concentrations is due to filament breakage during the assay.

The filamin concentration at which the mechanical transition occurs is between 0.12 μM and 0.18 μM for a 24 μM actin solution (Fig. 2B). Because the equilibrium \(K_d\) of phosphorylated chicken gizzard filamin for F-actin (\(K_d = 6.9 \, \text{μM}\)) (27) is known, the number of filamin dimers attached to each filament in solution can be calculated. We find that that filamin concentrations of 0.12 μM and 0.18 μM correspond to molar ratios of F-actin-bound filamin dimers to actin of 1:515 and 1:129. Because the pitch of F-actin contains 28 actin subunits and is 72-nm long, these molar ratios correspond to average distances
Fig. 6. Mechanical behavior of filamin (Fil) compared with α-actinin (α-A) and fascin (F)-response to oscillatory deformations of small amplitude. A, frequency-dependent elastic modulus of F-actin/filamin, F-actin/α-actinin, and F-actin/fascin networks for concentrations in cross-linking proteins 0.24 μM. The control for actin alone is shown in Fig. 2A. Elastic modulus (B) and phase angle (C) were estimated at a frequency of 1 radian/s for the same networks for concentrations in cross-linking proteins of 0, 0.12, and 0.24 μM. Mechanical properties in panels A–C were evaluated at a strain amplitude of 1%. Actin concentration is 24 μM is all panels (A–C).

The Assay Traditionally Used to Probe the Effect of Large Deformations on Cytoskeletal Networks Is Ill-suited—The strain-sweep assay, which is traditionally used to assess the mechanical response of F-actin/filamin networks to shear. This assay showed that the resilience and the strain-hardening response of actin networks were dramatically enhanced by the presence of filamin compared with α-actinin and fascin.

In the absence of cross-linkers, actin filaments strain harden slightly (22) due to the unfavorable shear-induced bending that occurs around topological overlaps between filaments (22, 29). The presence of cross-linkers further enhances this effect by reducing the possibility of filaments to slide past one another (22). The mechanism by which networks of the intermediate filaments display strain hardening is fundamentally different (29). Keratin intermediate filament is more flexible than F-actin, which should decrease the propensity of keratin networks to strain harden, but interactions between keratin polymers are pronounced (30, 31). Therefore, points of polymer entanglements in keratin networks are extremely long-lived, which promotes pronounced strain hardening when networks are subject to large shear deformations.

It is important to note that, over the tested range of filamin concentrations, the linear regime of deformations extends well beyond 1%, the strain amplitude at which our dynamic measurements were conducted. This justifies our use of 1% strain deformation amplitudes in dynamic measurements (shown in Fig. 2). It also suggests that the reduced network elasticity at low filamin concentrations and low frequency (Fig. 2A) is not due to shear-induced polymer breakage during the experiments as suggested previously (13, 14) but reflects the dynamic nature of filamin subject to low, non-destructive, shear deformation amplitudes.

Mechanical Behavior of Filamin Compared with α-Actinin and Fascin—The gelation profile of F-actin in the presence of high concentrations of filamin goes through a distinct maximum. Interestingly, a similar non-monotonic gelation kinetics is displayed by polymerizing actin networks in the presence of the stereotypical F-actin-bundling protein fascin (16). This singular non-monotonic gelation reflects a partial collapse of the network structure profile due to the formation of actin bundles (21). This gelation behavior is not displayed by F-actin networks in the presence of the stereotypical F-actin cross-linking/bundling protein α-actinin even at α-actinin molar ratios >1:10 (19, 32) for which α-actinin bundles F-actin, presumably because the α-actinin-mediated F-actin bundles themselves are cross-linked (33).

Despite the fact that they seem to possess very similar structures and functions (34), α-actinin and filamin display different mechanical responses at different frequencies, strain amplitudes, and concentrations. The fact that these two cytoskeleton proteins localize to the base of cell surface protrusions and in stress fibers of adherent cells (1) suggests that α-actinin and filamin do not have redundant mechanical functions. Our recent work suggests instead that these two proteins may work synergistically to provide the cytoskeleton with enhanced stiffness and resilience (32, 33).

The mechanical properties of actin networks will depend not only on the \( k_{on} \) and \( k_{off} \) of the actin-binding protein for F-actin and the relative concentrations of the auxiliary proteins and actin (which are controlled), but also on the intrinsic molecular flexibility of the cross-linker. Clearly, electron microscopy is unsuitable for probing the flexibility of the F-actin cross-linkers, especially when these cross-linkers are under mechanical tension. The flexibility of cross-linking proteins is just starting to be investigated through single-molecule techniques. Atomic force microscopy studies of the flexibility of fragments of α-actinin (the spectrin-like repeat domain) and filamin (the immu-
noglobulin-like domain) have just appeared (35, 36). Therefore, we ought to postpone the description of a predictive model that would relate the structure, binding kinetics, and molecular flexibility of F-actin cross-linking molecules (e.g. α-actinin, fascin, and filamin) to the F-actin networks morphology and rheology when these biochemical and biophysical parameters are known.

The dual role of filamin in controlling simultaneously the architecture and mechanics of F-actin networks suggests an important physiological function. Filamin localizes both in actin-rich lamellae and actin stress fibers of adherent cells (37). At the edge of the cell where filamin has been shown to be present (8), F-actin forms orthogonal networks containing X, Y, and T junctions mediated by F-actin cross-linking proteins. This is the region of the cell where fast remodeling of the actin cytoskeleton is required, but where reasonably high stiffness is also required to allow for net pushing forces produced by polymerizing actin against the cell membrane (38). This combined orthogonal architecture and stiffness could readily be provided by low concentrations in filamin. In contrast, stable F-actin bundles lying on the ventral side of adherent cells require high concentrations in filamin. As assessed by immunoﬂuorescence microscopy (8, 37) and immunogold staining (38), the concentration in filamin at the edge of the cells is indeed not as high as the local concentration in F-actin bundles. Hence, the biphasic behavior observed in the architecture and mechanical function may be due in part to the spatial distribution and dual mechanical function of filamin.

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REFERENCES
1. van der Flier, A., and Sonnenberg, A. (2001) Biochim. Biophys. Acta. 1538, 99–117
2. Stossel, T. P., Condeelis, J., Cooley, L., Hartwig, J. H., Nuegel, A., Schleicher, M., and Shapiro, S. S. (2001) Nat. Rev. Mol. Cell Biol. 2, 138–145
3. Matsudaira, P. (1994) Semin. Cell Biol. 5, 165–174
4. Nunnally, M. H., D’Angelo, J. M., and Craig, S. W. (1980) J. Cell Biol. 87, 219–226
5. Fox, J. W., Lamerti, E. D., Eksingul, Y. Z., Hong, S. E., Feng, Y., Graham, D. A., Scheffer, J. E., Debney, W. B., Hirsch, B. A., Radtke, R. A., Berkove, S. F., Huttenlocher, P. R., and Walsh, C. A. (1998) Neuron 21, 1315–1325
6. Santi, M. R., and Golden, J. A. (2001) J. Neuropathol. Exp. Neurol. 60, 856–862
7. Cunningham, C. C. (1995) J. Cell Biol. 129, 1589–1599
8. Flanagan, L. A., Chou, J., Falet, H., Neujahr, R., Hartwig, J. H., and Stossel, T. P. (2001) J. Cell Biol. 155, 511–517
9. Glogauz, M., Arora, P., Chou, D., Janney, P. A., Downey, G. P., and McCulloch, C. A. G. (1998) J. Biol. Chem. 273, 1689–1698
10. Stossel, T. P. (1993) Science 260, 1086–1094
11. Sato, M., Schwarz, W. H., and Pollard, T. D. (1987) Nature 325, 828–830
12. Taner, K. S. (1986) J. Biol. Chem. 261, 7615–7620
13. Janney, P. A., Hvidt, S., Lamb, J., and Stossel, T. P. (1990) Nature 345, 89–92
14. Janney, P. A., Hvidt, S., Kas, J., Lerche, D., Magg, A., Sackmann, E., Schiwa, M., and Stossel, T. P. (1994) J. Biol. Chem. 269, 32503–32513
15. Tang, J. X., Janney, P. A., Stossel, T. P., and Itu, T. (1999) Biophys. J. 76, 2208–2215
16. Tseng, Y., Fedorov, E., McCaffery, J. M., Alme, S. C., and Wirtz, D. (2001) J. Mol. Biol. 310, 351–366
17. Feramisco, J. R., and Burridge, K. (1980) J. Biol. Chem. 255, 1194–1199
18. Hartwig, J. H., and Stossel, T. P. (1981) J. Mol. Biol. 145, 563–581
19. Tseng, Y., and Wirtz, D. (2001) Biophys. J. 81, 1643–1656
20. Dui, M., and Edwards, S. F. (1998) The Theory of Polymer Dynamics, Claren-don Press, Oxford
21. Ferry, J. D. (1980) Viscoelastic Properties of Polymers, John Wiley & Sons, Inc., New York
22. Xu, J., Tseng, Y., and Wirtz, D. (2000) J. Biol. Chem. 275, 35886–35892
23. Morse, D. C. (1998) Macromolecules 31, 4704–4707
24. Wachstock, D., Schwarz, W. H., and Pollard, T. D. (1993) Biophys. J. 65, 205–214
25. Palmer, A., Xu, J., and Wirtz, D. (1998) Rheologica Acta 37, 97–108
26. Xu, J., Wirtz, D., and Pollard, T. D. (1998) J. Biol. Chem. 273, 9570–9576
27. Ohta, Y., and Hartwig, J. H. (1995) Biochemistry 34, 6745–6754
28. Xu, J., Casella, J. F., and Wirtz, D. (1999) Cell Motil. Cytoskeleton 42, 75–81
29. Coulombe, P. A., Bousquet, O., Ma, L., Yamada, S., and Wirtz, D. (2000) Trends Cell Biol. 10, 420–428
30. Yamada, S., Wirtz, D., and Coulombe, P. A. (2003) J. Struct. Biol. 143, 45–45
31. Tseng, Y., An, K. M., and Wirtz, D. (2002) J. Biol. Chem. 277, 18143–18150
32. Tseng, Y., Kole, T. P., and Wirtz, D. (2002) Biophys. J. 83, 3162–3176
33. Roux, T., and Vale, R. (1999) Guidebook to the Cytoskeletal and Motor Proteins, Oxford University Press, Oxford
34. Putikka, S., Ito, T., and Yamazaki, M. (2001) FEBS Lett. 498, 72–75
35. Tseng, Y., Fedorov, E., and McCaffery, J. M. (1998) The Theory of Polymer Dynamics, Claren-don Press, Oxford
36. Rief, M., Paschal, J., Saraste, M., and Gaub, H. E. (1999) J. Mol. Biol. 286, 553–561
37. Langanger, G., de Mey, J., Moeremans, M., Danes, G., de Brabander, M., and Small, J. V. (1994) J. Cell Biol. 99, 1324–1334
38. Pollard, T. D., and Borisy, G. G. (2000) Cytoskeleton 112, 453–465