DOI: 10.1002/cphc.200900604

Structural and Viscoelastic Properties of Actin Networks Formed by Espin or Pathologically Relevant Espin Mutants

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Dedicated to Erich Sackmann on the occasion of his 75th birthday

The structural organization of the cytoskeleton determines its viscoelastic response which is crucial for the correct functionality of living cells. Both the mechanical response and microstructure of the cytoskeleton are regulated on a microscopic level by the local activation of different actin binding and/or bundling proteins (ABPs). Misregulations in the expression of these ABPs or mutations in their sequence can entail severe cellular dysfunctions and diseases. Here, we study the structural and viscoelastic properties of reconstituted actin networks cross-linked by the ABP espin and compare the obtained network properties to those of other bundled actin networks. Moreover, we quantify the impact of pathologically relevant espin mutations on the viscoelastic properties of these cytoskeletal networks.

1. Introduction

Actin filaments are key structural and mechanical components in the cytoskeleton of eukaryotic cells. These filaments are organized by actin binding proteins (ABPs) into supramolecular assemblies such as bundles or cross-linked networks.[1–3] To reduce the overwhelming complexity found in living cells, it is a main strategy in the field of cytoskeletal mechanics to reconstitute actin filament assemblies with well-defined properties.[4] Indeed, this approach has been very successful for our understanding of how cross-linking of semi-flexible actin filaments into isotropically cross-linked networks determines the ensuing viscoelastic properties.[5–7] In contrast, the physical principles which lead to the formation of more complicated structural arrangements[8–10] are less well understood. Although the mechanical properties of single actin bundles have been described for several ABP systems[11,12] and considerable progress has been made in quantitatively describing viscoelastic properties of purely bundled actin/fascin networks,[13] an overall understanding of bundled actin networks has still to be achieved. This calls for a detailed investigation of other bundled actin systems to allow for a thorough test of theoretical predictions or simulations on bundled cytoskeletal networks.[14,15] Espins constitute a family of actin bundling proteins which are enriched in the parallel actin bundle of stereocilia in hair cells in the inner ear and are the target of mutations associated with deafness and vestibular dysfunction.[16–18] Mice with one such mutation, jerker, fail to accumulate espins and exhibit stereocilium degeneration.[16] Two human espin deafness mutations affecting the sequence of the espin C-terminal F-actin-binding site have been examined to date and found to significantly alter parallel actin bundle structure by small angle X-ray scattering.[19] Discovered originally in Sertoli cell junctions and brush border microvilli, espins are also enriched in the microvilli of a variety of sensory cells and in the dendritic spines of cerebellar Purkinje cells.[20] Espins differ from two other major classes of actin bundling proteins found in the vertebrates, namely, fimbrins/plastins and villin, in that they are not inhibited by physiological concentrations of calcium ion.[16] Thus, it is believed that espin cross-links confer calcium-ion insensitivity to F-actin bundles or networks in cells.[20]

Herein, we analyze the structural and viscoelastic behavior of reconstituted actin/espin networks. We compare networks of actin bundled by wildtype (WT) espin to actin filament assemblies which are organized by pathologically relevant espin mutants. Our findings show that WT espin forms cross-linked bundle networks whose properties are highly similar to those of actin/fascin bundle networks. Mutations in the F-actin-binding domain of espin not only prevent the correct formation of actin bundles but also significantly weaken the viscoelastic properties of the formed networks.

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2. Results and Discussion

Espin is reported to bundle actin filaments at high ratios between the cross-linker and actin, $R = \frac{c_{espin}}{c_{actin}}$.[19] Thus, we first analyze the microstructure of reconstituted actin/espin networks at high espin concentrations. Espin forms long and straight bundles which assemble into a well-ordered network (Figure 1 A). The overall structure of this bundle network closely resembles the network structure of purely bundled actin/fascin networks.[13] This underlines the generic trend of small ABPs showing a high bundling propensity.[21] For a further quantification of this striking resemblance of actin/espin and actin/fascin bundles, we study the formation of actin bundle ring structures in confined geometries employing an emulsion droplet systems.[12] As depicted in Figure 1 B, espin forms a continuous actin bundle ring at small droplet diameters. For larger emulsion droplets, however, the thickness of these bundle rings does not grow further. Instead, side branches or secondary rings are formed. The critical droplet diameter at which the first formation of a bundle side branch occurs is very similar for actin/espin and actin/fascin structures (yellow/orange symbols in Figure 1 B). This suggests that these two bundle systems have comparable finite thicknesses in the range of about 20 actin filaments/bundle.[22]

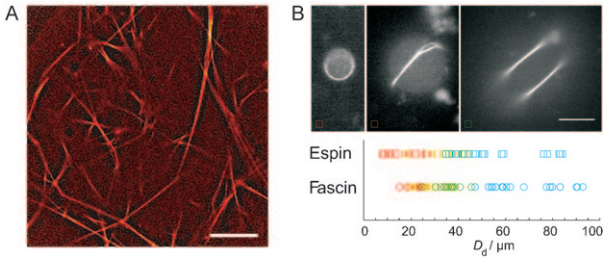

Figure 1. A) Overall network structure for an actin/espin network at $R = 1$ ($c_{actin} = 0.4$ mg mL^{-1}). Espin forms a bundle network with well-ordered straight and long bundles. The scale bar denotes 10 µm. B) Epi-fluorescence micrographs of TRITC phalloidin-labeled F-actin/espin bundles ($R = 1$, $c_{actin} = 0.4$ mg mL^{-1}) confined in emulsion droplets. The colors depicted in the diagram represent the different structures observed for espin (squares) and fascin (circles): For small droplet diameters, D_d, the filaments organize into a single ring (red), for larger droplets, forks (orange) and small side branches appear (yellow), and for very large droplets, several rings (green) or more complicated structures are found (blue). Scale bar: 10 µm.

While in the emulsion droplet system the formation of actin bundles is facilitated by the confinement effect of the spherical geometry,[12] a higher cross-linker concentration is necessary in bulk to induce a transition into a bundle network. In fact, the bundling transition for actin/espin networks is reported to occur at a similar concentration regime as for fascin,[13,19] which underlines the generic nature of this structural transition as parameterized in ref. [13]. It has been well established that such a structural transition always entails significant changes in the viscoelastic network response. Thus, in the following part of the manuscript, we address the influence of espin and its mutants on the viscoelastic response of reconstituted actin/espin networks.

Depending on the type and concentration of the cross-linking molecule, actin networks show different mechanical properties. While the cross-linking molecules HMM[6] and scruin,[8] as well as fascin[13] and α-actinin[10] in the bundle phase, strongly enhance the network elasticity in the linear regime, a comparably small effect is observed for the bundling protein filamin.[9] As shown in Figures 2 A and 2 B, the addition of espin results in a strong increase of the network elasticity. The elastic modulus is enhanced up to 1000-fold at 0.4 mg mL^{-1} actin, while at a lower actin concentration (namely, 0.2 mg mL^{-1} actin) this effect is less pronounced. In addition, the shape of the frequency-dependent viscoelastic moduli $G'(f)$ and $G''(f)$ drastically changes: For both actin concentrations investigated here, $G'(f)$ becomes gradually flatter with increasing espin concentrations. Moreover, at a high espin concentration a weak minimum in $G''(f)$ appears. Both effects have also been reported for actin networks cross-linked by the ABP fascin. However, for actin/fascin networks, the minimum in $G''(f)$ is more pronounced.

In the nonlinear regime, that is, at high deformations, the viscoelastic network response depends on many parameters including the cross-linker density, the shear rate, and the cross-linker microstructure. For instance, actin solutions and weakly

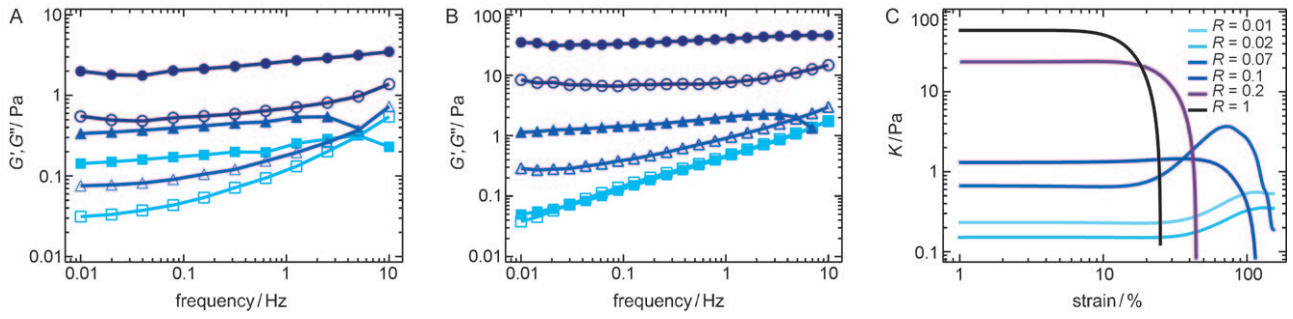

Figure 2. Viscoelastic response for actin networks cross-linked by wild-type (WT) espin. A, B) Frequency spectra of actin/espin networks for different espin concentrations [$R = 0.01$ (squares), $R = 0.1$ (triangles) and $R = 1$ (circles)] at 0.2 mg mL^{-1} (A) and 0.4 mg mL^{-1} actin (B). The closed symbols represent $G'(f)$ and the open ones $G''(f)$. WT espin increases the network elasticity and induces a flattening of the frequency spectrum. C) Nonlinear stiffness $K = \frac{\partial \sigma}{\partial \gamma}$ as a function of strain for actin/WT espin networks (0.4 mg mL^{-1} actin) at a shear rate of $\frac{d\gamma}{dt} = 12.5\,\%\,s^{-1}$. With increasing WT espin concentration, the nonlinear response changes from strain hardening to strain weakening.

cross-linked actin networks show a strain hardening behavior at sufficently high shear rates[3,23] whereas strain weakening is observed for fascin networks at low shear rates or high fascin concentrations.[24] We now investigate the nonlinear properties of actin/espin networks by applying a constant shear rate of 12.5 % s^{-1} and evaluating the resulting stress–strain relation as described in ref [23]. Similar to actin/fascin networks, actin/espin networks show a strain hardening response at low espin concentrations up to $R = 0.07$ while strain weakening is observed for high espin concentrations, that is, for $R = 0.1$ and above (Figure 2C). Thus, the microstructure, the onset of bundle formation, and the linear and the nonlinear viscoelastic response of actin/espin networks resemble the behavior observed for actin/fascin networks. The high tunability of this nonlinear network response arises from forced cross-linker unbinding events occurring between distinct actin bundles.[13,24]

Yet, the absence of a pronounced minimum in the viscous dissipation of actin/espin networks suggests that the density of cross-links between distinct espin bundles might be rather low[6,7]—that is, if the small espin protein is able to effectively cross-link distinct bundles at all.

To clarify this question, we investigate distinct bundle/bundle intersections as putative loci for cross-link points more in detail. For bundle networks induced by filamin it has been demonstrated that the network acquires internal stress during polymerization.[25] Due to the complicated branched structure of actin/filamin bundle networks, this internal stress cannot be released by transient cross-linker unbinding events, which results in a kinetically trapped network. However, for the much simpler network morphology observed for actin/espin bundle networks, such a stress-induced bundle unbinding and rebinding event might be observable by microscopy. In Figure 3A, a bundle/bundle intersection is depicted right before such an unbinding and rebinding event occurred for the left-hand bundle. As can be seen from the corresponding kymograph (Figure 3C), unbinding of the bundle/bundle cross-link is followed by an intermediate state of diffusive search for a new binding site until the bundle finally locks in into a new stable position (Figure 3B). This demonstrates that espin is indeed capable of forming bundle/bundle cross-links in reconstituted actin networks. However, such events are extremely rare to observe which underlines the low degree of bundle/bundle cross-linking as already inferred from macrorheology.

For cross-linked and bundled actin networks the binding affinity of the cross-linker crucially affects the structural organization and viscoelastic response.[10] The F-actin-binding domains of espin are crucial for the correct formation of cross-links within actin/espin bundles.[19] In the remaining part of this Article we aim to address the question: how do pathologically relevant mutations in the actin binding sites of wild-type (WT) espin affect the viscoelastic response of reconstituted actin/espin networks?

The mildest form of a mutation which can occur in a protein is a point mutation. The first espin mutant investigated here, hE3AdelK, shows exactly such a point mutation in the actin binding domain of espin, in a region which is believed to encompass one of its two F-actin binding sites.[16,18] In ref. [19] it has been shown that at molar ratios below $R = 0.1$, where WT espin can effectively organize actin into hexagonally packed bundles, hE3AdelK only induces the formation of aggregates with a short-range positional ordering. Highly ordered bundles are only formed at high concentrations of hE3AdelK (Figure 4A). Nevertheless, the effect of hE3AdelK on the static network elasticity is surprisingly similar to what is observed for WT espin (Figure 4B): the increase of the apparent plateau modulus, G_0, as a function of the cross-linker concentration and the maximal observed network stiffness are comparable in magnitude. This suggests that the structural properties of single actin/espin bundles have a rather small effect on the macroscopic network stiffness. At high cross-linker concentrations, where both WT espin and hE3ADelK induce the formation of bundles, also the viscoelastic spectra of actin/hE3AdelK networks resemble the spectra observed for actin/WT espin (Figure 4C): $G'(f)$ is rather independent of frequency and $G''(f)$ exhibits a shallow minimum. Moreover, the espin mutant modulates the nonlinear network response in a similar way as observed for WT espin: no strain hardening is detectable any more at high cross-linker concentrations (Figure 4D). However, this effect occurs already at a lower cross-linker concentration, $R = 0.02$ (at this R value strain hardening is still observed in networks cross-linked by WT espin).

Altogether, the viscoelastic properties of hE3AdelK/actin networks are very similar to those of WT espin/actin networks. However, much stronger alterations in the viscoelastic network response can be expected for a frame-shift mutation which affects an F-actin-binding domain. Such a frame-shift mutation should completely abolish the specific binding of the corresponding espin domain towards actin.

Surprisingly, it has been reported from X-ray scattering ex-

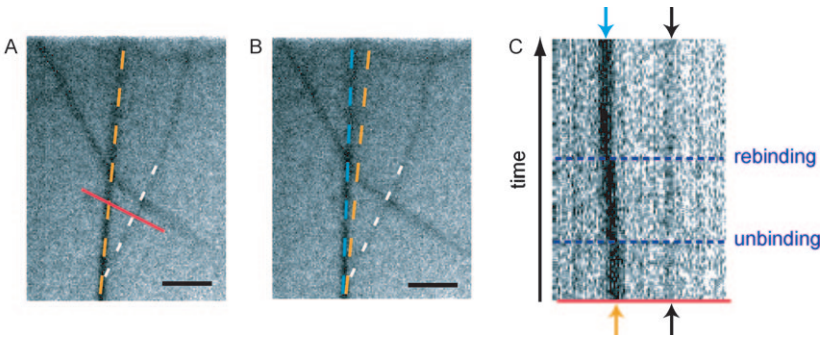

Figure 3. Unbinding and rebinding of a bundle/bundle cross-link point visualized by fluorescence microscopy. Two neighboring bundle/bundle intersection points are shown at times $t = 0$ (A) and $t = 28$ s (B). C) Kymograph showing a time lapse of 28 s for the red section depicted in (A). The position of the left bundle switches from its original position (orange) to a new final position (blue) while the position of the right bundle remains constant. The scale bars represent 2 μm.

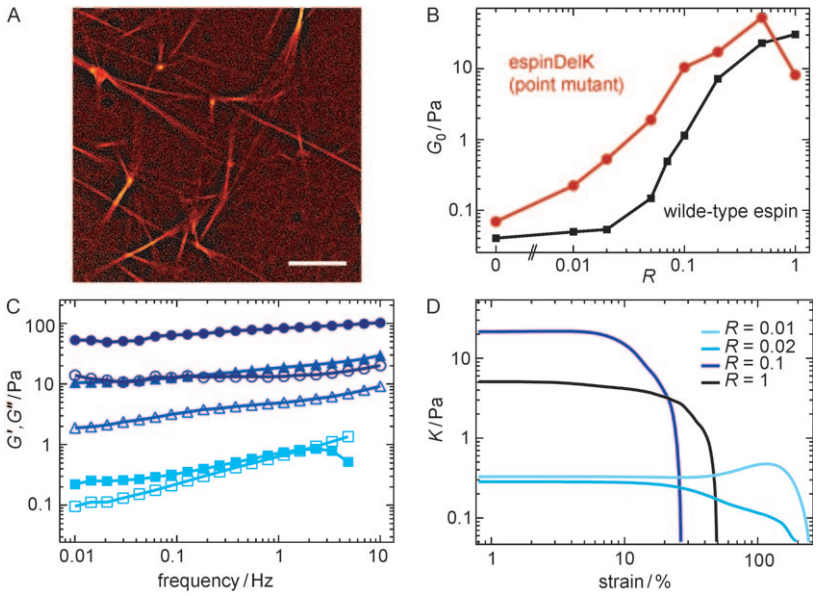

Figure 4. A) hE3AdelK induces the formation of a bundle network which resembles the networks induced by WT espin ($R=1$, $c_{actin}=0.4$ mg mL^{-1}). The scale bar denotes 10 μm. B) Apparent plateau moduli $G_0:=G'(10\ mHz)$ as a function of the relative cross-linker concentration for WT espin (black squares) and hE3AdelK espin (red circles). C) Frequency spectra of actin/hE3AdelK networks for different hE3AdelK concentrations [$R=0.01$ (squares), $R=0.1$ (triangles), and $R=0.5$ (circles)] at 0.4 mg mL^{-1} actin. The closed symbols represent $G'(f)$ and the open ones $G''(f)$. Also hE3AdelK espin increases the network elasticity and induces a flattening of the frequency spectrum. D) Nonlinear stiffness $K=\frac{\partial\sigma}{\partial\gamma}$ as a function of strain for actin/hE3AdelK espin networks (0.4 mg mL^{-1} actin) at a shear rate of $\frac{d\gamma}{dt}=12.5\,\%\,s^{-1}$. With increasing hE3AdelK concentration, the nonlinear response changes from strain hardening to strain weakening.

periments that the frame shift espin mutant hE3AdelCt is still able to mediate a perceivable interaction between actin filaments.[19] Such an interaction is also detectable in the linear viscoelastic response of actin/hE3 AdelCt networks. As depicted in Figure 5 A, the apparent plateau elasticity $G_0:=G'(10\ mHz)$ of an entangled actin solution (0.2 mg mL^{-1} actin) can be increased up to five times by the addition of hE3AdelCt. Also, the frequency spectra of actin/hE3 AdelCt networks gradually change from a power-law-like shape at $R=0$ to a rather flat spectrum at high R (Figure 5 B), as generically observed for cross-linked systems.[6,27] WT espin is known to be monomeric in solution.[26] Thus, one might speculate that the frame-shift espin mutant could dimerize by its mutated domains, thereby forming a relatively effective cross-linking (but not bundling) ABP. An alternative explanation for the unexpected cross-linking ability of hE3AdelCt could be that WT espin contains an additional third actin binding site which is unaffected by the frame-shift mutation. Finally, non-specific binding to actin (possibly mediated by the His tag) might also account for the observed behavior in the viscoelastic frequency response.

3. Conclusions

In summary, we have shown that the microstructure and the linear and nonlinear viscoelastic properties of actin/WT espin networks closely resemble those reported for purely bundled actin/fascin networks. The main difference between these two bundle networks seems to be given by a lower degree of bundle interconnectivity in the actin/espin system. Furthermore, we have analyzed the impact of two pathologically relevant espin mutations on the viscoelastic network response. While the point mutation investigated here is known to disturb the packing order of actin/espin bundles, we find that the ensuing viscoelastic properties of the bundle network are rather insensitive to this mutation. For the much more drastic frame-shift mutation, we detect a weak but significant cross-linking activity, which is surprising given that one F-actin-binding domain is eliminated.[19] The data presented herein nicely fit into the current understanding of how the cross-linker properties dictate the structure and viscoelastic reponse of reconstituted actin networks. However, the results obtained for the two espin mutants demonstrate that a detailed correlation of cross-linker properties, bundle organization, network structure, and mechanical properties is far from being trivial. Yet, the investigation of a broad spectrum of actin cross-linking proteins and associated mutants by experimental and theoretical means might enable us to understand the molecular

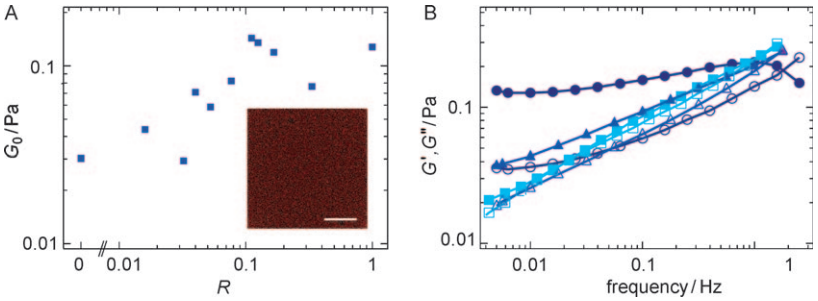

Figure 5. A) Apparent plateau moduli $G_0:=G'(10\ mHz)$ as a function of the relative cross-linker concentration for hE3AdelCt. The inset shows an actin network in the presence of hE3AdelCt ($R=1$, $c_{actin}=0.2$ mg mL^{-1}). No bundles are detectable. The scale bar denotes 10 μm. B) Frequency spectra of actin/hE3AdelCt networks for different hE3AdelCt concentrations [$R=0$ (squares), $R=0.016$ (triangles) and $R=1$ (circles)] at 0.2 mg mL^{-1} actin. The closed symbols represent $G'(f)$ and the open ones $G''(f)$. Surprisingly, even hE3AdelCt slightly increases the network elasticity and induces a flattening of the frequency spectrum.

basis of cytoskeleton-related diseases and predict the impact of dysfunctional actin cross-linkers in vivo.

Experimental Section

Materials: G-actin was obtained from rabbit skeletal muscle and stored in lyophilized form at $-21^{\circ}C$.[28] The G-actin solution was prepared by dissolving lyophilized actin in deionized water and dialyzing against G-buffer (2 mM Tris, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.2 mM DTT, and 0.005% NaN_3, pH 8) at 4°C. The G-actin solution was kept at 4°C and used within ten days. The average length of the actin filaments was controlled to 21 μm by adjusting the molar ratio between actin and gelsolin[29] obtained from bovine plasma serum, as reported in ref. [30]. Human espin 3A (MW: 30.9 kDa) and its two mutants hE3AdelK (MW: 30.9 kDa) and hE3AdelCt (MW: 29.8 kDa) were expressed in bacteria and purified as described in ref. [19].

Methods: The viscoelastic response of actin/espin networks was determined by measuring the frequency-dependent viscoelastic moduli $G'(f)$ and $G''(f)$ with a stress-controlled rheometer (Physica MCR 301, Anton Paar, Graz, Austria) over a frequency range of three decades. Polymerization was initiated by adding 10% volume 10x F-buffer (20 mM Tris, 5 mM ATP, 20 mM $MgCl_2$, 2 mM $CaCl_2$, 1 M KCl, 2 mM DTT, pH 7.5). The sample (approximately 480 μL) was loaded within 1 min into the rheometer using a 50 mm plate–plate geometry with 160 μm plate separation. Actin polymerization was carried out in situ, and the measurements were performed after full polymerization. To ensure a linear response, only small torques ($\approx$0.5 μNm) were applied. In all the experiments, the relative cross-linker concentration $R = \frac{c_{ABP}}{c_{actin}}$ was controlled as a key parameter. To investigate the network structures, actin was labeled with phalloidin-TRITC (Sigma–Aldrich, Germany). Pictures and movies were acquired on an Axiovert 200 microscope (Zeiss, Oberkochen, Germany). Emulsion droplets containing F-actin/espin bundles were prepared as described previously.[12]

Acknowledgements

We thank M. Rusp for the actin preparation. This work was supported by the Deutsche Forschungsgemeinschaft, through the DFG-Cluster of Excellence Nanosystems Initiative Munich and through Grant No. Ba2029/8-1, and the National Institutes of Health (NIH) grant R01 DC004314 (to J.R.B.). O. Lieleg acknowledges a postdoc fellowship from the German Academic Exchange Service (DAAD).

Keywords: actin · biophysics · fluorescence · mutation · proteins

[1] M. Tempel, G. Isenberg, E. Sackmann, *Phys. Rev. E* **1996**, *54*, 1802.
[2] M. L. Gardel, J. H. Shin, F. C. MacKintosh, L. Mahadevan, P. Matsudaira, D. A. Weitz, *Science* **2004**, *304*, 1301.
[3] M. L. Gardel, F. Nakamura, J. H. Hartwig, J. C. Crocker, T. P. Stossel, D. A. Weitz, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 1762.
[4] A. R. Bausch, K. Kroy, *Nat. Phys.* **2006**, *2*, 231.
[5] F. C. MacKintosh, J. Käs, P. A. Janmey, *Phys. Rev. Lett.* **1995**, *75*, 4425.
[6] R. Tharmann, M. M. A. E. Claessens, A. R. Bausch, *Phys. Rev. Lett.* **2007**, *98*, 088103.
[7] O. Lieleg, M. M. A. E. Claessens, Y. Luan, A. R. Bausch, *Phys. Rev. Lett.* **2008**, *101*, 108101.
[8] J. H. Shin, M. L. Gardel, L. Mahadevan, P. Matsudaira, D. A. Weitz, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9636.
[9] K. M. Schmoller, O. Lieleg, A. R. Bausch, *Phys. Rev. Lett.* **2008**, *101*, 118102.
[10] O. Lieleg, K. M. Schmoller, C. J. Cyron, Y. Luan, W. A. Wall, A. R. Bausch, *Soft Matter* **2009**, *5*, 1796.
[11] J. H. Shin, L. Mahadevan, P. T. So, P. Matsudaira, *J. Mol. Biol.* **2004**, *337*, 255.
[12] M. M. A. E. Claessens, M. Bathe, E. Frey, A. R. Bausch, *Nat. Mater.* **2006**, *5*, 748.
[13] O. Lieleg, M. M. A. E. Claessens, C. Heussinger, E. Frey, A. R. Bausch, *Phys. Rev. Lett.* **2007**, *99*, 088102.
[14] C. Heussinger, M. Bathe, E. Frey, *Phys. Rev. Lett.* **2007**, *99*, 048101.
[15] J. Astroem, P. B. Sunil Kumar, I. Vattulainen, M. Karttunen, *Phys. Rev. E* **2008**, *77*, 051913.
[16] L. Zheng, G. Sekerkova, L. Tilney, E. Mugnaini, J. R. Bartles, *Cell* **2000**, *102*, 377.
[17] S. Naz, A. J. Griffith, S. Riazuddin, L. L. Hampton, J. F. Battey, S. N. Khan, S. Riazuddin, E. R. Wilcox, T. B. Friedman, *J. Med. Genet.* **2004**, *41*, 591.
[18] F. Donaudy, L. Zheng, R. Ficarella, E. Ballana, M. Carella, S. Melchionda, X. Estivill, J. R. Bartles, P. Gasparini, *J. Med. Genet.* **2006**, *43*, 157.
[19] K. R. Purdy, J. R. Bartles, G. C. L. Wong, *Phys. Rev. Lett.* **2007**, *98*, 058105.
[20] G. Sekerkova, L. Zheng, P. A. Loomis, E. Mugnaini, J. R. Bartles, *Cell. Mol. Life Sci.* **2006**, *63*, 2329.
[21] B. Wagner, R. Tharmann, I. Haase, M. Fischer, A. R. Bausch, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 13974.
[22] M. M. A. E. Claessens, C. Semmrich, L. Ramos, A. R. Bausch, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6590.
[23] C. Semmrich, R. J. Larsen, A. R. Bausch, *Soft Matter* **2008**, *4*, 1675.
[24] O. Lieleg, A. R. Bausch, *Phys. Rev. Lett.* **2007**, *99*, 158105.
[25] K. M. Schmoller, O. Lieleg, A. R. Bausch, *Soft Matter* **2008**, *4*, 2365.
[26] J. R. Bartles, L. Zheng, A. Li, A. Wierda, B. Chen, *J. Cell Biol.* **1998**, *143*, 107.
[27] M. L. Gardel, J. H. Shin, F. C. MacKintosh, L. Mahadevan, P. A. Matsudaira, D. A. Weitz, *Phys. Rev. Lett.* **2004**, *93*, 188102.
[28] J. A. Spudich, S. Watt, *J. Biol. Chem.* **1971**, *246*, 4866.
[29] P. A. Janmey, J. Peetermans, K. S. Zaner, P. S. Stossel, T. Tanaka, *J. Biol. Chem.* **1986**, *261*, 8357.
[30] H. Kurokawa, W. Fujii, K. Ohmi, T. Sakurai, Y. Nonomura, *Biochem. Biophys. Res. Commun.* **1990**, *168*, 451.

Received: July 29, 2009
Revised: September 2, 2009
Published online on September 24, 2009

