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Regulating Contractility of the Actomyosin Cytoskeleton by pH

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SUMMARY

The local interaction of F-actin with myosin-II motor filaments and crosslinking proteins is crucial for the force generation, dynamics, and reorganization of the intracellular cytoskeleton. By using a bottom-up approach, we are able to show that the contractility of reconstituted active actin systems is tightly controlled by the local pH. The pH-dependent intrinsic crossbridge strength of myosin-II is identified to account for a sharp transition of the actin/myosin-II activity from noncontractile to contractile by a change in pH of only 0.1. This pH-dependent contractility is a generic feature, which is observed in all studied crosslinked actin/myosin-II systems. The specific type and concentration of crosslinking protein allows one to sensitively adjust the range of pH where contraction occurs, which can recover the behavior found in *Xenopus laevis* oocyte extracts. Small variations in pH provide a mechanism of controlling the contractility of cytoskeletal structures, which can be expected to have broad implications in our understanding of cytoskeletal regulation.

INTRODUCTION

The contraction of cytoskeletal structures driven by movement of myosin-II motors along crosslinked actin filaments is a generic principle that is broadly used by cells (Vavylonis et al., 2008; Martin et al., 2009; Cai et al., 2010; Kasza and Zallen, 2011). It is essential for various processes such as cell division, cell locomotion, and tissue morphogenesis. To effectively employ the contractility for multiple tasks, actomyosin activity in cells has to be controlled precisely in space and time. In vitro studies with reconstituted systems have been proven useful to gain insight into the mechanisms of actomyosin contraction and reveal parameters that allow tuning of the contractile behavior: it has been shown that not only a critical amount of motor activity but also a sufficiently high interconnectivity between actin filaments, which can be mediated by additional crosslinking proteins, is crucial for contraction (Bendix et al., 2008). Therefore, a variation of the concentrations of myosin-II, the crosslinkers (Bendix et al., 2008) but also of actin itself (Köhler et al., 2011), allows tuning of the contractile behavior of the system. In vivo, not necessarily the concentrations but rather the activities of these proteins will be regulated: it has been demonstrated that modulation of actomyosin contractility can be achieved by a change of the length distribution of actin filaments (Janson et al., 1991) or by adjusting the activity of myosin-II (e.g., by phosphorylation) (Ezzell et al., 1983). Variation of the crosslinker binding strength, as it can be achieved for α-actinin by a regulation of the calcium concentration (Burridge and Feramisco, 1981), can be expected to tune the network contractility. Moreover, it has been speculated that by the regulation of the myosin light-chain kinase, even pH indirectly controls the actomyosin contractility (Stock and Schwab, 2009). Along that line, experiments performed by Condeelis and Taylor (1977) decades ago suggested that the contractile behavior of *Dictyostelium discoideum* cell extracts is strongly pH-dependent; yet, the potential dependence of actomyosin contractility itself on pH has not been further addressed.

Although changes in intracellular pH are crucially involved in many important physiological processes such as cell migration (Denker and Barber, 2002; Stock and Schwab, 2006; Srivastava et al., 2007; Frantz et al., 2008), cell division (Sano et al., 2009), apoptosis (Matsuyama et al., 2000; Lagadic-Gossmann et al., 2004), and tumor progression (Cardone et al., 2005; Stock and Schwab, 2009; Webb et al., 2011), little is known still about the molecular mechanisms that are involved in pH-sensitive regulation pathways (Srivastava et al., 2007; Casey et al., 2010). Recently, it has been shown that cells employ local and transient pH variations to regulate the localized cytoskeletal response during invadopodium protrusion and retraction (Magalhaes et al., 2011). However, for most systems it is largely unknown over which pathway pH is affecting the cytoskeleton.

To unambiguously identify the mechanisms by which a change in pH can provoke a cytoskeletal response, a bottom-up approach is needed, allowing the tight control of all constituents. Here we show that pH acts as a direct regulator of actomyosin activity. Already a small variation of pH is sufficient to sharply switch the contractile behavior of the active cytoskeletal structures. We reconstitute a minimal model system consisting of F-actin, myosin-II filaments, and a broad variety of different crosslinking proteins. The presence of any of the studied

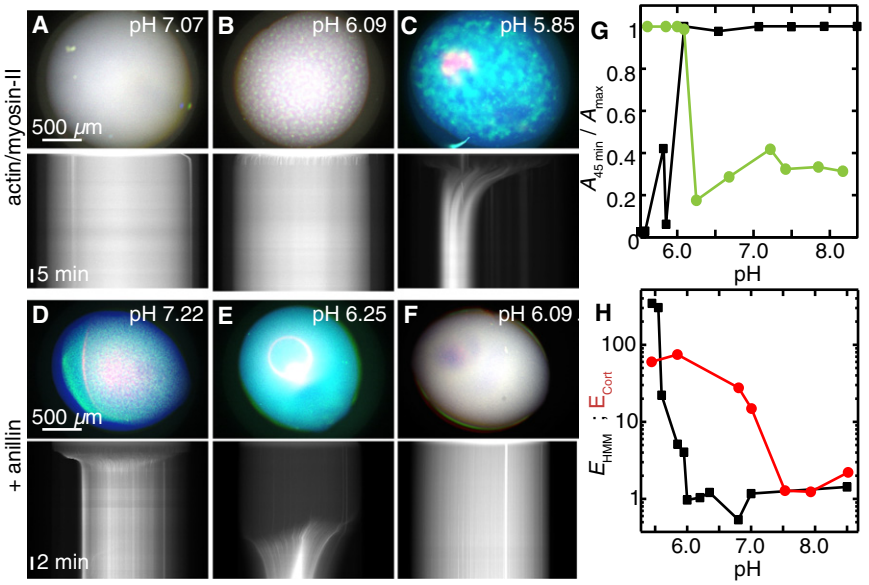

Figure 1. pH-Induced Contractility in Active Actin Systems

(A–F) Actin/myosin-II systems (10 μM actin, 0.1 μM myosin) do not contract at neutral pH (A, pH 7.07) down to pH 6.09 (B) as can be seen in the colored time overlay (blue: 0 min, green: 2 min and red: 20 min; upper image) and the kymograph of the maximal intensity projection (lower image). Yet, at lower pH (C, pH 5.85) a macroscopic contraction is observed. On the other hand, in presence of the crosslinker anillin (1.5 μM), contraction is observed only at the higher pH values (D, pH 7.22 and E, pH 6.25) but not at lower pH (F, pH 6.09). See also Movies S1–S4.

(G) Quantitative analysis of the contractile behavior in the absence (squares) or presence (green circles) of anillin. The fraction of the area which is still covered by the fluorescent gel after 45 min of contraction ($A_{45\,min}$) with respect to the maximal area (A_{max}) is shown as a function of pH.

(H) Macrorheology is used to determine the enhancement of the elastic modulus of F-actin systems by HMM (squares) and cortexillin (circles). The ratio of the elastic modulus in the presence of HMM or cortexillin and pure F-actin solutions ($E_{HMM/Cort} = G'_{HMM/Cort}$ [0.5 Hz]/$G'_{F\text{-}actin}$ [0.5 Hz]) is shown as a function of pH. Data points shown in (G) and (H) correspond to single experiments.

See also Figure S1.

crosslinking proteins inverts the pH-dependence of the contractility of pure actin/myosin-II networks and is required to reproduce the contractile behavior that we find for mitotic *Xenopus laevis* oocytes extracts. The pH sensitivities of myosin-II and crosslinking proteins themselves define a pH range where the network elasticity matches the motor activity, which in turn is mandatory for any long range reordering and active contraction. Using micropipette injection, we directly demonstrate that a local variation of pH allows generating a spatially confined contractile response. Our results identify a generic and simple mechanism that allows cells to precisely control their contractile processes in space and time.

RESULTS

In vitro macroscopic contraction assays provide a powerful tool to gain insights into microscopic mechanisms of actomyosin contractility (Bendix et al., 2008). The minimal reconstituted systems studied here consist of actin, which is polymerized in the presence of myosin-II, and the respective crosslinking proteins by addition of 10% of 10× AB buffer (see Experimental Procedures). In a solution of actin filaments, where no crosslinking proteins are present, the myosin-II filaments are unable to exert forces between the actin filaments. Thus at neutral pH (≈7) no contraction is observed by fluorescence microscopy (Figure 1A; Movie S1). Upon addition of crosslinking proteins, the active actin network contracts, as now the motors can act against the network elasticity (Figure 1D; Movie S2). During the force exertion of the myosin-II filaments, at least two heads need to be attached simultaneously to different actin filaments. By that, myosin-II filaments themselves become effectively transient crosslinkers for a short period of time. Recently, it has been suggested that this dwell time of myosin-II in the actin bound state increases with decreasing pH (Debold et al., 2008), which accounts for the early finding that the speed of actin filaments in a gliding assay decreases with decreasing pH (Kron and Spudich, 1986). The increased dwell time could lead to an effective increase of the connectivity in the actin solution even in the absence of any additional crosslinking protein. Thus also pure actin/myosin-II solutions might be able to contract at lower pH. Indeed, although pure actin/myosin-II solutions are still noncontractile at pH 6.09, already a decrease of the pH to 5.85 results in a significant contraction (Figures 1B, 1C, and 1G, squares; Movie S3). This pH-induced transition from a noncontractile to a contractile system is also observed if nonmuscle myosin-II is used instead of muscle myosin-II (Figures S1A–S1C), and can also be visualized by phase contrast microscopy in the absence of labeled phalloidin (Figures S1D–S1F).

In addition to the motor activity, it is the network elasticity that sets the contractile behavior of an active actin system (Bendix et al., 2008). Thus, revealing how pH affects the network elasticity would be the most direct way to test whether it is indeed an increase of the intrinsic crosslinking activity of myosin-II that enables contractility at low pH. Using a macrorheometer, the mechanical properties of reconstituted actin networks can be measured on a length scale in the order of 100 μm (Lieleg et al., 2010). In order to prevent any network contractions during rheological measurements, nonfilamentous heavy meromyosin (HMM) is used instead of the filament-forming full-length myosin-II. Above pH 6, the presence of HMM does not affect the elastic modulus of an F-actin solution (Figure 1H, squares). Yet, below pH 6 the ratio of the elastic modulus of active actin/HMM networks and pure actin solutions, E_{HMM}, sharply increases over more than two orders of magnitude. The fact that this increase of the network elasticity directly correlates with the transition from noncontractile to contractile

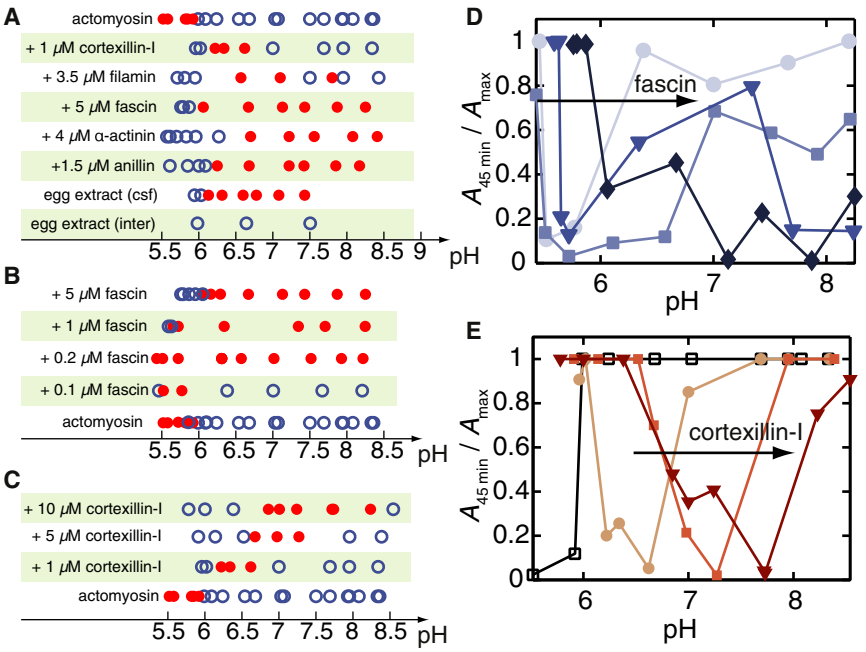

Figure 2. pH-Dependent Contractility of Crosslinked Active Actin Networks

(A–C) The contractility of reconstituted actin/myosin-II systems (10 μM actin, 0.1 μM myosin) is shown as a function of pH (red closed circles correspond to contractile systems, blue open circles correspond to noncontractile systems). (A) In the presence of crosslinking proteins the pH dependence of contractility resembles that of mitotic *Xenopus laevis* oocyte extract (csf). Interphase *Xenopus laevis* oocyte extracts do not contract within 45 min regardless the pH. (B) The pH-dependent transition from noncontractile to contractile is a function of motor- and crosslinking activity and is controlled by the fascin crosslinker concentration. (C) In active actin/myosin-II/cortexillin-I systems, the pH-dependent crosslinking activity of cortexillin-I results in a well-defined, cortexillin-I concentration-dependent window of contractility. (D and E) The fraction of the area that is still covered by the fluorescent gel after 45 min of contraction ($A_{45\ min}$) with respect to the maximal area (A_{max}) is shown for varying fascin (D, 0.1 μM [circles], 0.2 μM [squares], 1 μM [triangles], and 5 μM [diamonds]) and cortexillin concentrations (E, 0 μM [open squares], 1 μM [circles], 5 μM [closed squares], 10 μM [triangles]), respectively, as a function of pH. All data points shown correspond to single experiments.

See also Figure S2.

actin/myosin-II systems strongly suggests that the pH-dependent crosslinking activity of myosin-II itself accounts for the observed behavior.

Compared to the reconstituted actin/myosin-II system, an inverse pH dependence exists for *Dictyostelium discoideum* cell extracts: contraction occurs only at high pH but not below pH 7 (Condeelis and Taylor, 1977). A qualitatively similar behavior can be observed for mitotic *Xenopus laevis* oocyte extracts, where contraction occurs only above pH 6.3 (Figures 2A and S2A). A pH dependence of contractility that is almost identical to that of mitotic cell extract can be obtained in the reconstituted system by the addition of the crosslinker anillin, which indeed reverses the pH dependence of the actin/myosin-II system (Figures 1D–1G, circles; Movie S4). The additional crosslinking proteins enable contractility at high pH, where pure actin/myosin-II systems lack the critical amount of interconnectivity. Although a certain degree of elasticity is essential for contraction of actomyosin gels, contraction can also be suppressed if the concentration of crosslinkers is too high (Bendix et al., 2008). By lowering the pH, the crossbridge strength of the myosin-II is increased and thus the overall connectivity of the system becomes too high for the motor forces to be able to induce contraction (Figures 1E and 1F). This transition from a contractile to a noncontractile system caused by the pH dependence of myosin-II itself should be a generic feature of crosslinked actin/myosin-II gels. Indeed, also all other types of crosslinking proteins tested here (cortexillin, filamin, fascin, and α-actinin) reverse the pH dependence of the pure actin/myosin-II contractility similar to anillin (Figures 2A and S2A). The pH-dependent transition observed for mitotic *Xenopus laevis* oocyte extract can therefore be reproduced using any of the tested crosslinking proteins. This strongly suggests that it is the same mechanism, namely the increased network connectivity at low pH, which accounts for the behavior in the cell extract as well as in the reconstituted system. On the other hand, interphase *Xenopus laevis* oocyte extracts are noncontractile at all pH values tested (Figures 2A and S2A). Consistent with the observation that addition of crosslinking proteins does not enable contraction in interphase extracts (Field et al., 2011), this suggests that it is not a lack of network connectivity that prevents interphase extracts from contracting.

For the crosslinker fascin, the dependence of the network contractility on the crosslinker concentration can be explained considering only the pH dependence of the myosin-II crossbridge strength and the fact that contraction is only possible within a certain range of overall network elasticity. For pure actin/myosin-II systems or networks crosslinked by very low concentrations of fascin (0.1 μM), contraction is only possible at low pH. Here, myosin-II significantly contributes to the network elasticity and thus allows for contraction even at very low fascin concentrations (Figures 2B and 2D). This behavior is reversed at high fascin concentrations (1 or 5 μM). Interestingly, an intermediate fascin concentration (0.2 μM) can be found where contraction is observed over the whole range of pH values tested. There, enough fascin is present to allow for contraction at intermediate and high pH, but the critical network elasticity is still not exceeded at low pH, where myosin-II also acts as a crosslinker. The fact that the pH-dependent contractility of actin/fascin/myosin-II networks can be explained considering only the pH dependence of myosin-II suggests that the variation of

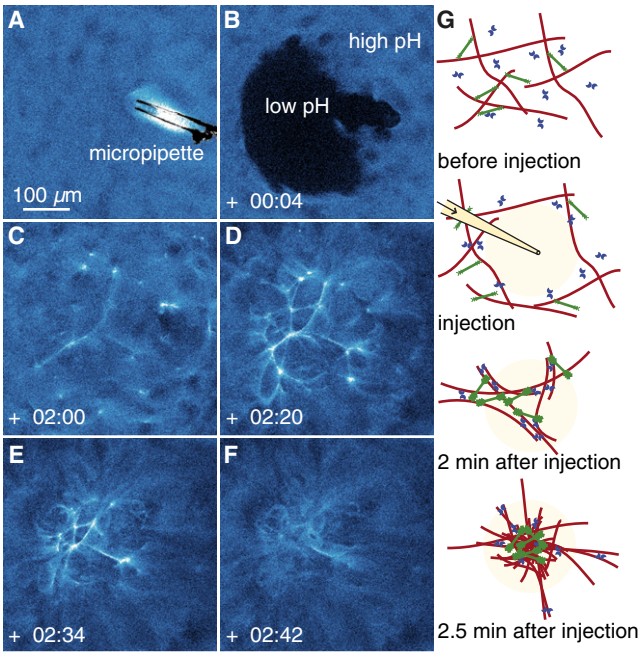

Figure 3. The pH Dependence of Actomyosin Contractility Is Employed to Induce Local Contraction in Active Actin/Myosin-II/Cortexillin Networks

(A–F) Confocal images (maximum projections of 40 μm z-stacks; in [A] an overlay of the fluorescence maximum projection and a bright field micrograph is shown) of 10 μM actin, 1 μM cortexillin, and 0.1 μM myosin-II at pH ≈7.75 after 30 min of polymerization show a homogeneous solution. Actin is visualized by the presence of Alexa488-phalloidin. Bundles are not observed (A). Using a micropipette, a small volume (in the order of 20 nL as estimated from confocal images) of 250 mM MES, pH 5.0 is injected and the micropipette is removed thereafter (B). Two minutes after injection of low pH buffer, bundles emerge at the spot of low pH (C). Due to myosin activity, the bundles contract (C–F).

(G) Illustration of the experiment. If polymerized at high pH, actin/myosin-II/cortexillin forms a homogeneous, noncontractile network. Upon injection of a small amount of low pH buffer, the crosslinking activity of cortexillin as well as the crossbridge strength of myosin-II increases and the network locally contracts.

See also Figure S3 and Movie S5.

pH does not significantly alter the effect of fascin. Indeed, with confocal imaging, we did not observe any pH-dependent changes in the structure of passive actin/fascin networks (Figures S2B–S2E) (Schmoller et al., 2012).

In the case of actin/myosin-II gels crosslinked by cortexillin or filamin, a second transition occurs at high pH, above which the system gets noncontractile again (Figure 2A). Variation of the cortexillin concentration reveals not only that contraction is limited to a certain range of pH, but also that for increasing cortexillin concentrations this range is significantly shifted to higher pH (Figures 2C and 2E). Both effects can be attributed to the fact that not only the crossbridge strength of myosin-II but also the crosslinking activity of cortexillin is pH-dependent: pull-down assays already indicated that cortexillin binds actin in a pH-dependent manner (Faix et al., 1996). Macrorheology is used to directly determine the pH dependence of the relevant parameter, namely the effect cortexillin has on the network elasticity. Indeed, the enhancement of the elastic modulus induced by addition of cortexillin to an F-actin solution, E_{cort}, strongly decreases with increasing pH (Figure 1H, circles). Thus, only within a certain range of pH is the effective network connectivity in actin/myosin-II/cortexillin systems suitable for contraction. This window of contractility is given by the balance of motor activity and the network elasticity, which is set by the pH dependence of both the myosin-II crossbridge strength and the crosslinking activity of cortexillin. For increasing cortexillin concentrations, the window of contractility shifts to higher pH (Figures 2C and 2E), as the effective crosslinker activity decreases with increasing pH.

To test whether a spatially confined variation of pH even allows local tuning of the contractile activity, a noncontractile actin/cortexillin/myosin-II gel is polymerized at pH ≈7.75 (Figure 3A). After 30 min, the gel appears homogeneous and no large structures such as bundles are detected with confocal microscopy. Using a micropipette, a small volume of low pH buffer is injected and within 2 min locally contracting bundles emerge in the area of low pH, whereas the rest of the network is not affected (Figure 3; Movie S5). A control experiment, where buffer with the same pH as used for the polymerization of the sample is injected (Figure S3), demonstrates that the observed contraction is not an artifact due to the mechanical perturbation of the network.

DISCUSSION

In summary, we have shown that due to the pH-dependent increase of its actin-bound dwell time, the myosin-II motor can also act as an effective crosslinker that enables contraction of actin/myosin-II systems at low pH. In combination with the distinct pH dependencies of actin crosslinking proteins, this results in an actomyosin contractility, which is highly sensitive to pH. Although the phenomenon of pH-dependent contractility occurs independently of the present type of crosslinking protein, the specific properties and concentrations of the different crosslinking proteins allow fine-tuning the pH where the transitions occur.

The generic existence of sharp pH-dependent transitions for all studied actomyosin systems strongly indicates that switching (on or off) of contractility via pH is an important mechanism in cells that acts in unison with other signaling pathways such as myosin-light chain phosphorylation. Vice versa, pH modulation allows adjustment of the actomyosin system such that a small variation of the concentration of active crosslinking proteins (e.g., induced via biochemical pathways) can also trigger the contraction. The results presented here demonstrate that local changes of pH can tune the contractile activity of the actin cytoskeleton. It has been suggested that during different physiological processes such as cell migration, pH gradients (0.2 pH units) are built up and maintained (Martin et al., 2011). These transient pH gradients are thought to play a major role for these cellular functions by mediating cytoskeletal reorganizations (Stock and Schwab, 2009; Casey et al., 2010). For example, such a pH gradient could enable cells to induce contractions at the retracting back of the cell while maintaining a flow at the leading front, with reduced overall contractility. Given the plasma membrane localization of Na^+/H^+ exchangers (Webb et al., 2011), local, submembranous intracellular pH gradients may

be of importance for cortical flows (Mayer et al., 2010) and cortex mechanics (Sedzinski et al., 2011). Our results also suggest a plausible simple causal explanation for the relation of acidification and muscle contraction during defecation in *Caenorhabditis elegans* (Beg et al., 2008; Pfeiffer et al., 2008). It remains for future studies to explore how pH regulates these and other cellular or developmental processes such as cytokinesis or morphogenesis, which also depend critically on contractility (Martin et al., 2009).

Our observations demonstrate that local variation of pH can provide an extremely powerful way for cells to control contractility in space and time. The interplay between the activation of cytoskeletal reorganizations by specific biochemical pathways and their modulation by small variations of the pH may, therefore, lead to a new perspective on cellular activation pathways.

EXPERIMENTAL PROCEDURES

Protein Purification

G-actin (Spudich and Watt, 1971; MacLean-Fletcher and Pollard, 1980), muscle myosin-II, and its subfragment HMM (Margossian and Lowey, 1982) are purified from rabbit skeletal muscle. Muscle myosin-II is used for the experiments shown in this work. A similar pH dependence is observed for nonmuscle myosin-II (Figures S1A–S1C). Nonmuscle myosin-II is purified from human platelets essentially as described in Pollard (1982) and stored in 0.6 M KCl, 1 mM dithiothreitol (DTT), and 10 mM imidazole (pH 7.0) at 3.7 μM. The fragment of *Xenopus laevis* anillin spanning amino acids 1–428 (Kinoshita et al., 2002) is cloned into pET-28a and purified from *Escherichia coli* with His-tags on both termini. Anillin 1–428 is stored in 25 mM imidazole pH 6, 25 mM KCl, 4 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT at −80°C. Recombinant *Dictyostelium discoideum* cortexillin-I (gift from G. Gerisch, Max Planck Institute of Biochemistry, Germany) is purified from *E. coli* BL21-CodonPlus-RP using a C-terminal His6-Tag (Faix et al., 1996). Cortexillin-I is stored at −80°C in cortexillin buffer (20 mM TRIS pH 8, 100 mM NaCl, 4 mM $CaCl_2$, and 2 mM DTT). Muscle filamin is isolated from chicken gizzard and further purified as reported in Shizuta et al. (1976). Recombinant human fascin is prepared as described by Ono et al. (1997) and Vignjevic et al. (2003). Alpha-actinin is isolated from turkey gizzard smooth muscle following Craig et al. (1982).

Xenopus laevis oocyte extracts are prepared as described in Field et al. (2011) (gift from I. Mattaj, EMBL Heidelberg, Germany).

Sample Preparation

G-actin is polymerized in presence of the respective actin binding proteins by addition of 10% of 10× AB buffer with different pH values (40 mM $MgCl_2$, 10 mM EGTA, 10 mM ATP, 20 mM DTT, 250 mM KCl [only in the case of rheological measurements], 250 mM MES for pH 3.5–6.5, imidazole for pH 7–7.5, or TRIS for pH 7.5–9). The different buffer compounds used here do not result in obvious changes of the properties of the actin networks, such as the elastic modulus, and thus do not account for the drastic pH dependencies observed. All data points shown correspond to single experiments. In order to obtain a consistent set of data, each series of experiments are performed with a single actin preparation. Different actin preparations result in only small differences in the exact value of the transition pH (see Figure 2B, where experiments with two different actin preparations are shown for actomyosin and with 5 μM fascin).

For contraction experiments, an ATP regeneration system consisting of 20 mM creatine phosphate and 0.1 mg/ml creatine phosphokinase (Sigma Aldrich) and additional buffer of the respective crosslinking proteins are added. Alexa488-phalloidin (0.3 μM; Invitrogen) is added to visualize the actin-containing areas. The presence of phalloidin does not influence the contraction behavior in presence or absence of crosslinking proteins as demonstrated in Figures S1D–S1F. The crosslinking proteins anillin, filamin, and α-actinin are diluted in filamin buffer (20 mM TRIS pH 7.5, 250 mM KCl, 0.5 mM EDTA, and 0.5 mM DTT) to a concentration 5-fold higher than the final concentration in the sample. Cortexillin is diluted in cortexillin buffer to the 2-fold of the final concentration. Fascin is diluted in fascin buffer (2 mM TRIS pH 8, 150 mM KCl) to the 5-fold of the final concentration. In the case of pure actin/myosin-II systems, either 20% filamin or fascin buffer or 50% cortexillin buffer is added to ensure comparable buffer conditions. No significant difference between these conditions is observed. In Figures 1G and 2E only the data obtained with filamin and cortexillin buffers, respectively, are shown. All crosslinker concentrations denote monomer concentrations.

After sample preparation, a part of the sample is used to measure the pH using a microelectrode (Schott Instruments). Note that the actual pH in the sample can deviate significantly from the pH of the AB buffer.

Microinjection experiments were performed with a homemade glass needle (22 μm diameter) using a Femto-Jet microinjector (Eppendorf). A small volume of low pH buffer (250 mM MES pH 5) is injected into a 40 μl sample (10 μM actin, 1 μM cortexillin, 0.1 μM myosin polymerized in 2 mM TRIS pH 8.2, 4 mM $MgCl_2$, 1 mM EGTA, 1 mM ATP, 2 mM DTT, 25 mM KCl) embedded in dodecane.

Fluorescence Microscopy

For macroscopic contraction assays, a 1.5 μl droplet of the solution (10 μM actin, 0.1 μM myosin-II, and indicated crosslinker concentration) is put in a dodecane phase immediately after initiation of polymerization and observed over time with fluorescence microscopy. Macroscopic contraction assays with cytoplasmic myosin-II contain 15 μM actin and 0.3 μM myosin-II.

Macroscopic contraction is imaged using either a Zeiss Axiovert 200 inverted fluorescence microscope (10× objective, NA 0.2) or a Leica DMI6000B inverted fluorescence microscope (10x objective, NA 0.3). Images are captured at 0.85 frames/s with a CCD (charge-coupled device) camera (Orca ER, Hamamatsu) attached to the microscope by a 0.4 (Zeiss) and 0.35 (Leica) camera mount, respectively. Confocal images are taken with a Leica TCS SP5 point-scanning confocal microscope (20× oil immersion objective, NA 0.7).

Image Processing

The total area of actin-containing regions in the droplet is determined by binarizing the fluorescence images. To this end, fluorescence intensity values of time lapse images are normalized to the range of 0–1. Binary images are obtained by applying a dynamic cutoff. The initial cutoff value is globally set to 0.057 or 0.067. The cutoff value is adjusted if regions of at least 200 pixels with increasing fluorescence intensity (compared to the image with the maximal actin containing region) are determined. The minimal intensity value in these regions is then used as the new cutoff value. Samples are defined as contractile if this area decreases to <80% of its maximal value within 45 min. For comparison of unlabeled systems and phalloidin-containing systems, phase contrast and fluorescence images are evaluated manually by measuring the actin containing area using a polygon in ImageJ. Except for the kymographs shown in Figure S3, kymographs are calculated from 1D-maximal intensity projections of 2D micrographs to minimize artifacts of asymmetric contractions.

Macrorheology

To confirm that the pH dependence of myosin-II significantly increases the network connectivity and thereby accounts for switching the contractile behavior, macrorheological measurements are performed. Macrorheology is not well suited to measure mechanical properties of actomyosin gels that globally contract during the measurement. As an alternative, we determine the elastic modulus of actin networks in the presence of HMM. In contrast to full length myosin-II, the nonprocessive HMM does not form filaments and can therefore hardly mediate forces between actin filaments. A stress-controlled macrorheometer (Physica MCR 301, Anton Paar, Graz, Austria) is used to determine the elastic modulus G' (0.5 Hz) of pure actin solutions (10 μM) and actin networks in the presence of 5 μM cortexillin or 1 μM HMM. After initiation of polymerization ~450 μl sample volume is loaded within 1 min into the rheometer using a 50 mm plate-plate geometry with 160 μm plate separation. Actin polymerization is carried out in situ, and followed by recording the elastic modulus G' at 0.5 Hz by applying only small torques

(≈0.5 μNm) to ensure a linear response. In the case of pure actin solutions and actin/cortexillin networks, the elastic modulus after 180 min of polymerization is used for the data shown in Figure 1H. For actin/HMM networks, the elastic modulus prior to ATP depletion, which results in a sharp increase of the elastic modulus, is used.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and five movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.08.014.

LICENSING INFORMATION

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