Scaling behaviour in steady-state contracting actomyosin networks

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Contractile actomyosin network flows are crucial for many cellular processes including cell division and motility, morphogenesis and transport. How local remodelling of actin architecture tunes stress production and dissipation and regulates large-scale network flows remains poorly understood. Here, we generate contracting actomyosin networks with rapid turnover in vitro, by encapsulating cytoplasmic Xenopus egg extracts into cell-sized ‘water-in-oil’ droplets. Within minutes, the networks reach a dynamic steady-state with continuous inward flow. The networks exhibit homogeneous, density-independent contraction for a wide range of physiological conditions, implying that the myosin-generated stress driving contraction and the effective network viscosity have similar density dependence. We further find that the contraction rate is roughly proportional to the network turnover rate, but this relation breaks down in the presence of excessive crosslinking or branching. Our findings suggest that cells use diverse biochemical mechanisms to generate robust, yet tunable, actin flows by regulating two parameters: turnover rate and network geometry.

The dynamic organization of cellular actin networks emerges from the collective activities of a host of actin-associated proteins, including factors that stimulate actin assembly and disassembly, various crosslinkers and filament-binding proteins that define the local network architecture, and myosin motors that generate contractile forces4–6. Myosin activity can drive global contraction and generate actin network flows, which play a crucial role in cell division, polarization and movement. For example, cell division is driven by the contraction of the cytokinetic ring, which is a quasi-one-dimensional actomyosin cable network. Contractile flows in the actin cortex are essential for cell polarization, while bulk actin network flows are crucial for localization of cellular components during the early stages of embryo development7. Continuous retrograde actin flows were further shown to provide the basis for amoeboid cell motility, the primary mode of motility observed in vivo for cells moving in a confined three-dimensional geometry8–10. In all of these examples, there is limited quantitative understanding of what determines the contractile network behaviour, and in particular what governs the rate of network flow. Both the force generation within actomyosin networks and their viscoelastic properties depend in non-trivial ways on the architecture and turnover dynamics of the network11–17. Conversely, the network architecture and dynamics are influenced by the stress distribution in the network and the flows it generates17–20. As such, understanding how the microscopic dynamics of actin and its associated proteins shape the large-scale structure and flow of contracting actomyosin networks remains an outstanding challenge.

The study of contracting actomyosin networks has primarily focused on the interplay between myosin activity and network connectivity15,16,21. Experiments with reconstituted networks assembled from purified actin, crosslinkers and myosin motors established the conditions required for large-scale global contraction12,18,22–25. However, in the absence of rapid network turnover, myosin-motor activity led to transient and essentially irreversible contraction; this is very different from the persistent actin flows in living cells, which exhibit continuous recycling of network components. Recent theoretical modelling and simulation studies have started to explore how rapid turnover influences force production, dissipation and the dynamic spatio-temporal organization of contracting actomyosin networks15,16,27–29. In particular, these simulations suggest that rapid turnover is essential for generating persistent flows, allowing the network to continuously produce active stress and dissipate elastic stress, while maintaining its structural integrity16.

Experimental efforts to study contracting actomyosin networks have been hampered by the lack of suitable model systems. In vitro reconstituted networks with limited turnover cannot sustain persistent network flows, so their contraction is necessarily transient, whereas in vivo studies on contracting networks are limited by the difficulties of measuring actin dynamics in complex cell geometries and the ability to control and vary system parameters. Here we leverage the unique benefits of an in vitro reconstitution system based on cell extracts that provides both myosin activity and physiological actin turnover rates, to investigate how the interplay between actin assembly and disassembly, myosin motor activity and network connectivity determines large-scale structure and dynamics of contracting actomyosin networks. Importantly, the rapid turnover in our system allows the networks to attain a dynamic steady-state characterized by persistent contractile flows and a self-organized, radially symmetric density distribution.

Using this system, we quantitatively characterize actin network turnover and flow and systematically investigate the emerging network properties. We find that under a broad range of physiological conditions, the networks contract homogeneously, despite large spatial variations in network density. The emergence of a density-independent contraction rate is surprising since the internal force...
generation in actin networks and their viscoelastic properties are strongly dependent on network density\textsuperscript{15,20,31}. Theoretical modelling using ‘active fluid theory’ suggests that the homogeneous contraction rate arises from a scaling relation between the active stress and the effective viscosity in these self-organized networks. We further find that the contraction rate is roughly proportional to the network turnover rate as we alter the dynamics of the system by changing its composition. This correlation breaks down when we add branching or crosslinking factors in comparable amounts to their endogenous concentrations\textsuperscript{12}. Together, these results show how physiological rates of actin turnover influence force production and dissipation in cellular actomyosin networks, demonstrating that the rate of network flow depends both on the actin turnover rate and on the local network architecture.

**Steady-state contracting actomyosin networks**

To generate contractile actin networks, we encapsulate cytoplasmic *Xenopus* egg extract in water-in-oil emulsion droplets\textsuperscript{10,13,20}. Endogenous actin nucleation activities induce the formation of a bulk network, which undergoes myosin-driven contraction\textsuperscript{10,16,31}. Within minutes after droplet formation, the system assumes a dynamic steady-state characterized by an inward network flow and a stationary network density that decreases toward the periphery (Fig. 1, Supplementary Fig. 1 and Supplementary Video 1). This steady-state is made possible by the rapid turnover in the system, such that the inward network flux is balanced by a diffusive flux of disassembled network components back to the periphery. The inward network velocity approaches zero at the boundary of a dense spherical ‘exclusion zone’ that forms at the centre of the droplet. This exclusion zone appears within minutes, as the network condensing this material into a spheroid (Fig. 1b and Supplementary Fig. 1e). The simple geometry and persistent dynamics of our system facilitate quantitative analysis of actin network turnover and flow. The network density and flow attain a radially symmetric pattern (Fig. 1b,c), which remains at steady-state for more than half an hour (Supplementary Fig. 1).

The network contraction rate is density-independent

Interestingly, the inward flow velocity, $V$, varies nearly linearly as a function of distance from the inner boundary, $V \approx -k(r-r_0)$, with $k$ being a constant, $r$ being the radial coordinate and $r_0$ being the radius of the exclusion zone (Fig. 1e). A linear velocity profile with $V \sim kr$ is a signature of uniform global contraction; in a homogeneously contracting network, the relative velocity between two points is directly proportional to their distance, so the radial network flow velocity depends linearly on the radial distance from the stationary contraction centre (see Supplementary Information). The contraction rate in this case can be determined from the slope of the linear fit for the inward radial velocity as a function of distance, and is equal to $k = 0.65 \pm 0.15$ min$^{-1}$ (mean $\pm$ s.d., $N = 39$; Supplementary Fig. 2a). This constant rate of contraction is a global characteristic of the self-organized network dynamics, and is not dependent on the geometry of the droplet (Supplementary Fig. 3).

In general, the relationship between the network contraction rate and network density is non-trivial, since the active stress driving contraction and the viscoelastic properties of the network are both density-dependent\textsuperscript{15,20,31}. Our observation that the self-organized dynamics in the system lead to a local contraction rate that is uniform (Supplementary Fig. 3e), despite large spatial variations in network density, is thus surprising. As shown below, homogeneous network contraction is observed for a range of different conditions, suggesting that the density-independent contraction is a manifestation of an inherent scaling relation in the system, rather than the result of fine-tuning of parameters.

**Measurements of net actin turnover**

We can infer the net rate of actin turnover taking into account the conservation of actin subunits described by the continuity equation, $\rho \dot{V} + \nabla \cdot \mathbf{J} = \dot{N}$, where $\rho$ is the network density, $\mathbf{V}$ is the network velocity and $\mathbf{J} = \rho \mathbf{V}$ is the network flux (Fig. 1f). The network density and velocity fields in our system are at steady-state, so the net turnover (that is, the difference between local assembly and disassembly) will be equal to the divergence of the network flux. Using the measured network density and flow, we determine the spatial distribution of network turnover ($\mathbf{V} \cdot \mathbf{J}(r)$; Fig. 1g). We find that the network undergoes net disassembly (that is, $\mathbf{V} \cdot \mathbf{J} < 0$) closer to the contraction centre in regions with higher network density, and net assembly (that is, $\mathbf{V} \cdot \mathbf{J} > 0$) toward the periphery where the network is sparse. The net turnover can also be plotted as a function of the local network density, showing that disassembly increases with network density (Fig. 1h). We can approximate the relation between network turnover and density by a linear fit: $\mathbf{V} \cdot \mathbf{J}(\rho) \sim \alpha - \beta \rho$. This linear relation describes the simplest model for actin turnover, with a constant assembly term and disassembly that is proportional to local network density. The net turnover rate, $\beta$, can be estimated from the slope of the linear fit to the divergence of network flux as a function of network density (Supplementary Fig. 2a; see Methods), which is equal to $\beta = 1.4 \pm 0.3$ min$^{-1}$ (mean $\pm$ s.d., $N = 39$; Supplementary Fig. 2a).

**Contracting actomyosin networks as an active fluid**

To understand the origin of the homogeneous contractile behaviour, we turned to modelling using active fluid theory\textsuperscript{13,28}. The actomyosin network is described as an active isotropic viscous fluid characterized by a density $\rho$ and a velocity field $\mathbf{V}$. The network dynamics are governed by two equations, one for mass conservation (continuity equation) and one for momentum conservation (force balance equation). At steady-state, the force balance equation can be written as $\nabla \cdot \sigma + \dot{N} = f_0$, where $\sigma_\text{act}$ is the active stress, $\sigma_\text{ve}$ is the effective viscoelastic network stress and $f_0$ is the friction with the surrounding fluid. The viscoelastic contribution is dominated by the viscous stress (Supplementary Information), and estimates indicate that the friction with the fluid is negligible (Supplementary Information). Hence, the myosin-generated active stress that drives contraction is largely balanced by the viscous network stress resisting it: $\nabla \cdot \sigma + \dot{N} = 0$. Assuming that the actomyosin network is an isotropic compressible viscous continuum in a spherically symmetric system, we obtain $\frac{\lambda}{\mu} (\sigma_\text{act} + 2 \mu \nabla^2 \mathbf{V} + \lambda \mathbf{V} \cdot \nabla \mathbf{V} + 4 \mu \nabla^2 \mathbf{V}) = 0$, where $\lambda$ and $\mu$ are the bulk and shear network viscosities, respectively\textsuperscript{16}. The periphery of the network at large radii typically does not reach the water/oil interface and the friction with the cytosol is negligible, so we take stress-free boundary conditions for the outer rim of the network. Integrating over $r$ and taking into account this boundary condition at large $r$, we obtain: $-\frac{1}{r^2} \frac{\partial}{\partial r} (r^2 V) = -\frac{\sigma_\text{act}}{2r}$. Both the active stress and the network viscosities are expected to vary considerably, since the network density is spatially variable. However, if their ratio remains constant, $\frac{\sigma_\text{act}}{2r}$, the velocity profile will be linear $\frac{1}{r^2} (r^2 V) = -c \rightarrow V = -\frac{c}{r^3}$ and the network will contract homogeneously with a contraction rate equal to $k = c/3$. This analysis indicates that when the active stress and the effective viscosities scale similarly with density, so their ratio remains constant, the local contraction rate becomes density-independent and the network will contract homogeneously as observed (Fig. 1). The ratio between the active stress and the effective viscosity, $\frac{\sigma_\text{act}}{2r}$, can then be deduced from the measured velocity profile and is found to be $\sim$1 min$^{-1}$ (Supplementary Fig. 3e).
**Influence of auxiliary proteins on network dynamics**

The network dynamics can be modified by supplementing the extract with various components of the actin machinery. Actin disassembly can be enhanced by the combined action of the severing/depolymerizing protein cofilin together with coronin and Aip1, which have recently been shown to work in concert to enhance actin disassembly in vitro even under assembly-promoting conditions (for example, physiological conditions with high actin monomer disassembly in vitro even under assembly-promoting conditions which have recently been shown to work in concert to enhance actin depolymerizing protein cofilin together with coronin and Aip1, assembly can be enhanced by the combined action of the severing/extract with various components of the actin machinery. Actin disassembly is a function of the local actin network density. The net turnover decreases roughly linearly with actin network density.

**Fig. 1 | Quantitative analysis of actin network flow and turnover.** Bulk contracting actomyosin networks are formed by encapsulating Xenopus extract in water-in-oil droplets. The network is labelled using a low concentration of GFP-Lifeact, which does not significantly alter the network (see Methods), and imaged by time-lapse spinning-disc confocal microscopy. Images are acquired at the mid-plane of the droplets, where the network flow is approximately planar due to the symmetry of the set-up. The system reaches a dynamic steady-state characterized by a radially symmetric inward flow. a, Schematic of the actin network inside a water-in-oil droplet, illustrating the actin turnover dynamics and myosin-driven contraction. b, Top: spinning-disc confocal image of the equatorial cross-section of a network labelled with GFP-Lifeact. Bottom: bright-field images of a droplet showing the aggregate of particulates that forms an exclusion zone around the contraction centre. Scale bar, 20 μm. c, The network velocity field for the droplet shown in b, as determined by correlation analysis of the time-lapse video (Supplementary Video 1). d, The actin network density as a function of distance from the contraction centre. The thin lines depict data from individual droplets, and the thick line is the average density profile. The density is normalized to have a peak intensity equal to 1. e, The radial velocity as a function of distance from the contraction centre. The inward velocity increases linearly with distance. f–h, Analysis of net actin network turnover. f, Schematic illustration showing that, at steady-state, the divergence of the actin network flux is equal to the net network turnover rate. The network flux is equal to the product of the local network density and velocity \( J(r) = \rho(r)V(r) \). g, The divergence of the network flux \( \nabla \cdot J(r) \) (see Methods), plotted as a function of distance from the contraction centre, showing the spatial distribution of the net turnover. Negative values (at smaller \( r \)) correspond to net disassembly, while positive values (near the droplet’s periphery) indicate net assembly. h, The divergence of the network flux plotted as a function of the local actin network density. The net turnover decreases roughly linearly with actin network density.

Net turnover

\( \nabla \cdot J = J_{\text{in}} - J_{\text{out}} \)

**Actin network turnover**

\( J = \rho V \)

**Network flux**

\( J(r) = \rho(r)V(r) \)
Fig. 2 | Influence of assembly and disassembly factors on actin network architecture, flow and turnover. Contractile actin networks are generated by encapsulating Xenopus extract supplemented with different assembly and disassembly factors. In all cases, the system reaches a steady-state within minutes. The inward contractile flow and actin network density were measured (as in Fig. 1). a–c. The steady-state network behaviour is shown for samples supplemented with 12.5 μM coflin, 1.3 μM coronin and 1.3 μM Aip1 (see Supplementary Video 2) (a); 1.5 μM ActA (see Supplementary Video 3) (b); 0.5 μM mDia1 (c). For each condition, a spinning-disc confocal fluorescence image of the equatorial cross-section of the network labelled with GFP–Lifeact (left) is shown, together with graphs depicting the radial network flow and density as a function of distance from the contraction centre (middle), and the net actin turnover as a function of network density (right). The thin grey lines depict data from individual droplets, and the thick line is the average over different droplets. 

d e g. The concentration-dependent effects of adding ActA (d–f) and mDia1 (g) to network dynamics. d. The radial network flow plotted as a function of distance from the contraction centre. For each ActA concentration, the mean (line) and s.d. (shaded region) over different droplets are depicted. e. The network contraction rate in individual droplets is determined from the slope of the fit to the radial network flow as a function of distance. For each ActA concentration, the contraction rate was averaged over different droplets (0 μM, N = 15; 0.1 μM, N = 4; 0.5 μM, N = 3; 0.7 μM, N = 4; 1.5 μM, N = 4). To obtain the relative contraction rates, these values were divided by the average contraction rate for the unsupplemented control sample. The relative network contraction rate (mean ± s.d.) is plotted as a function of the added ActA concentration. f. The net actin turnover rate, determined from the divergence of the flux, plotted as a function of network density for the different ActA concentrations. g. The relative network contraction rates (mean ± s.d.; as in e) plotted as a function of the added mDia1 concentration. For each mDia1 concentration, the contraction rate was averaged over different droplets (0 μM, N = 14; 0.1 μM, N = 12; 0.5 μM, N = 12; 0.7 μM, N = 12; 1.5 μM, N = 10). In e and g, the measured contraction rates in each condition were compared to the control sample (0 μM) using the Mann–Whitney test. Conditions for which the contraction rates were statistically different from the control are indicated (*).
Influence of crosslinking on actin network dynamics. Contractile actin networks are generated by encapsulating Xenopus extract supplemented with different concentrations of the actin crosslinker α-actinin. The inward contractile flow and actin network density were measured (as in Fig. 1). a, The steady-state network behaviour for a sample supplemented with 10 µM α-actinin (see Supplementary Video 4). A spinning-disc confocal fluorescence image of the equatorial cross-section of the network labelled with GFP-Lifeact (left) is shown, together with graphs depicting the inward radial network flow and network density as a function of distance from the contraction centre (middle) and the net actin turnover as a function of network density (right). The thin grey lines depict data from individual droplets, and the thick line is the average over different droplets. The dashed lines show the results for the control unsupplemented sample. The network contracts in a non-homogeneous manner, reflected by the nonlinear dependence of the radial network flow on the distance from the contraction centre. b, The concentration-dependent effect of α-actinin on network density and flow. The network density (b) and radial flow (c) plotted as a function of distance from the contraction centre. For each α-actinin concentration, the mean (line) and s.d. (shaded region) over different droplets are depicted. The position of the network density peak moves towards the inner boundary with increasing α-actinin concentrations, and the radial velocity becomes a nonlinear function of the distance from the contraction centre. d, The derivative of the radial velocity, \(-\frac{\partial}{\partial r} \sqrt{V^2}\), plotted as a function of distance from the contraction centre. This function becomes position-dependent for α-actinin concentrations \(\geq 4\) µM. According to the model, this derivative should be approximately equal to the ratio between the active stress and the effective network viscosities, \(\frac{\sigma}{2\nu(r)\lambda}\). e, The derivative of the radial velocity, \(-\frac{\partial}{\partial r} \sqrt{V^2}\), plotted as a function of network density. According to the model, this derivative should be approximately equal to the ratio between the active stress and the effective network viscosities, \(\frac{\sigma}{2\nu(r)\lambda}\). For α-actinin concentrations \(\geq 4\) µM, this ratio becomes density-dependent, indicating that the scaling relation between the active stress and the effective viscosity no longer holds.

Influence of crosslinking on actin network dynamics. Contractile actin networks are generated by encapsulating Xenopus extract supplemented with different concentrations of the actin crosslinker α-actinin. The inward contractile flow and actin network density were measured (as in Fig. 1). a, The steady-state network behaviour for a sample supplemented with 10 µM α-actinin (see Supplementary Video 4). A spinning-disc confocal fluorescence image of the equatorial cross-section of the network labelled with GFP-Lifeact (left) is shown, together with graphs depicting the inward radial network flow and network density as a function of distance from the contraction centre (middle) and the net actin turnover as a function of network density (right). The thin grey lines depict data from individual droplets, and the thick line is the average over different droplets. The dashed lines show the results for the control unsupplemented sample. The network contracts in a non-homogeneous manner, reflected by the nonlinear dependence of the radial network flow on the distance from the contraction centre. b, The concentration-dependent effect of α-actinin on network density and flow. The network density (b) and radial flow (c) plotted as a function of distance from the contraction centre. For each α-actinin concentration, the mean (line) and s.d. (shaded region) over different droplets are depicted. The position of the network density peak moves towards the inner boundary with increasing α-actinin concentrations, and the radial velocity becomes a nonlinear function of the distance from the contraction centre. d, The derivative of the radial velocity, \(-\frac{\partial}{\partial r} \sqrt{V^2}\), plotted as a function of distance from the contraction centre. This function becomes position-dependent for α-actinin concentrations \(\geq 4\) µM. According to the model, this derivative should be approximately equal to the ratio between the active stress and the effective network viscosities, \(\frac{\sigma}{2\nu(r)\lambda}\). e, The derivative of the radial velocity, \(-\frac{\partial}{\partial r} \sqrt{V^2}\), plotted as a function of network density. According to the model, this derivative should be approximately equal to the ratio between the active stress and the effective network viscosities, \(\frac{\sigma}{2\nu(r)\lambda}\). For α-actinin concentrations \(\geq 4\) µM, this ratio becomes density-dependent, indicating that the scaling relation between the active stress and the effective viscosity no longer holds.

The different dependence of the contraction rate on the amount of protein added for the two types of nucleation-promoting factor is probably related to the inherent difference in the connectivity of the nucleated filaments. Arp2/3-mediated nucleation creates a filament that is physically connected to an existing filament through a branch junction. In contrast, mDia1 nucleates filaments in solution, which must subsequently be crosslinked to other filaments to become associated with the network. As such, high levels of Arp2/3 activation generate highly connected networks consisting of densely branched filaments, whereas enhanced mDia1 activity results in many unconnected filaments.

Our results (Figs. 1 and 2) demonstrate that confined contractile actin networks with rapid turnover can attain a dynamic steady-state over a wide range of parameters. Reaching a steady-state does not require fine-tuning of parameters; rather the system dynamically adapts the overall assembly/disassembly rates to be equal. This can be done, for example, through variations in the fraction of actin associated with the network, which will induce changes in the actin monomer concentration and assembly dynamics, until the assembly processes balance disassembly. The characteristics of the steady-states obtained vary considerably as a function of system composition, whereby the rate of network contraction and/or the turnover rate changes over a few-fold range following the addition of different components of the actin machinery (Fig. 2 and Supplementary Figs. 4–6). Surprisingly, the network flow maintains a nearly linear velocity profile under a variety of different conditions. Thus, while the rate of network contraction depends on the composition of the system, the local contraction rate for a given condition (which is equal in the active fluid model to the ratio between the active stress and the effective viscosities) is homogeneous, across a range of network densities.

The active fluid model also implies that a nonlinear contraction velocity profile indicates that the ratio between the active stress and the effective viscosity varies in space and is, hence, density-dependent. We find such behaviour when we supplement the extract with sufficiently high concentration of crosslinkers such as α-actinin (Fig. 3 and Supplementary Video 4). We can infer the density-dependence of the ratio between the active stress and the effective viscosity from the spatial variation in the local contraction velocity (Fig. 3c). We find that the ratio between the active stress and the viscosity, for high α-actinin concentration (>4 µM), decreases with density, indicating that nonlinear contributions as a function of density in the active stress and/or the viscosity become important. Addition of the filament bundler fascin had a different effect; actin network contraction remained homogeneous but at a (~1.5 µM), the contraction rate returns to values close to the control sample, but the network appearance is very different, with a strong diffuse background signal, probably due to the presence of many dissociated filaments. In both cases, the net turnover rate remains largely unchanged (Supplementary Fig. 7a,b).
The contraction and turnover rates were determined for each droplet from the slopes of linear fits to the radial network flow as a function of distance, and the net turnover as a function of network density, respectively (see Methods and Supplementary Fig. 2). The measured network density profile for the control sample is compared to the predictions of a model that assumes constant turnover and contraction rates (Supplementary Information). The measured density distribution (mean and s.d.) nicely matches the model-predicted distribution based on the average values of the turnover and contraction rates, $\beta = 1.4\, \text{min}^{-1}$ and $k = 0.65\, \text{min}^{-1}$ (dashed line). The relative width of the network profile (quantified as the distance between the inner boundary and the position where the network reaches half its maximal value, normalized by the droplet’s radius) plotted as a function of the ratio between the net turnover rate and the contraction rate. The dots depict values for individual droplets and the error bars show the mean and s.d. for all of the droplets examined for each condition. The model results (dashed line; Supplementary Information) predict the increase of the network width as a function of the ratio between the net turnover rate and the contraction rate. Increasing the contraction and turnover rates proportionally leads to faster network dynamics, but the network density profile remains the same, whereas changes in the ratio between the contraction and turnover rates lead to significant modifications in network structure (Supplementary Information).

Contracted networks formed under different conditions, including 80% extract (control; grey) and samples supplemented with 1.5 μM ActA (purple); 2.6 μM fascin (magenta); 0.5 μM mDia1 (cyan); 10 μM α-actinin (red); 0.5 μM capping protein (green); or 12.5 μM cofilin, 1.3 μM coronin and 1.3 μM Aip1 (orange). a. The radial network flow rate plotted as a function of distance from the contraction centre. For each condition, the mean (line) and s.d. (shaded region) over different droplets are depicted. b. The net actin turnover as a function of network density plotted for different conditions. The contraction rate and turnover rate are determined for each droplet from the slopes of linear fits to the radial network flow as a function of distance, and the net turnover as a function of density, respectively (Fig. 2).  

The network dynamics can thus be characterized by two timescales: the contraction time ($k^{-1}$) and the actin turnover time ($\beta^{-1}$), which have the same order of magnitude (~1 min). The diffusion time across the system provides a third timescale ($\tau_D \approx R^2/6D$, where $R$ is the droplet radius and $D \approx 15\, \mu\text{m}^2\text{s}^{-1}$ is the diffusion coefficient of actin monomers in the cytosol). However, for droplets in the size range considered here ($R < 65\, \mu\text{m}$), the diffusion time is $< 1\, \text{min}$. In this case, diffusion is sufficient to redistribute network components across the system, and the behaviour of the system is primarily governed by the contraction and turnover timescales.

We consider the characteristic network dynamics over a range of conditions (Fig. 4). For many conditions (for example, addition of CCA, fascin, mDia1 and capping protein), the network contraction rate is correlated with the net turnover rate (dashed line; Fig. 4e), such that their ratio remains nearly constant. As a result, the large-scale density profiles of the contracting networks are similar, while the dynamics are faster or slower depending on the magnitude of the contraction and turnover rates (see Supplementary Information and Fig. 4g). Adding crosslinkers (α-actinin) or enhancing branching activity (ActA) leads to deviations in the ratio between the contraction rate and turnover rate (Fig. 4f and Supplementary Fig. 8), which are reflected by large-scale changes in network structure (Fig. 4g); a lower ratio (stronger contraction) results in compact networks concentrated around the contraction centre as observed with α-actinin (Fig. 3), whereas a larger ratio results in more extended networks as seen with ActA (Fig. 2b). The local modifications in network architecture generated by increasing the density of filament branch junctions or crosslinks are thus translated to global changes in the overall network structure.

**Fig. 4 | Characteristics of contracting actin networks with turnover.** The contracting flows and actin turnover were measured for contractile actin networks formed under different conditions, including 80% extract (control; grey) and samples supplemented with 1.5 μM ActA (purple); 2.6 μM fascin (magenta); 0.5 μM mDia1 (cyan); 10 μM α-actinin (red); 0.5 μM capping protein (green); or 12.5 μM cofilin, 1.3 μM coronin and 1.3 μM Aip1 (orange). First, the radial network flow rate plotted as a function of distance from the contraction centre. For each condition, the mean (line) and s.d. (shaded region) over different droplets are depicted. b. The radial network flow rate plotted as a function of distance from the contraction centre. For each condition, the mean (line) and s.d. (shaded region) over different droplets are depicted.
The behaviour of contracting actomyosin networks arises from the interplay between the active stress generated within the network and the viscoelastic stress of the network. Both the force generation and the dissipation depend on the microscopic details of the network organization, which are determined by the activity of a host of actin regulatory proteins. We have developed an experimental system where we can systematically investigate the emergent behaviour of contracting networks at steady-state and quantitatively characterize network flow and turnover in the presence of physiological rates of actin turnover. Our findings imply that the active stress and the effective network viscosity scale similarly with network density, so their ratio becomes density-independent, leading to homogeneous network contraction. We further find that the contraction rate is roughly proportional to the actin turnover rate for a range of conditions (Fig. 4c). Simulation studies of actomyosin networks in the presence of rapid turnover show that viscous dissipation in the network will be dominated by filament breaking and disassembly, so the effective viscosity is expected to be inversely proportional to the turnover rate. This is consistent with our results since, according to the active fluid model, the contraction rate should be inversely proportional to the effective viscosity. Thus, if viscosity is inversely proportional to the turnover rate, the contraction rate will be proportional to the turnover rate.

Our observations provide new insights into the relationship between network density and flow in contracting actomyosin networks. This relationship has been studied in vivo, for example, in the context of the cytokinetic ring. Our results suggest that a constant contraction rate can increase, even if the network density changes over time, if the production and dissipation of internal stress in the cytokinetic ring exhibit similar scaling with network density as observed here. Furthermore, the rate of constriction would be expected to decrease when actin disassembly processes are slowed down, as observed. More generally, the ability to study contracting actomyosin networks with physiological actin turnover rates in vitro, as demonstrated here, can provide further insights into the behaviour of actomyosin networks in vivo and promote quantitative understanding of cellular dynamics.

Online content
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References
1. Blanchoin, L., Boujemaa-Paterski, R., Sykes, C. & Plastino, J. Actin dynamics, architecture, and mechanics in cell motility. Physiol. Rev. 94, 235–263 (2014).
2. Pollard, T. D. Actin and actin-binding proteins. Cold Spring Harb. Perspect. Biol. 8, a018226 (2016).
3. Brieher, W. Mechanisms of actin disassembly. Mol. Biol. Cell 24, 2299–2302 (2013).
4. Ono, S. The role of cyclase-associated protein in regulating actin filament dynamics—more than a monomer-sequestration factor. J. Cell Sci. 126, 3249–3258 (2013).
5. Ono, S. Functions of actin-interacting protein 1 (AIP1)/WD repeat protein 1 (WDR1) in actin filament dynamics and cytoskeletal regulation. Biochem. Biophys. Res. Commun. 306, 315–322 (2003).
6. Carvalho, A., Desai, A. & Oegema, K. Structural memory in the contractile ring makes the duration of cytokinesis independent of cell size. Cell 137, 926–937 (2009).
7. Mayer, M., Depken, M., Bois, J. S., Julicher, F. & Grill, S. W. Anisotropies in cortical tension reveal the physical basis of polarizing cortical flows. Nature Cell Biol. 16, 617–621 (2014).
8. Yi, K. et al. Dynamic maintenance of asymmetric meiotic spindle position through Arp2/3-complex-driven cytoplasmic streaming in mouse oocytes. Nat. Cell Biol. 13, 1252–1258 (2011).
9. Hawkins, R. J. et al. Spontaneous contractility-mediated cortical flow generates cell migration in three-dimensional environments. Biophys. J. 101, 1041–1045 (2011).
10. Liu, Y. J. et al. Confinement and low adhesion induce fast amoeboid migration of slow mesenchymal cells. Cell 160, 659–672 (2015).
11. Lenz, M., Thoresen, T., Gardel, M. L. & Dinner, A. R. Contractile units in disordered actomyosin bundles arise from F-actin buckling. Phys. Rev. Lett. 108, 238107 (2012).
12. Murrell, M. P. & Gardel, M. L. F-actin buckling coordinates contractility and severing in a biometric actomyosin cortex. Proc. Natl Acad. Sci. USA 109, 20820–20825 (2012).
13. Linsmeier, I. et al. Disordered actomyosin networks are sufficient to produce cooperative and telescopic contractility. Nat. Commun. 7, 12615 (2016).
14. Ennoumi, H. et al. Architecture and connectivity govern actin network contractility. Curr. Biol. 26, 616–626 (2016).
15. McFadden, W. M., McCall, P. M., Gardel, M. L. & Munro, E. M. Filament turnover tunes both force generation and dissipation to control long-range flows in a model actomyosin cortex. PLoS Comput. Biol. 13, e1005811 (2017).
16. McCall, P. M., MacKintosh, F. C., Kovar, D. R. & Gardel, M. L. Cofilin drives rapid turnover and fluidization of entangled F-actin. Preprint at https://www.biorxiv.org/content/early/2017/06/26/156224 (2017).
17. Tan, T. H. et al. Self-organization of stress patterns drives state transitions in actin cortices. Sci. Adv. 4, eaar2847 (2018).
18. Bendix, P. M. et al. A quantitative analysis of contractility in active cytoskeletal protein networks. Biophys. J. 94, 3126–3136 (2008).
19. Hayakawa, K., Tatsumi, H. & Sokabe, M. Actin filaments function as a tension sensor by tension-dependent binding of cofilin to the filament. J. Cell Biol. 195, 721–727 (2011).
20. Galkin, V. E., Orlova, A. & Egelman, E. H. Actin filaments as tension sensors. Curr. Biol. 22, R96–R101 (2012).
21. Alvarado, J., Steinman, M., Sharma, A., MacKintosh, F. C. & Koenderink, G. H. Force percolation of contractile active gels. Soft Matter 13, 5624–5644 (2017).
22. Merkle, D., Kahya, N. & Schwille, P. Reconstitution and anchoring of cytokinetic ring inside giant unilamellar vesicles. ChemBioChem 9, 2673–2681 (2008).
23. Jessen, Y., Sonn Segev, A., Roichman, Y. & Bernheim-Grouswasser, A. Myosin II does it all: assembly, remodeling, and disassembly of actin networks are governed by myosin II activity. Soft Matter 9, 7127–7137 (2013).
24. Kohler, S., Schmoller, K. M., Crevenna, A. H. & Rausch, A. R. Regulating contractility of the actomyosin cytokinesis by pH. Cell Rep. 2, 433–439 (2012).
25. Rivas, G., Vogel, S. K. & Schwille, P. Reconstitution of cytoskeletal protein assemblies for large-scale membrane transformation. Curr. Opin. Chem. Biol. 22, 18–26 (2014).
26. Carvalho, K. et al. Actin polymerization or myosin contraction: two ways to build up cortical tension for symmetry breaking. Phil. Trans. R. Soc. B 368, 20120005 (2013).
27. Mak, M., Zanan, M. H., Kamm, R. D. & Kim, T. Interplay of active processes modulates tension and drives phase transition in self-renewing, motor-driven cytoskeletal networks. Nat. Commun. 7, 10323 (2016).
28. Hirata, T. & Salbreux, G. Role of turnover in active stress generation in a filament network. Phys. Rev. Lett. 116, 188101 (2016).
29. Banerjee, D. S., Munjal, A., Lecuit, T. & Rao, M. Actomyosin pulsation and flows in an active elastomer with turnover and network remodeling. Nat. Commun. 8, 1121 (2017).
30. Gardel, M. L. et al. Elastic behavior of cross-linked and bundled actin networks. Science 304, 1301–1305 (2004).
31. Lieleg, O., Schmoller, K. M., Claessens, M. M. A. E. & Bausch, A. R. Cytoskeletal polymer network: viscoelastic properties are determined by the microscopic interaction potential of cross-links. Biophys. J. 96, 4725–4732 (2009).
32. Wühr, M. et al. Deep proteomics of the Xenopus laevis egg using an mRNA-derived reference database. Curr. Biol. 24, 1467–1475 (2014).
33. Hirose, A. M. et al. Confinement induces actin flow in a meiotic cytoplasm. Proc. Natl Acad. Sci. USA 109, 11705–11710 (2012).
34. Abu-Shah, E. & Keren, K. Symmetry breaking in reconstituted actin networks. eLife 3, e01433 (2014).
35. Abu-Shah, E., Malik-Garbi, M. & Keren, K. in Building a Cell from its Component Parts (eds Ross, J. & Marshall, W.) (Elsevier, Amsterdam, 2014).
36. Field, C. M., Nguyen, P. A., Ishihara, K., Groen, A. C. & Mitchison, T. J. in Methods in Enzymology: Reconstituting the Cytoskeleton (ed. Vale, R. D.) Vol. 540, 399–415 (Elsevier, Amsterdam, 2014).
37. Prost, J., Julicher, F. & Joanny, J. F. Active gel physics. Nat. Phys. 11, 111–117 (2015).
38. Lewis, O. L., Guy, R. D. & Allard, J. F. Actin–myosin spatial patterns from a simplified isotropic viscoelastic model. Biophys. J. 107, 863–870 (2014).
39. Landau, L. D. & Lifshitz, E. M. Theory of Elasticity Vol. 7 (Elsevier, New York, 1986).
40. Jansen, S. et al. Single-molecule imaging of a three-component ordered actin disassembly mechanism. *Nat. Commun.* **6**, 7202 (2015).
41. Courson, D. S. & Rock, R. S. Actin crosslink assembly and disassembly mechanisms for alpha-actinin and fascin. *J. Biol. Chem.* **M110**, 123117 (2010).
42. Raz-Ben Aroush, D. et al. Actin turnover in lamellipodial fragments. *Curr. Biol.* **27**, 2963–2973.e14 (2017).
43. Kim, T., Gardel, M. L. & Munro, E. Determinants of fluidlike behavior and effective viscosity in cross-linked actin networks. *Biophys. J.* **106**, 526–534 (2014).
44. Pinto, I. M., Rubinstein, B., Kucharavy, A., Unruh, J. R. & Li, R. Actin depolymerization drives actomyosin ring contraction during budding yeast cytokinesis. *Dev. Cell.* **22**, 1247–1260 (2012).

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**Author contributions**

M.M.-G., E.A.-S. and K.K. designed the experiments. M.M.-G., N.I., S.J. and K.K. performed the experiments. M.M.-G., E.A.-S., S.I., B.L.G., N.I. and K.K. provided reagents for the experiments. M.M.-G. and K.K. analysed the data. A.M., M.M.-G. and K.K. developed the model. M.M.-G., A.M. and K.K. wrote the paper. All co-authors discussed the results and commented on the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Methods

Cell extracts, proteins and reagents. Concentrated M-phase extracts were prepared from freshly laid Xenopus laevis eggs as previously described [1–3]. Briefly, Xenopus eggs were injected with hormones to induce ovulation and laying of unfertilized eggs for extract preparation. The eggs from different frogs were pooled together and washed with 1X MMR (100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 0.1 mM EDTA, 5 mM Hepes, pH 7.8), at 16 °C. The jelly envelope surrounding the eggs was dissolved using 2% cysteine solution (in 100 mM KCl, 1 mM MgCl₂, 0.1 mM CaCl₂, pH 7.8). Finally, the eggs were washed with CSF-XB (10 mM K-Hepes pH 7.7, 100 mM KCl, 1 mM MgCl₂, 5 mM EGTA, 0.1 mM CaCl₂ and 50 mM sucrose) containing protease inhibitors (10 µg/ml each of leupeptin, pepstatin and chymostatin). The eggs were then packed using a clinical centrifuge and crushed by centrifugation at 15,000g for 15 min at 4 °C. The crude extract (the middle yellowish layer out of three layers) was collected, supplemented with 50 mM sucrose containing protease inhibitors (10µg/ml each of leupeptin, pepstatin, chymostatin), snap-frozen as 10µl aliquots and stored at −80 °C. Typically, for each extract batch, a few hundred aliquots were made. Different extract batches exhibit similar behaviour qualitatively, but the values of the contraction and net turnover rates vary (Supplementary Fig. 9). All comparative analysis between conditions was done using the same batch of extract.

ActA-His was purified from strain JAT084 of Listeria monocytogenes (a gift from J. Theriot, Stanford University) expressing a truncated actA gene encoding amino acids 1–613 with a COOH-terminal six-histidine tag replacing the transmembrane domain, as described previously [4–6]. Purified proteins were aliquoted, snap-frozen in liquid N₂ and stored at −80 °C until use.

Emulsion preparation. Emulsions were made by adding 1–3% (v/v) extract mix to degassed mineral oil (Sigma) containing 4% cetyl PEG/PPG-10/1 dimethiocone (Abil EM90, Evonik Industries) and stirring for 1 min on ice. The mix was then incubated for an additional 10 min on ice to allow the emulsions to settle. Samples were then made in chambers assembled from two passivated coverslips separated by 30-µm-thick double stick tape (3M), sealed with vaseline/lanolin/paraffin (at 1:1:1) and attached to a glass slide by incubating cleaned coverslips in silanization solution (5% dichlorodimethylsilane in heptane) for 20 min, washing in heptane, sonicking twice in double-distilled water for 5 min and once in ethanol for 5 min, and drying in an oven at 100 °C. Droplets with radii in the range of 25–65 µm were imaged 10–60 min after sample preparation.

Microscopy. Emulsions were imaged on a 31 spinning-disc confocal microscope running Slidebook software, using a 63x oil objective (NA = 1.4). Images were acquired using 488 nm laser illumination and appropriate emission filters at room temperature. Images were collected in an EM-CCD (QuantEM; Photometrics). Time-lapse videos of emulsions were taken at the equatorial plane, so the network velocity is within the imaging plane.

Analysis. The steady-state density and velocity distributions were determined from time-lapse videos of contracting networks. The videos were acquired at the droplets’ equatorial planes where the distributions exhibit radial symmetry for the squished geometry and spherical symmetry for the spherical geometry (Supplementary Fig. 3). Time-lapse videos were taken at time intervals of 2.5–10 s (depending on network speed) with 512 × 512 pixels per frame at 0.2054 µm per pixel. Background subtraction and corrections for uneven illumination field were performed by subtracting the mean intensity of images of droplets without a fluorescent probe, and dividing by a normalized image of the illumination field distribution. To determine the network field distribution, we considered very large droplets (larger than the field of view) with uniform probe distribution generated by inhibiting actin polymerization with 6.6 µM capping protein and no ATP regeneration mix. Bleach correction was performed by dividing the entire image by a constant factor determined from an exponential fit to the total image intensity as a function of time. The difference in the refractive index of the oil and the extract within the droplets distorts the intensity near the edge of the droplets. To correct for this, we measured the fluorescence intensity as a function of distance from the edge for droplets with uniform probe distribution (as above). The correction as a function of distance from the edge of the droplet was determined by averaging the measured intensity near the edge for 50 droplets. The fluorescence signal was corrected for edge effects by dividing by this correction function. The velocity distribution (Fig. 1g) was extracted using direct cross-correlation analysis based on PIVlab code [7] written in Matlab with modifications. The videos were first preprocessed to enhance contrast using contrast-limited adaptive histogram equalization and a high-pass filter in Matlab. Cross-correlation was performed on overlapping regions, 30 × 30 pixels in size, on a grid with 10×10 pixel intervals. To avoid wild artefacts produced by the edge of the droplets (larger than the field of view) with uniform probe distribution generated by inhibiting actin polymerization with 6.6 µM capping protein and no ATP regeneration mix. The contraction rate for each droplet was determined from the slope of the linear fit to the radial velocity as a function of distance from the contraction centre. The fit range was taken to be between the radius where the network intensity peaks near the inner boundary and the largest radius for which the network velocity could be determined reliably. The error in the contraction rate in individual droplets was estimated as the half-width of the 95% confidence interval for the slope values determined using Matlab (Supplementary Fig. 2). Since the maximal error in determining the slope of the fit was smaller than the variability between droplets made with the same composition, we report the s.d. among droplets as a measure of the 100 mM KCl, 2 each condition. The radius of the inner boundary (r₂) was determined as the intersection point of the linear fit to the velocity with the x-axis.

The network density was determined by averaging the corrected fluorescence signal over different angles and over time (typically 20–40 frames) to obtain

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the probe density distribution as a function of distance from the contraction centre. The measured probe distribution reflects the sum of signals emanating from the network-bound probe and from the diffusing probe. To approximate the network-bound-probe density we subtracted the measured signal near the periphery of the droplet, which is equivalent to assuming that the network density approaches zero near the periphery and that the diffusing probe is distributed uniformly.

Analysis of the net turnover of the network is based on the continuity equation at steady-state, $\nabla \cdot \mathbf{J} = \text{net turnover}$. The network flux is the product of the network density and velocity, $J(r) = f(r)v = \rho(r)v(r)$. The divergence of the flux is calculated assuming spherical-symmetry as $\nabla \cdot J(r) = \frac{d}{dr} [\frac{1}{r} \rho(r)v(r)]$. The divergence of the flux was also plotted as a function of the local density $\rho$, and fitted to a linear function $\nabla \cdot J(\rho) = a - b\rho$. The fit was performed on the density range for which the system undergoes net disassembly (that is, $\nabla \cdot J(\rho) < 0$). The net turnover rate reported was determined as the slope of the linear fit of the divergence of the flux as a function of the network density ($\rho$). The error in the turnover rate in individual droplets was estimated as the half-width of the 95% confidence interval for the slope values determined using Matlab (Supplementary Fig. 2). Since the typical error in determining the slope of the fit was smaller than the variability between droplets made with the same composition, we report the s.d. among droplets as a measure of the variability for each condition. The contraction and turnover rates between the unsupplemented control sample and other conditions were statistically compared using the Mann–Whitney test.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The data that support the plots within this paper and other findings of this study are available from the corresponding author upon request.

**References**
45. Thielicke, W. & Stamhuis, E. PIVlab: towards user-friendly, affordable and accurate digital particle image velocimetry in MATLAB. J. Open Res. Software 2, p.e30 (2014).
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- [ ] For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted
  - Give $P$ values as exact values whenever suitable.
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Life sciences study design

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| Sample size                                      | Data was collected from several batches of extract. Comparative analysis was done with one extract. Multiple droplets were taken from a few different samples. |
|--------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions                                   | Analysis was done in droplets were the contraction center remained stationary for the duration of the movie                                 |
| Replication                                       | experiments were replicated in different samples. Qualitative results were verified in more than one batch of extract                  |
| Randomization                                     | N/A                                                                                                                                       |
| Blinding                                          | N/A                                                                                                                                       |

Reporting for specific materials, systems and methods

| Materials & experimental systems                  |
|--------------------------------------------------|
| n/a                                              |
| ☒ Involved in the study                          |
| ☒ Unique biological materials                    |
| ☐ Antibodies                                     |
| ☒ Eukaryotic cell lines                          |
| ☒ Palaeontology                                  |
| ☐ Animals and other organisms                    |
| ☒ Human research participants                    |

| Methods                                           |
|--------------------------------------------------|
| n/a                                              |
| ☒ Involved in the study                          |
| ☒ ChIP-seq                                       |
| ☒ Flow cytometry                                 |
| ☒ MRI-based neuroimaging                         |