Table S1, related to Figure 3. Key parameter values of the ring simulation.

Symbol	Meaning	Value	Legend
d	Ring binding zone width	0.2 μm	**(A)**
ρ_{for}	Mean density of formin dimers along the ring	15 μm^{-1} ($\pm 2.5\ \mu m^{-1}$)	**(A)**
k_{off}^{for}	Formin off rate	0.023 s^{-1} ($\pm 0.007\ s^{-1}$)	**(B)**
ρ_{myo}	Initial mean density of myosin clusters along the ring	7.5 μm^{-1} ($\pm 1\ \mu m^{-1}$)	**(C)**
k_{off}^{myo}	Myosin cluster off rate	0.026 s^{-1} ($\pm 0.001\ s^{-1}$)	**(D)**
f_{myo}	Myosin II cluster force	4 pN (±3.5 pN)	**(E)**
r_{myo}	Myosin II cluster capture radius for actin filaments	0.1 μm	**(F)**
ρ_x	Mean density of α-actinin crosslinks along the ring	25 μm^{-1} ($\pm 5\ \mu m^{-1}$)	**(A)**
k_{off}^{x}	α-actinin crosslink off rate	3.3 s^{-1} (1.9 - 9.6 s^{-1})	**(G)**
r_x^0	α-actinin crosslink rest length	30 nm (28 - 36 nm)	**(H)**
k_x	α-actinin crosslink stiffness	25 pN/μm	**(I)**
l_p	Actin filament persistence length	10 μm (6 – 25 μm)	**(J)**
r_{sev}	Actin severing rate per filament length by cofilin	1.8 $\mu m^{-1}\ min^{-1}$	**(K)**
v_{pol}	Formin-mediated barbed end actin polymerization rate	70 nm/s	**(K)**
γ_{myo}	Myosin II cluster drag coefficient	1.3 nN·s/μm	**(L)**
γ_{for}	Formin drag coefficient	1.9 nN·s/μm	**(L)**
γ_{ring}	Total ring-membrane drag coefficient in protoplasts	2805 nN·s/μm (±45 nN·s/μm)	**(M)**

Legend:

Values in parentheses are standard deviations or ranges for experimentally measured values, and standard errors for fitting parameters.

(A) (Wu and Pollard, 2005).

(B) (Yonetani et al., 2008), Fig. S3C. Together with the value for ρ_{for}, this gives a formin binding rate of $r_{for} = 0.35\ \mu m^{-1} \cdot s^{-1}$.

(C) Assumed the same as the density of nodes at the end of ring assembly (Wu and Pollard, 2005). Since there are ~3000 Myo2p myosin-II heavy chains in a ring of ~10 μm in length, this implies ~40 Myo2 heavy chains per myosin cluster.

(D) From FRAP measurements of myosin light chain Cdc4p (Pelham and Chang, 2002). Together with the value for ρ_{myo}, this gives a myosin binding rate of $r_{myo} = 0.20\ \mu m^{-1} \cdot s^{-1}$.

(E) From our measurements of node motions in this work.

(F) Estimated from single-molecule high resolution colocalization (SHREC) measurements of the distance that myosin heads extend from precursor nodes (Laporte et al., 2011).

(G) (Kuhlman et al., 1994; Miyata et al., 1996; Xu et al., 1998).

(H) (Meyer and Aebi, 1990).

(I) Estimated from in vitro actin bundles (Claessens et al., 2006).

(J) (Ott et al., 1993; Riveline et al., 1997).

(K) Fit to previous measurements of rings in intact cells (see Fig. S3 and Extended Experimental Procedures). Polymerization rate corresponds to 26 subunits/s.
(L) Fit to our measurements of the speeds of myosin II and formin in the ring (Fig. 2).
(M) Fit to our measured protoplast ring constriction curves (Fig. 6E and S4C).

Table S2, related to Experimental Procedures. *S. pombe* strains used in this study

Strain	Genotype	Source/Reference
CL4	*h- rlc1-3GFP ade6-M216 his3-D1 leu1-32 ura4-D18*	(Vavylonis et al., 2008)
CL5	*h- rlc1-tdTomato-NatMX6 sad1-mEGFP-KanMX6 ade6-M216 his3-D1 leu1-32 ura4-D18*	(Vavylonis et al., 2008)
CL54	*h+ myo2-E1 rlc1-3GFP ade6-M216 his3-D1 leu1-32 ura4-D18*	(Balasubramanian et al., 1998)
CL110	*h+ nmt41-GFP-CHD (rng2)-leu1+ rlc1-tdTomato-natMX6 ade6-M210 leu1-32 ura4-D18*	(Martin and Chang, 2006)
JW1114	*h+ cdc12-3YFP-KanMX6 sad1-CFP-KanMX6 ade6-M210 leu1-32 ura4-D18*	(Wu et al., 2006)

Movie S1, related to Figure 1. Diffusion of nodes marked with Rlc1p-3GFP in a protoplast.
Confocal fluorescence micrographs taken at 2 s intervals for a total of 100 s. Width of the field of view: 13.2 µm.

Movie S2, related to Figure 1. Directed, stop-go motions of nodes marked with Rlc1p-3GFP in a protoplast.
Confocal fluorescence micrographs taken at 8 s intervals for a total of 592 s. Width of the field of view: 10.6 µm.

Movie S3, related to Figure 1. Sliding and constriction of a contractile ring marked with Rlc1p-3GFP in a compressed protoplast.
Confocal fluorescence micrographs taken at 3 min intervals. Bar: 5 µm.

Movie S4, related to Figure 2. Motions of formin Cdc12p-3YFP in the contractile ring of a compressed protoplast.
Reversed contrast confocal fluorescence micrographs taken at 2 s intervals. Bar: 2 µm.

Movie S5, related to Figure 2. Motions myosin-II marked with Rlc1p-tdTomato in the contractile ring of a compressed protoplast.
Reversed contrast confocal fluorescence micrographs taken at 4 s intervals. Bar: 2 µm.

Movie S6, related to Figure 4. Ring simulation under standard, "wild type" conditions.
Actin filaments: gray; myosin cluster capture zone: orange; formins: blue; α-actinin crosslinks: green. Horizontal dashed lines: boundaries of formin and myosin binding zone. The field of view is 5 µm wide, a portion of a 10 µm long ring. Starting from an initial condition with no actin filaments, the ring reaches a fluctuating steady-state in ~30 s.

Movie S7, related to Figure 6. Comparison of observed and predicted ring constriction in a compressed protoplast with dimensions h = 1.60 µm and R = 1.96 µm.
(Left) Confocal fluorescence images at 3 min intervals of a constricting and sliding contractile ring marked with Rlc1p-3GFP. (Top) Overhead view and (bottom) side view of maximum projection images reconstructed from confocal stacks.
(Right) Simulated contractile ring (blue) constricting in the compressed protoplast. (Top) Overhead view and (bottom) side view. Solid red line: cell boundary. Dotted red line: boundary of the flat portion of the protoplast. Parameters, as in Table S1.

EXTENDED EXPERIMENTAL PROCEDURES

1. Measurement of ring tension

2. Simulation of the fission yeast contractile ring

3. Quantitative characterization of the ring organization: the sarcomericity, the bundling coefficient, and their relation to ring tension

4. Sliding-constriction model of rings in protoplasts, and comparison to observed ring shapes and constriction rates

1. Measurement of ring tension

Protoplast membrane tension was measured using micropipette aspiration (Evans, 1989; Hochmuth, 2000). Pipettes with long tapers >1 cm were drawn from borosilicate capillaries (WPI, Sarasota, FL, 1 mm OD, 0.58 mm ID) using a Sutter P-97 puller (Novato, CA). Tips were cut at 1.5-3 μm diameter using a Narishige MF-830 microforge (Tokyo, Japan) using a small molten glass bead. Observation chambers consisted of a pair of glass coverslips (#1.5, Waldemar Knittel Glasbearbeitungs- GmbH, Braunschweig, Germany) separated by 3 mm, attached to a metal block using vacuum grease. The chamber and pipettes were filled with 50% EMM5S in E buffer with either 0.6, 0.8, or 1.2 M sorbitol, and 0.5% BSA. Freshly prepared protoplasts, suspended in the same solution, were introduced into a corner of the observation chamber. Gentle suction was applied with the micropipette (mounted on a Sutter MP285 3-axis manipulator) to pick up and lift a protoplast above the coverslip. Pressure was increased in steps every 15-110 s and bright field images were recorded every 0.5 s.

Aspiration pressure was controlled using a hydrostatic system. Before aspiration, the zero pressure was set by vertically moving a reservoir (open to atmospheric pressure) and observing the movement of debris into or out of the pipette. When such motion stops, the reservoir and pipette opening are at the same hydrostatic pressure. A second, reference reservoir was then placed at the same level as the first reservoir. The pressure difference between the reservoirs was continuously read using a pressure sensor (Validyne DP-15) and transducer (Model CD223, Validyne Engineering, Northridge, CA). The desired aspiration pressure was then set either by lowering the first reservoir (for pressures up to ~20 cm H_2O) or by isolating it from atmospheric pressure and withdrawing the air above it using a programmable syringe pump (KDS230, KD Scientific). Pressure data (in the form of voltages) were digitized and recorded using a HEKA EPC 10 patch amplifier (HEKA Instruments, Inc., Bellmore, NY). Digital videos were synchronized with pressure data by a trigger signal from the EM-CCD camera to the amplifier.

Image analysis was performed in ImageJ (http://rsb.info.nih.gov/ij/). For each protoplast (n = 9), the diameter of the spherical protoplast cap $2R_c$, the pipette diameter $2R_p$, and the length l of the protoplast aspirated into the micropipette (Fig. 2B) were measured from kymographs. Because protoplasts lack a cortical actin cytoskeleton, we assumed that the tension opposing aspiration into the pipette originates from the plasma membrane. The membrane tension σ can then be determined from $\sigma = \Delta P R_p/[2(1 - R_p/R_c)]$, where ΔP is the suction pressure required to aspirate an equilibrium length $l = R_p$ of protoplast into the pipette (Fig. 2B) (Evans and Yeung, 1989).

Assuming that the perturbation due to aspiration has a small effect on the membrane tension, this expression then represents the membrane tension of the unaspirated cell that we seek. The suction pressure ΔP was identified by overlaying plots of suction pressure and aspirated protoplast length as functions of time (Fig. S2H). We measured the tension σ under 3 different osmotic conditions, with sorbitol concentrations 0.6, 0.8, or 1.2 M. The mean membrane tensions in each case were similar, and showed no clear dependence on osmotic conditions: 0.33 ($n = 4$), 0.17 ($n = 2$), and 0.30 mN/m ($n = 3$), respectively. Thus we used the mean of the 9 measured membrane tensions, $\sigma = 0.28 \pm 0.28$ mN/m (mean ± SD), to calculate the ring tension, as follows.

We inferred ring tension from protoplasts in a range of in 0.6 – 1.2 M sorbitol that contained sliding rings that furrowed the membrane (n = 12) (Fig. 2A). The ring tension is determined by a force balance at the furrow in the radial direction, $T = R_{ring}(\sigma_1 \cos\theta_1 + \sigma_2 \cos\theta_2)$ (Fig. 2A). Here, R_{ring} is the ring radius, θ_1 and θ_2 are the angles the membrane makes with the plane of the ring at the furrow, and σ_1 and σ_2 are the membrane tensions of the two protoplast lobes. Since the ring sliding was very slow, with constriction timescales of ~30-60 min (Fig. 6E), we assumed that cytosolic pressure gradients produced by the moving membrane furrow had sufficient time to relax. Thus the pressure is uniform within the cell, so the membrane of each lobe supports the same pressure difference. It thus follows from Laplace's law that the ratio of the membrane tensions in the lobes equals the ratio of the lobe radii. Since the relative difference in lobe radii was <50%, we took the mean of the membrane tensions in the two lobes of a furrowed protoplast to be equal to the value, σ, that we measured in unfurrowed protoplasts using micropipette aspiration. Hence, $\sigma_1 = 2\sigma/(1+R_2/R_1)$ and $\sigma_2 = 2\sigma/(1+R_1/R_2)$, where R_1 and R_2 are the lobe radii. Thus, by measuring the ring radius R_{ring} and the lobe radii R_1 and R_2 from images of furrowed protoplasts, and then calculating the resulting angles θ_1 and θ_2, we could directly calculate ring tension T. The values we obtained give a mean value of $T = 391 \pm 154$ pN (mean ± SD).

2. Simulation of the fission yeast contractile ring

We developed a computer simulation to study the organization and tension of the ring. The ring tensions generated in our simulation were then used as input to our model of ring sliding-constriction in protoplasts (described in section 4). Our simulation represents the contractile ring at some instant during constriction, when its length L is less than or equal to its initial maximum value at the onset of constriction. Importantly, constriction lasts much longer (~30-60 min, Fig. 6E and Pelham and Chang, 2002) than the time required for the organization and tension of the simulated ring to attain steady state. A good measure of the latter timescale is the memory time for tension fluctuations, ~30 s (Fig. 4A). Thus, during the time required for the statistical properties of the ring to reach steady state, the ring length changes very little. This justifies our procedure in which we ran the ring simulation and calculated the tension for a fixed ring length L, and then repeated this for different values of L corresponding to the shortening ring throughout constriction.

As our experimental results suggest that protoplast rings are functional (Figs. 1 and 2), the model is assumed to be equally applicable to rings in intact fission yeast cells and in protoplasts, and accordingly we compared its predictions both to literature measurements on intact cells and to measurements on protoplasts in the present study. In intact fission yeast, the length of the ring at the onset of constriction is $L \approx 10$ μm (Pelham and Chang, 2002), while in protoplasts we

observed ring lengths up to a maximum of $L = 22.1$ μm (Fig. 6E). Table 1 summarizes the key parameter values used in the simulation. All simulations were performed in MATLAB.

Ring geometry

The simulated ring lies on the inner surface of the plasma membrane and has circumference, or length, L. Our experimental measurements showed that the thickness of the ring perpendicular to the membrane (<0.4 μm, Figs. 1J and S3A,B) is significantly less than the mean actin filament length in the simulation (~1.3 μm). Moreover, in a previous study actin filaments were estimated to make a small ~8º angle with the membrane during ring assembly (Laporte et al., 2011). Thus, in our simulations we approximated the ring as a quasi-two-dimensional structure in the plasma membrane. Since the mean filament length of ~1.3 μm is much less than the initial ring length of ~10-20 μm, we simulated the ring as a flat band with periodic boundary conditions (Movie S6). This assumption did not significantly affect ring tension, since the bending of actin filaments imposed by the curvature of the ring would generate negligible forces (see below).

Newly arriving formins and myosin-II clusters were assumed to bind randomly, with uniform spatial distribution, within a zone of the membrane of width $d = 0.2$ μm. This choice of width resulted in steady state rings with formins randomly distributed around the ring (Fig S3E) and components extending across a band ~0.2 μm wide (Fig. 4F), in agreement with present (Fig. 2H-J) and previous (Wu and Pollard, 2005) experiments, respectively.

Actin filaments and formins

We described actin filaments as semi-flexible polymers, accounting for the fact that actin filaments are stiff but can bend, according to the bending modulus $\kappa = k_B T l_p$, where $k_B T$ is the thermal energy and $l_p = 10$ μm the persistence length (Ott et al., 1993; Riveline et al., 1997). We used a standard method to follow filament configurations, tracking a subset of the actin filament's subunits separated by 0.1 μm, measured along the filament's length. Thus, every 37th actin subunit is labeled, and in the following the "i^{th} subunit" denotes the ith labeled subunit. The filament segments connecting labeled subunits are represented by springs that also have a local energy penalty for bending, determined by the bending modulus κ (Laporte et al., 2012) (Gauger and Stark, 2006). Thus, the springs have rest length $l_{act}^0 = 0.1$ μm. Note this is less than the myosin capture diameter (see below), ensuring stable actin-myosin connections. Each spring has stiffness $k_{act} = 1000$ pN/μm, equivalent to a filament tensile modulus of $k_{act} l_{act}^0 / A_{fil} = 4$ pN/nm^2, where $A_{fil} \approx 25$ nm^2 is the actin filament cross-sectional area. This value was chosen to be smaller than the true modulus, ~1800 pN/nm^2 (Kojima et al., 1994), to allow the use of longer simulation time steps while still ensuring very small typical extensions of <10%. The simulated ring was relatively insensitive to the value of the actin filament bending modulus κ, as varying κ by a factor of 3 created small variations in tension T (<12%), bundling coefficient Ψ_{bund} (<1%), and sarcomericity Ψ_{sarc} (<14%) (Fig. 5H).

Each actin subunit had an effective drag coefficient representing the drag coefficient of the segment of length l_{act}^0 separating it from the next labeled subunit along the filament's length. This drag coefficient was $\gamma_{subunit} = 0.2$ pN·s/μm, corresponding to a cytoplasmic viscosity of $\eta \approx \gamma_{subunit}[\ln(l_{act}^0/d_{act}) + 0.35]/(3\pi l_{act}^0) = 0.6$ Pa·s (Tirado and Garciadelatorre, 1979), on the order of previous measurements (Howard, 2001), where d_{act} is the actin filament diameter. Simulation

results were not sensitive to the value of $\gamma_{subunit}$ because the typical drag force $\gamma_{subunit}v_{pol}$ = 0.014 pN is much smaller than the myosin force f_{myo} = 4 pN. The barbed end of each actin filament was capped by a formin dimer anchored to the membrane. The subunit at the barbed end of each actin filament represented the formin, and was assigned drag coefficient γ_{for}, whose value (Table S1) was determined by fitting to the mean speed we experimentally measured for formin in the ring (Fig. 2).

The actin filament diameter of ~5-9 nm is likely much less than thickness of the ring perpendicular to the membrane, which we measured to be of order 0.4 μm or less (Figs. 1J and S3A,B), and actin filaments in the assembling ring were estimated to dip a small ~8º angle out of the plane of the membrane (Laporte et al., 2011). These findings suggest that actin filaments can cross one another in the ring with relatively little mutual interference. Thus our simulations neglected excluded volume interactions between different actin filaments, which could freely cross one another. We accounted for excluded volume interactions between myosin clusters (see below), but such interactions between formins were omitted, as our measurements suggest they exist as single dimers (Fig. S2E-G) and are therefore likely much smaller than myosin clusters.

Myosin clusters
The structure of myosin-II in the fission yeast contractile ring is unknown. Our simulations assumed that myosin forms clusters, each containing 40 Myo2p myosin heavy chains, the same number of Myo2p molecules per node from which the ring is assembled (Wu and Pollard, 2005). Each myosin cluster is anchored to the membrane with drag coefficient γ_{myo}, whose value (Table S1) was determined by fitting to the mean speed we experimentally measured for myosin clusters in the rings of protoplasts (Fig. 2). Myosin clusters interact in two ways with actin filament subunits that are within its capture radius of r_{myo} = 0.1 μm from the center of the cluster (Table S1). First, myosin pulls on the subunit with force f_{myo} = 4 pN (Table S1) in the direction of the filament's pointed end. We imposed a maximum net force of 40pN that a myosin cluster could exert on all filaments that it binds to, based on an assumed force of 2 pN per myosin dimer. Thus the force per filament is reduced if a cluster interacts with >10 filaments (see below). Second, the actin subunit is captured and drawn toward the center of the cluster, an effect we modeled with a spring with zero rest length connecting the center of the cluster with the subunit. In addition, myosin clusters interact with one another through a short range excluded volume repulsion if they move within a distance d_{myo} = 50 nm of one another. These interactions and their implementation are described in more detail below.

Turnover in the simulated ring
The cytokinetic ring is an open system, constantly exchanging components with its environment. The simulated turnover of actin, formin, and myosin-II followed the reactions depicted in Fig. 3A. Formin and myosin binding and unbinding, and actin filament severing were implemented stochastically, and the values of the corresponding rate constants (Table S1) were constrained by published experimental observations.

Formin binding. New formin proteins bind with uniform probability at all locations within a zone of width d = 0.2 μm. The number entering the ring during a time step of duration Δt follows a Poisson distribution with mean $r_{for}L\Delta t$, where r_{for} is the binding rate per ring length. Each newly bound formin instantly nucleates an actin filament, so a binding event results in a formin

subunit connected to an actin filament subunit by a spring with an initial rest length of approximately zero ($v_{pol}\Delta t \approx 3.5\times10^{-6}$ μm), where v_{pol} is the actin polymerization velocity. The new filament adopts any orientation θ with respect to the ring axis with equal probability. The value of the binding rate was $r_{for} = \rho_{for} k_{off}^{for} = 0.35$ μm^{-1}·s^{-1}, where ρ_{for} =15 μm^{-1} is the formin density (Wu and Pollard, 2005), and $k_{off}^{for} = 0.023$ s^{-1} is the formin unbinding rate (Yonetani et al., 2008, Fig. S3C) (Table S1).

Actin polymerization. Each actin filament elongates from the formin that caps its barbed end. To implement the increasing filament length, while still describing the filament as a series of springs with fixed rest length, new springs are inserted at the barbed end at a rate corresponding to the polymerization rate. This is done as follows. At each time step, a length $v_{pol}\Delta t$ of filament is added to the rest length of the spring at the barbed end (Laporte et al., 2012), where v_{pol} is the formin-mediated polymerization velocity. Once the rest length of the spring at the barbed end exceeds a threshold value of 0.12 μm, the spring is split into two springs of lengths 0.02 μm (1st spring at the barbed end) and $l_{act}^{0} = 0.1$ μm (2nd spring) as a new subunit enters the actin filament. Thus, each actin filament consists of a spring connected to the barbed end whose rest length is growing due to polymerization, connected to a series of springs of constant rest length $l_{act}^{0} = 0.1$ μm.

Severing by cofilin. Every actin filament is severed by cofilin with probability $r_{sev} l_{fil} \Delta t$ each time step, where l_{fil} is the filament length and r_{sev} the severing rate per filament length. After each subunit of yeast ATP-actin polymerizes, the bound ATP hydrolyzes and the γ-phosphate dissociates in <10 s (Ti and Pollard, 2011). Since we predict that actin turns over in ~11 s (Fig. 7C), and since cofilin binding can accelerate phosphate release >10 fold (Blanchoin and Pollard, 1999), we assume that most polymerized subunits are ADP-actin and that cofilin severs with equal probability at any location along each filament. Thus, the *net* severing probability for the filament increases with length, consistent with the kinetics of cofilin severing of ADP-actin filaments in vitro (Andrianantoandro and Pollard, 2006). After severing, all subunits and springs between the point of severing and the pointed end are deleted from the simulation.

Formin unbinding. Each formin dissociates from the ring with probability $k_{off}^{for}\Delta t$ in each time step, where k_{off}^{for} is the formin off rate. If a formin dissociates, both the formin and its associated actin filament are deleted from the simulation.

Myosin turnover. In the simulation, myosin-II clusters bind with uniform probability at all locations in the binding zone of width $d = 0.2$ μm. The number entering during a time step of duration Δt follows a Poisson distribution with mean $r_{myo} L \Delta t$, where r_{myo} is the binding rate per ring length. The value of the binding rate was $r_{myo} = \rho_{myo} k_{off}^{myo} = 0.20$ μm^{-1}·s^{-1}, where ρ_{myo} =7.5 μm^{-1} is the myosin-II cluster density assuming 40 heavy chains per cluster (Wu and Pollard, 2005), and $k_{off}^{myo} = 0.026$ s^{-1} is the myosin unbinding rate (Pelham and Chang, 2002) (Table S1). Each myosin-II cluster dissociates from the ring with probability $k_{off}^{myo}\Delta t$ in each time step.

FRAP simulations. To simulate a formin FRAP assay (Figs. 7C and S3C), we assigned all formin subunits in the ring at some instant to a 'bleached' population, and then tracked the arrival of new, 'unbleached' formins. To simulate an actin FRAP assay (Fig. 7C), we assigned all actin filaments in the ring at some instant to a bleached population, and then tracked the arrival of new unbleached filaments and the growth of new unbleached portions of bleached filaments.

Forces and velocities

The ith actin filament subunit in the simulated ring has the 2-component position vector $\mathbf{r}_i^{\mathrm{act}}$, where $i = 1,2,3...n_{\mathrm{subunit}}$ and n_{subunit} is the total number of actin filament subunits. Within each actin filament, subunits are counted from the barbed to the pointed end. Similarly, myosin cluster i has position $\mathbf{r}_i^{\mathrm{myo}}$, where $i = 1,2,3...n_{\mathrm{myo}}$ and n_{myo} is the total number of myosin clusters. The velocities of the actin subunits and myosin clusters are determined by the forces acting upon them, summarized in Fig. 3B:

$$\frac{\partial \mathbf{r}_i^{\mathrm{act}}}{\partial t} = \frac{1}{\gamma_i}\left(\mathbf{f}_i^{\mathrm{spr}} + \mathbf{f}_i^{\mathrm{bend}} + \mathbf{f}_i^{\mathrm{x}} + \mathbf{f}_i^{\mathrm{pull}} + \mathbf{f}_i^{\mathrm{cap}}\right) \tag{S1}$$

$$\frac{\partial \mathbf{r}_i^{\mathrm{myo}}}{\partial t} = \frac{1}{\gamma_{\mathrm{myo}}}\left(\mathbf{f}_i^{\mathrm{pull,myo}} + \mathbf{f}_i^{\mathrm{cap,myo}} + \mathbf{f}_i^{\mathrm{excl}}\right) . \tag{S2}$$

Here γ_{myo} is the myosin drag coefficient, and γ_i is equal to the formin drag coefficient γ_{for} if subunit i is at the barbed end of a filament, and is equal to the actin subunit drag coefficient $\gamma_{\mathrm{subunit}}$ otherwise. At each simulation time step of duration Δt, Eqs. S1 and S2 were used to determine the component velocities, allowing update of positions. Below we describe the forces in Eqs. S1 and S2.

Actin filament springs. The force on actin subunit i from actin filament springs is

$$\mathbf{f}_i^{\mathrm{spr}} = -\sum_j k_{\mathrm{act}}\left(\left|\mathbf{r}_i^{\mathrm{act}} - \mathbf{r}_j^{\mathrm{act}}\right| - l_{\mathrm{act}}^0\right)\frac{\mathbf{r}_i^{\mathrm{act}} - \mathbf{r}_j^{\mathrm{act}}}{\left|\mathbf{r}_i^{\mathrm{act}} - \mathbf{r}_j^{\mathrm{act}}\right|}, \tag{S3}$$

where the sum is over actin subunits j that neighbor subunit i, and are part of the same filament. For interior subunits, $j = i - 1, i + 1$, while for subunits at barbed and pointed ends, $j = i + 1$ and $j = i - 1$, respectively.

Actin filament bending. The force on actin subunit i from filament bending is

$$\mathbf{f}_i^{\mathrm{bend}} = -\frac{\partial}{\partial \mathbf{r}_i^{\mathrm{act}}}\left[\frac{\kappa}{l_{\mathrm{act}}^0}\sum_{j=1}^{n_{\mathrm{spring}}-1}\left(1 - \hat{\mathbf{t}}_{j+1}\cdot\hat{\mathbf{t}}_j\right)\right] , \tag{S4}$$

where the quantity in square brackets is the total bending energy of the actin filament containing subunit i, the sum is over all of the n_{spring} springs in this actin filament, κ is the actin filament bending modulus, and $\hat{\mathbf{t}}_j$ is the unit vector parallel to spring j (Gauger and Stark, 2006; Laporte et al., 2012).

Actin crosslinking forces due to α*-actinin.* When actin subunits from two different filaments are within distance r_x^{bind} = 50 nm of each other, α-actinin Ain1p forms a crosslink between the subunits with a binding rate tuned so the density of α-actinin in the ring matched the experimentally measured value (Table S1). The α-actinin is modeled as a spring connecting the two actin subunits having spring constant k_x = 25 pN/μm (Table S1). α-actinin crosslinks unbind at rate k_{off}^{x} (Table S1) and are also assumed to unbind if the separation between the linked actin subunits exceeds r_x^{bind}. The force on actin subunit i from α-actinin crosslinks is

$$\mathbf{f}_i^{x} = -\sum_j k_x \left(\left| \mathbf{r}_i^{\text{act}} - \mathbf{r}_j^{\text{act}} \right| - r_x^0 \right) \frac{\mathbf{r}_i^{\text{act}} - \mathbf{r}_j^{\text{act}}}{\left| \mathbf{r}_i^{\text{act}} - \mathbf{r}_j^{\text{act}} \right|}, \tag{S5}$$

where r_x^0 is the rest length of the spring (Table S1), and the sum is over actin subunits j that are linked to actin subunit i by α-actinin.

Myosin-II pulling. An actin filament subunit that enters within the capture radius of a myosin cluster is pulled by the myosin cluster with force f_{myo} in a direction tangent to the filament at that point (i.e., parallel to the spring connecting that subunit to its neighbor) and acting toward the filament's pointed end. The value of this force was f_{myo} = 4 pN, from our measurements of node motions in this work (Table S1). However, each myosin cluster is assumed to exert a maximum net force of 40 pN on all the actin filaments to which it binds (assuming 2 pN per myosin dimer), so that the force per filament is reduced accordingly if a cluster interacts with >10 filaments. The total myosin pulling force on actin filament subunit i is therefore

$$\mathbf{f}_i^{\text{pull}} = n_i^{\text{myo}} f_{\text{myo}} \frac{\mathbf{r}_i^{\text{act}} - \mathbf{r}_{i-1}^{\text{act}}}{\left| \mathbf{r}_i^{\text{act}} - \mathbf{r}_{i-1}^{\text{act}} \right|}, \tag{S6}$$

where n_i^{myo} is the effective number of myosin clusters interacting with subunit i, and subunit i-1 is the adjacent subunit in the direction of the barbed end. The effective number of clusters is $n_i^{\text{myo}} = \sum_j \alpha_j$, where the sum is over myosin clusters j that interact with filament i. Here $\alpha_j = 1$ if myosin cluster j interacts with $n_j^{\text{int}} \le 10$ actin filaments, and $\alpha_j = 10/n_j^{\text{int}}$ if the cluster interacts with $n_j^{\text{int}} > 10$ filaments. From the above expression, myosin pulls locally along the direction of the filament's contour, and this force does not locally change the actin filament's orientation. Each actin-myosin pulling interaction also produces an equal and opposite force on the myosin cluster due to Newton's third law, and the net pulling force on myosin cluster i is named $\mathbf{f}_i^{\text{pull, myo}}$. We assume that all of these forces due to myosin are independent of actin and myosin cluster velocities, since these velocities are much less than the measured unloaded actin filament gliding velocity produced by fission yeast Myo2p, v_{myo}^0 = 0.4 μm/s (Lord and Pollard, 2004).

Myosin-II capture. In addition to pulling, a myosin cluster also binds to, or captures, actin subunits within the capture radius r_{myo} and draws them toward the center of the cluster. This binding is modeled as a spring with zero rest length connecting the center of the cluster with the actin filament subunit. The total myosin capture force on actin subunit i is

$$\mathbf{f}_i^{\text{cap}} = -\sum_j k_{\text{myo}} \left[\left(\mathbf{r}_i^{\text{act}} - \mathbf{r}_j^{\text{myo}} \right) - \left(\left(\mathbf{r}_i^{\text{act}} - \mathbf{r}_j^{\text{myo}} \right) \cdot \hat{\mathbf{t}}_i \right) \hat{\mathbf{t}}_i \right], \tag{S7}$$

where $\hat{\mathbf{t}}_i$ is the unit vector in the direction of spring i, and the sum is over all myosin clusters j that are within distance r_{myo} of actin subunit i. The first term is the spring force, while the second term subtracts off the component parallel to the actin filament. Thus the capture force only acts perpendicular to the filament, and therefore perpendicular to the myosin pulling force. Each capture interaction also produces an equal and opposite force on the myosin cluster due to Newton's third law, and the net capture force on myosin cluster i is named $\mathbf{f}_i^{\text{cap,myo}}$. The spring constant k_{myo} = 5 pN/µm was set to allow stable actin-myosin connections while still allowing myosin to transfer to the next actin subunit in the filament as the filament grows. Significantly larger values of k_{myo} caused permanent pinning of actin subunits to myosin clusters, while significantly smaller values prevented stable actin-myosin binding.

Myosin-II excluded volume. Myosin-II clusters experience a short range excluded volume repulsion if they move within distance d_{myo} = 50 nm of each other. The net force on myosin cluster i is

$$\mathbf{f}_i^{\text{excl}} = -\sum_j k_{\text{myo}}^{\text{excl}} \left(\left| \mathbf{r}_i^{\text{myo}} - \mathbf{r}_j^{\text{myo}} \right| - d_{\text{myo}} \right) \frac{\mathbf{r}_i^{\text{myo}} - \mathbf{r}_j^{\text{myo}}}{\left| \mathbf{r}_i^{\text{myo}} - \mathbf{r}_j^{\text{myo}} \right|}, \tag{S8}$$

where the sum is over all myosin clusters j that are within d_{myo} of cluster i, and the spring constant of repulsion, $k_{\text{myo}}^{\text{excl}}$ = 10,000 pN/µm, was chosen to be extremely stiff to approximate a "hard sphere" interaction. The value of d_{myo} was set to be on the order of the myosin capture radius, but smaller than the assumed excluded volume radius for nodes (Laporte et al., 2012; Vavylonis et al., 2008) because myosin clusters in the ring likely lack some node components.

Calculation of ring tension in the simulation

As mentioned above, we performed simulations at fixed ring length. To calculate the ring tension as a function of ring length, $T(L)$, for each value of L we ran the simulation to steady state and then recorded the time-averaged value of the tension over a time >25-fold larger than the formin turnover time. At each simulation time step, ring tension was calculated using the formula:

$$T = \sum_{i=1}^{N_{\text{spring}}} \frac{\cos^2(\theta_i^{\text{spring}}) T_i^{\text{spring}} l_i^{\text{spring}}}{L}, \tag{S9}$$

where T_i^{spring}, l_i^{spring}, and θ_i^{spring} are the tension, length, and orientation of spring i, and the sum is over all springs in the simulation (actin filament springs, α-actinin springs, myosin capture force springs, and springs representing myosin cluster excluded volume). The angle θ_i^{spring} is measured relative to the ring axis, and the $\cos^2(\theta_i^{\text{spring}})$ factor selects the components of spring length and tension parallel to the ring axis. Thus, T is the component of tension of ring components parallel to the ring axis, averaged over the ring length. This is the relevant component that works to constrict the ring. The expression of Eq. S9 is valid provided drag forces between actin filaments and the cytoplasm can be neglected, i.e., the actin subunit drag coefficient γ_{subunit} is sufficiently small. In our simulations this condition was satisfied, as the characteristic drag force $\gamma_{\text{subunit}} v_{\text{pol}}$ =

0.014 pN was far smaller than the myosin force f_{myo} = 4 pN. Thus we expect the contribution to the tension from these drag forces is small.

Time step

The simulation time step was $\Delta t = 5\times10^{-5}$ s. We chose this value to be significantly less than the relaxation time of the actin filament subunit-spring system, $\gamma_{subunit}/k_{act} = 2\times10^{-4}$ s. The time step was therefore also far smaller than the formin and myosin turnover time of ~30 s. This choice lengthened an actin filament by 3.5×10^{-3} nm per time step, far less than the actin subunit size ~2.7 nm. When we used a time step Δt = 0.04 s, giving a length increase per time step close to the length of an actin subunit, we found no changes in ring properties (tension, sarcomericity, organization). For example, ring tension at the onset of constriction was 355 ± 50 pN, within the range of tensions 340 ± 57 pN that we obtained using our usual, much smaller, simulation time step.

Determination of unknown turnover parameter values: r_{sev} and v_{pol}

The parameters r_{sev} and v_{pol} are the cofilin-mediated severing rate per length of actin filament, and the formin-mediated polymerization rate of actin filaments, respectively. Unlike many other simulation parameters, these have not been directly measured in the ring. However, their values help to determine the amount of actin and the turnover rates of components in the simulation. Thus, we could fix their values by insisting that the results of the simulation were consistent with experiments that have probed these features. We estimated r_{sev} and v_{pol} by tuning them simultaneously so that two properties of the simulated ring matched data from previous experiments: (1) from published electron micrographs, we estimated the number of actin filaments in parallel across the ring to be ~20 (Kanbe et al., 1989); (2) the contractile ring disintegrated in ~55 s after treatment with a large dose of the toxin Latrunculin A that apparently completely inhibited actin polymerization (Yonetani et al., 2008), Fig. S3D. To simulate the Latrunculin treatment experiment, we set the polymerization rate v_{pol} to zero at t = 0 and recorded the time at which 90% of the actin originally in the ring was lost due to formin unbinding and cofilin severing (Fig. S3D, dashed line). We then set the parameter values so that this time matched the experimentally observed time for 90% of rings to disintegrate. We could not compare directly to the amount of actin in the ring, as this was not reported. In the experiments, a ring was defined to have disintegrated when the actin became indistinguishable from background, so we assumed that the amount of actin remaining served as a good guide for the disintegration time.

The parameter values that reproduced these two experimental results, (1) and (2) above, were v_{pol} = 70 nm/s (26 subunits/s) and r_{sev} = 1.8 $\mu m^{-1}min^{-1}$ (Table S1). These best-fit parameter values produce a mean actin filament length of ~1.3 µm (Movie S6).

The best-fit value of v_{pol}, 70 nm/s, is one third the rate of filament extension from precursor nodes (Vavylonis et al., 2008). The value of r_{sev}, 1.8 $\mu m^{-1}min^{-1}$, is ~6-fold faster than the rate at which the optimal concentration of cofilin Adf1p severs muscle actin filaments in vitro (Andrianantoandro and Pollard, 2006). Similarly to the cofilin severing kinetics, a previous model of actin patch dynamics also predicted rates for several reactions in live fission yeast that were faster than those measured *in vitro* using muscle actin (Berro et al., 2010), some of which have been verified (Arasada and Pollard, 2011; Ti and Pollard, 2011).

Testing the simulation turnover mechanisms: comparison with experiments in fission yeast cells expressing mutant formin

To validate our assumed actin and formin turnover mechanisms (Fig. 3A), we tested whether our simulations could recapitulate the behavior observed in fission yeast cells with altered actin or formin turnover rates.

Yonetani et al. (2008) reported that deletion of both profilin binding sites in the FH1 domain of formin Cdc12p resulted in dramatically reduced actin polymerization activity in vitro. FRAP measurements showed that the dissociation of the mutant formin Cdc12p ($k_{\mathrm{off}}^{\mathrm{for}}$ = 0.009 s^{-1}) from contractile rings of cells also expressing wild-type Cdc12p was slower than the dissociation of wild-type Cdc12p from rings in wild-type cells (0.023 s^{-1}, Fig. S3C). Further, treatment with a large dose of Latrunculin A to inhibit all actin polymerization disintegrated rings more slowly in cells expressing mutant Cdc12p (138 s) than in wild-type cells (55 s, Fig. S3D Inset) (Yonetani et al., 2008).

To test if our simulation could capture this result, we simulated the Latrunculin A ring disintegration assay assuming that all formins in the ring were mutant formins. Using the measured mutant $k_{\mathrm{off}}^{\mathrm{for}}$ value, and treating the mutant actin polymerization rate v_{pol} as a fitting parameter, we ran the simulation to steady state. Then we set $v_{\mathrm{pol}} = 0$ (to mimic Latrunculin addition) and recorded the time for 90% of the actin to disappear. Note that the simulated disintegration time depends on the mutant actin polymerization rate because the latter determines the filament length distribution in the steady state ring just before polymerization is switched off, and this distribution in turn affects the disintegration time. To reproduce the observed disintegration time, we found a best-fit value for the mutant actin polymerization rate of $v_{\mathrm{pol}} = 12$ nm/s, much less than our model's best-fit value in wild-type cells, 70 nm/s (Fig. S3D and Table S1). This best-fit value is an upper bound, and would have been even lower had we assumed that wild-type formins were present in the ring along with the mutant proteins. This lower mutant polymerization rate is consistent with the reduced polymerization activity seen experimentally (Yonetani et al., 2008). Thus, the assumed turnover mechanisms in our simulation (Fig. 3A) are able to capture the behavior of contractile rings with perturbed actin and formin turnover kinetics.

Calculation of actin and formin FRAP curves

The analytical solution for the formin FRAP curve of Fig. 7C has the format $I_{\mathrm{for}}(t)/I_{\mathrm{for}}^{\infty} = 1 - \exp(-k_{\mathrm{off}}^{\mathrm{for}} t)$, where I_{for} is the formin fluorescence and $I_{\mathrm{for}}^{\infty}$ is the final recovery value, because of the assumed simple 1st order unbinding of formin from the ring.

The actin FRAP curve is calculated from $I_{\mathrm{act}}(t) = I_{\mathrm{l}} v_{\mathrm{pol}} N_{\mathrm{fil}} \int_0^t dt'\, p(t')$, where I_{l} is the actin fluorescence per unit filament length, N_{fil} is the number of filaments in the ring and $p(t')$ is the "survival probability" of a monomer at time t' that was polymerized at time 0, i.e. the probability that such a monomer still belongs to the ring after time t'. Based on the turnover mechanisms described above, the survival probability obeys $\frac{dp}{dt} = -k_{\mathrm{off}}^{\mathrm{for}} p - r_{\mathrm{sev}} v_{\mathrm{pol}} t p$. Evaluating the

integral above then yields $I_{act}(t)/I_{act}^{\infty} = [\text{erf}(a + bt) - \text{erf}(a)]/[1 - \text{erf}\,(a)]$, where $a = k_{off}^{for}(\frac{r_{sev}v_{pol}}{2})^{-1/2}$ and $b = (r_{sev}v_{pol}/2)^{1/2}$. This expression was used in Fig. 7C.

Tension and organization of the simulated ring are robust to parameter variations
Within a range about the experimentally determined "wild type" parameter values of Table S1, the simulations robustly self-organized components into a functional ring whose tension was close to the value we measured experimentally (Fig. 4A). To test the robustness of the model, we varied the parameter values in simulations of the ring at the onset of constriction, and we measured the ring tension, and the sarcomericity ψ_{sarc} and bundling coefficient ψ_{bund} that quantify the ring's organization (Fig. 5).

Production of a functional ring with essentially the same bundled organization and tension as in the wild type simulations required that formin and myosin-II clusters bind in a zone <0.4 μm wide, and that that myosin clusters have a capture radius >70 nm (Fig. 5E,F) and an anchor mobility >0.2 nm/pN s (Fig. 5A-C). The wild type simulations assumed there was no directional bias in the actin filaments nucleated by formins, the simplest possible assumption; introducing bias had little effect, and the rings were similar to those in wild type simulations. (Fig. 5G). This suggests that the spontaneous rotation and bundling of actin filaments is such an efficient process that possible orientational bias of actin nucleation in the circumferential direction of the ring may be redundant. Production of functional rings was similarly insensitive to the actin filament bending modulus, myosin turnover rate and actin polymerization and severing rates at fixed mean filament length (Fig. 5H and data not shown). For the latter, for each value of the cofilin severing rate r_{sev} we tuned the actin filament polymerization rate v_{pol} to compensate in such a way as to maintain the actin filament length fixed.

Bending forces in actin filaments due to ring curvature are negligible
The simulation ignores forces due to actin filament bending imposed by the curvature of the ring as it follows the curved cell membrane. Here, we show that these forces are negligible compared to other forces in the ring. The bending energy per unit length of an actin filament due to this curvature is $E = \kappa/(2R_{ring}^2)$, where R_{ring} is the filament's radius of curvature (equal to the ring's radius of curvature) and κ is the bending modulus (Landau and Lifshitz, 1986). The force per unit length acting in the radial direction is then $-\partial E/\partial R_{ring} = \kappa/R_{ring}^3$. In a ring whose cross section is intersected by N actin filaments, the total outward radial force per unit length due to filament bending is thus $\sigma_{bend} = N\kappa/R_{ring}^3$. In Laplace's law, the filament bending energy contributes an extra term that translates into a negative effective contribution to the ring tension given by

$$T_{bend} = -\sigma_{bend} R_{ring} = -N\kappa/R_{ring}^2 . \tag{S10}$$

Using $N = 20$ (Kanbe et al., 1989), $\kappa = k_B T l_p = 0.041$ pN·μm^2 (where $k_B T$ is thermal energy and l_p is the actin filament persistence length, Table S1), and $2\pi R_{ring} = 2$-20 μm, gives -8 pN < T_{bend} < -0.08 pN. These values are much smaller than the ring tension of ~350 pN.

Once the ring has a length <1 μm (the approximate filament length in our simulations), its diameter is ~0.3 μm, comparable to the thickness of the ring and to the size of myosin-II clusters. Thus, no matter what model one might adopt of the contractile ring, physically it cannot continue

to constrict and retain the same form with the same components. We consider it very likely that a distinct process takes over at this stage, perhaps related to the process of abscission.

3. Quantitative characterization of the ring organization: the sarcomericity, the bundling coefficient, and their relation to ring tension

Derivation of approximate expression for the simulated ring tension in terms of statistical properties of the ring organization

Here we derive the expression for the ring tension T presented in the main text in Results, in the subsection "*Relationship between organization and tension in the ring*." This approximate expression relates the tension to the sarcomericity and bundling coefficient that characterize the organization and will be defined below. We will show that the ring tension can be expressed as a simple function of several key properties of the ring after adopting the following simplifying assumptions. (*i*) We calculate only the tension in actin filaments, as this accounts for >99% of the tension in the simulation, while tension in α-actinin crosslinks, myosin excluded volume forces, and myosin capture account for the remainder. (*ii*) We assume that each actin filament is straight, so its orientation with respect to the ring axis is characterized by a single angle. This is expected to produce fairly small errors, because the mean magnitude of actin filament curvature due to bending in the simulation is ~0.17 μm^{-1}, giving a mean radius of curvature of ~6 μm, more than 4-fold larger than the mean actin filament length. (iii) Similarly to our derivation of the near-exact formula for tension in the ring given by Eq. S9, we ignore the very small contribution from drag forces between actin filaments and the cytoplasm (see discussion following Eq. S9).

Consider first the population of actin filaments in the ring that have length l and orientation θ, where $-\pi < \theta < \pi$, and $\theta = 0$ for a filament aligned with the ring axis that has barbed end to the left. We define $c_{\text{myo},l\theta}(s)$ to be the mean linear number density (per unit filament length) of myosin clusters interacting with filaments in this population a distance s from the filament's anchored barbed end, measured along the filament in the direction of its pointed end. The barbed end is at $s = 0$, while the pointed end is at $s = l$. This myosin density is an average for filaments of length l and orientation θ. Since each myosin cluster exerts a total force f_{myo}, the mean tension generated by myosin-II clusters in a filament of length l and orientation θ, averaged over its length, is given by

$$T_{l\theta} = \frac{f_{\text{myo}}}{l} \int_0^l s c_{\text{myo},l\theta}(s) ds \ . \tag{S11}$$

The s in the integrand of Eq. S11 is present because myosin at location s produces tension in the portion of the filament of length s between the myosin and the anchored barbed end. Note that there is no contribution to tension from drag forces that act on the barbed end anchor, $s = 0$, due to filament motion. Each such filament makes a contribution to the mean tension averaged along the ring of length L that is equal to $T_{l\theta}\cos^2(\theta)l/L$. Here the factor $\cos(\theta)l/L$ gives the fraction of the ring's length that the filament's length projects onto, and the second factor of $\cos(\theta)$ gives the component of the filament's tension that contributes to the ring tension which acts parallel to the ring circumference. For a ring with a total of N_{fil} filaments, the total ring tension is then

$$T = f_{\text{myo}} \frac{N_{\text{fil}}}{L} \left\langle \cos^2(\theta) \int_0^l s c_{\text{myo},l\theta}(s) ds \right\rangle_{l,\theta} , \tag{S12}$$

where the angular brackets with subscript l,θ denotes the average over the distributions of both actin filament length and orientation. This is rewritten

$$T = f_{\text{myo}} c_{\text{fil}} \langle l \rangle \langle \cos^2(\theta) \rangle \frac{\left\langle \cos^2(\theta) \int_0^l s c_{\text{myo},l\theta}(s) ds \right\rangle_{l,\theta}}{\langle l \rangle \langle \cos^2(\theta) \rangle} , \tag{S13}$$

where $c_{\text{fil}} = N_{\text{fil}}/L$ is the mean linear density of actin filaments. Thus the tension can be expressed in the form presented in "*Relationship between organization and tension in the ring"* in Results:

$$T = f_{\text{myo}} c_{\text{fil}} \langle l \rangle \psi_{\text{bund}} \psi_{\text{sarc}} . \tag{S14}$$

Here we defined the bundling coefficient Ψ_{bund} to be

$$\psi_{\text{bund}} = \langle \cos^2(\theta) \rangle , \tag{S15}$$

and we defined the "sarcomericity" Ψ_{sarc} as

$$\psi_{\text{sarc}} = \frac{\left\langle \cos^2(\theta) \int_0^l s c_{\text{myo},l\theta}(s) ds \right\rangle_{l,\theta}}{\langle l \rangle \langle \cos^2(\theta) \rangle} \approx \frac{\left\langle \int_0^l s c_{\text{myo},l}(s) ds \right\rangle_l}{\langle l \rangle} , \tag{S16}$$

where $c_{\text{myo},l}(s)$ is the mean linear density of myosin clusters engaged with filaments of length l. To obtain the simplified final expression for Ψ_{sarc} in Eq. S16, we assumed that filament length and orientation are independent. In practice this is a good approximation, as most filaments make small angles with the ring axis ($\Psi_{\text{bund}} = 0.8$, Fig. 4J). In the main text, we simplified the notation by dropping the l subscript, $c_{\text{myo},l}(s) \rightarrow c_{\text{myo}}(s)$. Note that the contribution of each myosin cluster to the number density $c_{\text{myo},l}(s)$ takes into account the fact that the myosin-II heads in the cluster are saturated by 10 actin filaments, when the maximum cluster force of 40 pN is reached. Thus, $c_{\text{myo},l}(s)$ represents the density of myosin-II heads that are bound at a distance s from the barbed end to actin filaments of length l, divided by the number of heads per cluster. The contribution of a myosin cluster j to this density is $\alpha_j = 1$ if myosin cluster j interacts with $n_j^{\text{int}} \leq 10$ actin filaments, and $\alpha_j = 10/n_j^{\text{int}}$ if the cluster interacts with $n_j^{\text{int}} > 10$ filaments.

Ψ_{bund} and Ψ_{sarc} are dimensionless numbers that embody the features of ring organization that determine the ring tension. The bundling coefficient is bounded between 0 and 1, where $\Psi_{\text{bund}} = 1$ indicates perfect alignment of all filaments with the ring axis, and $\Psi_{\text{bund}} = 0$ indicates that all filaments are aligned perpendicular to the ring axis. An important principle that emerges from this simple calculation is that the mean tension in an actomyosin organization depends not only on the amount of myosin that binds to the actin filaments, but also on the *locations* of the myosin along the filaments: it is the *moment* of the myosins that governs the amount of tension, not simply the total amount. This is quantified by the sarcomericity, which is the mean myosin moment per actin filament length, i.e., a sum over the myosin-II clusters interacting with a filament, weighted by the distances of these interactions from the barbed end, and then averaged over all filaments. The moment is then normalized by the mean actin filament length, <l>. Thus,

$\Psi_{\text{sarc}} = 1$ indicates that the average tension-producing effect of the myosins is the *equivalent* of a single myosin cluster situated at each actin filament pointed end. This could be produced, for example, by 2 myosin clusters bound half-way along each actin filament, or, more realistically, by a distribution of myosin locations whose net normalized moment gives unity.

In "wild type" simulations, we found a value of $\Psi_{\text{sarc}} = 4.2$ (Fig. 4K). Thus, the tension-producing effect of myosin-II clusters is equivalent to about 4 clusters acting at the pointed end of actin filaments, at the maximum possible distance from the anchored barbed end. This provides a quantitative measure, in terms of tension-producing capability, of the high concentration of components in the self-organized actomyosin bundle produced by self-organization.

Calculation of the bundling coefficient Ψ_{bund} and sarcomericity Ψ_{sarc} in ring simulations
The averages in Eqs. S15 and S16 are time averages over all filaments in the ring. To calculate Ψ_{bund} in the ring simulation, at each time step we estimated θ for each actin filament as the angle of the straight line connecting the barbed and pointed ends, averaged over all filaments as in Eq. S15, and then averaged over all simulation time steps once the ring had reached steady state. To calculate Ψ_{sarc}, at each time step we averaged over all actin filaments as in Eq. S16, with each actin filament-myosin cluster interaction giving a contribution proportional to its distance from the barbed end, and then averaged over all simulation time steps once the ring had reached steady state.

4. Sliding-constriction model of rings in protoplasts, and comparison to observed ring shapes and constriction rates

Sliding-constriction model for ring constriction in protoplasts
The ring simulation outputs the ring tension as a function of ring length, $T(L)$. We used this ring tension to calculate the length $L(t)$ and the shape of constricting rings as a function of time t as the rings constricted while sliding along the cell membrane of protoplast cells. The model assumed that anchors attach the ring to the membrane and endow the ring with total drag coefficient γ_{ring}. We assume that the drag coefficient is distributed evenly along the arc length of the ring (uniformly distributed anchors), which may be maintained by the rapid turnover of ring components that occurs on a timescale much shorter than constriction. The sliding velocity and shape of the ring are then determined by balancing two forces (Fig. 3D). (i) The component tangent to the membrane of the centripetally directed force on the ring due to ring tension T. The value of this centripetal force is given by Laplace's law. (ii) Drag forces due to the sliding of ring-membrane anchors through the membrane.

Spherical protoplasts. First, we consider the simple case of a spherical protoplast. In the absence of external constraints, protoplasts would adopt a spherical shape due to the loss of cell wall. In our model, constriction is driven by ring tension that exerts at each point of the ring an inward radial force per ring length equal to $T/(L/2\pi)$, following Laplace's law in two dimensions. The component of this force tangent to the membrane is $2\pi T\sin\theta/L$, where θ is the angle between the membrane tangent in the steepest direction and the normal to the plane of the ring. Ring sliding is resisted by viscous forces due to anchors with force per unit length $(\gamma_{\text{ring}}/L)v$, where $v =$

$(\mathrm{d}L/\mathrm{d}t)/(2\pi\sin\theta)$ is the sliding velocity of the ring and γ_{ring} is the total drag coefficient of all the anchors in the membrane. Equating these forces gives the ring length dynamics as

$$\gamma_{ring}\frac{\mathrm{d}L}{\mathrm{d}t} = -4\pi^2 T(L)(1 - L^2/L_{proto}^2) \ , \tag{S17}$$

since $\sin^2\theta = 1 - L^2/L_{proto}^2$, where L_{proto} is the circumference of the spherical protoplast. In this simple geometry, due to symmetry considerations, the ring would maintain a circular shape and lie in a plane at any instant.

Compressed protoplasts. In the present study, ring constriction was measured in protoplasts that were compressed between a microscope slide and coverslip to deform them from spheres into a range of partially flattened shapes (Fig. 6A), which allowed us to test the effect of cell shape on ring constriction. We compared measurements of constricting rings in these deformed protoplasts to our sliding-constriction model in which we describe the cell shape by the height of the cell membrane $z(x,y)$ above the location (x,y) on the slide:

$$\begin{aligned} z(x,y) &= \pm h && 0 < x^2 + y^2 < R^2 \\ z(x,y) &= \pm\left[h^2 - \left(\sqrt{x^2+y^2} - R\right)^2\right]^{1/2} && R^2 < x^2 + y^2 < (R+h)^2 \end{aligned} \ , \tag{S18}$$

Here R is the radius of the parallel discs that constitute the upper and lower surfaces of the protoplast in contact with the coverslip and slide, respectively, and h is one half of the protoplast height (Fig. 6A). The slide is at $z = -h$ and the coverslip deforming the cell is at $z = h$. The profile of the protoplast surface extending beyond the parallel discs is a semicircle (Fig. 6A). In this geometry, the length of the ring when confined to the plane $x = 0$ is equal to the protoplast equatorial circumference, $L_{proto} = 4R + 2\pi h$.

Contractile ring dynamics. The configuration of the ring in compressed protoplasts is described by the vector function $\mathbf{r}(s,t) = \langle x(s,t), y(s,t), z(s,t)\rangle$, where $\mathbf{r}(s,t)$ is the position vector at time t of the element of the ring that was located an arc length s along the ring at $t = 0$. The parameter s therefore has values in the range $0 < s < L(0)$ at all times, with $\mathbf{r}(0,t) = \mathbf{r}(L(0),t)$ since the ring is a closed loop. Note that s is defined with respect to *initial* arc length and thus labels a material element of the ring; at later times s does not represent arc length, due to stretching or compression of the ring. The ring length at time t is

$$L(t) = \int_0^{L(0)} \left|\frac{\partial \mathbf{r}}{\partial s}\right| \mathrm{d}s \ . \tag{S19}$$

The dynamics of the ring are governed by a balance of forces generated by ring tension and drag forces from ring anchors that resist ring sliding. The force balance per unit length of ring is

$$\frac{\gamma_{ring}}{L(t)}\frac{\partial \mathbf{r}}{\partial t} = T\kappa\left[\mathbf{n} - \mathbf{N}(\mathbf{N}\cdot\mathbf{n})\right] \ , \tag{S20}$$

where $\kappa(s) = |\mathbf{r}'\times\mathbf{r}''|/|\mathbf{r}'|^3$ is the ring curvature and $\mathbf{n}(s) = \mathbf{r}''/|\mathbf{r}''|$ the principal unit normal to the ring at s. $\mathbf{N}(\mathbf{r}(s,t))$ is the inward directed unit normal vector to the protoplast surface, calculated from Eq. S18. Primes denote partial derivates with respect to s. The right hand side of Eq. S20 is due to the force per unit ring length that drives ring sliding, namely the local inward force on the ring

due to tension that is equal to $T\kappa$ in the direction of the principal normal vector to the ring (Laplace's law). The factor in square brackets selects the component of this force tangential to the protoplast surface. The tension is uniform along the ring. The left hand side of Eq. S20 is the drag resistance force per unit ring length from ring anchors. Eq. S20 is therefore a generalization of Eq. S17 to arbitrary cell shapes that we applied to compressed protoplasts whose shape is given in Eq. S18. In contrast to a spherical cell where the ring would maintain a circular shape, the ring curvature now varies at different points along its contour and the ring no longer lies in a plane.

Implementation of the sliding-constriction model. Using time-lapse confocal microscopy we observed 17 compressed protoplasts expressing Rlc1p-3GFP that had constricting rings. For each cell we measured the protoplast shape parameters R and h (Eq. S18, Fig. 6A), the ring length as a function of time $L(t)$ (Fig. 6E and Fig. S4C), and the myosin-II concentration in the ring as a function of ring length (Fig. S1H). To simulate ring constriction in each of these seven protoplasts, we first input the mean measured myosin-II concentration into the ring simulation which output ring tension as a function of ring length $T(L)$ (see above). We then simulated ring constriction by representing the ring as a series of discrete points which were initially equally spaced around the ring, which was confined to the plane at $x = 0.1$ μm. (We chose this initial ring configuration as all of our experimental observations in protoplasts commenced after the ring had slid >0.1 μm away from the center of the cell.) At each timestep, Eq. S20 was solved for the velocity of each point using the $T(L)$ value from the ring simulation, and κ, $\mathbf{n}$, and $\mathbf{N}$ were computed using finite differences with respect to neighboring points. After evolving the position of each point on the ring, the ring length was updated (i.e., a discrete version of Eq. S19 was executed). We found that after a brief transient ring shapes were independent of the initial ring configuration.

Predicted ring shapes do not depend on model parameters. In Eq. S20, the rate of ring sliding $\partial\mathbf{r}/\partial t$ is proportional to the ratio of the ring tension to the total anchor drag coefficient. However, since both T and γ_{ring} are independent of s, the series of shapes that the ring adopts is independent of T and γ_{ring}. This is because the factor T/γ_{ring} can be eliminated from the equation by rescaling time $t \rightarrow tT/\gamma_{\text{ring}}$. Thus, the tension and drag coefficient do not affect the evolution of ring shapes, but only the rate at which the evolution occurs. Because no other quantities that determine ring dynamics in Eq. S20 depend on model parameters, the predicted ring shapes are independent of all model parameters and the comparison between predicted and observed ring shapes (Figs. 6C and S4A) requires no fitting parameters. The ring shapes are a consequence only of the broad model assumptions, i.e., a ring with uniform tension working against drag forces from uniformly distributed anchors.

Comparison of predicted and observed ring shapes. Calculated ring shapes are compared to ring shapes in the seven observed protoplasts in Figs. 6C and S4A and Movie S7, and display remarkable agreement independently of any model parameter values. Unlike rings in spherical cells which would be circular, the rings in compressed protoplasts adopt a bent out-of-plane shape due to the deformed shape of the protoplast. From the top view, the central portion of the ring on the flat surface ($0 < x^2 + y^2 < R^2$, Eq. S18) lags behind the "edges" of the ring (Figs. 6C and S4A, and Movie S7). This is because the tensile forces driving constriction are caused by ring curvature (Eq. S20, Laplace's law). Thus on the upper and lower flat portions of the

protoplast the ring must have curvature in order for the tension to have a component that can pull the ring across the flat portion.

Comparison of model to measurements of the time course of ring constriction

Predicted ring lengths from the sliding-constriction model, $L(t)$, calculated using the procedure described above, were fit to the 17 measured constriction curves (Figs. 6E and S4C, model I). A brute force search of parameter space was used to minimize the residual sum of squares and determine the values of 18 parameters: a single value of the ring drag coefficient γ_{ring} and 17 initial ring lengths for the 17 observed protoplasts. The best-fit drag coefficient was γ_{ring} = 2805 ± 45 nN·s/μm, where the standard error was calculated from the diagonal of the covariance matrix $\mathbf{V} = \sigma^2 \left(\sum_i \mathbf{J}_i^{\mathrm{T}} \mathbf{J}_i \right)^{-1}$. Here, σ^2 is the sum of squared residuals divided by the degrees of freedom, $\mathbf{J} = \partial L / \partial \boldsymbol{\beta}$ is the Jacobian, which was evaluated numerically at each data point i, and $\boldsymbol{\beta}$ is the vector of fitting parameters.

We also tested three alternative models, which were fit to the $L(t)$ measurements using the same procedure. Model II used the identical sliding-constriction model I but assumed that the drag coefficient is proportional to ring length ($\gamma_{ring} \sim L$). Models I and II (Figs. 6E and S4C) assumed that the ring operates close to its isometric tension limit, i.e., even though the ring is shortening the tension is very close to its value at fixed length calculated by our ring simulation. Our simulations suggest that this quasi-static limit is realized in protoplasts, since relaxation of ring properties occurred on the ~30 s timescale of component turnover whereas constriction occurs over ~30-60 min. In other words, the anchor forces resisting ring sliding are so strong that the ring operates close to stalling conditions where the load would be sufficient to prevent constriction. Thus, in Models I and II the constriction rate is highly sensitive to the external load.

We compared these models that assumed isometric tension conditions with two models where the ring set its own constriction rate, rather than its own tension. In model III we assumed the constriction rate was proportional to initial ring length, as in *C. elegans* (Carvalho et al., 2009). In model IV we assumed that the constriction rate was proportional to the relative myosin concentration, which we measured as a function of ring length in protoplasts (Fig. S1H, Inset). When fitting these models to the full set of constriction data (Figs. 6E and S4C), the additional fitting parameter (in addition to the initial length of each ring) was the constriction rate per unit ring length and the constriction rate per relative myosin concentration, respectively. The ring in models III and IV is analogous to a muscle fiber working close to its zero load velocity; this velocity is an intrinsic property of the muscle, unrelated to properties of the load the muscle works against. For example, in model III the ring length changes at a constant rate despite the varying load from anchor drag forces, which decrease over time as the ring slides more slowly along steeper sections of the protoplast to maintain a constant constriction rate. Thus, the constriction rate is insensitive to the load on the ring anchors.

The sliding-constriction model with constant γ_{ring} (model I) fit the experimental data better than the three alternative models (Fig. 6F and S4D). Model I captured the observed increase in constriction rate with time (Fig. 6E and S4C), mostly because the constriction rate is higher when the ring is on a steep surface where the component of tension parallel to the surface is greater (Fig. 3D). The high tensions generated in shorter rings due to the high myosin concentration (Fig. 4B) made only a small contribution to the increase of constriction rate over

time (Fig. 4B), since these high tensions occur in rings shorter than those observed (Fig. 4B and 6E). Model II, with γ_{ring}~L, produced $L(t)$ curves with larger curvature than observed because constriction accelerates even more strongly over time due to the decreasing total drag on the shortening ring. Models III and IV produced less curvature than observed (Fig. 6E and S4C).

To compare the fits quantitatively, we used chi-squared tests for goodness of fit. We first estimated the variance of L due to measurement error to be the variance of the experimental data about the best fitting model (Fig. 6F and S4D), i.e. we assumed that model I was correct. Using this value for the variance, the tests indicated poor fits for models II-IV relative to model I ($p<10^{-5}$, 531 degrees of freedom) (Fig. 6F). Chi-squared tests also indicated that the distribution of residuals from the basic sliding-constriction model (model I) were most consistent with a normal distribution (Fig. S4D).

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