Supplemental Materials

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Supplemental Information for

A theoretical model of cytokinesis implicates feedback between membrane curvature and cytoskeletal organization in asymmetric cytokinetic furrowing

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Supplementary Model Description

We established a theoretical model describing the mechanical aspects of the cytokinetic ring and its coupling with the furrowing plasma membrane. The central notion is that membrane curvature affects the alignment of the contractile/cytoskeletal filaments, which in turn governs the contractility that further drives furrowing. Our model employing this curvature-mediated feedback loop not only can recapitulate asymmetric furrowing, but can also predict the functions of the essential cytokinetic proteins. Below, in Section I, we provide further theoretical considerations that justify the modeling strategy, including 1) geometric layout and 2) discrete representation of the model, 3) elaborations on curvature-mediated filament alignment, 4) the definition of order parameter for filament alignment, and 5) the near-equilibrium treatment of the dynamic evolution. In Section II, we provide the table of the quantitative model parameters followed by their estimation based on previous experimental measurements summarized in Section III, which also provides a brief account of the scheme used for model parameter fitting with the experimental data in the our work.

Section I. Further theoretical considerations

1. Dimensions of cytokinetic ring

Without explicit representation of individual molecules, we model the ring as a circle of connected contractile segments bound to the interior of a membrane tubule, which is 50 microns long and which has a diameter of 30 microns. This is based on the dimensions of the *C. elegans* zygote. The ring is centered on and perpendicular to a membrane tubule according to assumptions from the literature (Rappaport, 1996) that the division plane is perpendicular to, and often roughly halfway along, the cell’s long axis. The specific dimensions of the cytokinetic ring in the model are based on the previous measurements including demonstrations that 1) the average actin filament is ~200-300 nm long in cytokinetic ring; 2) the cortex thickness is 100-300 nm; 3) the dimensions of myosin II in actomyosin bundles (see parameter derivations for details); 4) the initial diameter of the ring is 30 microns in our system. To conform to these measured sizes, the cytokinetic ring in the model thus assumes a composite structure comprising ~250 ring segments that are 250 nm × 250 nm in cross section, and initially 250 nm long. Note that the essential model results on asymmetric furrowing is insensitive to the dimension of this initial ring segment (Fig. S1D). Within each segment, 85 myosin II bipolar mini-filaments (Burns *et al.*, 1995a; Burns *et al.*, 1995b; Yumura *et al.*, 2008; Zhou and Wang, 2008; Vale *et al.*, 2009) span two groups of actin filaments, which occupy the two circumferential ends of each segment (adjacent to adjoining segments) (Fig. 1C). The external, barbed ends of the actin filaments can be thought of as connected to the neighboring segment by actin crosslinkers, which are themselves represented by the spring-like connections between the segments.
2. Discrete model representation

To elaborate on our reasoning for using discrete representation, we first derive the mechanical energy directly related to the cytokinetic ring. Let us focus on a small segment of the ring in the discrete representation of contraction (see the schematics below, also see Fig. 1D in the main text). There are two sources of energy: (1) The effect of contraction from the local segment on the positions of its neighbors; (2) The effect of the neighboring segments’ contraction on the position of the local segment.

Here, \( \mathbf{f}_+ (s,t) \) and \( \mathbf{f}_- (s,t) \) are the internal contractile forces on the left and right end for the \( s \)th segment, respectively. \( \mathbf{f}_+ (s) + \mathbf{f}_- (s) = 0 \), \( \mathbf{f}_+ (s) = \mathbf{f}_- (s) = f(s) \). \( l_+ (s,t) = l_- (s,t) = \frac{1}{2} l(s,t) \). \( l(s,t) \) is the effective contractile length of the actomyosin filament, which essentially reflects the portion of the actin filaments overlapping with the myosin heads within the ring segment. \( f(s) \) has the unit of force per length. The contraction of the \( s \)th segment consists of the inward opposing forces from its two ends, which also pulls the neighboring segments in the direction of contraction. The direction of contraction is tangential to the ring segment: The left half of the ring segment contracts from left to right whereas the right half contracts from the right to the left. Without losing generality, this energy potential term can be written as

\[
-\mathbf{f}_+ (s,t) l_+ (s,t) \cdot \left( \mathbf{r}(s-\Delta s,t) - \mathbf{r}(s+\Delta s,t) \right),
\]

where \( \Delta s = 1 \) corresponds to the discrete form. On the other hand, the neighboring segments pull the \( s \)th segment in the direction of their combined contractions, which results in the energy potential term:

\[
-\mathbf{r}(s,t) \cdot \left( \mathbf{f}_- (s-\Delta s,t) l_- (s-\Delta s,t) + \mathbf{f}_+ (s+\Delta s,t) l_+ (s+\Delta s,t) \right).
\]

Combined together, the energy potential of the contraction is:
\[-\frac{1}{2} \sum_{i=1}^{N} \left[ \bar{f}(s,t)l(s,t) \cdot (\bar{r}(s-\Delta s,t) - \bar{r}(s+\Delta s,t)) + \bar{r}(s,t) \cdot (\bar{f}(s-\Delta s,t)l(s-\Delta s,t) + \bar{f}(s+\Delta s,t)l(s+\Delta s,t)) \right] \]

where the factor \( \frac{1}{2} \) is needed to prevent double counting the energy contribution.

In the continuous limit (small \( \Delta s \)), the discrete summation becomes an integral, and the energy potential from contraction is:

\[-\frac{1}{2} \int ds \left\{ \delta(s,t)l(s,t) \cdot (\bar{r}(s-\Delta s,t) - \bar{r}(s+\Delta s,t)) + \bar{r}(s,t) \cdot (\bar{f}(s-\Delta s,t)l(s-\Delta s,t) + \bar{f}(s+\Delta s,t)l(s+\Delta s,t)) \right\} \]

This is essentially a line tension-like term, although the force here may not be uniform. The problem is: as \( \Delta s \to 0 \), the segment lengths \( (l_s^+ \text{ and } l_s^-) \) also approach zero, which renders the above integral to be zero. This result arises from the fact that the contraction is tangential to the ring and scales with ring segment length. If the ring segment is infinitesimally small, so is local contraction. This is different from line tension in lipid phase segregation, wherein the unbalanced force is normal to the interfacial boundary, and not tangential. Therefore, in order to faithfully preserve the effect of actomyosin contraction, we describe the cytokinetic ring in the discrete form. This discrete representation dictates not only the rest of the ring mechanics (see Eq. [3]), including the bending energy of the ring and ring segment connectivity, and the interaction between the ring and the midzone spindle, but also the membrane mechanics.

### 3. Curvature-mediated filament alignment

The driving force for curvature-dependent filament alignment in the circumferential direction comes from difference in free energy \( \Delta E_{\text{attachment}} \) between filaments aligned with, and filaments perpendicular to, the membrane furrow. Consider \( E_{\text{attachment}} \) as the attachment energy when the filament is fully attached to the membrane. A filament attached to a flat membrane, as well as a filament aligned in the direction of the furrow, can both fully attach to the membrane. A filament that is oriented perpendicular to the furrow will not be able to attach to the membrane along its full length, because its stiffness precludes it from following the membrane shape. To the simplest (linear) approximation, the difference between the attachment energy of a filament oriented perpendicular to the furrow that of a parallel filament is

\[ \Delta E_{\text{attachment}} = E_{\text{attachment}} \cdot \frac{\Delta r}{r^*} \]

where \( \Delta r \) is the deviation of the membrane from the baseline (see the schematics below), and \( r^* \) is the characteristic distance over which filament-membrane attachment is effective (a constant). When the membrane is flat, \( \Delta r \) and consequently, \( \Delta E \), are zero, which means that there is no difference in attachment energy due to filament orientation.
In the furrow, the increase in filament-membrane distance $\Delta r$ relates to the longitudinal curvature $r_{zz}$ by a quadratic expansion in the cylindrical coordinate system (see the schematics below): $\Delta r = \frac{1}{8} r_{zz} \left( \frac{\Delta z}{r} \right)^2$, where $\Delta z$ is the width of the ring (~250nm). Therefore, the attachment energy difference between filaments oriented parallel and perpendicular to the furrow, respectively, is proportional to $E_{attachment} \times$ the longitudinal curvature $r_{zz}$:

$$\Delta E_{attachment} = E_{attachment} \cdot \frac{\Delta r}{r} = E_{attachment} \cdot r_{zz} \cdot \left( \frac{\Delta z}{8r^2} \right).$$

In addition, $\Delta E_{attachment}$ depends on the radial curvature: the densities of adaptor proteins along the ring (amount per unit length) increase throughout ring closure, similar to that of myosin ((Maddox et al., 2007), and Fig. S2C-D). That is, the attachment energy per unit length along the ring perimeter and, hence, the energy difference between filaments oriented in parallel and perpendicularly to the furrow, increases as the ring radius decreases and thus radial curvature increases ($E_{attachment} \propto 1/r$). Consequently, $\Delta E_{attachment}$ is proportional to the product of the curvatures in the longitudinal direction and the radial direction – the Gaussian curvature ($\Delta E_{attachment} \propto r_{zz}/r$). This is reflected in the “$A$” term in Eq. [4]. The term $D$ in Eq. [4] is positive, which tends to smooth the variations in filament ordering along the ring circumference. Therefore, it promotes the propagation of filament ordering. Due to the angular definition of the order parameter $\psi$, $D$ is also related to the bending resistance of the filaments. That is, the larger the local bending modulus of the filament, the more the local filament ordering can be propagated to neighboring regions. The fourth order term is mainly dictated by entropy, which favors disorder in filament orientation. Its coefficient $B$ thus represents an energy associated with entropy and is positive.

4. The definition of the order parameter for filament ordering

The order parameter $\psi$ describes filament ordering within each ring segment. The physical interpretation of this order parameter in the context of cytokinesis takes into account 1) the agreement of the orientation of filaments in each group with the circumferential direction of contractility, and 2) the polarity of actin filaments with respect to that of the myosin mini-filaments (pointed ends inward and barbed ends outward).
Consider the sarcomere-like structure of each ring segment. An actin filament has its own polarity with pointed and barbed ends $\vec{q}$ with $|\vec{q}| = 1$. The model describes the cytokinetic ring on a coarse-grained level, considering the characteristics of the ensemble of actin filaments within the ring segment, rather than describing individual filaments. The values for average polarity of the two groups of actin filaments within each ring segment are $\vec{p}_1$ and $\vec{p}_2$ (see schematics below, analogous to Fig. 1E in the main text), similarly to the concept of a liquid crystal. Here $\vec{p}_i = \frac{1}{N} \sum_{j=1}^{N} \vec{q}_{ij}$, where $\vec{q}_{ij}$ is the polarity for the $j$th actin filament in the $i$th group, $i = 1, 2$, and $j = 1, 2, \ldots, N$. The magnitude of the polarity $|\vec{p}_i|$ reflects the order within each collection of filaments, or how well the filaments align with each other, in which $0 \leq |\vec{p}_i| \leq 1$. $|\vec{p}_i| = 0$ when the filaments are in total disorder, while $|\vec{p}_i| = 1$ reflects perfect filament alignment. The vector direction of the polarity $\vec{p}_i$ is the mean orientation of the filaments.

In order to effectively bind to the actin filaments, the myosin II heads must bind in a specific orientation with regard to the polarity of actin filament (Nagy et al., 2013). For actin filaments aligned in the opposite direction of contraction, the myosin II-actin filament binding is very weak, with a turnover rate $> 100$/second as opposed to 0.016/sec in the ideal orientation (Nagy et al., 2013). Even if actin is perfectly aligned but in the wrong polarity orientation, there is no effective actin-myosin binding, just as when filaments are totally disordered. For instance, if the two groups of actin filaments within the ring segment have their pointed ends pointing away from each other, then the myosin II mini-filaments will not bind to them. Thus, the order parameter is defined so that it captures this property of actin-myosin II interactions.

![Diagram of Direction of myosin II contraction](image)

The order parameter of filament ordering $\psi$ for each ring segment is defined as

$$\psi = \frac{1}{4} \left( \vec{p}_1 \cdot \vec{r} \cdot \vec{p}_2 \cdot (\vec{r} \cdot \vec{r}) + \vec{p}_2 \cdot (\vec{r} \cdot \vec{r}) \cdot |\vec{p}_1 \cdot \vec{r}| \right) + \frac{1}{2} \vec{p}_1 \cdot \vec{r} \cdot \vec{p}_2 \cdot (\vec{r} \cdot \vec{r})$$

where $\vec{r}$ is the myosin II mini-filament contraction direction on the left half of the ring segment, and is also the clockwise tangential direction of the ring. Here, $|\psi| \leq 1$. The magnitude of the order parameter reflects how well the actin filaments within the ring segment align in the direction of myosin II contractions and, hence, the capacity of effective binding to the myosin II mini-filaments. When $\psi$ is positive, the two groups of actin filaments are in the
functionally antiparallel orientation; when it is negative, they are in the parallel orientation. Specifically, when \( \psi = 1 \), the two groups of actin filaments are in perfect alignment with the antiparallel direction of the local myosin II mini-filament contractions. \( \psi = -1/2 \) describes the two groups of actin filaments perfectly aligned in the circumferential direction of the ring segment, but in the parallel orientation (see the schematics above).

5) Near-equilibrium treatment of the dynamic evolution

For the dynamic equations (Eqs. [5]-[8]), we provide the following considerations. First, to calculate furrow ingress dynamics, we numerically integrate the dynamic equations (Eqs. [5]-[8]) over time from the initial state in which filament orientation is random and contraction force is zero everywhere, the cytokinetic ring and the midzone are centered on the origin, and the membrane tubule is not constricted and at mechanical equilibrium. For simplicity, but without losing the essence of the system, we consolidated all the randomness of the system into the phase ordering process with a white Gaussian noise with the variance \( |\zeta_0|^2 \) (Eq. [7]). We chose \( |\zeta_0| \sim 0.001 \) in the model (Eq. [7]), which seeded the spatial heterogeneity in filament alignment. We can show that the exact value of \( |\zeta_0| \) will not affect the qualitative results as long as it is within a physically relevant range (<0.1). Second, the model only focuses on the mechanical behavior and filament ordering of ring segments at a longer timescale. This treatment assumes that such prolonged behavior reflects the average of the dynamics at shorter timescales, over which proteins constantly bind and unbind from the actomyosin filament within the cytokinetic ring. Third, cytokinetic process in reality consumes and dissipates energy; consequently, it is a non-equilibrium system in the truest sense. Our model is built upon the energy consideration at/near equilibrium, and the ensuing dynamic evolution of the model variables follows the driving force from the corresponding variation of such energy. Hereby, the implicit model assumption is that the energy dissipating biochemical pathway is slow enough such that the system can quickly relax to mechanical equilibrium. In this limit, our near-equilibrium treatment is valid. Last, the effective contractile length \( l \) will shrink upon local contraction force to confer the ring contraction (Eq. [6]). This shrinkage is mediated by actin depolymerization, which consumes ATP and is promoted by protein factors including coflin. While myosin II contraction initiates the filament shrinkage process, it is the biochemistry that gates the pace of actin filament shrinkage, whose effect is lumped into the effective viscous drag coefficient \( \lambda_2 \). In reality, the mechanical resistance force stemming from the deformations of the membrane shape and the ring could also impinge upon the actin filaments. However, myosin II contraction is known to work against resistance: The more it is resisted, the more the myosin II contracts – a catch bond. Therefore, the additional mechanical force from the deformation of the membrane and the ring could be cancelled out by this property of myosin II contraction. To be consistent with this experimental observation, the model only envisages the simplest scenario and describes the dynamics of ring segment shrinkage as driven by the contractility alone.

Section II. Model parameter table
| Model parameters | Description | Values measured from experiments | Values used in the model | References | Notes |
|------------------|-------------|----------------------------------|--------------------------|------------|-------|
| $\kappa_1$       | Membrane bending modulus | 50-400 k$_B$T | 100 k$_B$T | (Bruinsma et al., 2000; Roux et al., 2005; Simson et al., 1998) | 1 |
| $\sigma$         | Effective membrane surface tension | $10^{-7}$-$10^{-4}$ N/m | $1.5\times10^{-2}$ N/m | (Bruinsma et al., 2000; Israelachvili, 1991; Matzke et al., 2001; Roux et al., 2005; Simson et al., 1998) | 1 |
| $P_0$            | Osmotic pressure | $\sim$ 1000 Pa | 1000 Pa | (Rauch and Farge, 2000) | 1 |
| $f_0$            | The maximum contractile force per unit ring length | 7.7-77.0 pN/nm | 20.0 pN/nm | (Elliott et al., 1963; Rome, 1967; Matsubara and Elliott, 1972; Davey and Graafhuis, 1975; Robert et al., 1977; Hibberd et al., 1985; Applegate and Pardee, 1992; Finer et al., 1994; Molloy et al., 1995; Uyeda et al., 1996; Bendix et al., 2008) | 2 |
| $K_1$            | The elastic constant of the linkage between neighboring ring segments | 2.6 - 430 pN/nm | 10.0 pN/nm | (Tawada and Sekimoto, 1991; Huxley and Tideswell, 1996; Barclay, 1998; Piazzesi et al., 2002; Claessens et al., 2006; Takagi et al., 2006) | 3 |
| $K_2$            | The spring constant of midzone spindle-ring repulsion | $\sim$ 4pN/µm | 4pN/µm | (Grill et al., 2005) | |
| $\kappa_3$       | The ring bending modulus | $(0.3-10.6)\times10^{-21}$ N·m$^2$ | $2.0\times10^{-21}$ N·m$^2$ | (Janson et al., 1991; Janson et al., 1992; Gittes et al., 1993; Shin et al., 2004; Deguchi et al., 2005; Claessens | 4, 5 |
| Symbol | Description | Value | Reference(s) |
|--------|-------------|-------|--------------|
| $\alpha$ | Inhibition factor of contraction from midzone spindle | 0.2-0.8 (0.6) | (Grill et al., 2005) |
| $D$ | Energetic penalty for spatial variation of order parameter | Comparable to but less than $\kappa_3$ | The same as for $\kappa_3$ |
| $A$ | Gaussian curvature-dependent free energy potential for filament alignment | $1.7 \times 10^3$ pN⋅nm$^2$ | (Brochard-Wyart et al., 2006; Diz-Munoz et al., 2010) |
| $a_0$ | The intrinsic driving force for filament alignment during cytokinesis | $-8.5 \times 10^3$ pN⋅nm | (Nakamura et al., 2005; Sawyer et al., 2009; Wachsstock et al., 1993) |
| $B$ | The fourth order coefficient of the free energy of filament alignment | $8 \times 10^5$ pN⋅nm | |
| $\lambda_1$ | Effective viscous drag coefficient of ring contraction | $34.0 - 2.5 \times 10^6$ pN⋅second/µm | (Bausch et al., 1998; Bernheim-Groswasser et al., 2005; Marcy et al., 2004; Reinhart-King et al., 2005; Rieu et al., 2005; Tawada and Sekimoto, 1991; Zumdieck et al., 2007) |
| $\lambda_2$ | Effective viscous drag coefficient for filament shrinkage | $20-160$ pN⋅second/nm | (Pelham and Chang, 2002; Gromley et al., 2005; Murthy and Wadsworth, 2005; Wu and Pollard, 2005; Medeiros et al., 2006; Kamasaki et al., 2007; Haviv et al., 2008b; Vavylonis et al., 2008; Carvalho et al., 2009) |
| $\lambda_3$ | The effective viscous drag | $(4.6-16.4) \times 10^6$ | (Broersma, 1981; Li and Tang, 2006; Mukhina et al., 2007; Lu et al., 2008) |
Section III. Derivations of model parameters

1. Membrane surface tension $\sigma$, bending modulus $\kappa$, and osmotic pressure $P_0$

The surface tension for pure membrane is on the order of $10^{-7}$-$10^{-4}$ N/m as measured by membrane height fluctuation correlation (Bruinsma et al., 2000; Roux et al., 2005; Simson et al., 1998). If we approximate the cell as a membrane tube of radius $r$ and the length $l$, we can find the equilibrium force balance prior to anaphase by minimizing $\text{Eq. [2]},$ which leads to $r r \sigma - \kappa l / r - P_0 r^2 = 0$. For a typical cell radius of $r=15\mu m$, the membrane bending contribution, $\kappa / r$, is on the order of $10^{-14}$ N, and thus negligible compared to the contribution of osmotic pressure $P_0 r^2$, which is estimated to be $\sim 2.3 \times 10^{-7}$ N with $P_0 \sim 1000$ Pa (Rauch and Farge, 2000). To balance the outward osmotic pressure, the required effective membrane surface tension $\sigma$ has to be $\sim 1.5 \times 10^{-2}$ N/m or at least two orders of magnitude larger than the surface tension of pure membrane. Most likely, this means that the membrane surface tension is strongly influenced by the underlying actomyosin cytoskeleton. However, a similar value for surface tension is also reached by assuming that the membrane becomes stretched during cytokinesis: the elastic energy penalty from the large change in lipid area $\sim \frac{1}{2} k (a-a_0)^2$ yields an effective surface tension of $\sim 10^{-2}$-$10^{-1}$ N/m (Israelachvili, 1991), similar to the measured Young’s modulus of the cell cortex during cytokinesis (Matzke et al., 2001).

Since the energy penalty from membrane bending is negligible in determining cell shape, sharp membrane curvatures become possible, which are indeed observed during cytokinesis in the *C. elegans* zygote (Maddox et al., 2005).

2. Estimation on contraction forces $f_0$

We estimate the contraction force $f_0$ by two means: Based on single-molecule data, and based on measurements on muscle fibers.

A single myosin II motor protein can exert a contraction force of 0.3 - 3.0 pN (Finer et al., 1994; Molloy et al., 1995; Uyeda et al., 1996; Bendix et al., 2008). Up to 4 myosin molecules can occupy each 14.3nm interval of an actin filament (Pepe and Drucker, 1979). Therefore, the contraction force for a single actomyosin filament is in the range of $4 \times (0.3 \text{pN} - 3.3 \text{pN})/14.3\text{nm} = 0.09 - 0.9 \text{pN/nm}$. With 85 filaments in the cross-section of the cytokinetic ring, we estimate the contraction force $f_0$ as $85 \times (0.09 - 0.9) \text{pN/nm} = 7.7 - 77 \text{pN/nm}$.
The isometric force produced by a skinned muscle fiber is \( \sim 2 \times 10^5 \) N/m\(^2\), in which the density of actomyosin filament is \( \sim 4.8 \times 10^{14} \) filaments/m\(^2\) (Hibberd \textit{et al.}, 1985). Each actomyosin filament thus can generate \( \sim 420\) pN contraction force. Given that the actomyosin filament is \( \sim 2 \) microns long (Matsubara and Elliott, 1972), the contraction force per unit length \( f_0 \) is \( \sim 21\) pN/nm, which is within the estimated range of 7.7 - 77 pN/nm. In the model, we choose the value of the contraction force \( f_0 \) to be 20 pN/nm.

With this value for \( f_0 \) we find that total contraction force peaks at \( \sim 150\) nN, which is within the range of the measured values (Rappaport, 1967; Hiramoto, 1975; Rappaport, 1996; Burton and Taylor, 1997) (Fig. S1E).

3. \textit{Derivation of the elastic spring constant between neighboring ring segments} \( K_1 \)

In our model, the segments making up the cytokinetic ring are connected by elastic springs. Depending on the nature of the linker, its elastic spring constant can vary from 0.03 pN/nm for the actin cross linker fascin (Claessens \textit{et al.}, 2006), over 0.2 pN/nm for myosin (Tawada and Kimura, 1986) to \( \sim 1-5\) pN/nm (Huxley and Tideswell, 1996; Barclay, 1998; Piazzesi \textit{et al.}, 2002; Takagi \textit{et al.}, 2006) that was measured for the stiffness of an actomyosin crossbridge. As there are 85 actomyosin filaments in the cross-section of the cytokinetic ring acting in parallel, we arrive at a possible range of 85x(0.03-5) = 2.6-430 pN/nm for the elastic spring constant \( K_1 \) between neighboring ring segments. In the model, we choose \( K_1 \) to be 10 pN/nm. As long as \( K_1 \) is above a critical threshold (\( K_1 > 5\) pN/nm), the choice of \( K_1 \) will not affect the essential model results on asymmetric furrowing.

4. \textit{Derivation for the bending modulus of ring segments} \( \kappa_3 \)

We estimate the bending modulus of ring segments \( \kappa_3 \) in three ways: from sperm acrosomal processes, from single actin filaments, and from stress fibers.

The bending modulus of a horseshoe crab sperm acrosomal process consisting of 80 tightly linked actin filaments has been measured \textit{in vitro} as (0.3-10.6)\times10^{-21} N\cdot m^2 (Shin \textit{et al.}, 2004). This is likely an upper bound, since this actin bundle is highly crosslinked and not capable of contraction. Indeed, it has been reported that over-expression of the actin cross linker \( \alpha \)-actinin inhibits closure of the cytokinetic ring (Janson \textit{et al.}, 1991; Janson \textit{et al.}, 1992; Mukhina \textit{et al.}, 2007).

The bending modulus of a bundle is proportional to the square of the number of filaments within the bundle (Claessens \textit{et al.}, 2006). Given that the bending modulus of a single actin filament is \( \sim 7.3\times10^{-26} \) N\cdot m^2 (Gittes \textit{et al.}, 1993), the bending modulus for a bundle of 85 actin filaments can be estimated as 5.3\times10^{-22} N\cdot m^2.

In a uniform beam, the relation between the bending modulus and elastic modulus of the bundle is \( \kappa_3 = E\pi r_{\text{filament}}^4 / 4 = ES^2 / 4 \), where \( E \) is the elastic modulus, and \( S \) is the cross section area of the bundle. In endothelial cells, the transverse elastic modulus of
stress fibers with cross section \( \sim 0.05 \, \mu m^2 \) is \( \sim 10kPa \) (Lu et al., 2008), whereas the longitudinal elastic modulus is \( \sim 300kPa \) (Deguchi et al., 2005). If the cytokinetic ring is organized as a stress fiber like bundle, we expect the bending modulus of ring segments \( \kappa_3 \) to fall into the range of \( 3\times10^{-22} - 9.4\times10^{-21} \, N \cdot m^2 \).

Taken together, the bending modulus of the ring \( \kappa_3 \) is in the range of \( 3\times10^{-22} - 10.6\times10^{-21} \, N \cdot m^2 \). In our model, we choose a bending modulus of the ring \( \kappa_3 \) as \( 2.0\times10^{-21} \, N \cdot m^2 \).

5. **Derivation of energy penalty for spatial variation of order parameter \( D \)**

The order parameter in the model \( \psi \) is defined as the cosine of the angle between the orientation of the local ring segment and that of the cytokinetic ring plane. According to this definition, the energy penalty arising from the variation between order parameters of neighboring segments is part of the bending energy of the cytokinetic ring itself. Therefore the value of \( D \) in the model should be comparable to, but less than, the bending modulus of ring segments \( \kappa_3 \). In the model calculation, we choose it to be \( 1.8\times10^{-21} \, N \cdot m^2 \).

6. **The effective viscous drag coefficient of ring contraction \( \lambda_1 \)**

The driving force of ring contraction is counter-balanced by viscous drag, *i.e.*, the hydrodynamics is in the regime of low Reynolds number. Consequently, the timescale of ring contraction is set by the effective viscous drag coefficient \( \lambda_1 \).

Viscous drag stems from three sources: (1) the viscous drag between a filament and its surrounding cytoplasm; (2) the friction from myosin attachment; and (3) the friction from crosslinkers other than myosin, such as \( \alpha \)–actinin, which contribute to the majority of the viscous drag in actin gel in *in vitro* experiments.

The friction coefficient for the attachment of a single myosin head is \( \sim 0.2pN\cdot s/\mu m \) (Tawada and Sekimoto, 1991). For a segment of 85 actomyosin filaments, the effective viscous drag coefficient is 170 times that of the single myosin head, which yields \( \sim 34pN\cdot s/\mu m \). The friction from myosin attachment is two orders of magnitude higher than that from cytoplasm. Therefore, we ignore the viscous drag from cytoplasm.

The effective viscous drag coefficient is likely to be higher than \( 34 \, pN\cdot s/\mu m \), due to the presence of actin crosslinkers such as Anillin in the ring. Indeed, *in vitro* and *in vivo* experiments in which high concentrations of actin filament crosslinkers are present yield even larger viscous drag coefficients ranging from 100s to \( 10^6 \)s of \( pN\cdot s/\mu m \) (Bausch et al., 1998; Bernheim-Grosawser et al., 2005; Marcy et al., 2004; Reinhart-King et al., 2005; Rieu et al., 2005; Zumdieck et al., 2007). In the model, we set the effective viscous drag coefficient of ring contraction, \( \lambda_1 \), to be \( \sim 2.0 \times 10^4 \, pN\cdot s/\mu m \), which ensures that ring contraction can complete within 4-5 minutes, in accordance with experimental observation.
7. **Effective viscous drag coefficient for actomyosin filament shrinkage \( \lambda_2 \)**

The effective viscous drag coefficient for actomyosin filament shrinkage \( \lambda_2 \) relates to the effective shrinkage rate of actomyosin filaments due to contractile force (Eq. [8]). The effective actomyosin filament shrinkage rate in the model is the net result of filament de-polymerization minus filament polymerization. Filament shrinkage can be triggered by myosin contraction (Gromley *et al.*, 2005; Murthy and Wadsworth, 2005; Medeiros *et al.*, 2006; Haviv *et al.*, 2008a). In our model, we assume a linear dependence between the effective shrinkage rate and contraction force. This dependence is described by the effective viscous drag coefficient for actomyosin filament shrinkage \( \lambda_2 \), which has the unit of viscous drag (force\*time/length).

We derive a range for \( \lambda_2 \) from measurements in fission yeast. Filament depolymerization can be estimated by the rate of fluorescence recovery after photobleaching of actin filaments. The half-time of actin recovery has been measured in both fission yeast and mammalian cultured cell cytokinetic rings to be \(~ 30 \) seconds (Pelham and Chang, 2002; Guha *et al.*, 2005; Murthy and Wadsworth, 2005), translating to a turnover rate \( ~ 0.023 \ s^{-1} \). With an average actin filament and ring segment length in fission yeast of \(~ 600 \) nm (Kamasaki *et al.*, 2007), a turnover rate of \( 0.023 s^{-1} \) gives rise to an effective depolymerization rate of \(~ 13.8 \) nm/s per ring segment. Experiments in fission yeast also showed that actin typically polymerizes at the rate of 200nm/s (Vavylonis *et al.*, 2008). With \(~ 20 \) filaments within each bundle in fission yeast, the average polymerization rate of the bundle is thus \(~ 10 \) nm/s. The effective filament shrinkage rate in our model is thus \(~ (13.8-10) =3.8 \) nm/s.

The contraction force in the cytokinetic ring in fission yeast can be estimated from the number of myosin proteins \( (~20) \) per node, which are connected by single actin filaments (Wu and Pollard, 2005). A single myosin head can produce a contraction force of \(~ 2pN \). Since each myosin has two heads, the contraction force per actin filament is 80 pN. We thus estimate the effective viscous drag coefficient for filament shrinkage \( \lambda_2 \) to be \(~ 80pN/(3.8nm/s) ~20pN\cdot second/nm \).

Alternatively, we can estimate the effective viscous drag coefficient for filament shrinkage \( \lambda_2 \) from cell-level dynamics in fission yeast. The initial ring perimeter in fission yeast is \(~ 10 \) microns, and its contraction rate is \(~ 500 \) nm/min \( (~ 8nm/s) \) (Pelham and Chang, 2002). With an average actin filament length of \(~ 600 \) nm (Kamasaki *et al.*, 2007), there are effectively \(~ 16 \) ring segments along the perimeter, leading to a shrinkage rate of \(~ 0.5 \) nm/s per segment. Given the 80 pN contraction upon each segment, the effective viscous drag coefficient in the model \( \lambda_2 \) can be estimated at \(~ 160pN\cdot second/nm \), leading to an overall range of 20 - 160pN\cdot second/nm for \( \lambda_2 \). In the model, we choose \( \lambda_2 \) to be \(~ 100 \) pN\cdot second/nm.

8. **The effective viscous drag coefficient for filament alignment \( \lambda_3 \)**

Filament alignment within the ring is resisted by the effective viscous drag coefficient for filament alignment, \( \lambda_3 \), which, is dominated by two-dimensional rotational
viscous drag. Theoretical studies show that the 2-D rotational diffusion of a filament is $D_r = 3k_B T [\ln (L/d) + \gamma_r] / (\pi \eta L^3)$, where $L$ and $d$ are the length and the diameter of the filament, $\gamma_r$ is -0.447 for $L/d \to \infty$ and $\eta$ is the viscous drag of the solution (Broersma, 1981). The effective viscous drag coefficient for filament alignment is thus $\lambda_3 \sim k_B T / D_r$.

For a single F-actin filament, $L \sim 1 \text{micron}$ and $d \sim 8 \text{ nm}$. In water, with $\eta = (1.41\text{-}4.99) \times 10^{-3} \text{ Pa} \cdot \text{s}$ (Li and Tang, 2004), its rotational diffusion coefficient is thus $\sim 2 \text{ rad}^2/\text{second}$. We use these measured values as references to estimate the effective rotational viscous drag in the model.

The length of a filament in our model is initially 250 nm, and the diameter of a single actin filament is $\sim 8 \text{ nm}$. The viscous drag of the solution $\eta$ can be calculated from the effective viscous drag coefficient of ring contraction $\lambda_1$ by division by the diameter of the ring segment, which comes to $8 \times 10^4 \text{ Pa} \cdot \text{s}$. With these values, we can calculate the rotational viscous drag coefficient as $(5.4\text{-}19.3) \times 10^4 \text{ pN nm s}$. The rotational diffusion of a bundle of 85 filaments should be $1/85$ of that of a single filament; thus, we need to multiply the above range by 85, yielding a range of $(4.6\text{-}16.4) \times 10^6 \text{ pN nm s}$. Furthermore, as the filaments get shorter as time progresses, the average drag coefficient is likely to be at the lower end of the range. Thus, we choose the effective viscous drag coefficient for filament alignment, $\lambda_3$, to be $6 \times 10^6 \text{ pN nm s}$.

9. **Gaussian curvature-dependent free energy potential for filament alignment $A$**

When the underlying membrane is curved, filaments in a ring segment can align circumferentially along the contractile plane and therefore fully attach to the membrane, presumably lowering the free energy. However, if filaments are oriented out of the division plane, their attachment to the membrane is less favorable. The curvature-dependent free energy for filament alignment in the model relates to the free energy difference between these two states. Consequently, the Gaussian curvature-dependent free energy term $A \cdot C_G$ is in the units of energy in Equation [3]. Since the Gaussian curvature is in units of the inverse of length$^2$, $A$ is in units of (energy-length$^2$); i.e., the free energy term $A \cdot C_G$ approximates the filament-membrane binding energy times the attachment area. For a ring segment with the attachment area $(250 \text{ nm})^2$ as in the model, the value for $A$ leads to an effective binding energy between the actomyosin filaments and the membrane of $\sim 1.7 \times 10^7 \text{ pN nm}^3/(250 \text{ nm})^2 \sim 2.7 \times 10^2 \text{ pN nm} \sim 70 k_B T$. Here, the energy $70 k_B T$ corresponds to the binding energy per ring segment. Given that there are typically $600\text{-}1000$ per $(\mu\text{m})^2$ filament-membrane binding sites per segment (Brochard-Wyart et al., 2006; Diz-Munoz et al., 2010), the number of binding sites for the cross section of $(250 \text{ nm})^2$ ranges from 38 to 63. Thus, the binding energy per site in the calculation is $\sim 1\text{-}2 k_B T$, which is physically reasonable (Brochard-Wyart et al., 2006; Diz-Munoz et al., 2010).

10. **Considerations on the intrinsic driving force for filament bundling ($a_0$), and the fourth order term ($B$)**
We assume that the intrinsic driving force for filament alignment is governed by upstream cell cycle- and spindle-based signals, including the Rho GTPase pathway. We combine all curvature-independent factors affecting filament alignment into the factor $a_0$ in the model. $a_0$ is a phenomenological parameter, and originates from enthalpy. Its value is negative unless otherwise mentioned; free energy is lowered when filaments align. Consequently, it drives initial filament alignment and, hence, early ring contraction.

The fourth order coefficient of filament alignment free energy is also a phenomenological parameter, which is always positive to maintain the stability of the system. Mathematically, this parameter limits the amplitude of the order parameter $\psi$ during phase ordering. Physically, the fourth order term originates from entropy, which opposes the enthalpic driving force for ordering (filament alignment). Our calculations show that the qualitative results of the model do not critically depend on the exact value of the fourth order coefficient of the filament alignment free energy.

We set the free energy change for filament bundling ($a_0$) in Eq. [5] at $\sim 2125 \ k_B T$. For each of the $\sim 85$ filaments per segment, the favorable binding energy for bundling or alignment is $\sim 25 \ k_B T$. This value is physically reasonable based on the following considerations: The mesh size in actin gel has been estimated/measured to be $\sim 50 \text{nm}$ (Plastino et al., 2004), which could be taken as the spacing between crosslinkers. Given the $250 \text{nm}$ length of the filament, there shall be $\sim 5$ binding sites by the crosslinkers. This estimation gives rise to the binding energy per site $\sim 5 \ k_B T$. The dissociation constants for F-actin binding affinity of crosslinkers ranges from $\sim 5 \ \mu M$ ($\alpha$-actinin) to $\sim 17 \ \mu M$ (filamin) (Nakamura et al., 2005; Sawyer et al., 2009; Wachsstock et al., 1993), which gives rise to the free energy changes for binding is $\sim 8-12 \ k_B T$. In addition, these crosslinkers are dimers when bound to F-actin; thus the free energy change per binding site is $\sim 4-6 \ k_B T$. Therefore, $5k_B T$ per binding site is within this range, and physically reasonable (Nakamura et al., 2005; Sawyer et al., 2009; Wachsstock et al., 1993).

11. Modeling parameter fitting scheme

We use the least-square-displacement method to determine the best fit. To consistently compare with experimental results, the model outputs of ring closure and asymmetry over time were converted to the curves of the corresponding timing and asymmetry as functions of ring closure percentage. Following this protocol, the data from both model output and the experiments were re-plotted every 5% of ring closure. Following least-square-displacement method, we then computed the difference between the model and experiment results. To fit the experimental data of control cells, we first used the average value for each model parameter from the ranges determined from published measurements (see model parameter table). For the unknown phenomenological parameters, we estimated them as elaborated above. In fitting, we used these estimated values without varying them. We then varied the rest of the model parameters at $\sim 10\%$ intervals from the starting parameter set within the parameter range. The smallest difference from the least-square-displacement method thus yields the nominal parameter set that gives the best fit of control experimental data. The goodness of this fit is thus $\sim 10\%$. This nominal model parameter set is listed as “Values used in
the model” in the model parameter table. To fit the experimental data from perturbations, we varied the four key model parameters (\(a_0\) and \(A\) in Eq. [4], and \(\lambda_1\) in Eq. [5] and \(\lambda_3\) in Eq. [7]) at intervals of 0.5% from their respective nominal values corresponding to the control fit. The goodness of fit for the four key model parameters is thus \(\sim 0.5\%\).

References

Applegate, D., and Pardee, J.D. (1992). Actin-facilitated assembly of smooth muscle myosin induces formation of actomyosin fibrils. The Journal of Cell Biology 117, 1223-1230.

Barclay, C.J. (1998). Estimation of cross-bridge stiffness from maximum thermodynamic efficiency. Journal of Muscle Research and Cell Motility 19, 855-864.

Bendix, P.M., Koenderink, G.H., Cuvelier, D., Dogic, Z., Koeleman, B.N., Brieher, W.M., Field, C.M., Mahadevan, L., and Weitz, D.A. (2008). A Quantitative Analysis of Contractility in Active Cytoskeletal Protein Networks. Biophys. J. 94, 3126-3136.

Brochard-Wyart, F., Borghi, N., Cuvelier, D., and Nassoy, P. (2006). Hydrodynamic narrowing of tubes extruded from cells. Proceedings of the National Academy of Sciences 103, 7660-7663.

Broersma, S. (1981). Viscous force and torque constants for a cylinder. J. Chem. Phys. 74, 6989-6990.

Burns, C.G., Larochelle, D.A., Erickson, H., Reedy, M., and De Lozanne, A. (1995a). Single-headed myosin II acts as a dominant negative mutation in Dictyostelium. Proceedings of the National Academy of Sciences of the United States of America 92, 8244-8248.

Burns, C.G., Reedy, M., Heuser, J., and De Lozanne, A. (1995b). Expression of light meromyosin in Dictyostelium blocks normal myosin II function. The Journal of Cell Biology 130, 605-612.

Burton, K., and Taylor, D.L. (1997). Traction forces of cytokinesis measured with optically modified elastic substrata. Nature 385, 450-454.

Carvalho, A., Desai, A., and Oegema, K. (2009). Structural memory in the contractile ring makes the duration of cytokinesis independent of cell size. Cell 137, 926-937.

Claessens, M.M.A.E., Bathe, M., Frey, E., and Bausch, A.R. (2006). Actin-binding proteins sensitively mediate F-actin bundle stiffness. Nat Mater 5, 748-753.

Davey, C.L., and Graafhuis, A.E. (1975). Paracrystallization of actomyosin. Cellular and Molecular Life Sciences 31, 441-443.

Deguchi, S., Ohashi, T., and Sato, M. (2005). Evaluation of Tension in Actin Bundle of Endothelial Cells Based on Preexisting Strain and Tensile Properties Measurements. Mol. Cell. Biomech. 2, 125-134.

Diz-Munoz, A., Krieg, M., Bergert, M., Ibarlucea-Benitez, I., Muller, D.J., Paluch, E., and Heisenberg, C.-P. (2010). Control of Directed Cell Migration In Vivo by Membrane-to-Cortex Attachment. PLoS Biol 8, e1000544.
Elliott, G.F., Lowy, J., and Worthington, C.R. (1963). An X-ray and light-diffraction study of the filament lattice of striated muscle in the living state and in rigor. Journal of Molecular Biology 6, 295-305, IN298-IN299.

Finer, J.T., Simmons, R.M., and Spudich, J.A. (1994). Single myosin molecule mechanics: piconewton forces and nanometre steps. Nature 368, 113-119.

Gardel, M.L., Shin, J.H., MacKintosh, F.C., Mahadevan, L., Matsudaira, P., and Weitz, D.A. (2004). Elastic Behavior of Cross-Linked and Bundled Actin Networks. Science 304, 1301-1305.

Gittes, F., Mickey, B., Nettleton, J., and Howard, J. (1993). Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. The Journal of Cell Biology 120, 923-934.

Grill, S.W., Kruse, K., uuml, and licher, F. (2005). Theory of Mitotic Spindle Oscillations. Physical Review Letters 94, 108104.

Gromley, A., Yeaman, C., Rosa, J., Redick, S., Chen, C.-T., Mirabelle, S., Guha, M., Sillibourne, J., and Doxsey, S.J. (2005). Centriolin Anchoring of Exocyst and SNARE Complexes at the Midbody Is Required for Secretory-Vesicle-Mediated Abcission. Cell 123, 75-87.

Guha, M., Zhou, M., and Wang, Y.-l. (2005). Cortical Actin Turnover during Cytokinesis Requires Myosin II. Current Biology 15, 732-736.

Haviv, L., Gillo, D., Backouche, F., and Bernheim-Grosfasser, A. (2008a). A Cytoskeletal Demolition Worker: Myosin II Acts as an Actin Depolymerization Agent. Journal of Molecular Biology 375, 325-330.

Haviv, L., Gov, N., Ideses, Y., and Bernheim-Grosfasser, A. (2008b). Thickness distribution of actin bundles in vitro. European Biophysics Journal 37, 447-454.

Helfrich, W. (1973). Elastic properties of lipid bilayers – Theory and possible experiments. Z. Naturforsh (C) 28, 693-703.

Hibberd, M.G., Dantzig, J.A., Trentham, D.R., and Goldman, Y.E. (1985). Phosphate Release and Force Generation in Skeletal Muscle Fibers. Science 228, 1317-1319.

Hiramoto, Y. (1975). Force exerted by the cleavage furrow of sea urchin eggs. Development, Growth & Differentiation 17, 27-38.

Huxley, A.F., and Tideswell, S. (1996). Filament compliance and tension transients in muscle. Journal of Muscle Research and Cell Motility 17, 507-511.

Israelachvili, J. (1991). Intermolecular and Surface Forces.

Janson, L.W., Kolega, J., and Taylor, D.L. (1991). Modulation of contraction by gelation/sololation in a reconstituted motile model. The Journal of Cell Biology 114, 1005-1015.

Janson, L.W., Sellers, J.R., and Taylor, D.L. (1992). Actin-binding proteins regulate the work performed by myosin II motors on single actin filaments. Cell Motil Cytoskeleton 22, 274-280.

Kamasaki, T., Osumi, M., and Mabuchi, I. (2007). Three-dimensional arrangement of F-actin in the contractile ring of fission yeast. J Cell Biol 178, 765-771.
Landau, L.D., and Lifshitz, E.M. (1980). Statistical Physics. Butterworth Heinemann: Oxford.
Li, G., and Tang, J.X. (2004). Diffusion of actin filaments within a thin layer between two walls. Physical Review E 69, 061921.

Lu, L., Oswald, S.J.,Ngu, H., and Yin, F.C.P. (2008). Mechanical Properties of Actin Stress Fibers in Living Cells. Biophys. J. 95, 6060-6071.

Maddox, A.S., Habermann, B., Desai, A., and Oegema, K. (2005). Distinct roles for two C. elegans anillins in the gonad and early embryo. Development 132, 2837-2848.

Maddox, A.S., Lewellyn, L., Desai, A., and Oegema, K. (2007). Anillin and the septins promote asymmetric ingress of the cytokinetic furrow. Dev Cell 12, 827-835.

Matsubara, I., and Elliott, G.F. (1972). X-ray diffraction studies on skinned single fibres of frog skeletal muscle. Journal of Molecular Biology 72, 657-669.

Matzke, R., Jacobson, K., and Radmacher, M. (2001). Direct, high-resolution measurement of furrow stiffening during division of adherent cells. Nat Cell Biol 3, 607-610.

Medeiros, N.A., Burnette, D.T., and Forscher, P. (2006). Myosin II functions in actin-bundle turnover in neuronal growth cones. Nat Cell Biol 8, 216-226.

Mendes Pinto, I., Rubinstein, B., Kucharavy, A., Unruh, J.R., and Li, R. (2012). Actin depolymerization drives actomyosin ring contraction during budding yeast cytokinesis. Dev Cell 22, 1247-1260.

Miller, A.L., and Bement, W.M. (2009). Regulation of cytokinesis by Rho GTPase flux. Nat Cell Biol 11, 71-77.

Minoshima, Y., Kawashima, T., Hirose, K., Tonozuka, Y., Kawajiri, A., Bao, Y.C., Deng, X., Tatsuka, M., Narumiya, S., May, W.S., Nosaka, T., Semba, K., Inoue, T., Satoh, T., Inagaki, M., and Kitamura, T. (2003). Phosphorylation by Aurora B Converts MgcRacGAP to a RhoGAP during Cytokinesis. Developmental Cell 4, 549-560.

Molloy, J.E., Burns, J.E., Kendrick-Jones, J., Tregear, R.T., and White, D.C.S. (1995). Movement and force produced by a single myosin head. Nature 378, 209-212.

Mukhina, S., Wang, Y.-l., and Murata-Hori, M. (2007). [alpha]-Actinin Is Required for Tightly Regulated Remodeling of the Actin Cortical Network during Cytokinesis. Developmental Cell 13, 554-565.

Murthy, K., and Wadsworth, P. (2005). Myosin-II-Dependent Localization and Dynamics of F-Actin during Cytokinesis. Current Biology 15, 724-731.

Nagy, A., Takagi, Y., Billington, N., Sun, S.A., Hong, D.K., Homsher, E., Wang, A., and Sellers, J.R. (2013). Kinetic characterization of nonmuscle myosin IIb at the single molecule level. J Biol Chem 288, 709-722.

Pelham, R.J., and Chang, F. (2002). Actin dynamics in the contractile ring during cytokinesis in fission yeast. Nature 419, 82-86.
Pepe, F.A., and Drucker, B. (1979). The myosin filament: VI. Myosin content. Journal of Molecular Biology 130, 379-393.

Piazzesi, G., Lucii, L., and Lombardi, V. (2002). The size and the speed of the working stroke of muscle myosin and its dependence on the force. The Journal of Physiology 545, 145-151.

Plastino, J., Lelidis, I., Prost, J., and Sykes, C.c. (2004). The effect of diffusion, depolymerization and nucleation promoting factors on actin gel growth. European Biophysics Journal 33, 310-320.

Rappaport, R. (1967). Cell Division: Direct Measurement of Maximum Tension Exerted by Furrow of Echinoderm Eggs. Science 156, 1241-1243.

Rappaport, R. (1996). Cytokinesis in Animal Cells. Cambridge University Press: Cambridge, UK.

Rauch, C., and Farge, E. (2000). Endocytosis Switch Controlled by Transmembrane Osmotic Pressure and Phospholipid Number Asymmetry. Biophysical Journal 78, 3036-3047.

Robert, T.H., Wallace Ip, B.S., Cayer, M.L., and Smith, D.S. (1977). Actin-myosin interaction. Self-assembly into a bipolar "contractile unit". Journal of Molecular Biology 111, 159-171.

Rome, E. (1967). Light and X-ray diffraction studies of the filament lattice of glycerol-extracted rabbit psoas muscle. Journal of Molecular Biology 27, 591-594.

Schroeder, T.E. (1972). The contractile ring. II. Determining its brief existence, volumetric changes, and vital role in cleaving Arbacia eggs. J Cell Biol 53, 419-434.

Shin, J.H., Mahadevan, L., So, P.T., and Matsudaira, P. (2004). Bending Stiffness of a Crystalline Actin Bundle. Journal of Molecular Biology 337, 255-261.

Takagi, Y., Homsher, E.E., Goldman, Y.E., and Shuman, H. (2006). Force Generation in Single Conventional Actomyosin Complexes under High Dynamic Load. Biophysical Journal 90, 1295-1307.

Tawada, K., and Kimura, M. (1986). Stiffness of carbodiimide-crosslinked glycerinated muscle fibres in rigor and relaxing solutions at high salt concentrations. J Muscle Res Cell Motil. 7, 339-350.

Tawada, K., and Sekimoto, K. (1991). A physical model of ATP-induced actin-myosin movement in vitro. Biophys. J. 59, 343-356.

Uyeda, T.Q., Abramson, P.D., and Spudich, J.A. (1996). The neck region of the myosin motor domain acts as a lever arm to generate movement. Proceedings of the National Academy of Sciences of the United States of America 93, 4459-4464.

Vale, R.D., Spudich, J.A., and Griffith, E.R. (2009). Dynamics of myosin, microtubules, and Kinesin-6 at the cortex during cytokinesis in Drosophila S2 cells. J Cell Biol 186, 727-738.

Vavylonis, D., Wu, J.Q., Hao, S., O'Shaughnessy, B., and Pollard, T.D. (2008). Assembly mechanism of the contractile ring for cytokinesis by fission yeast. Science 319, 97-100.

Wilson, C.A., Tsuchida, M.A., Allen, G.M., Barnhart, E.L., Applegate, K.T., Yam, P.T., Ji, L.,
Keren, K., Danuser, G., and Theriot, J.A. (2010). Myosin II contributes to cell-scale actin network treadmilling through network disassembly. Nature 465, 373-377.

Wu, J.-Q., and Pollard, T.D. (2005). Counting Cytokinesis Proteins Globally and Locally in Fission Yeast. Science 310, 310-314.

Yumura, S., Ueda, M., Sako, Y., Kitanishi-Yumura, T., and Yanagida, T. (2008). Multiple mechanisms for accumulation of myosin II filaments at the equator during cytokinesis. Traffic 9, 2089-2099.

Zhou, M., and Wang, Y.-L. (2008). Distinct Pathways for the Early Recruitment of Myosin II and Actin to the Cytokinetic Furrow. Mol. Biol. Cell 19, 318-326.
Supplemental Figure Legends

Supplemental Figure 1. Phase diagrams and graphical output describe the robustness of the theoretical model. A) Calculated phase diagram of the dependence of furrow ingression on contractility and filament shrinkage. The model predicts that there exists a threshold filament shrinkage rate, only above which successful furrow closure is possible. Otherwise, the sliding of filaments will result in neither ring contraction nor furrow ingression. On the other hand, successful furrow closure entails sufficiently large contractility to overcome the resistance from the contractile apparatus itself together with that from membrane elasticity and midzone spindle. The red cross indicates the parameters of controls. This model prediction is in line with the established role of actin depolymerization in furrow ingression (Nagaoka et al., 1995; Abe et al., 1996; Hotulainen et al., 2005; Zumdieck et al., 2007). B) Calculated phase diagram of the dependence of furrow fate on contraction and filament alignment. The model predicts that, for successful furrow closure, either contractility or alignment needs to be fast so that the initial ingression can propagate around the entire cell. The red cross indicates the parameters of controls. Both contraction and filament alignment rate can change by at least an order of magnitude before the ring can no longer close successfully, which is reflected by the robustness ring closure in perturbed cells. C) The theoretical model predicts that the asymmetry of furrow ingression decreases with the cell size. Size was varied by varying the number of ring segments, without changing segment dimensions. D) The theoretical model predicts that the asymmetry of furrow ingression is insensitive to the initial ring segment length. For (C) and (D), asymmetry factor corresponds to when the ring has reduced to 20% of its starting circumference. E) The theoretical model
predicts the time trace of the contractile force during asymmetric furrow ingression for the case of control cells. Note that the peak contractile force is ~ 150 nN, which is within the range of the measured values (Rappaport, 1967; Y., 1975; Rappaport, 1996; Burton and Taylor, 1997).

Supplemental Figure 2. Experimental rationale for model fittings. A) F-actin is bundled by the actin bundling domain of *C. elegans* ANI-1 anillin and by α-actinin (a positive control). B) Depletion of septins causes over-accumulation of myosin in the cell equator. Total intensity of GFP-tagged myosin was measured as in (B) a Bars = SEM. C) Depletion of myosin or septins does not change levels of anillin in the cell equator. Total intensity of GFP-tagged anillin was measured in a region at the equatorial cortex and normalized to the intensity in the anterior of the same cell, per timepoint, and to anaphase onset. Bars = SEM. D) GFP-tagged myosin, anillin and septin all persistently increase in abundance per micron of the cytokinetic ring throughout its closure. Bars = SD.

Supplemental Figure 3. The spindle midzone is a weak barrier to contractile ring ingression. A, C, and D) Plots of ring closure and concentricity with respect to time for cells thoroughly depleted of the microtubule bundler SPD-1 (A), partially depleted of ANI-1 anillin (6 hours of feeding versus 24-48 for thorough C) and partially depleted of ANI-1 while simultaneously thoroughly depleted of SPD-1 (D). Error bars = SD. Gray lines = control averages (see Fig. 2C). B) The kinetics of the separation of the two nascent nuclei in anaphase were measured in cells expressing mCherry-tagged histone
and GFP-tagged myosin. Plots reveal the rapid separation when thorough SPD-1
depletion disrupts force coupling across the spindle by the midzone. Scale bar = 5 µm.

Supplemental Figure 4. Asymmetric cytokinesis occurs with random directionality in C. elegans zygotes. A) The direction of furrowing varies among control C. elegans zygotes. The position of the contractile ring at 5% intervals from zero to 80% closed is shown for the population of control cells to demonstrate the variability in the direction and character (straight versus spiraling) of the path taken by the center of the contractile ring. Black bars at the bottom for each cell represent the location of the coverslip. Some cells appear smaller because ring size and location are shown, and for some cells, annotation had only begun after furrow initiation. B) Comparison between the variability (standard deviation) in model realizations and experimental variability in ring closure (blue) and asymmetry (green). C) The two tips of the cytokinetoric furrow progress with different speeds. Left: Furrow tip progress was estimated from the intersection (orange and red dots) between the contractile ring and a circle of 90% cell diameter (black dashed circle). Right: Furrow tip progress for a population of control cells (n = 13). Line colors indicate corresponding size of the contractile ring. Dashed line indicates equal progression of both furrow tips.

Supplemental Movie 1. The contractile ring (labeled with functional GFP-tagged myosin heavy chain (NMY-2) closes asymmetrically (non-concentrically) in a C. elegans embryo that has been mounted such that its division plane was parallel with the coverslip. Overlaid with the myosin-GFP fluorescence is the annotation of the cell outline (magenta) and contractile ring (cyan), as defined by a best-fit circle through three or more
manually chosen points (dots). The centers of the cell outline and contractile ring are shown as +s of the corresponding color; the dash-dotted line connecting them and extending outward is the axis of asymmetry. The elapsed time is approximately 5 minutes; the cell is approximately 30 microns wide.
Supplemental Experimental Procedures

Expression and purification of recombinant protein

The predicted F-actin bundling domain (ABD; aa.s 234-377) of *C. elegans* Anillin (ANI-1, Y49E10.19) was tagged with MBP and a His tag using a modified pET vector (pETM44) (Fethiere *et al.*, 2004). BL-21 (DE3) *E. coli* bearing this plasmid were cultured in TB-medium, 0.1% glucose, 30µg/ml kanamycin. Protein expression was induced with 0.2 mM IPTG at 0.8 OD600nm overnight at 18°C. Cells were harvested by centrifugation and resuspended in lysis buffer (50 mM Tris-HCl [pH 8.0], 500 mM NaCl, 5 mM β-mercaptoethanol) supplemented with 1 mM PMSF and complete EDTA-free protease inhibitor tablets. Cells were lysed by two cycles of freezing and thawing, and then sonication. Cell lysate was centrifuged at 50,000 x g for 45 min and the recombinant protein was extracted from the supernatant by metal chelating affinity chromatography using a POROS MC20 column pre-equilibrated in lysis buffer. After extensive washing, protein was eluted with a 0-0.5 M imidazole gradient. Fractions containing recombinant MBP-Anillin were pooled and purified further by gel filtration on a superdex-200 column equilibrated in 20 mM HEPES [pH 7.4], 150 mM NaCl, 1 mM β -mercaptoethanol. Protein concentration was determined by Bradford method using bovine serum albumin for the standard.

Actin bundling visual assay

An F-actin bundling assay was performed as published (Kinoshita *et al.*, 2002). Briefly, 2 µM of rabbit skeletal muscle actin (Cytoskeleton) was resuspended in Buffer A (2mM
potassium phosphate [pH 7.2], 75mM KCl, 10% glycerol, 2mM MgCl₂, 2mM EGTA, 0.5 mM ATP, 0.1 mM DTT). The solution was centrifuged at 16,000 x g at 4°C for 10 min to remove aggregates. Phalloidin was added to the supernatant for a final concentration of 2 µM and the mixture was incubated 20 min at room temperature. Stabilized F-actin was incubated with or without 1 µM α-actinin (Cytoskeleton) or Anillin-ABD. Reactions were incubated on poly-L-lysine coated coverslips for 20 min at room temperature, the excess was removed and the reaction was fixed for 10 min with 3.7% formaldehyde in PBS. Excess fixative was removed by gentle aspiration and washed with PBS. After a 30 min block with a solution of 2% BSA and 0.01% Tween-20 in PBS, actin filaments were stained with Alexa Fluor 546 phalloidin (Molecular Probes) for 1 h at room temperature. All reactions and incubations were done in a humid chamber. Coverslips were mounted on slides and imaged by swept-field confocal microscopy (Prairie, Nikon) with a 100x, 1.4 NA oil-immersion objective.

**Myosin and anillin early recruitment (for Figures S2B and C)**

JJ1473 or OD159 embryos were mounted on agarose cushions and imaged on a compound microscope equipped with a spinning-disc confocal head as published (Maddox *et al.*, 2007). Maximum intensity projections of the cortex apposed to the coverslip were generated. Anaphase specific myosin of anillin recruitment to a rectangular region occupying much of the equatorial cortex was measured as published (Maddox *et al.*, 2007).
Levels of myosin, anillin and septins in the ring throughout cytokinesis (for Figure S2D)

Levels of fluorescently-tagged cytokinetic ring proteins were determined in a 10-pixel wide band just outside the ring annotated using cyanRing (see Figure 2A). Ring intensity was normalized by subtracting cytoplasmic background in a rectangular region away from the cytokinetic ring. Strains JJ1473, OD159 and OD26 were used for these analyses.

Spindle elongation measurements

The distance between segregating masses of chromatin reflects the force coupling across the spindle, from the cortical pulling forces acting on astral microtubules at one pole of the cell to those at the other pole. The distance between the centroids of the chromatin masses was measured with a custom kymograph tool written in Matlab. In each maximum-projected frame of a time-lapse image sequence of *C. elegans* zygotes expressing mCherry-tagged histone (OD122), the user clicked on the apparent centroid of each chromatin mass. Through these two points, a 50 micron long line was drawn, and the fluorescence intensity was integrated in a 5 micron band perpendicular to the line. The centroid of each chromatin mass along the spindle axis could thus be estimated in two ways: As the distance between the user-clicked points, and as the distance between the centroids of the integrated intensities. As the two measurements agreed within <0.5 microns in our tests, we report only the first of the two measurements, which is easier to obtain.
**Estimation of furrow tip progress**

For Figure S4C, progress of the two tips of the cytokinetic furrow was measured as the angle from the furrow initiation point and the up to two intersections of the contractile ring and a circle of 90% cell diameter. The furrow initiation point was estimated as the center between the first two intersections of the contractile ring with the circle of 90% cell diameter.
Table S1. *C. elegans* strains used in this study

| Strain | Genotype                                                                 |
|--------|--------------------------------------------------------------------------|
| JJ1473 | unc-119(ed3) III; zuIs45[nmy-2::NMY-2::GFP + unc-119(+)] V.              |
| OD122  | JJ1473 x unc-119(ed3) III; itIs37[pAA64(Ppie-1::mCherry::his-58) unc-119(+)] |
| OD159  | unc-119(ed3) III; ltIs86 [pASM65; pie-1::ANI-1 (fl cDNA)::GFP; unc-119 (+)] |
| OD26   | unc-119(ed3) III; ltIs20 [pASM10; pie-1/GFP::unc-59; unc-119 (+)]         |
Table S2. dsRNAs used in this study

| Gene #    | Name | Oligo #1           | Oligo #2           | Template         | RNA conc. (mg/ml) |
|-----------|------|--------------------|--------------------|------------------|------------------|
| For injection |      |                    |                    |                  |                  |
| Y49E10.19 | ani-1| TAATACGACTCATA     | AATTAACCCCTCATA    | yak488c11        | 1.28             |
|           |      | TAGGTCAAAACCTCAAT  | AAGGCATTGTGCTTC    |                  |                  |
|           |      | GGAGAGGACAA        | AAATTCCTCAC        |                  |                  |
| K07C5.1   | arx-2| TAAATACGACTCATA    | AATTAACCCCTCATA    | N2 genomic DNA   | 0.31             |
|           |      | TAGGCCGAGCTTGCCTA  | AAGGTGCAATACGC     |                  |                  |
|           |      | AAATGCTTG          | GATCCAAAATA        |                  |                  |
| Y34D9A.4  | spd-1| TAAATACGACTCATA    | AATTAACCCCTCATA    | N2 genomic DNA   | 0.24             |
|           |      | TAGGCCGAGCTTGCCTA  | AAGGTGCAATACGC     |                  |                  |
|           |      | AAATGCTTG          | GATCCAAAATA        |                  |                  |
| Y34D9A.4  | spd-1| TAAATACGACTCATA    | AATTAACCCCTCATA    | N2 genomic DNA   | 0.24             |
|           |      | TAGGCCGAGCTTGCCTA  | AAGGTGCAATACGC     |                  |                  |
|           |      | AAATGCTTG          | GATCCAAAATA        |                  |                  |
| W09C5.2   | unc-59| TAAATACGACTCATA    | AATTAACCCCTCATA    | yak465c12        | 1.81             |
|           |      | TAGGCCGAGCTTGCCTA  | AAGGTGCAATACGC     |                  |                  |
|           |      | AAATGCTTG          | GATCCAAAATA        |                  |                  |
| Y50E8A.4  | unc-61| TAAATACGACTCATA    | AATTAACCCCTCATA    | yak411f2         | 2.79             |
|           |      | TAGGCCGAGCTTGCCTA  | AAGGTGCAATACGC     |                  |                  |
|           |      | AAATGCTTG          | GATCCAAAATA        |                  |                  |
| For feeding |      |                    |                    |                  |                  |
| Y49E10.19 | ani-1| CATGTTCACTGACAA    | CAAAACCTCAATGGGAG  | N2 genomic DNA   |                  |
|           |      | CTGGGATA           | AGGACAATC          |                  |                  |
| K07C5.1   | arx-2| TCCGAAAAGGAAGTT    | TTTTCAGGCGAAATG    | N2 genomic DNA   |                  |
|           |      | CCGAGA             | GATTC              |                  |                  |
Supplemental References

Abe, H., Obinata, T., Minamide, L.S., and Bamburg, J.R. (1996). Xenopus laevis actin-depolymerizing factor/cofilin: a phosphorylation-regulated protein essential for development. The Journal of Cell Biology 132, 871-885.

Burton, K., and Taylor, D.L. (1997). Traction forces of cytokinesis measured with optically modified elastic substrata. Nature 385, 450-454.

Fethiere, J., Venzke, D., Diepholz, M., Seybert, A., Geerlof, A., Gentzel, M., Wilm, M., and Bottcher, B. (2004). Building the stator of the yeast vacuolar-ATPase: specific interaction between subunits E and G. J Biol Chem 279, 40670-40676.

Hotulainen, P., Paunola, E., Vartiainen, M.K., and Lappalainen, P. (2005). Actin-depolymerizing Factor and Cofilin-1 Play Overlapping Roles in Promoting Rapid F-Actin Depolymerization in Mammalian Nonmuscle Cells. Molecular Biology of the Cell 16, 649-664.

Kinoshita, M., Field, C.M., Coughlin, M.L., Straight, A.F., and Mitchison, T.J. (2002). Self- and Actin-Templated Assembly of Mammalian Septins. Developmental Cell 3, 791-802.

Maddox, A.S., Lewellyn, L., Desai, A., and Oegema, K. (2007). Anillin and the septins promote asymmetric ingress of the cytokinetic furrow. Dev Cell 12, 827-835.

Nagaoka, R., Abe, H., Kusano, K.-I., and Obinata, T. (1995). Concentration of cofilin, a small actin-binding protein, at the cleavage furrow during cytokinesis. Cell Motility and the Cytoskeleton 30, 1-7.

Rappaport, R. (1967). Cell Division: Direct Measurement of Maximum Tension Exerted by Furrow of Echinoderm Eggs. Science 156, 1241-1243.

Rappaport, R. (1996). Cytokinesis in Animal Cells. Cambridge University Press: Cambridge, UK.

Y., H. (1975). Force exerted by the cleavage furrow of sea urchin eggs. Development, Growth & Differentiation 17, 27-38.

Zumdieck, A., Kruse, K., Bringmann, H., Hyman, A.A., and Julicher, F. (2007). Stress generation and filament turnover during actin ring constriction. PLoS One 2, e696.
A

Effective Contraction Rate \( f_0/\lambda_1 \) (1/sec)

Effective Filament Shrinkage Rate \( f_0/\lambda_2 \) (1/sec)

Unsuccessful Closure

Successful Closure

B

Effective Contraction Rate \( f_0/\lambda_1 \) (1/sec)

Effective Alignment Rate \( f_0L^2/\lambda_3 \) (1/sec)

Unsuccessful Closure

Successful Closure

C

Asymmetry (% maximal) vs. Initial ring radius (\( \mu m \))

D

Asymmetry (% maximal) vs. Initial ring segment length (nm)

E

Total contraction force (nN) vs. Time (minutes)
Dorn et al., MBoC revision Figure S3
A

Counterclockwise furrow tip progress

θ1, 2, 3 CCW CW θ1, 2, 3

B

Variance

Ring closure (% starting size)

Time (min. after ana. onset)

Experimental (n=17)

Fits (n=10)

Asymmetry (%)

C

CCW furrow tip progress (θ; deg)

CW furrow tip progress (θ; deg)

90% starting radius

Contractile ring

Contractile ring size (% starting radius)