

Actin turnover protects the cytokinetic contractile ring from structural instability

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Abstract

In common with other actomyosin contractile cellular machineries, actin turnover is required for normal function of the cytokinetic contractile ring. Cofilin is an actin-binding protein contributing to turnover by severing actin filaments, required for cytokinesis by many organisms. In fission yeast cofilin mutants, contractile rings suffer bridging instabilities in which segments of the ring peel away from the plasma membrane, forming straight bridges whose ends remain attached to the membrane. The origin of bridging instability is unclear. Here we used molecularly explicit simulations of contractile rings to examine the role of cofilin. Simulations reproduced the experimentally observed cycles of bridging and reassembly during constriction, and the occurrence of bridging in ring segments with low myosin II Myo2 density. The lack of cofilin severing produced ~ 2-fold longer filaments and, consequently, ~ 2-fold higher ring tensions. Simulations identified bridging as originating in the boosted ring tension, which increased centripetal forces that detached actin from Myo2 that anchored actin to the membrane. Thus, cofilin serves a critical role in cytokinesis by providing protection from bridging, the principal structural threat to contractile rings.

Summary statement:

Molecularly explicit simulations showed that cofilin-mediated actin severing protects the fission yeast cytokinetic contractile ring from instabilities in which actin peels away into straight bridges.

Introduction

Turnover of actin and other components is a universal feature of actomyosin contractile machineries in cells. Actin turns over through nucleation of filamentous actin by formins, Arp2/3 or other factors (Pollard, 2007), and by dissociation and/or disassembly of filaments. Cofilin is an actin-binding protein whose severing activity is critical for actin turnover in many machineries (Elam et al., 2013), including the actomyosin contractile ring that drives cell division during cytokinesis (D'Avino et al., 2015; Pollard and O'Shaughnessy, 2019; Pollard and Wu, 2010a). Cofilin is required for cytokinesis in many organisms (Abe et al., 1996; Gunsalus et al., 1995; Ono et al., 2003) including fission yeast (Nakano and Mabuchi, 2006), but many aspects of the mechanisms that make cofilin essential to cytokinesis remain poorly understood.

Fission yeast assembles a contractile ring from several hundred precursor protein complexes called nodes, each node incorporating myosin II Myo2, formin Cdc12 that nucleates and grows actin filaments, and other components (Wu et al., 2006). Normal ring assembly requires cofilin, as shown by studies of cytokinesis with mutants (Chen and Pollard, 2011; Nakano and Mabuchi, 2006). In cofilin mutants with reduced actin binding and severing activity, contractile rings failed to assemble normally from nodes, but instead assembled rings more slowly via diverse intermediate structures (Chen and Pollard, 2011). It was argued (Chen and Pollard, 2011) that cofilin-mediated actin filament severing is required for assembly from the nodes which are pulled together by pairwise actin filament connections that need regular renewal by severing to avoid node clumping and assembly failure (Vavylonis et al., 2008).

The role of cofilin during constriction of the assembled ring is much less clear. Is cofilin needed for constriction, and if so what is the mechanism? Fission yeast contractile rings with cofilin mutants constricted at normal mean rates, but with more variability (Chen and Pollard, 2011). However, during constriction rings exhibited remarkable structural irregularities, in which segments of the ring peeled away from the plasma membrane into straight segments we call bridges (Cheffings et al., 2019; Chen and Pollard, 2011; Malla et al., 2021). As bridging tended to occur in ring segments with lower Myo2 density, it was suggested the instability originated in a combination of reduced local Myo2-mediated actin anchoring and reduced local ring tension in the bridging region (Cheffings et al., 2019).

The contractile ring is a complex cellular machine of many components, so understanding the mechanisms of constriction and force production can benefit from mathematical modeling (Glotzer, 2005; O'Shaughnessy and Thiyagarajan, 2018; Pollard and O'Shaughnessy, 2019). Fission yeast is uniquely positioned for realistic quantitative modeling

due to a wealth of available data on the identities, amounts, biochemical properties and organization of ring components (Chatterjee and Pollard, 2019; Chen et al., 2015; Courtemanche et al., 2016; Friend et al., 2018; Hayakawa et al., 2020; Pollard et al., 2017; Pollard and Wu, 2010a; Stark et al., 2010; Takaine et al., 2015; Wu and Pollard, 2005). This data was used to constrain models of the contractile ring which reproduced structural features and ring tensions measured in live cells (Alonso-Matilla et al., 2019; McDargh et al., 2021; Stachowiak et al., 2014; Thiyagarajan et al., 2017). In contrast to the classic sarcomeric force production mechanism of striated muscle (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954), a sliding node mechanism emerged, where Myo2 and the unconventional myosin Myp2 (Bezanilla et al., 1997) pull nodes around the ring via attached actin filaments barbed-end-anchored to nodes via formins (McDargh et al., 2021). This generates tension in filaments, and a net ring tension. Experimental and modeling studies of septation, the growth of new cell wall in the wake of the constricting ring, suggest septation determines the constriction rate while the centripetal force from the ring mechanically regulates septation (Thiyagarajan et al., 2015; Zhou et al., 2015).

Here we used mathematical modeling to study the role of cofilin-mediated turnover in constricting contractile rings. Using a well-tested simulation framework (McDargh et al., 2021), we studied fission yeast contractile rings with the ADF-M3 cofilin mutant with reduced actin binding and severing activity (Chen and Pollard, 2011). Simulations quantitatively reproduced the experimentally observed bridges, the correlation with lower Myo2 densities and, remarkably, the cyclic character of bridging with 3 bridging-rehealing episodes during the course of constriction (Cheffings et al., 2019). The origin of the bridging instability is that actin filaments grow much longer without cofilin-mediated severing, so the ring tension is massively boosted, sufficient to pull actin filaments away from the Myo2 that anchors them to the membrane. In live cells (Laplante et al., 2015) and in simulations (McDargh et al., 2021), essentially the same bridging instability is seen in *myo2-EI* mutants with weakened binding to actin, showing that either increased centripetal loading or reduced anchoring provokes bridging. Thus, by maintaining actin filament lengths within a safe bound, cofilin protects the contractile ring from bridging instability, the principal structural threat to the contractile ring.

Results

Molecularly detailed simulation of the fission yeast cytokinetic ring

To examine the role of cofilin we adapted a molecularly explicit model of the fission yeast contractile ring we developed previously (McDargh et al., 2021), highly constrained by extensive experimental data that make the fission yeast contractile ring presently the most amenable to realistic mathematical modeling. Our model incorporates the measured amounts, turnover rates and biochemical properties of key components, and their organization in the ring. The amounts of many ring components were measured over time and many were biochemically characterized (Chatterjee and Pollard, 2019; Chen et al., 2015; Courtemanche et al., 2016; Friend et al., 2018;

Hayakawa et al., 2020; Pollard et al., 2017; Pollard and Wu, 2010b; Stark et al., 2010; Takaine et al., 2015; Wu and Pollard, 2005). Super-resolution FPALM microscopy showed the actomyosin organization in constricting rings is built from protein complexes called nodes (Laplante et al., 2016), anchored to the plasma membrane (PM). Each node contains myosin II Myo2, the actin filament nucleator formin Cdc12 and other components (Laplante et al., 2016; McDonald et al., 2017; Vavylonis et al., 2008; Wu et al., 2006). A second unconventional myosin II Myp2 does not belong to the nodes and is likely unanchored from the plasma membrane.

Here we briefly summarize the main points of the model (for details and parameter values see Materials and Methods and ref. (McDargh et al., 2021)). In the model each node is a coarse-grained representation of the organization revealed by FPALM, including Myo2 and formin Cdc12 (Laplante et al., 2016) (Fig. 1A). Formin Cdc12 dimers bind the node and nucleate and grow actin filaments, while the heads of the ~ 8 Myo2 dimers belonging to a node are represented by an ellipsoid with dimensions and location matching experiment. The semiflexible actin filaments are represented by chains of rods with bending stiffness corresponding to the ~ 10 μm persistence length (Ott et al., 1993), and Myp2 is represented as unanchored 200 nm clusters of 16 molecules each (Alonso-Matilla et al., 2019; Cheffings et al., 2019; Laplante et al., 2015; Takaine et al., 2015).

Filaments intersecting Myo2 or Myp2 clusters bind and are pulled (Fig. 1B), following linear force-velocity relations with total stall forces per cluster, $f_{\text{Myo2}}^{\text{stall}}$ and $f_{\text{Myp2}}^{\text{stall}}$. Drag forces resist lateral motion of the node parallel to the membrane, with drag coefficient chosen to reproduce the experimental lateral node velocity distribution (Laplante et al., 2016). Steric repulsions prevent crossing of actin filaments. Actin filaments are dynamically crosslinked by Ain1 α -actinin dimers (Wu and Pollard, 2005).

The component amounts follow the experimental values throughout constriction (Courtemanche et al., 2016; Wu and Pollard, 2005). For example, at constriction onset the ring contains 3300 Myo2 molecules, 230 formin dimers in ~ 210 nodes, 2300 Myp2 molecules in ~ 140 clusters, and 230 actin filaments of total length ~ 580 μm .

On binding a node, a formin nucleates a randomly oriented actin filament. Cofilin-mediated severing stochastically shortens filaments homogeneously with rate $r_{\text{sev}} = 0.93 \mu\text{m}^{-1} \text{min}^{-1}$ (see Table 1) (Elam et al., 2013). A whole-node turnover scheme is assumed, with a node dissociation time of 41 sec consistent with measured dissociation rates of node components. Myp2 clusters bind actin filaments with turnover time 46 s (Takaine et al., 2015). Rates of severing, binding and actin polymerization are set by demanding simulations reproduce the experimental densities of Myo2, Myp2 and formin and mean actin length, all evolving as constriction progresses (Fig. 1C).

Simulations reproduce forces and structural properties of experimental contractile rings

We ran simulations of the model with a septum growing inwards at 2.4 nm s^{-1} (Thiyagarajan et al., 2015) for ~ 25 min. Components were initially randomly positioned in a 200 nm wide circular band representing the membrane lining the inside of the septum. Constriction was

initialized after a preliminary 3 min run to allow the ring to self-organize. This dynamic organization maintained itself throughout constriction and reproduced many experimental features (Fig. 2A and Movie S1). Consistent with experiment, the actin filaments formed a ~ 130 nm thick bundle of ~ 47 filaments associated with a 100 nm thick Myo2 ring overlapping a concentric 200 nm thick inner Myp2 ring, separated by ~ 26 nm (Laplante et al., 2015; Laplante et al., 2016; McDonald et al., 2017; Swulius et al., 2018).

Ring tension was generated by a sliding node mechanism (Fig. 2B), very different to the classic sarcomeric mechanism of striated muscle (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). When incoming nodes bound the membrane, their formins nucleated actin filaments which were pulled by Myo2 belonging to other nodes. Since the membrane-anchored nodes host ~ 1 actin filament on average, they were pulled clockwise or counterclockwise around the ring depending on the actin orientation, with a node velocity distribution closely reproducing that measured using FPALM (Laplante et al., 2016; McDargh et al., 2021). In this way, filaments with barbed ends anchored to the membrane became tense, and a net ring tension was generated by the ~ 200 nodes (at constriction onset) (Fig. 2B). Unanchored Myp2 clusters also contributed by pulling filaments. Ring tensions increased from ~ 500 to ~ 1000 pN as rings constricted (Fig. 4C), with a mean of ~ 740 pN, close to the values measured in live fission yeast protoplasts (McDargh et al., 2021).

Bridging instabilities are the principal structural threat to the contractile ring

The contractile ring builds a specialized organization from actin, myosin and other components that efficiently exerts centripetal force to drive constriction. For functionality, this organization must be stable over the ~ 25 mins of constriction. Our simulations and experiments show that the biggest structural threat originates in the high tensions in filaments that must bend to form the circular bundle at the core of the ring (McDargh et al., 2021). Energetically, bent tensile filaments prefer to straighten by pulling away from the membrane into straight bridges (Fig. 3A).

Now the centripetal force per length tending to straighten a segment of a ring of radius R_{ring} and tension T into a bridge is T/R_{ring} (Fig. 3A). Further, the principal ring component anchoring actin to the membrane is the membrane-anchored Myo2, which binds actin filaments abundantly and provides the anchoring force F_{anchor} that opposes radial displacement of the actin filaments (McDargh et al., 2021). Myp2 makes a similar bundling contribution.

Thus we anticipate bridging will be precipitated by either (i) weakened actin-Myo2 binding (lower F_{anchor}), or (ii) increased ring tension T (higher centripetal force T/R_{ring}). To test this, we performed simulations (i) with the temperature-sensitive Myo2-E1 mutant, which binds actin weakly and has minimal ATPase activity (Lord and Pollard, 2004; Stark et al., 2013), and (ii) with artificially elevated ring tensions T by using unphysiologically higher values of $f_{\text{Myo2}}^{\text{stall}}$ (4-fold increase), the stall force parameter which sets the scale of the forces exerted by Myo2 clusters (see Materials and Methods). Indeed, in both cases, rings suffered significant bridging, with Myp2-containing actin bridges 0.5 – 1.5 μm in length (Fig. 3B, C). The bridging in simulated *myo2-E1* rings (McDargh et al., 2021) closely matched bridging observed in live

cells of the *myo2-E1* mutant, a vivid demonstration of the instability (Laplante et al., 2015) (Fig. 3C).

Contractile rings in *adf1-M3* mutants with reduced actin severing have longer actin filaments and higher ring tension

ADF/cofilin family proteins promote actin turnover by severing actin filaments (Andrianantoandro and Pollard, 2006; Elam et al., 2013; Pavlov et al., 2007). To study the effects of cofilin we ran simulations to mimic the *adf1-M3* mutant, which has strongly reduced actin binding and severing activity (Chen and Pollard, 2011), by setting the severing rate to zero, $r_{\text{sev}} = 0$. In the model, actin then dissociates only as whole filaments when formins dissociate from nodes (McDargh et al., 2021) (Fig. 1C).

In *adf1-M3* simulations (Fig. 4A) actin filaments were ~ 2 -fold longer than in wild-type simulations (Fig. 4B). Further, ring tension was ~ 2 -fold increased over much of the course of constriction (Fig. 4C). Given reduced severing, longer actin filaments are expected, but the enhanced tension is less obvious. To understand this, we measured tensions in individual actin filaments and found that the maximal tension at the anchored barbed end increased roughly linearly with filament length in wild-type simulations (Fig. 4D). Thus, longer filaments have higher tension, because they are bound by more myosin-II dimers. Since the total ring tension is a sum of all filament contributions over the bundle cross-section (Fig. 2B), the net ring tension is higher with longer filaments (McDargh et al., 2021).

Simulations reproduce bridging observed in contractile rings of *adf1-M3* mutants

Interestingly, bridging instabilities are seen experimentally for the *adf1-M2* and *adf1-M3* mutants with reduced severing activity (Cheffings et al., 2019) (Fig. 4A). Why reduced severing activity triggers bridging is unknown. Our simulation results above have already provided a strong clue: longer actin filaments lead to higher ring tension T (Fig. 4B-D), and higher tension generates higher centripetal Laplace forces $\sim T/R_{\text{ring}}$ which promote bridging (Fig. 3A).

This line of reasoning was indeed confirmed by simulations, which showed dramatic bridging instabilities in simulated cofilin mutants. The simulated bridges were remarkably similar to those observed in live cells (Fig. 4A and Movie S2). As demonstrated in (Cheffings et al., 2019), bridges contained Myp2, but not Myo2. Leaving Myo2 behind, bridges pulled away from the membrane and comprised several actin filaments bundled by Myp2, as expected since Myp2 is unanchored and its localization to the ring depends on actin (Laplante et al., 2015; Takaine et al., 2015). Each bridge had a few tenuous attachments to the ring at the bridge ends, mediated by barbed-end-anchored actin filaments.

Bridge formation is negatively correlated with Myo2 concentration

In experimental images of *adf1-M3* contractile rings the Myo2 density is clearly lower in regions of the membrane from which a bridge has pulled away (Cheffings et al., 2019) (Fig. 4A).

Consistent with these experiments, in simulated cofilin mutants bridging was more frequent in regions of lower Myo2 density around the ring. Since Myo2 is hosted by nodes, this was reflected by a negative correlation between node density and bridge location: the node density was ~ 2-fold lower in regions of the ring where a bridge occurred than in regions without a bridge ($n = 30$ rings, Fig. 5A and Movie S2).

These experimental and simulation results are consistent with the hypothesis that bridging is a failure of Myo2-mediated anchoring, so that bridging is more likely where fewer anchors are present.

Simulations reproduce cyclic bridging observed in contractile rings of cofilin mutants

In *adf1-M3* cells, bridging in contractile rings was cyclic with 3 cycles over the course of ring constriction (Cheffings et al., 2019) (Fig. 5B). Remarkably, our simulations quantitatively reproduced this behavior: fitting our simulated data to a sum of Gaussians using the identical statistical procedure to that used in the experimental study (Cheffings et al., 2019), we find 3 cycles of bridging and self-assembly occur during constriction (Fig. 5B and Movie S2). Thus, simulations reproduce cyclic bridging and the number of cycles per constriction observed in live cells, albeit with a somewhat shorter time interval between bridging episodes (~ 4.5 min in simulations versus ~ 7 min experimentally, Fig. 5B). This may be because the experimental measurements span a longer time period including the maturation episode that begins ~ 20 min before constriction (Wu and Pollard, 2005), whereas all simulations begin at constriction onset (time zero in Fig. 5B).

We argue that the reproduction of this very specific experimental phenotype strongly suggests the simulation recapitulates the essential mechanisms governing the fission yeast contractile ring. In particular, we conclude that cofilin-mediated turnover that maintains actin filament lengths within a certain range are essential to protect the ring from bridging instability.

Discussion

Here we studied the role of ADF/cofilin family proteins that sever actin filaments in the contractile ring by simulating rings in cofilin mutants and making detailed comparisons with experiments on the same mutant. Two candidates are the *adf1-M2* and *adf1-M3* mutants. In both mutants, the severing activity of cofilin is reduced but constriction rates are normal (Cheffings et al., 2019; Chen and Pollard, 2011). Adf1-M3 has two mutations (E132A, K133A), binds actin filaments ~ 10-fold more slowly than wild-type and severs actin defectively (Chen and Pollard, 2011). In a severing assay (Chen and Pollard, 2011), Adf1-M3 required a ~ 10-fold higher concentration than wild-type Adf1 for maximal actin severing activity, and even then the

severing rate was ~ 3 -fold reduced from wild-type. Thus to study the effects of cofilin we ran simulations that mimic the *adf1-M3* mutant in which we set the severing rate to zero, $r_{sev} = 0$.

Our results provide evidence that actin turnover is essential for structural stability of the actomyosin contractile ring. By regulating the length of actin filaments, ADF/cofilin-mediated severing protects the ring from bridging, its principal structural threat manifested in live cells in *myo2-E1* (Laplante et al., 2015) and *adf1* mutants (Cheffings et al., 2019; Chen and Pollard, 2011). Our modeling results explain why ring disruption due to this instability is expected in these two apparently unrelated contexts: in one case due to weakened anchoring, in the other due to increased unanchoring force.

The principal job of the contractile ring is to generate tension and exert inward centripetal force that directs constriction, accomplished by myosin II pulling on the bundled curved actin filaments in the ring and making them tense. However, a curved actin filament under tension has an enormous energetic preference to become straight. For example, 500 sec after constriction onset a typical filament has length $l_{act} \sim 2 \mu\text{m}$ and tension $T_{fil} \sim 15 \text{ pN}$ (Figs. 4B,D) since the mean filament tension is one half the barbed end value (McDargh et al., 2021). Given the ring radius of $\sim 1.3 \mu\text{m}$, its end-to-end distance increases by $\Delta L \sim 0.2 \mu\text{m}$ on straightening, giving an energy advantage whose order of magnitude is $\sim T_{fil} \Delta L \sim 750 kT$. Summed over a bundle this yields an enormous thermodynamic driving force for actin filament bundles in the ring to straighten into bridges. Thus the contractile ring is vulnerable to straightening instability and must be stabilized by anchoring. We find the principal anchoring agent is the membrane-anchored Myo2 which binds actin filaments (McDargh et al., 2021) (see Fig. 3A, C).

The contractile ring has presumably evolved to efficiently generate tension T , given the available myosin II in the ring. However, above a certain level tension is structurally dangerous, as the force $\sim T/R_{ring}$ that powers bridging will exceed the opposing Myo2-mediated anchoring forces. Cofilin-mediated severing plays a critical role because the tension depends on the actin filament length, $T \sim \rho_{myo} l_{act}$ (McDargh et al., 2021). Thus, for a given density ρ_{myo} of myosin II along the ring, longer filaments lead to higher ring tension and above a certain length provoke bridging. The principal conclusion of our study is that it is the job of cofilin to prune the actin filaments sufficiently that their length never exceeds this dangerous threshold (Fig. 4B).

Consistent with this conclusion, bridging is more likely in segments of the ring where the Myo2 density is lower. This has been observed in experimental studies of *adf1-M3* contractile rings (Cheffings et al., 2019) (Fig. 4A), and we found the same is true in *adf1-M3* simulations (Fig. 5A). Thus, bridging is a failure of Myo2-mediated anchoring in which tense actin filaments pull away from Myo2 that normally binds them to the membrane. Bridges peel away from the plasma membrane at locations where the inward Laplace forces exceed the local anchoring force from Myo2, which depends on the Myo2-actin unbinding threshold and the number of Myo2 heads that bind filaments. Thus, bridging is most likely in regions with low Myo2 density.

We remark that other conceivable sources of structural instability include fracture of individual actin filaments or filament pull-away from formins, given filament tensions reach tens of pN (Fig. 4D). However, fracture tensions $\sim 100 \text{ pN}$ measured for rabbit skeletal muscle actin

filaments (Kishino and Yanagida, 1988) suggest filaments can withstand such loads, and measured formin-actin dissociation constants in budding yeast (Pruyne et al., 2002) and mouse (Harris et al., 2004) are of order 10 nM, indicating strong formin-actin barbed end binding.

Actin turnover as a necessary stabilizing process to protect the structure of actomyosin contractile assemblies is an emerging theme. Component turnover was proposed to allow the fission yeast ring to rapidly reassemble itself without trauma as it gradually constricts (McDargh et al., 2021), and actin turnover is required for homeostasis of the ring (Chew et al., 2017). In *Drosophila* embryos, actomyosin assemblies driving apical constriction were observed to recoil from adherens junctions when cofilin was inhibited by depleting cofilin phosphatase (Jodoin et al., 2015). Following breakage, the mechanical connections between the actomyosin structures and the adherens junctions were re-established on a ~ 2 min timescale (Jodoin et al., 2015). This rupture-repair cycle is reminiscent of the ~ 7 min bridging-repair cycles in the fission yeast contractile ring (Cheffings et al., 2019).

Materials and Methods

A brief summary of the model of the fission yeast contractile ring is presented below. For further details see ref. (McDargh et al., 2021).

Node organization. The model uses a coarse-grained representation of the membrane-anchored nodes (Laplante et al., 2016) (Fig. 1A). (i) Near the membrane, a sphere represents Cdc15 and IQGAP Rng2, with size and location matching the experimental 70 nm wide Cdc15 distribution; (ii) formin Cdc12 dimers, binding the node 44 nm from the membrane, nucleate and grow actin filaments; (iii) an ellipsoid with the experimental $132 \times 102 \times 102$ nm dimensions located 94 nm from the membrane represents the heads of the ~ 8 Myo2 dimers.

Actin filaments. Actin filaments are represented as chains of 100 nm long rods (actin subunits). At the chain hinges, torsional springs impose a bending stiffness consistent with the reported persistence length $\sim 10 \mu\text{m}$ (Ott et al., 1993).

Myosin forces. Actin filaments intersecting Myo2 or Myp2 clusters bind and are pulled parallel to the filament (Fig. 1B). We use linear force-velocity relations for Myo2 and Myp2, with stall forces per cluster, $f_{\text{Myo2}}^{\text{stall}}$ and $f_{\text{Myp2}}^{\text{stall}}$, and with the measured load-free velocity v_{myo}^0 for the estimated 25 myosin II heads per actin filament, $v_{\text{myo}}^0 = 0.24 \mu\text{m s}^{-1}$ (Stark et al., 2010). The same load-free velocity was used for Myp2. The pulling force experienced by the actin filaments captured by a Myo2 node or a Myp2 cluster depends on the number of captured filaments: below a critical number n^* (myosin heads in excess), each filament experiences a maximum force $f_{\text{filament}} = 8 \text{ pN}$ from a node/cluster; above the critical number n^* (actin filaments in excess), the maximal force of 16 myosin heads is distributed equally amongst the filaments. The critical number $n^* = 8.75$, is the ratio of the total force exerted by 16 heads,

$16f_{\text{Myo2/Myp2}}^{\text{stall}}$, over the maximum force exerted on a filament. **Actin-myosin binding and unbinding.** When an actin filament subunit intersects a Myo2 ellipsoid ($132 \times 102 \times 102$ nm), it experiences a force perpendicular to the filament segment, pulling the filament towards the center of the ellipsoid. The force depends linearly on the distance between the center of the Myo2 ellipsoid and the actin subunit, with rest length $d_{\text{Myo2}} = 25.5$ nm. The spring constant is k_{Myo2} . A similar binding rule is applied to Myp2 clusters, with $d_{\text{Myp2}} = 50$ nm, and spring constant k_{Myp2} . The values of these two spring constants were obtained by fitting the unbinding threshold forces of Myo2 and Myp2, $f_{\text{unbind}}^{\text{Myo2}}$ and $f_{\text{unbind}}^{\text{Myp2}}$, to the experimental ring thickness and Myo2-Myp2 separation (see ref. (McDargh et al., 2021)).

Actin crosslinking. Actin filaments are dynamically crosslinked by Ain1 α -actinin dimers, which act as springs of rest length 30 nm and spring constant $25 \text{ pN } \mu\text{m}^{-1}$ taken from experiment (Claessens et al., 2006; Meyer and Aebi, 1990). α -actinin stochastically binds pairs of actin subunits separated by less than the α -actinin length (30 nm) and unbinds stochastically after a mean time 0.3 s (Li et al., 2016) or when overstretched (length > 50 nm).

Drag forces and steric forces. The membrane-anchored nodes experience drag forces resisting lateral motion parallel to the membrane, with a drag coefficient chosen to reproduce the experimental node velocity distribution, whose mean value is $22 \pm 10 \text{ nm s}^{-1}$ (Laplante et al., 2016). Node-node, Myp2-Myp2, node-Myp2 and actin-actin steric forces oppose overlapping of clusters or filaments. For node-node, Myp2-Myp2 and node-Myp2 interactions, the excluded volume interactions are soft elastic potentials with cut-off scales (see Table 1). Given the typical node-node separation in simulations is ~ 50 nm, less than the cut-off scale of node-node interactions (132 nm), Myo2 of adjacent nodes overlaps. These excluded volume interactions between nodes produce an effective node-node friction adding to the node-membrane drag resistance to node motion, a necessary contribution to reproduce the experimental node velocity distribution (Laplante et al., 2016). For the actin-actin interaction, the steric force takes the exponential form with a decay length. **Amounts and turnover of ring components.** The component amounts follow the experimental values throughout constriction (Courtemanche et al., 2016; Wu and Pollard, 2005). Since the detailed kinetics governing the building and dismantling of nodes are unknown, for simplicity the model assumes a whole node turnover scheme, in which Myo2 binds and dissociates from the ring as a cluster of 8 Myo2 dimers together with its host node. The node dissociation time is 41 s, consistent with the measured dissociation times of node components (Clifford et al., 2008; Pelham and Chang, 2002; Yonetani et al., 2008). Nodes stochastically bind a $0.2 \mu\text{m}$ wide zone, representing the ingrowing septum edge (Cortes et al., 2007), with a binding rate chosen to yield the experimentally determined density of nodes along the ring (Laplante et al., 2016; Wu and Pollard, 2005). On binding a node, a formin nucleates and grows an actin filament that is stochastically shortened by cofilin-mediated severing (Elam et al., 2013). The initial orientation of the nucleated filament is random,

within limits such that the orientation is inward and within an angle θ_{\max} of the ring direction. Results were insensitive to the nucleated filament orientation distribution. Myp2 clusters bind actin filaments with turnover time 46 s (Takaine et al., 2015). Cofilin-mediated severing stochastically shortens actin filaments homogeneously with rate $r_{\text{sev}} = 0.93 \mu\text{m}^{-1} \text{min}^{-1}$ (Elam et al., 2013). Rates of binding, actin polymerization and actin filament severing are set by demanding simulations reproduce the experimental densities of Myo2, Myp2 and formin and mean actin length, all evolving as constriction progresses (Courtemanche et al., 2016; Wu and Pollard, 2005).

Running the simulation. A dynamic boundary condition represents the ingrowing septum whose diameter, initially $3.7 \mu\text{m}$, decreases at 2.4 nm s^{-1} (Thiyagarajan et al., 2015) for a total constriction time $\sim 25 \text{ min}$. The width of the contractile ring is self-determined by the simulation.

Model parameters. Parameters used are shown in Table 1. For simulations with artificially boosted Myo2 forces (Fig. 3B), the stall force $f_{\text{Myo2}}^{\text{stall}}$ was increased 4-fold, from 1.75 pN to 7 pN. For simulations of *myo2-E1* mutants (Fig. 3C), the Myo2 stall force $f_{\text{Myo2}}^{\text{stall}}$ was set to zero, and the unbinding threshold $f_{\text{unbind}}^{\text{Myo2}}$ was reduced from 40 pN to 12 pN. For simulations of *adf1-M3* mutants the severing rate r_{sev} was set to zero.

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Competing interests

The authors declare no competing or financial interests.

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Author Contributions

B.OS. conceived the study; Z.M. and B.OS. designed the model; Z.M., T.Z. and H.Z. performed simulations and analysis; B.OS. wrote the paper.

Data Availability

All codes (MATLAB) of our molecular explicit model are available on our public GitHub page (https://github.com/OShaughnessyGroup-Columbia-University/constriction_of_the_fission_yeast_contractile_ring).

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Figures and Table

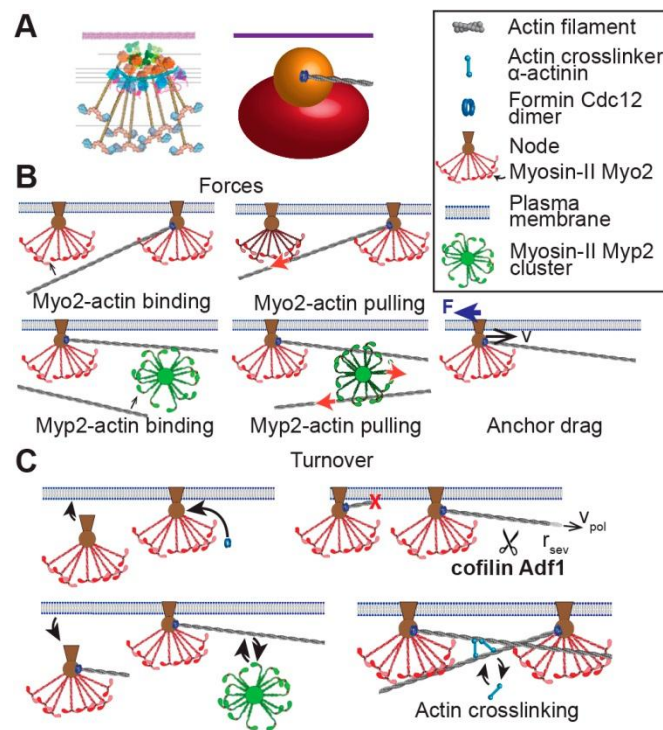


Fig. 1. Molecularly explicit model of the *S. pombe* contractile ring. (A) Left: schematic of membrane-anchored node organization, adapted with permission from Ref (Laplante et al., 2016). Plasma membrane (purple). Myosin II Myo2 (heads, blue; C-terminus, light green; light chains, pink). Formin Cdc12 (magenta). IQGAP Rng2 (orange). F-BAR Cdc15 (light blue). Mid1 (dark green). Right: coarse-grained representation of a node in the model. Red ellipsoid positioned 94 nm from the plasma membrane represents the heads of the 8 Myo2 dimers. Orange sphere represents Rng2, Cdc15 and other components. (B) Forces in the model. (C) Turnover of ring components. Formin Cdc12 dimers bind nodes 40 nm from the membrane and polymerize randomly oriented actin filaments at rate v_{pol} . Nodes bind to and dissociate from the plasma membrane. Formin Cdc12 dimers bind nodes and polymerize randomly oriented actin filaments at rate v_{pol} . Cofilin Adf1 (scissors) stochastically severs filaments at rate r_{sev} . Myp2 clusters bind and unbind actin filaments.

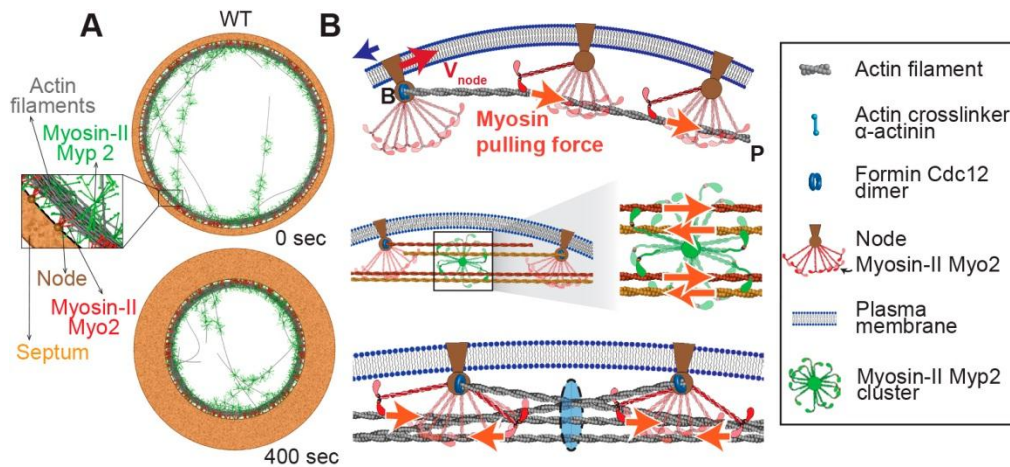


Fig. 2. Sliding node mechanism of ring tension production. Representations of nodes, Myo2, Myp2 and actin filaments as in Fig. 1. (A) Simulated wild-type contractile rings at constriction onset and 400 sec after onset. The ingrowing septum is shown as the brown region outside the ring. Actin filaments, grey. In images of simulated rings in this and other figures, Myo2 (red) and Myp2 (green) are rendered with explicit molecular detail for clarity. (B) Sliding node ring tension mechanism. Top: Individual actin filaments hosted by a node are made tense by pulling forces from Myo2 belonging to other nodes. Filament tension requires drag forces (blue arrow) that oppose motion of the node anchor relative to the membrane. Middle: unanchored Myp2 clusters also contribute to ring tension, by pulling on roughly equal numbers of oppositely oriented actin filaments. Bottom: the sum of the tensions of all actin filaments passing through a given cross-section of the ring gives the net ring tension.

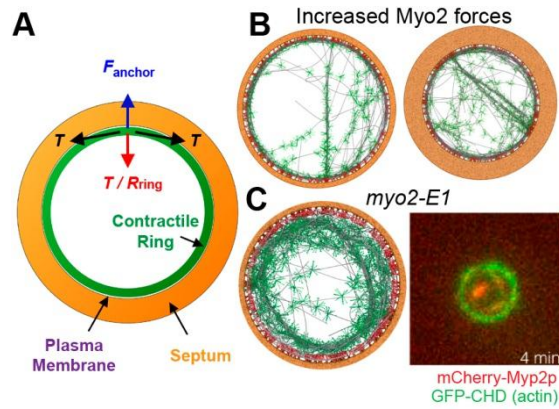


Fig. 3. Mechanism of bridging instability. (A) For a ring of radius R_{ring} the ring tension T produces a centripetal force per unit length T/R_{ring} , normally balanced by anchoring force F_{anchor} provided by membrane-anchored Myo2 belonging to nodes. If Myo2-actin binding is weakened (reduced F_{anchor} , as in *myo2-E1* mutants) or tension is boosted (increased T/R_{ring} , as in cofilin *adfl-M3* mutants) actin filaments may peel off the membrane into straight bridges. (B) Simulations with 4-fold increased Myo2 stall force $f_{\text{Myo2}}^{\text{stall}}$ show significant bridging. (C) Significant bridging is seen in simulated (McDargh et al., 2021) and experimental *myo2-E1* rings, where Myo2 heads bind actin weakly. The experimental image is adapted with permission from Ref (Laplante et al., 2015).

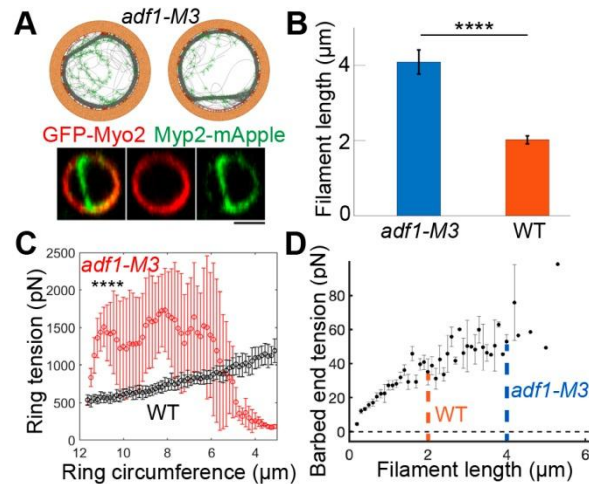


Fig. 4. Bridging instabilities are triggered in cofilin *adf1-M3* mutants because longer actin filaments produce higher ring tension. (A) Simulated *adf1-M3* rings (top row) reproduce bridging observed experimentally in *adf1-M3* mutant cells (bottom row). Simulated and experimental bridges contain Myp2 clusters. Experimental images adapted with permission from Ref. (Cheffings et al., 2019). Scale bar: 2 μm . (B) Actin filament length (mean \pm s.d.) in simulated *adf1-M3* ($n = 30$) and wild-type ($n = 30$) rings 500 sec after constriction onset. ****, $p < 0.0001$, Welch's unequal variances two-tailed t test. (C) Ring tensions (mean \pm s.d.) during constriction in simulated *adf1-M3* ($n = 30$) and wild-type ($n = 30$) rings. ****, $p < 0.0001$, Welch's unequal variances two-tailed t test for every ring circumference exceeding 8 μm . (D) Actin filament tension at the node-attached barbed end (mean \pm s.d.) versus filament length in simulated wild-type rings 500 sec after constriction onset ($n = 30$). Dashed lines indicate mean lengths of (B).

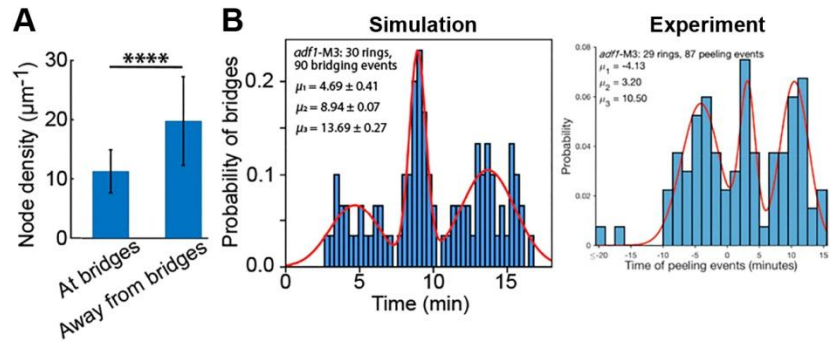


Fig. 5. Simulated *adf1-M3* rings show cyclic bridging and negative correlation of bridging location with local Myo2 concentration. (A) Node density (mean \pm s.d.) at and away from bridging sites in simulated *adf1-M3* rings ($n = 20$ bridging events). ****, $p < 0.0001$, paired two-tailed t test. (B) Probability of bridging at different times during constriction of simulated *adf-M3* rings and of experimental *adf-M3* rings from (Cheffings et al., 2019). Histogram shows number of bridging events in each 20 sec time bin divided by total number of bridging events in $n = 30$ simulations (left). Red curves: best fit Gaussian mixture models, three Gaussians with means μ_1, μ_2, μ_3 . For the simulations, the best fit widths are 1.68 ± 0.44 , 0.56 ± 0.07 and 1.84 ± 0.28 min, respectively. Simulation data are for constriction only (0 min and later), while experimental data include the maturation episode preceding constriction (negative times).

Table 1. Key parameter values of the ring simulation.

Symbol	Meaning	Value at onset of constriction	Legend
	Ring binding zone width	0.2 μm	(A)
	Initial ring length	11.6 μm	(B)
	Ring constriction rate	70 nm min^{-1}	(B)
ρ_{Cdc12p}	Density of formin Cdc12p dimers along the ring	20 μm^{-1}	(C)*
ρ_{Myo2}	Density of Myo2 nodes along the ring	18 μm^{-1}	(D)*
ρ_{Myp2}	Density of Myp2 clusters along the ring	12.5 μm^{-1}	(E)*
$k_{\text{Myo2}}^{\text{off}}$	Myo2 off rate	0.0245 s^{-1}	(F)
$k_{\text{Myp2}}^{\text{off}}$	Myp2 off rate	0.026 s^{-1}	(G)
$f_{\text{Myo2}}^{\text{stall}}$	Myo2 stall force per head	1.75 pN	(H)
$f_{\text{Myp2}}^{\text{stall}}$	Myp2 stall force per head	1.0 pN	(H)
	Major axis of Myo2 capture zone	132 nm	(I)
	Minor axes of Myo2 capture zone	102 nm (both)	(I)
	Diameter of Myp2 capture zone	200 nm	(J)
ρ_{actinin}	Density of α -actinin dimers along the ring	25 μm^{-1}	(K)
$k_{\text{actinin}}^{\text{off}}$	α -actinin off rate	3.3 s^{-1}	(L)
r_{sev}	Cofilin-mediated severing rate per unit length on actin filament	0.93 $\mu\text{m}^{-1} \text{min}^{-1}$	(M)*
v_{pol}	Formin-mediated actin polymerization rate	127 nm s^{-1}	(M)*
l_{p}	Actin filament persistence length	10 μm	(N)

γ_{anch}	Anchor drag coefficient per node	500 pN·s μm^{-1}	(O)
$k_{\text{ex}}^{\text{Myp2-Myp2}}$	Myp2-Myp2 excluded volume spring constant	0.32 pN nm^{-1}	
$k_{\text{ex}}^{\text{Myo2-Myo2}}$	Myo2-Myo2 excluded volume spring constant	0.12 pN nm^{-1}	
$k_{\text{ex}}^{\text{Anchor-Myp2}}$	Anchor-Myp2 excluded volume spring constant	0.095 pN nm^{-1}	
$r_0^{\text{Myp2-Myp2}}$	Myp2-Myp2 excluded volume cutoff distance	200 nm	
$r_0^{\text{Myo2-Myo2}}$	Myo2-Myo2 excluded volume cutoff distance	132 nm	
$r_0^{\text{Anchor-Myp2}}$	Anchor-Myp2 excluded volume cutoff distance	202 nm	
f_{act}	Actin-Actin excluded volume maximum force	10 pN	
r_{ex}	Actin-Actin excluded volume distance	15 nm	
f_{filament}	Actin-limited stall force	8 pN	(P)
$f_{\text{unbind}}^{\text{Myo2}}$	Myo2-actin unbinding threshold force	40 pN	(Q)
$f_{\text{unbind}}^{\text{Myp2}}$	Myp2-actin unbinding threshold force	30 pN	(Q)
v_{myo}^0	Load-free Myo2/Myp2 velocity	0.24 $\mu\text{m s}^{-1}$	(R)
k_x	Crosslinker spring constant	25 pN μm^{-1}	(S)
r_x	Crosslinker rest length	30 nm	(S)
θ_{max}	Maximum angle between new actin filament and ring	30°	(T)

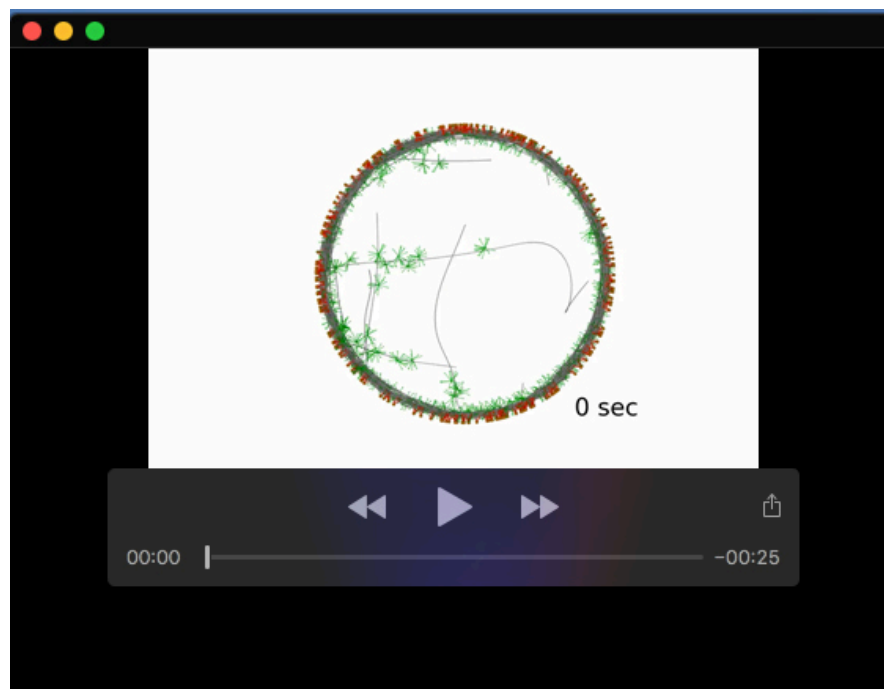
Legend:

* indicates that these parameters were tuned throughout constriction to ensure the time course of component numbers matched previous experimental measurements.

- (A) We assume that new constriction nodes bind the leading edge of the septum (in-growing cell wall). The septum has width $\sim 0.2 \mu\text{m}$ (Cortes et al., 2007).
- (B) Measured in (Pelham and Chang, 2002).
- (C) 200 dimers of Cdc12 were measured at constriction onset for a ring with circumference $\sim 10 \mu\text{m}$. (Courtemanche et al., 2016). For simplicity we neglect formin For3 that is also present, since For3 grows actin cables (Feierbach and Chang, 2001) and *Δfor3* cells have normal actin levels in the ring and lack observable cytokinesis defects.
- (C) (Yonetani et al., 2008). Together with the value for ρ_{Cdc12} , this gives a formin binding rate of $r_{\text{Cdc12p}} = 0.46 \mu\text{m}^{-1} \cdot \text{s}^{-1}$ at the onset of constriction.
- (D) Calculated from 2900 Myo2p in a $10 \mu\text{m}$ long ring at the onset of constriction (Wu and Pollard, 2005), and 8 Myo2 dimers per node (Laplante et al., 2016).
- (E) Calculated from 2000 Myp2p in a $10 \mu\text{m}$ long ring at the onset of constriction (Wu and Pollard, 2005), and 16 Myp2 heads per cluster, determined in Ref (McDargh et al., 2021)r.
- (F) Lifetimes of myosin light chains Cdc4 (Pelham and Chang, 2002) and Rlc1 (Clifford et al., 2008) and the formin Cdc12 (Yonetani et al., 2008) were measured previously using FRAP. We calculated the off-rate from the Rlc1 lifetime and used it for the Myo2 off rate as this was in the middle of the range of experimental values.
- (G) Consistent with off-rates of 0.022 s^{-1} obtained using previous FRAP measurements of Myp2 (Takaine et al., 2015).
- (H) Fitted in (McDargh et al., 2021).
- (I) From the distribution of Myo2 heads measured in FPALM (Laplante et al., 2016).
- (J) From the apparent size of Myp2 clusters measured in deconvolution microscopy (Takaine et al., 2015).
- (K) (Wu and Pollard, 2005).
- (L) (Li et al., 2016).
- (M) Fitted in (McDargh et al., 2021).
- (N) (Ott et al., 1993; Riveline et al., 1997).
- (O) Fitted in (McDargh et al., 2021) to reproduced the node velocity ($22 \pm 10 \text{ nm s}^{-1}$) measured by FPALM (Laplante et al., 2016).
- (P) (Vavylonis et al., 2008)
- (Q) Fitted in (McDargh et al., 2021).
- (R) Calculated in (McDargh et al., 2021).
- (S) Estimated from *in vitro* measurements on crosslinked actin bundles (Claessens et al., 2006) and from measurements of α -actinin length using electron microscopy (Meyer and Aebi, 1990).
- (T) Chosen to minimize the fraction of actin in whiskers. The overall structure of the ring was qualitatively insensitive to increases in this parameter, though the whisker fraction increases with larger maximum angles.



Movie 1. Simulation of a constricting wild-type ring. Components represented according to scheme of Figs. 1, 2. No bridging occurs.



Movie 2. Simulation of a constricting *adf1-M3* ring. Three bridging events occur during constriction (arrows).