Robust gap repair in the contractile ring ensures timely completion of cytokinesis

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Cytokinesis in animal cells requires the constriction of an actomyosin contractile ring, whose architecture and mechanism remain poorly understood. We use laser microsurgery to explore the biophysical properties of constricting rings in Caenorhabditis elegans embryos. Laser cutting causes rings to snap open. However, instead of disintegrating, ring topology recovers and constriction proceeds. In response to severing, a finite gap forms and is repaired by recruitment of new material in an actin polymerization–dependent manner. An open ring is able to constrict, and rings repair from successive cuts. After gap repair, an increase in constriction velocity allows cytokinesis to complete at the same time as controls. Our analysis demonstrates that tension in the ring increases while net cortical tension at the site of ingression decreases throughout constriction and suggests that cytokinesis is accomplished by contractile modules that assemble and contract autonomously, enabling local repair of the actomyosin network. Consequently, cytokinesis is a highly robust process impervious to discontinuities in contractile ring structure.

Introduction

Cytokinesis, the process that completes cell division by partitioning the contents of the mother cell to the two daughter cells, requires the assembly and constriction of a contractile ring (Green et al., 2012). The contractile ring is composed of a network of actin filaments and nonmuscle myosin II motor proteins, whose architecture and mechanism of constriction remain poorly understood. The contractile ring could be composed of a series of contractile units (Bement and Capco, 1991; Savoia et al., 1999; Carvalho et al., 2009), although a modular organization has so far not been observed by EM (Kamasaki et al., 2007). Alternatively, the ring could be a dynamic actomyosin bundle based on work in fission yeast protoplasts, as proposed by Stachowiak et al. (2014). Laser microsurgery has provided powerful insight into cellular actomyosin structures and has been used to study wound healing (Mandato and Be- ment, 2001), stress fibers (Kumar et al., 2006; Colombelli et al., 2009), cortex dynamics (Tinevez et al., 2009), cohesion of the epithelial tissue during cell division (Founouenou et al., 2013; Guillot and Lecuit, 2013; Herszterg et al., 2013), and shape changes during tissue morphogenesis (Collinet et al., 2015; Munjal et al., 2015). In this study, we combine laser microsurgery with live imaging in the Caenorhabditis elegans early embryo to probe the biophysical properties of constricting contractile rings during cytokinesis.

Results and discussion

In the four-cell embryo, two cells (ABa and ABp) undergo contractile ring constriction parallel to the focal plane, which provides an end-on view of the constricting ring when made visible under the microscope and facilitates the monitoring of shape change and ring component dynamics (Fig. S1 A). Furthermore, these cells are relatively large, with a contractile ring perimeter of 55.8 ± 0.4 µm (mean ± 95% confidence interval [CI]) at constriction onset, and divide with highly reproducible kinetics, enabling spatiotemporal measurements and analysis (Fig. S1, D and E). The velocity of ring constriction is roughly constant until the ring approaches the spindle midzone, when ring closure slows down until constriction completes (Fig. S1 E; Carvalho et al., 2009).

Using nanosecond laser pulses, we severed rings of ABa and ABp cells expressing myosinNMY-2::GFP, a fluorescent nonmuscle myosin II heavy chain that localizes in the ring and in the remaining cell cortex at different stages of constriction, without damaging the adjacent plasma membrane (Fig. S1, A and B; Fig. S2, A–C; and Video 1). Contractile rings exhibited a fast mechanical change of length and curvature, snapping

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Abbreviations used: CI, confidence interval; PH, pleckstrin homology.
open immediately after the cut. Surprisingly, rings neither regressed completely nor showed signs of disintegration. Instead, after finite retraction of the severed ends, the gap was repaired (Fig. 1, A and B; and Video 1). These results are similar to those obtained in laser-cutting experiments for actomyosin rings bordering plasma membrane wounds in *Xenopus* oocytes (Mandato and Bement, 2001). In our experiments, the gap was rapidly filled in with myosinNMY-2::GFP and ring constriction resumed. Severed rings always successfully completed constriction \((n = 75)\), and embryos continued to develop (7 out of 11 tracked embryos hatched, and the remaining embryos arrested during elongation, a late stage of embryogenesis). A striking finding is that despite locally destroyed ring topology, the resulting ring fragment retained most of its shape, which implies that it is anchored to the plasma membrane. Moreover, a mechanism is in place to repair gaps in the contractile ring at any stage of constriction, invariably allowing for the successful conclusion of cytokinesis.

To assess the robustness of contractile ring repair, we performed sequential cuts on the same constricting ring. In one set of experiments \((n = 6; \text{Fig. 1 C})\), the second cut was made at the
site of the first cut after the gap had been repaired (Video 2). In another set of experiments, sequential cuts were made in spatially separate regions 20–25 s apart (n = 8; Fig. 1 D). Similar to rings that were cut once, rings cut twice with either cutting regimen repaired the gaps and completed constriction. These results demonstrate that ring gap repair is history independent and is a locally autonomous process that is not influenced by events in adjacent regions of the ring.

To test whether gaps in contractile ring structure must be repaired in order for constriction to proceed, we made 22 consecutive cuts on the same side of a constricting ring, thus preventing it from resealing for 300 s. Strikingly, the ring fragment shortened from 30 μm to 10 μm during this time (Fig. 1 E and Video 3). Although the ring fragment constricted at a reduced velocity compared with uncut control rings, this experiment shows that a continuous contractile ring structure is not a prerequisite for constriction and is in agreement with the early observations that isolated fragments of cleavage furrows from dividing newt eggs are able to constrict in a buffer containing ATP (Mabuchi et al., 1988).

Next, we followed the initial response of severed rings with high temporal resolution. Immediately after the laser cut, ring snapping implied that the ring was under tension. Moreover, the severed ends no longer followed the curvature of the ring, instead adopting a straight conformation (71 out of 75 cut rings; Fig. 2 A), implying that the ring is working against cortical or membrane tension, as ring constriction is influenced by the mechanical properties of the remaining cell cortex (Zhang and Robinson, 2005; Turlier et al., 2014; Sain et al., 2015). We consistently observed this change in curvature in rings marked with other contractile ring components, including lifeAct::GFP (marking actin) and GFP::septinUNCE-59 (Fig. S2 E). Moreover, we observed a corresponding deformation of the plasma membrane at the cut site using GFP::P·PLC1 as a marker (Fig. S2 F), which revealed local relaxation of the plasma membrane at the tip of the septum. The curvature of the plasma membrane recovered 10–15 s after the cut (Fig. S2 F).

We were able to accurately follow the severed ends of cut rings, which allowed us to determine the gap size and the length of the cut ring (arc length) for 10 s after the cut (Fig. 2). The response to the laser cut resembled a viscoelastic mechanical relaxation, similar to what has been described for rings in an epithelial tissue (Guillot and Lecuit, 2013). At least two phases of gap formation after microsurgery were detectable within our temporal resolution: a fast phase of elastic recoil and a slow (“creep”) phase that lasted for ~6 s after the cut. Then the gap size remained roughly constant for ~4 s and subsequently decreased in a phase of repair (Figs. 2 C and S2 D). Plotting gap size at a time when the gap had widened to its maximum (~6 s after laser cutting; Fig. 2 C) and reached 20.2 ± 0.9% of the initial perimeter (Fig. S2 D) revealed a proportional increase between gap size and initial ring perimeter (Fig. 2 D).

To gain mechanical insight, we estimated ring recoil and cortical relaxation for cut rings with different initial perimeters (see Materials and methods for details). Ring recoil after laser cutting (Δl) is the change in arc length caused by tension and equals the initial ring perimeter minus the ablated length minus the arc length. The ablated length corresponds to the laser material removed by the laser spot, which is ~0.5 μm, and the arc length was measured 3.6 or 3.9 s after laser cutting (see Materials and methods for details). We found that Δl decreases as the perimeter decreases (Fig. 2 E, blue, orange, and green circles). The strain of the ring (ε), which corresponds to the tension in the intact ring according to Hooke’s law, is defined as Δl divided by the arc length (in the absence of tension). We found an increase in ring tension with decreasing perimeter (Fig. 2 F, blue, orange, and green circles). In contrast to the prediction of a static theoretical model whereby tension in the ring decreases proportionally to ring perimeter (Sain et al., 2015), our result reflects the dynamic and static force components that the constraining ring has to work against. One of the dynamic components could be the viscous drag forces caused by septum area growth that are expected to increase with constriction (Fig. S1 B). An increase in the strain of the ring at the last stages of constriction would be in agreement with the increase in the concentration of contractile ring components that is observed for smaller perimeters (Carvalho et al., 2009).

Gap size is the consequence of (a) ablated material, (b) ring recoil, and (c) stress relaxation in the cortex or membrane of the two forming cells behind the contractile ring, which changes ring curvature. Therefore, cortical relaxation corresponds to gap size minus Δl, and we found that it decreases proportionally to the decrease in ring perimeter (Fig. 2 G, blue, orange, and green circles; n = 22; R² = 0.84). We found that for large perimeters, the gap size is mostly determined by cortex relaxation (~5 μm for a perimeter of 40 μm; Fig. 2 G) rather than ring recoil (~2 μm, 5% of 40 μm; Fig. 2 E). For smaller perimeters, the contributions of cortex relaxation and ring recoil are roughly equal (~1 μm).

The prediction of this mechanical dissection is that, in an embryo with reduced or no cortical tension, the difference between gap size and ring recoil should vanish, but the tension in the ring should still be nonzero and should increase during constriction. To test this prediction, we calculated ring strain (ε) and the gap size minus Δl in laser ablation experiments with embryos partially depleted of mlc-4, the regulatory light chain of nonmuscle myosin II. The regulatory light chain of nonmuscle myosin II is essential for myosin filament assembly and actomyosin contractility, which in turn determines cortical tension (Tinevez et al., 2009; Vicente-Manzanares et al., 2009). As expected, embryos partially depleted of mlc-4 exhibited a significant drop of cortical tension throughout constriction (Fig. 2 G, gray circles), and severed ends did not straighten (i.e., the curvature of the open ring did not change when compared with that of the intact ring; Fig. 2 H). Notably, in agreement with our prediction, the strain in the ring still increased during constriction (Fig. 2 F, gray circles). Collectively, our mechanical dissection suggests that at the onset of ring constriction, the tension born by the ring is predominantly determined by balancing cortical tension. In the course of constriction, when the perimeter decreases and the septum area increases, cortex and membrane tension are still present but decreasingly resist ring constriction. Instead, the ring works increasingly against dynamic force components, such as viscous friction and surface production. The net sum of static (Sain et al., 2015) and dynamic force components increases for decreasing perimeters along with time before it drops to zero at the end of constriction (Fig. 2 I).

After the immediate elastic recoil of the cut ring, the arc length of the initially larger rings continued to decrease while that of smaller rings did not show a clear tendency to further constrict until complete repair (Fig. 2 J). Nevertheless, this, together with evidence from the repeated ablation experiment (Fig. 1 E), indicates that the presence of the gap did not impact the ability of the open ring to constrict. It also suggests that
Figure 2. Mechanical dissection of ring tension and cortical relaxation throughout ring constriction. (A) Kymograph of the cut ring region showing the snapping event and straightening of the severed ends (a). The time interval between frames 3 and 4 is 2.6 s; other frames are 1 s apart. The overlay (b) shows frames immediately before (red) and after (cyan) the cut. (B) Schematic defining initial ring perimeter before the cut, arc length, and gap size. (C) Gap size measured during the first 10 s after the cut (n = 24). Cut rings were divided into three groups with initial perimeter averages of 35.8 µm (group I), 23.6 µm (group II), and 14.6 µm (group III). (D) Maximal gap size plotted against the initial perimeter for control rings cut once (black circles, n = 69) or twice, as in Fig. 1C (first cut in purple, second cut in green; n = 6). Maximum gap size and initial perimeter are proportional (R² = 0.81 and r = 0.90; P < 0.0001). Maximum gap size for rings of mlc-4–depleted embryos are in gray. (E–G) Ring recoil (Δl; E), strain of the ring (ε; F), and the difference between gap size and Δl reflecting ring curvature change (G) plotted against the initial perimeter for control (blue, orange, and green circles, color coded as in C; n = 22) or mlc-4–depleted rings (gray circles; n = 20). In G, a clear proportionality is observed (R² = 0.84 and r = 0.92; P < 0.0001). (H) As in A, but for a cut ring of an embryo partially depleted of mlc-4. The time interval between frames 3 and 4 is 3.92 s; other frames are 2.32 s apart. (I) Schematic illustrating how the net tension results from the sum of static and dynamic components and increases for decreasing perimeters. (J) Rate of change in arc length during the first 8 s after cut against the initial perimeter (left). Significant values are in red and nonsignificant in gray. Error bars represent the 95% CI. An inset of the red points is shown (right). Bars, 5 µm.
either some other mechanical component, such as the cell cortex or the plasma membrane, assists ring constriction or that the remaining ring arc is still able to do work.

After gap widening (i.e., 6 s after the cut), a 12-s period followed when we could not accurately identify the severed ends. During this time, gaps were completely filled in with myosinNMY-2::GFP, lifeAct::GFP, and GFP::septinUNC-59 (Fig. 1, A and B; and Fig. S2 E) so that 21.9 ± 1.0 s after the laser cut, all gaps were undetectable and a closed ring topology was reestablished (Fig. 3, A and B). We considered this to be the time point when gap repair was complete. Therefore, the time required for cut rings to completely repair gaps ranging from 2.1 to 8.9 µm was constant. Interestingly, the perimeter at which repair completed matched the initial perimeter (Fig. 3 C), indicating that the previous ring tension was established. In agreement with this, in cases where constricting rings were cut sequentially (Fig. 1 C), maximum gap size after the second cut was proportional to the initial perimeter before the second cut. This demonstrates that the tensions at the time of gap repair had been reestablished (Fig. 2 D, green and purple circles).

The invariable time to complete ring repair suggests that contractile ring components were recruited along their entire length of the gap, as gap closure from severed ends would be predicted to take longer for larger gaps. In agreement with this, myosin intensity profiles along the circumference of the ring revealed no significant lateral mobility from within the ring toward the gap (Fig. 3, D and E). Analysis of a minority of cut rings (2 out of 71), in which the first attempt at gap repair failed and the plasma membrane regressed more than usual, lent further support to this idea. In these two cases, myosin recruitment could be observed throughout the membrane at distinct foci (Fig. 3 F, arrows; and Video 4), and the gap was completely filled in with

Figure 3. Analysis of repair after laser microsurgery in contractile rings. (A) Kymograph of the cut ring region showing that ring curvature is recovered by the end of gap repair. (B) Time between the laser cut and completion of gap repair plotted against the initial perimeter (n = 38). (C) The perimeter at the time of completion of repair plotted against the initial perimeter, showing a strong correlation (n = 38; R² = 0.99 and r = 0.99; P < 0.0001). (D) Schematic illustrating how heat maps of myosinNMY-2::GFP distribution along the contractile ring circumference over time shown in E were generated. (E) Heat maps for myosinNMY-2::GFP as described in D. (F) Stills of two cut rings that fail the first repair attempt. Red arrows point to foci of myosinNMY-2::GFP that accumulate de novo along the length of the gap. Time is in seconds, and time zero corresponds to the frame before the laser cut. Bars, 5 µm.
myosin\textsuperscript{NMY-2::GFP} 20 s after the first failed repair (35 s after the cut). We conclude that within \(\sim\)22 s of being severed by laser microsurgery, constricting contractile rings have repaired their gaps from multiple sites along the exposed plasma membrane.

The multiple sites of myosin recruitment along the gap are likely to correspond to foci where new actin filament nucleation occurs. To test whether actin polymerization is required for gap repair, we treated permeabilized four-cell embryos with low doses of latrunculin A, which prevents new actin polymerization by sequestering actin monomers (Fig. 4, A and B). If added before furrow ingression, latrunculin A inhibits ring constriction altogether (Fig. 4 C, EMS cell). When added after ring constriction initiates, latrunculin A allows control rings to keep constricting (albeit slower than untreated rings; Fig. 4 C) but completely prevents ring resealing after laser microsurgery (Fig. 4 D). This shows that actin filament polymerization is essential for ring repair.

To examine the impact of the repair process on the kinetics of ring constriction, we analyzed the rate of change in ring perimeter after repair. Remarkably, this analysis revealed that cut rings took the same time to constrict to completion as uncut control rings (Fig. 5 A and Video 1). Inspection of individual traces revealed that the constriction velocity of cut rings was on average 35\% higher than that of uncut control rings (\(n = 15\); Fig. 5 B). Cut rings eventually caught up with uncut control rings likely because of their proximity to the spindle midzone.

The spindle midzone has been shown to slow down contractile ring constriction (Carvalho et al., 2009), suggesting that it imposes a limit on the interval during which repaired rings can close with an increased constriction velocity. In agreement with this, repaired rings with larger initial perimeters, which started further away from the spindle midzone, closed with an increased constriction velocity for a longer time before their perimeters caught up with the mean perimeter of uncut control rings (\(R^2 = 0.59\); Fig. 5 C). Along with accelerated constriction, we observed transient myosin accumulation in the repaired region greater than the mean myosin levels in that region before the cut. Myosin levels peaked 45.2 ± 1.9 s after the cut and then decreased and stabilized at \(\sim\)0.6 times the initial value, 73.9 ± 4.7 s after the cut (Figs. 5 D and S3, B and F). Actin also accumulated in the same region, with levels peaking 26.2 ± 1.5 s after the cut (Figs. S2 E and S3 B). Interestingly, the increase in myosin levels beyond the stabilization level roughly coincided with the time of repair completion, always occurring within the interval of increased constriction velocity (Fig. 5, B and D; and Fig. S3 F). We conclude that, after complete gap repair and recovery of ring topology, cut rings transiently recruit an excess of myosin to the repair site and speed up constriction, allowing them to complete cytokinesis in the same time as uncut rings.

Our analysis of contractile ring dynamics after laser microsurgery provides strong support for the previously proposed model that constriction is powered by a series of contractile units of equal size that locally constrict the plasma membrane at a constant velocity and make additive contributions to the overall constriction velocity (Carvalho et al., 2009). We propose that contractile units are connected to one another such that the contractile ring is able to sustain tension. The functional autonomy of individual contractile units explains the observation that constriction proceeds despite the presence of a gap in the ring circumference. Furthermore, units must be anchored to the membrane and/
Figure 5. Repaired rings close faster and catch up with uncut control rings. (A) Perimeter versus time plot of four cut rings with initial perimeters of 41, 36, 30, and 26 µm. The dashed portion of colored lines corresponds to time points from 0 to ~22 s, for which part of the measured perimeter corresponds to the outline of cortical myosin\(^{NMY-2::GFP}\) labeling. The mean perimeter for uncut rings is in black, with gray lines indicating the 95% CI [data as in Fig. S1 C]. Arrows indicate moment of laser cut. (B) Normalized ratio of perimeter change over time after cut \((n = 15)\); see Fig. S1 F and Materials and methods for details). Colored lines represent individual examples, and the black line shows the mean. The dashed lines indicate normalized ratios of 1 and 1.35 (mean of normalized velocities >1 over the time period of 22–55 s after the laser cut). The gray area represents the 95% CI of the mean curve. (C) Time required for cut rings to catch up with the perimeter of uncut rings plotted against initial perimeter \((n = 21); R^2 = 0.59 \text{ and } r = 0.76; P < 0.0001)\). (D) Normalized myosin fluorescence intensity levels plotted against time after cut for rings with initial perimeters between 16 and 42 µm (see Fig. S3 A for method). Black dots represent mean intensities [see Materials and methods for details] with 95% CI error bars; gray circles represent individual measurements. (E) An increase in constriction velocity proportional to the gap size can be explained by the contractile unit model. When a ring is cut, the plasma membrane retracts at the site of the cut, and contractile units (green) assemble de novo at nucleation sites (green circles) distributed along the plasma membrane. This increases the total number of units in the ring and the overall velocity of constriction after gap repair relative to uncut control rings. We propose that subsequent myosin hyperaccumulation (thick green line) transiently increases tension locally along the new units and promotes faster shortening of the repaired ring, leading to an overall higher constriction velocity than expected by serial incorporation of new units.
or cortex and sustain tension in the severed ring, which explains why a severed ring does not fall apart and exhibits only minimal change in curvature. Our measurements of arc length and gap size after laser cutting reveal that tension within the contractile ring increases during constriction, concomitantly with a decrease in net cortical tension at the ingression site. Furthermore, we show that the increase in ring tension occurs even when cortical tension is substantially reduced and myosin activity is partially compromised. Our mechanical analysis suggests that in the early phases of constriction, the ring primarily resists cortical or membrane tension but works increasingly against other forces, which we suggest is caused by increased surface area behind the ring as it gets smaller. Therefore, contributions of additional force components should be considered in future models of ring constriction.

We show that at any time during constriction, gaps varying in size up to fourfold are efficiently repaired with identical kinetics in an actin filament polymerization–dependent manner, and that multiple sites of myosin recruitment are present during the repair of larger gaps. We speculate that those discrete recruitment sites correspond to nucleation foci on the “naked” plasma membrane exposed after a laser cut, from which new actin filaments are polymerized and contractile units are assembled. Such nucleation sites may correspond to points where contractile units are anchored to the plasma membrane, allowing for tension transmission between neighboring units and for lateral tension transmission to the membrane and remaining cortex.

The rapid kinetics of gap repair highlights the remarkable capacity for local remodeling of contractile ring structure throughout constriction, which makes cytokinesis a highly robust process unlikely to fail once the contractile ring has assembled. Importantly, the contractile units assembled de novo at the site of the gap increase the total number of units in the repaired ring. As the overall velocity of ring constriction is proportional to the total number of units, the incorporation of additional ring thereby increases during constriction, concomitantly with a decrease in surface tension, in a dynamic force balance at a faster shortening velocity. Thus, the acceleration of repaired rings may be accounted for parallel incorporation, increasing the cross-sectional force capacity in the newly assembled units. Myosin hyperaccumulation would increase ring tension transiently and thereby work against resistive mechanical components, such as viscous drag and surface tension, in a dynamic force balance at a faster shortening velocity. Therefore, the acceleration of repaired rings may be promoted by both the gain of additional contractile units and transient increase of local myosin density at the site of the laser cut. However, we note that in some cases, myosin levels drop to stabilization levels before ring slowdown (Fig. S3, D–F). This argues against higher myosin density and higher force capacity as the sole cause for increased constriction velocity.

The reason why myosin hyperaccumulates in the repair region remains unclear. Interestingly, a transient local overshoot of myosin was also reported after bleb retraction in metaphase cells (Biro et al., 2013). We hypothesize that, in analogy to what occurs after bleb retraction, the local spike of myosin in the contractile ring is triggered by local deformation of the plasma membrane at the cut site. Finally, our analyses fit with previous demonstrations of limited ring edge recoil and repair of contractile rings bordering plasma membrane wounds in Xenopus oocytes (Mandato and Bement, 2001). It would be interesting to assess whether local actomyosin hyperaccumulation and ring acceleration are also observed in this context, as these responses may be universal features of actomyosin contractile rings.

Materials and methods

C. elegans strains

Strains used in this study are listed in Table S1 and were maintained at 20°C.

Live imaging

Live imaging of one- or four-cell C. elegans embryos was performed under no compression at 23°C. Adult hermaphrodites were dissected in M9 buffer (86 mM NaCl, 42 mM Na2HPO4, 22 mM KH2PO4, and 1 mM MgSO4·7H2O), and embryos were placed in the wells of imaging chambers as shown in Fig. 4 B (Carvalho et al., 2011). In the experiments with latrunculin A (Sigma-Aldrich) or propidium iodide, adult hermaphrodites were dissected in meiosis medium (25 mM Hapes, pH 7.4, 5 mg/ml insulin, 20% heat-inactivated FBS, and 60% Leibowitz-15 medium; see Acute drug treatment method section for details). Images were acquired with a spinning-disk confocal system with a CSU-X1 confocal spinning-disk head (Yokogawa Electric Corporation) mounted on an inverted microscope (TE2000U; Nikon) equipped with an electron-multiplying charge-coupled device camera (iXon 897+; Andor Technology), a 100× 1.4 NA oil-immersion Plan-Apochromat objective (Nikon), and 488-nm and 561-nm lasers (Coherent). Laser shuttering and microscope hardware were controlled using NIS-Elements software (Nikon) and a digital-to-analogue converter card (PCI 8733; National Instruments).

Laser microsurgery

Laser microsurgery in constricting contractile rings was performed by using the imaging objective (1.4 NA) to focus the second harmonic beam (532-nm wavelength) of a pulsed Nd:YAG laser (FQ-500-532; Elforlight), which was injected into the microscope stand through a secondary filter turret (stage-up kit). Pulse width was 10 ns, and pulse energy was 1.5–2 μJ, with the sample being exposed to a 1-kHz pulse train for 800 ms. The laser spot at the sample was ∼0.5 μm wide in the x–y plane and at least ∼0.5 μm wide along the optical axis. The scale of the ablation volume is comparable but larger than the laser spot volume, which is a result of the requirement that the laser intensity surpass the ablation threshold intensity across the full contractile ring width. Laser microsurgery was performed at different stages of ring constriction at ring perimeters between 11 μm and 49 μm. Image acquisition was initiated before the ring reached the desired stage of constriction. By that time, the embryo was moved to have the ring positioned in the laser microsurgery target site, and the laser cut was performed. It took on average 1.6 s for the laser cut procedure, during which image acquisition was paused. For the experiments in Fig. 1 (C–E), two or more sequential cuts were made in the same constricting ring, either in the same region, with the second cut being inflicted where the repair from the first cut occurred (Fig. 1 C), or in neighboring regions along the ring (Fig. 1 D). To obtain cuts in neighboring regions, the cuts were inflicted ∼20 s apart. In this case, image acquisition was stopped before the first cut and only resumed after the second cut. To keep the ring continuously open, 22 consecutive cuts were performed in the same region of the ring (Fig. 1 E).

To monitor contractile ring behavior immediately after laser microsurgery, a single image was acquired in the 488-nm channel every second; to follow the repair and constriction after laser microsurgery, 5 × 0.5–μm, 7 × 0.5–μm, or 7 × 1.0–μm z stacks were collected in the 488-nm channel every 1.7 s, 2.32 s, or 3.2 s, respectively (fastest acquisition mode). To monitor contractile ring constriction of uncut rings, 7 × 0.5–μm or 11 × 1.0–μm z stacks were collected every 2.32 s or 10 s, respectively.
Plasma membrane behavior after laser cutting was monitored in four-cell embryos expressing a plasma membrane probe (pleckstrin homology [PH] domain PLC-1 Δ1) fused with GFP (PH::GFP; Audhya et al., 2005) and myosin

For cut rings, ring perimeter was determined as described in the first paragraph for uncut rings. In the period of time from the cut until repair completion (0–22 s), a portion of the measured perimeter corresponded to the outline of the cortex as judged by cortical myosin

Measurement of gap size, arc length of the severed ring, and time of gap repair. Gap size measurements were performed in single sections or in maximum-intensity z projections by manually tracing a straight line between the severed ends for each time point during the ~10 s after laser microsurgery (Fig. 2, B and C; and Fig. S2 D). After the first 10 s, the gap started being filled with contractile ring material, and measuring its size became impossible. Gap size was normalized to the ring perimeter before the cut (initial perimeter) in Fig. S2 D. The maximum gap size was plotted against the initial perimeter in Fig. 2 D. The arc length of the open ring within the first 10 s after the cut was measured by manually tracing a line from the left severed end to the right severed end along the ring (Fig. 2 B). Ring recoil (Δl; Fig. 2 E), strain of the ring (ε; Fig. 2 F), and cortical relaxation (gap size minus Δl; Fig. 2 G) were determined after measuring arc length or gap size at the time point of 3.6 or 3.9 s after laser cutting, which was the earliest possible time point (these measurements had to be done in z projections of at least five z sections). Any significant length changes caused by shortening between ablation and the first time point thereafter (Fig. 2 J) were not considered in the calculations. Ring recoil (Δl) was approximated by subtracting the ablated length (0.5 µm) and arc length from the initial ring perimeter. The strain of the ring—the length change caused by tension divided by the length at rest (loss of tension)—was approximated by dividing Δl by the arc length. The values for Δl and ε correspond to approximations because we were not able to determine Δl immediately after laser ablation but only a few seconds later, and a small part of the ring is destroyed during ablation. The rate of change of the arc length during the first 8 s after laser cutting was determined by linear regression and plotted against initial perimeter (Fig. 2 J). The time of gap repair was defined as the interval between the time point just before the laser cut and the time point when the gap was completely filled in with myosin

Measurement of instantaneous ring constriction velocities after laser cut. The instantaneous rate of change of perimeter Δp = (constriction velocity) was determined with the first order difference method for time points after completion of repair and with perimeters >19 µm (at which point rings approach the spindle midzone and slow down; Carvalho et al., 2009). The difference in perimeter between time points t[i + 1] and t[i] was divided by the interval between those time points for all frames i of a video: Δp(t[i]) = (p(t[i + 1]) − p(t[i]))/t[i + 1] − t[i]). The rate of change of the perimeter for the reference uncut ring, ΔP(t), and for every cut ring, Δp(j)[t], j = 1…n, was calculated. P(t) represents the mean of ABp rings. For cut rings, Δp[j][t] was normalized to ΔP, where P(T) = p[j][T]; i.e., the time point T for which the perimeter of the uncut ring matched the cut ring was determined by interpolation, and the velocity at this time point T was used for normalization: ΔΩ[j][t] = Δp[j][t]/ΔP(T), for all traces j = 1…n (Fig. S1 G). The velocity ΔP(T) was determined by interpolation of T within the discrete values of ΔP, P(t). A mean curve for ΔΩ[j][t] was calculated by temporally aligning the Ω[j] with respect to the time point for severing, overlaying all Ω[j] (Fig. 5 B), dividing the time axis in equal bins with size Δt = 4 s, and filling each bin with the normalized velocities Ω[j](k • Δt) < t < (k + 1) • Δt), k = 1…M, with M equaling the number of bins. Then, the arithmetic mean of the values in each bin was calculated. Similarly, the SEM = SD/sqrt(N[k]), with N[k] equaling the number of values in bin k, was determined for all M bins, and the 95% CI was calculated.
The time interval for cut rings to catch up with uncut rings was defined as the interval between the time point just before the laser cut and the time point when the repaired ring reached a perimeter that was within the region of the uncut ring perimeter mean plus 95% CI (Figs. 5 C and S3 D).

Quantification of myosin fluorescence levels after laser cut. The myosinNMY-2::GFP mean intensity level in the cut region was quantified within a box 0.7 µm thick and 2.0 µm wide drawn over the region where the cut was inflicted; the dimensions of the box were kept constant for all time points measured before and after the cut. A circular region in the cytoplasm, which decreased in size as the ring constricted, was used to quantify the mean intensity of the myosinNMY-2::GFP cytoplasmic signal. The cytoplasmic signal was subtracted from the mean intensity in the cut region, and values were normalized to the mean myosin intensity before the cut (Figs. 5 D and S3, A and F). Individual traces revealed that myosin levels increased to a maximum level and then decreased before the cut (Figs. 5 D and S3, A and F). The cytoplasmic signal was subtracted for each time frame ti. A 3-pixel-wide polyline was manually traced along the ring for each time point, and the intensity profile Ai(x) = A(x, ti) was generated. The profiles Ai(x) were normalized to the maximal value of the first profile A1 = max{Ai(x)} and smoothed using the exponential moving average with the smoothing constant 0.2. The sequence of the normalized smoothed profiles Ai(x) = Ai(x)/A1 corresponds to a surface which was presented as a heat map showing the levels Ai = constant. The length of each profile δi corresponds to the ring perimeter, and the time-ordered sequence of δi produces the perimeter dynamics.

Online supplemental material

Fig. S1 shows details about contractile ring constriction in the four-cell C. elegans embryo. Fig. S2 shows that our laser microsurgery experiments do not cause plasma membrane damaging and shows gap behavior in embryos expressing fluorescent probes for the contractile ring and plasma membrane. Fig. S3 shows characterization details of the myosin hyperaccumulation in the repaired region. Video 1 shows two contractile rings snapping open, getting repaired, and completing constriction in the same time as uncut rings after laser microsurgery. Video 2 (Fig. 1 C) shows the contractile ring’s ability to repair itself after two sequential cuts in the same region. Video 3 (Fig. 1 E) shows that a contractile ring that has been kept continuously open is able to keep constricting. Video 4 (Fig. 3 F) shows the new nucleation sites from which the gap seems to be filled in a cut ring in which the first attempt at gap repair failed but second succeeded. Table S1 contains a list of the worm strains used in this study.

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