Contractile ring mechanosensation and its anillin-dependent tuning during early embryogenesis

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Abstract

The contractile ring plays crucial roles in animal morphogenesis. Previous studies have explored how tissue mechanics controls the contractile ring; however, the mechanisms by which the ring senses tissue mechanics remain largely unknown. Here, we demonstrate the mechanism of contractile ring mechanosensation and its tuning during asymmetric ring closure of *Caenorhabditis elegans* embryos. High-resolution imaging of cortical flow revealed that local suppression of the ring-directed cortical flow is associated with a delay in furrowing. This suppression of cortical flow results from cortical compression. We found that the artificial inhibition of ring-directed cortical flow was sufficient to induce asymmetric ring closure in symmetrically dividing cells. Moreover, genetic analysis suggests that the positive feedback loop among ring-directed cortical flow, myosin enrichment, and ring constriction constitutes the anillin-dependent, mechanosensitive engine driving asymmetric ring closure. Our results suggest that the balance between RhoA-dependent and cortical flow-dependent myosin enrichment fine-tunes the ring's mechanosensitivity in tissues.

Introduction

The cytokinetic contractile ring plays crucial roles in morphogenesis during asymmetric cell division and epithelial morphogenesis. Previous studies have shown that intracellular and extracellular mechanics can influence contractile ring positioning, but how the contractile ring senses these mechanical cues remains largely unknown [1–3]. One important model system to study contractile ring mechanosensation is the asymmetric ring closure found in animal zygotes and epithelial tissues, termed unilateral or asymmetric cytokinesis [4,5]. Unilateral cytokinesis is ubiquitously observed in both invertebrates and vertebrates, including humans [6–10], and its dysregulation has been associated with reduced cytokinesis resilience, disrupted epithelial integrity, and defective lumen morphogenesis[6,11,12].

Contractile ring assembly is regulated by the activation of small GTPase RhoA. The mitotic spindle plays a central role in specifying the site of RhoA activation through the centralspindlin complex [13–16]. The activated RhoA then induces Rho-associated kinase (ROCK)-dependent myosin activation, as well as formin-dependent actin assembly, to initiate contractile ring formation [17–19]. Furthermore, the centrosome and astral microtubules at the polar region suppress ring assembly [20–27]. Pioneering studies have shown that asymmetric spindle positioning is sufficient to induce asymmetric ring closure in a few examples such as ctenophore zygotes and normally symmetrically-dividing sea urchin embryos, [28,29] and that the spindle-cortex proximity asymmetrically activates RhoA [30].

Conversely, unilateral cytokinesis in various vertebrate and invertebrate epithelial tissues and in the *C. elegans* zygote is spindle positioning-independent. In both systems,

the mere spindle-cortex proximity does not dictate the leading edge of the ring[6,31–34]. In

Drosophila embryonic and follicular epithelia, adherens junctions regulate asymmetric

ring closure [2,34]. In this system, passive mechanical resistance imposed by adherens

junctions anchors the contractile ring at the apical cell cortex. In contrast, unilateral

cytokinesis in the *C. elegans* zygote is regulated by contractile ring components anillin

and septin[6]. In this system, contractile ring components enrich at the leading edge,

forming a structurally asymmetric ring. However, the precise mechanisms of asymmetric

ring closure remain unclear in both systems. In *C. elegans* zygotes, knockdowns of anillin

and septin disrupt the asymmetric ring closure [6]; however, the *ani-1(RNAi)* phenotype is

rescued in a mutant background of a worm-specific RhoA activator, NOP-1 [35].

Furthermore, anillin and septin are dispensable for asymmetric ring closure at least in

Drosophila embryonic and adult dorsal thorax epithelial cells, leaving the role of anillin

cryptic [2,36].

Recent studies show that cortical flow, a concerted flow of cell cortex materials at

the cell surface, facilitates contractile ring assembly through cortical compression[37–39].

Therefore, we hypothesized that the contractile ring utilizes this mechanism to sense

tissue mechanics. To elucidate the mechanism of contractile ring mechanosensation, we

performed high-resolution 4D imaging. By developing analysis pipelines measuring

various parameters such as ring closure, ring eccentricity, cortical flow, and myosin

enrichment rate from the same datasets, we gained an integrative view of

mechanochemical contractile ring regulation during asymmetric ring closure.

Quantitative analysis of these parameters suggests that the furrowing delay at the lagging

cell cortex is induced by the mechanical retardation of cortical flow towards the ring. By

conducting an *in vitro* cell-bead adhesion assay, we artificially reduced the ring-directed

cortical flow, thereby confirmed the role of ring-directed cortical flow in asymmetric

contractile ring closure. Finally, through a genetic analysis, we identified the molecular

pathway that tunes contractile ring mechanosensitivity.

Results

High-resolution 4D imaging and quantitative analysis of contractile ring dynamics

To better understand the mechanism of asymmetric ring closure, we performed high-

resolution 4D imaging of GFP-fused non-muscle myosin II (NMY-2) in *C. elegans*

zygotes (Figures 1A and S1A). We applied a new imaging method combining the use of

spacer beads and a refractive index-matching sample medium to avoid the previously

reported compression-dependent cell rotation [40,41] and to improve image resolution [42],

respectively (see Methods for detail). These improvements allowed us to capture

uncompressed embryonic volumes (30 μm thickness) at 5.6-s intervals with a 1 μm step

size, without cell rotation (Figure S1B and Movie S1).

We developed image analysis pipelines to quantify the dynamics of contractile

ring closure (Figures 1B and S1A). First, we performed image segmentation to

computationally detect the contractile ring (Figures 1B and S1C). Using the segmentation

data, we measured the ring radius and ring center coordinates at every time point. From

these measurements, we calculated the ring closure indices $R_{closure}(t)$ (with 1.0

representing complete closure), ring eccentricities $E(t)$ (indicating off-center tendency),

and the degrees of leading-edge and lagging-edge advancement towards the cell center

$L_{lead}(t)$, $L_{lag}(t)$ (Figure 1D). Due to the natural variations in cytokinesis timing, we aligned

the time series data relative to the time around 10% contractile ring closure (Figure S1D).

We also defined cytokinesis onset based on the extrapolation of an initial linear part of

the ring closure curve to 0 (Figure S1D). Markedly, the newly developed imaging and

quantification methods allowed us to detect differences in sub-percentage ring closure

indices. The data obtained show that the contractile ring initially undergoes nearly the

upper limit of eccentric closure (Figure 1E; Movie S1), and unilateral furrow ingression

continues until the start of lagging edge furrowing (Figure 1E–F). These data indicate that

the primary factor contributing to ring off-centering is the time interval between the

onsets of furrowing at the leading edge and the lagging edge (Figure 1F; 0-117 sec).

Ring closure velocity and a delay in lagging edge furrowing determine the ring

eccentricity

We analyzed the relationship among peak eccentricity, time lag between leading

and lagging edge advancement, and ring closure velocity. The time lag, Δt, was

determined by the delay between the leading and lagging edges reaching 10%

advancement relative to the initial ring diameter (Figure 1F–G). Additionally, ring

closure velocity, v, was calculated based on the slope of the $R_{clousre}$ curve between 20%

and 70% closure points (Figure 1G). We found that ring closure velocity and peak

eccentricity have no clear correlation (Pearson's r = 0.352, p = 0.028, n = 39; Figure 2A).

On the contrary, the time lag is weakly correlated with the peak eccentricity (Pearson's r

= 0.54, p = 0.0003, n = 39; Figure 2A). Since a slowly closing ring might have a longer

time lag than a faster one, we calculated the normalized time lag, Δt_n, by multiplying the

ring closure velocity by the time lag (Figure 2B). We found that the normalized time lag

is strongly correlated with peak eccentricity (Pearson's r = 0.93, p < 0.0001, n = 39;

Figure 2B), indicating that both ring closure velocity and time lag control asymmetric

ring closure in normal cells.

To investigate whether ring closure velocity and time lag generally control

eccentricity, we analyzed these parameters in mutants or RNAi knockdowns of

actomyosin regulators (Figures 2C–G and S2A–F). In addition to the previously reported

ANI-1/Anillin, we found that POD-1/Coronin and MLC-4/myosin regulatory light chain

are required for ring eccentricity (Figure 2C–D). We also confirmed that a deletion

mutation of the worm-specific RhoA activator NOP-1 rescued the *ani-1(RNAi)* phenotype

[35] and that the knockdown of LET-502/ROCK did not cause eccentricity defects [6], as

reported previously (Figure 2C–D).

We calculated time lag, ring closure velocity, and normalized time lag in these

conditions. Time lag (raw values) and peak eccentricity were not particularly correlated

(Pearson's r = 0.54, p = 0.21; Figure S2G). However, we found that normalized time lag

is strongly correlated with peak eccentricity (Pearson's r = 0.99, p < 0.0001; Figure 2E).

The relationship between ring closure velocity and time lag also shows that conditions

with asymmetric closure and symmetric closures are separated within the parameter

space (Figure 2F). Therefore, cells that exhibit faster ring closure and longer time lag

tend to display asymmetric ring closure. Using these data, we investigated why the *nop-1*

mutation was able to rescue the *ani-1(RNAi)* phenotype. We found that the *nop-1*

mutation increased both time lag and ring closure velocity compared to *ani-1(RNAi)*

alone (Figure 2G), suggesting that the rescue is due to the increase of these parameters.

These results suggest that ring eccentricity is collectively determined by the ring closure

velocity and a delay in furrowing at the lagging edge.

Reduced ring-directed cortical flow at the lagging cell cortex accompanies

circumferential-axis cortical flow

We analyzed the mechanism of the lagging edge furrowing delay. As ring-directed

cortical flow is proposed to contribute to the positive feedback regulation of the

contractile ring assembly (Figure 3A)[37], we hypothesized that the delay is due to the

feedback loop having lower activity at the lagging edge than at the leading edge. To test

this hypothesis, we analyzed cortical flow at the leading and lagging cortices using

particle image velocimetry (PIV) [43] (Figures 3B and S3, and Movie S2).

First, we analyzed the mechanism of ring-directed cortical flow generation, which

is an unclear part of the proposed feedback model. Cortical flow is a phenomenon driven

by a gradient in actomyosin contractility [44]. This gradient is established during

cytokinesis, with higher contractility at the equator and relaxation at the pole [23,37,38].

Given that the contractility gradient continues to exist during ring assembly and closure,

the ring should consistently pull the cell cortex from the polar region. To confirm this

prediction, we plotted velocities of ring edge advancement (Figure 3C; solid lines) and

ring-directed cortical flow (Figure 3C; dotted lines) and found that they correlate well

both at the leading and lagging edges (leading edge: Pearson's r = 0.77, $p < 0.0001$,

lagging edge: Pearson's r = 0.89, $p < 0.0001$). Furthermore, ring closure velocity and

peak ring-directed flow are highly correlated in different RNAi and mutant conditions,

including *ani-1(RNAi)* (Figure 8D; Pearson's r = 0.94, $p = 0.002$). These results suggest

that the invaginating ring edge consistently pulls the nearby cell cortex to generate ring-

directed cortical flow to form a positive feedback loop (Figure 3A).

Next, we analyzed the difference in cortical flow between leading and lagging

cortices. To better understand the influx of the cortical flow within the equatorial region,

we calculated the convergence of cortical flow vectors along the A-P axis and the

orthogonal, circumferential-axis (Figure 3B). Furthermore, we tracked myosin foci to

confirm the PIV data (Figure 3E). At the leading cell cortex, A-P axis convergence

increased rapidly and reached to its peak before the onset of lagging edge furrowing

(Figure 3D; left). Consistently, myosin foci tracking showed continuous ring-directed

cortical flow (Figure 3D; top). Conversely, A-P axis convergence in the lagging cell

cortex decreased shortly after cytokinesis onset and reached a local minimum before the

onset of lagging edge furrowing (Figure 3D; left, Figure 3E; $t_n = 58$). During this period,

we observed increase in circumferential axis convergence only at the lagging cell cortex

(Figure 3D; right). These results suggest that circumferential-axis cortical flow negatively

correlates with ring-directed cortical flow.

Cells undergoing asymmetric ring closure exhibit polarized mechanical loading at

the lagging cortex

Circumferential cortical flow could inhibit ring-directed cortical flow, potentially through

a mechanical process (Figure 3A). To infer cortical mechanics from the flow vectors, we

used cortical convergence. Before furrowing onset, convergence at the surface signifies

cortical compression (Figure 4A, top). After the onset of furrow ingression, convergence

at the surface represents the sum of cortical compression and ingression, similar to how

converging surface sea water sinks towards the ocean floor (Figure 4B, bottom).

In control embryos, we observed that ring-directed cortical compression at the

lagging cell cortex decreased upon cytokinesis onset (Figures 4B; top left). However, this

decline was not apparent in *ani-1(RNAi)* (Figure 4B; top middle). The decline reemerged

with the introduction of a *nop-1* mutation (Figure 4B; top right). On the other hand, there

were no marked differences in circumferential axis convergence under these conditions

(Figure 4B; bottom row). The rate of reduction in ring-directed cortical compression,

estimated from the lines connecting local maxima and minima (red and blue arrowheads

in the Figure 4B), also confirmed a similar relationship (Figure 4C). We found that

conditions with asymmetric ring closure (control, *ani-1;nop-1*, and *let-502*) and

symmetric ring closure (*ani-1* and *nop-1*) exhibited a reduction and no change in ring-

directed compression, respectively (Figure 4C). Since control and *ani-1(RNAi)* displayed

a similar ring closure velocity (Figure 2F), the observed cortical relaxation cannot be

attributed to the ring closure rate. In contrast, peak circumferential-axis convergence

strongly correlates with ring closure velocity (Pearson's r = 0.90, *p* = 0.005) (Figure 4D–

E). These results suggest that in general, contractile ring closure induces circumferential

axis compression. This compression is then accompanied by reduced ring-directed

compression in cells undergoing asymmetric ring closure (Figure 4F). However, ring-

directed compression in *ani-1(RNAi)* does not respond to the compression along the

circumferential axis.

Non-ring-directed cortical compression suppresses ring-directed cortical flow

We hypothesized that any non-ring-directed cortical compression, like the

circumferential-axis compression we observed, inhibits ring-directed cortical flow. To

test this hypothesis, we induced ectopic cortical compression using a semi-dominant

temperature-sensitive mutation of actin, *act-2(or295)*, known to cause actin filament

stabilization and ectopic cortical contractility [45] (Figure 5A). In normal cells, the leading

cortex demonstrates consistent ring-directed flow, while the lagging cortex experiences a

period of delay followed by ring-directed flow (Figure 5A–B). In *act-2(or295)*, ring-

directed movement of myosin foci was suppressed during contraction and pronounced

during relaxation (Figure 5C). Ring closure is necessary for relaxation-induced ring-

directed cortical flow, as similar flow was not observed post-cytokinesis (Figure 5D).

These results suggest that non-ring-directed cortical compression inhibits ring-directed

cortical flow at the lagging cell cortex (Figure 5E).

Non-ring-directed compression delays ring edge advancement without suppressing

ring constriction

A previous study demonstrated that laser-induced ablation of the cell cortex behind the

ring did not accelerate the ring closure rate [37]. This suggests that ring closure is not

constrained by the mechanical resistance of the cell cortex behind the ring. If this is

indeed the case, the observed retardation of ring-directed flow would not limit the ring

closure rate, and therefore, would not influence the ring eccentricity. We tested this

possibility using highly contractile *act-2(or295)* mutants and found that the overall ring

closure curves were similar to those of the control, with the ring velocity even exceeding

than the control (Figure 2F). This confirms the first part of our prediction. However, the

contractile rings of the *act-2* mutant frequently displayed oscillatory movement during

constriction and exhibited a lower mean peak eccentricity compared to the control

(Figures 6A and 2D). These results suggest that cortical compression influences ring

position without altering the constriction rate.

We investigate how cortical compression behind the ring affects ring positioning.

To infer cortical compression, we measured myosin intensity changes at the cell surface

closest to the objective lens (referred to as the top surface) (Figure 6B). The upward and

downward movements of contractile ring along the top-bottom axis were also measured

using the same samples (Figure 6B). During contraction at the top surface, the downward

movement of the top ring edge was often halted, as though the top ring edge were

anchored (Figure 6C–E; labeled as "contraction," Movie S3). Conversely, relaxation of

the top surface often coincided with the bottomward movement of the top ring edge

(Figure 6C–E; labeled as "relaxation," Movie S3). Quantitative analysis of cortical

compression and acceleration of the top ring edge also confirmed these trends (Figure

6F–H; positive acceleration indicates movement towards the bottom surface). These

findings suggest that cortical compression behind the ring impedes the advancement of

the nearby ring edge.

How does the compression-dependent retardation of the ring edge occur without

delaying the overall ring constriction rate? Notably, we frequently observed upward

movement of the bottom ring edge when the top surface is compressed (Figure 6C–E, I).

Thus, it is likely that when a certain part of the ring edge advancement is prevented, other

areas of the ring edge pull more cortex from the nearby surface, maintaining a similar

constriction rate.

Artificial inhibition of ring-directed cortical flow is sufficient to induce ring

eccentricity

Our data suggest that the retardation of ring-directed cortical flow can trigger asymmetric

ring closure. To directly test this hypothesis, we inhibited ring-directed cortical flow in

symmetrically dividing cells. It is known that the two-cell stage AB cell undergoes

asymmetric ring closure, similar to the P_0 zygote [46] (Figures 7A and S5). We found that

unilateral ring closure requires cell contact with the neighboring P_1 cell (Figure 7B–C).

To inhibit ring-directed cortical flow, we attached adhesive polystyrene beads to the

symmetrically dividing isolated AB cell (Figure 7D–E). The beads, which were coated

with positively charged Rhodamine fluorescent dyes and salts, adhere to the plasma

membrane. This adhesion is presumed to occur through electrostatic interaction, similar

to the mechanism observed with poly-L-lysine coating. As shown in our previous study,

attachment of the 30 μm diameter beads reduced ring-directed flow in the area proximal

to the bead attachment site (Figure 7E) [1]. The response scaled with the bead diameter,

suggesting that passive mechanical resistance imposed by adhesive beads limits ring-

directed flow (Figure 7E). Notably, the attachment of the 30 μm beads increased peak

eccentricity, and about 50% of the cells exhibited a level of ring eccentricity comparable

to that of the intact AB cell *in vivo* (Figure 7C, F–H). Thus, we conclude that mechanical

retardation of ring-directed cortical flow is sufficient to induce asymmetric ring closure.

Anillin tunes contractile ring mechanosensitivity

Our analysis suggests that contractile rings in both the P_0 zygote and the two-cell stage

AB cell exhibit mechanosensitivity. We found that *ani-1(RNAi)* embryos exhibited

reduced ring eccentricity in both cell types, whereas *pod-1(RNAi)* showed defects only in

P_0 (Figure 8A; top panel). The observed loss of asymmetric closure in *pod-1(RNAi)* can

be explained by reduced circumferential-axis convergence (Figure 4D and 8A). These

results suggest that the contractile ring in *pod-1(RNAi)* retains its mechanosensitivity but

lacks the P_0 mechanical cue. Conversely, *ani-1(RNAi)* embryos had a normal level of

circumferential-axis convergence (Figure 4D and 8A), indicating that the contractile rings

in *ani-1(RNAi)* have impaired mechanosensitivity.

We observed that contractile rings in *ani-1(RNAi)* often retain a unusually high

level of myosin signal (Figure 8B). Indeed, the myosin enrichment rate in *ani-1(RNAi)* is

greater than any of the other RNAi groups that we tested (Figure 8C). Therefore, we

reasoned that the ectopic enrichment of myosin might disrupt the mechanosensitivity of

the *ani-1(RNAi)* ring. We hypothesized that a cortical flow-dependent positive feedback

loop in the contractile ring assembly sensitizes the contractile ring to mechanical stresses,

and that the feedback loop is disrupted in *ani-1(RNAi)* embryos (Figure 8G; central box).

We observed a strong correlation between ring closure velocity and peak ring-

directed cortical flow across different RNAi/mutant groups including *ani-1(RNAi)*, as

described above (Figure 8D). We also found that the myosin enrichment rate is highly

correlated with ring closure velocity and peak ring-directed cortical flow among sample

groups excluding *ani-1(RNAi)* (Figure 8E: Pearson's r = 0.93, *p* < 0.007, Figure 8F:

Pearson's r = 0.84, p < 0.03). Conversely, *ani-1(RNAi)* exhibited excess myosin

enrichment rate relative to the ring closure velocity and peak ring-directed cortical flow

(Figure 8E–F). These results suggest that the positive feedback loop between ring-

directed cortical flow, myosin enrichment, and ring constriction was disrupted in *ani-*

1(RNAi) embryos.

We further observed that the *nop-1* mutation rescued the asymmetric ring closure

defects in *ani-1(RNAi)* in both the P_0 and AB cells (Figure 8A), indicating that the *nop-1*

mutation restored the impaired mechanosensitivity of the contractile ring. The rescue of

polarized mechanical loading also supports this idea (Figure 4B–C). Analysis of myosin

levels revealed that the *nop-1* mutation reduced the ectopic myosin enrichment rate in

ani-1(RNAi) (Figure 8B–C). As a result, *ani-1(RNAi); nop-1(it142)* embryos regained a

normal level of myosin enrichment relative to ring closure velocity and peak ring-

directed flow (Figure 8D–F). Therefore, we concluded that impairment of the contractile

ring's mechanosensitivity is due to the disruption of the positive feedback loop caused by

excessive myosin enrichment.

Discussion

In this study, we have identified the mechanisms underpinning contractile ring

mechanosensation and the tuning of mechanosensitivity. Previous studies have shown

that ring-directed cortical compression promotes ring assembly, but our findings

demonstrate that non-ring-directed compression slows down ring-directed cortical flow

and the advancement of the ring edge. The suppression of ring-directed cortical flow was

sufficient to induce asymmetric ring closure in symmetrically dividing cells. The

mechanism of ring mechanosensation relies on a normal rate of myosin enrichment, in

relation to the velocities of ring closure and cortical flow. Finally, our results show that

anillin is required for the sensitization of the contractile ring against intercellular and

intracellular mechanics.

Based on our analysis and previous studies, we propose a cortical flow-dependent

contractile ring mechanosensation model (Figure 8G). The critical component of the

model is the positive feedback loop among ring constriction, ring-directed cortical flow,

and myosin enrichment. Khaliulin et al. (2018) proposed this feedback loop in the

contractile ring constriction process based on correlations among these parameters in

normal zygotes [37]. Our study further confirms these correlations in RNAi and mutant

backgrounds with varying ring closure velocities (Figure 8D–F). In our model,

intracellular and intercellular mechanical cues locally limit ring-directed cell cortex-

pulling at the lagging cell cortex (Figure 8G; arrows from the yellow boxes), thereby

suppressing the ring-directed cortical flow. The local suppression of the ring-directed

cortical flow should reduce compression-dependent myosin enrichment, resulting in the

formation of the structurally asymmetric ring observed in previous studies [6]. The local

reduction in myosin levels should then locally slow down ring edge advancement,

resulting in asymmetric ring closure.

Our analysis underscores the central role of the cortical flow pathway in ring

mechanosensation. Conversely, the RhoA pathway likely inhibits the mechanosensation

process. We found that reduced activities of the RhoA activator NOP-1 and effector LET-

502 did not influence ring eccentricity. However, we showed impaired

mechanosensitivity in *ani-1(RNAi)* embryos, in which myosin was highly enriched in the

contractile ring (Figure 8). Recent research has shown that ANI-1-binding proteins GCK-

1 and CCM-3 negatively regulate RhoA activity by promoting RhoGAP cortical

localization [47]. Therefore, it is likely that ANI-1 knockdown lifted this negative

regulation, leading to the activation of RhoA-dependent myosin enrichment. Indeed,

normal myosin enrichment as well as ring mechanosensitivity were restored in *ani-1;*

nop-1 (Figure 8A–C). We propose that the balance between RhoA-dependent and cortical

flow-dependent myosin enrichment tunes ring mechanosensitivity (Figure 8H). The

mechanosensitivity of the ring should decrease when the contribution of the cortical flow

pathway-dependent myosin enrichment is negligible compared to that of RhoA-

dependent pathway (Figure 8H).

The structural asymmetry of the contractile ring in the *C. elegans* zygote likely

arises from asymmetric activity in the cortical flow-dependent feedback mechanisms

between the leading and lagging cell cortices [37]. A similar structural asymmetry has been

reported in *Drosophila* pupal dorsal thorax epithelia [11]. However, *Drosophila* embryonic

epithelial cells [2], follicular epithelia [34], and pupal wing epithelia [11] do not display such

structural asymmetry. As our study identified that the cortical flow-dependent feedback

mechanism mediates ring mechanosensation, this variability suggests that the

mechanosensitivity of contractile ring can be tuned differently during development,

enabling tissue-specific morphogenic cytokinesis. The cortical flow pathway likely

translates subtle differences in tissue mechanics into asymmetry in the furrowing rate,

while excessive RhoA activity could suppress ring-dependent mechanosensing. Cell

types that lack structurally asymmetric rings may undergo asymmetry ring closure by

relying on extreme differences in tissue stiffness (e.g., a stiffer apical cortex connected to

adherens junction vs a softer basal cortex) rather than on high ring mechanosensitivty.

Alternatively, these cell types might employ other mechanosensitive mechanisms, such as

mechanosensitive recruitment of myosin regulators to neighboring cells [48,49].

The tuning of ring mechanosensitivity would be critical in animal development.

Here, we will use lumen morphogenesis as an example to explain this concept. A

previous study showed that 3D cultures of epithelial cells initially undergo symmetric

cytokinesis to form a central lumen [12,50–53]. Subsequent ring closures are asymmetric,

maintaining a solitary lumen. In the case of intraflagellar transport protein IFT88

knockdown, the first cytokinesis becomes asymmetric, leading to the formation of

multiple lumens [12]. Consistently, a mouse model with a loss of function mutation of *ift88*

exhibits a polycystic kidney phenotype [54]. In this context, the contractile ring in the first

cytokinesis should exhibit lower mechanosensitivity to prevent ectopic unilateral

cytokinesis, while the contractile ring in subsequent cytokinesis should be highly

mechanosensitive. Our study provides a theoretical framework for a deeper

understanding of tissue-specific mechanochemical regulation of the contractile ring,

which is crucial for further elucidating the role of cytokinesis in these morphogenetic

events.

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Author contributions

C.R.H.: Formal analysis, Investigation, Writing-Original Draft, Validation,

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Formal analysis, Investigation. **J.Z.**: Formal analysis, Investigation. **K.S.**:

Conceptualization, Formal analysis, Investigation, Methodology, Software, Validation,

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Declaration of interests

The authors declare no competing interests.

Methods

Experimental Model and Subject Details

All *C. elegans* strains were cultured using the standard method [55]. A temperature

sensitive-actin mutant *act-2(or295)* was cultured at 15 ºC until the L4 larval stage and

incubated at 25 ºC overnight before imaging. The following transgenes were used:

cp13[*nmy-2*::GFP + LoxP] (non-muscle myosin II) [56], *or1940*[GFP::*sas-7*] (centriole

marker) [57], *itIs37* (mCherry::histone H2B), *ruIs32*[*pie*-1p::GFP::H2B::*pie-1* 3'UTR +

unc-119(+)].

RNAi

Feeding RNAi was performed at 25 ºC using the standard method [58]. For control RNAi, a

bacterial strain carrying an empty vector (L4440) was used. To observe *let-502(RNAi)* P_0

zygotes, the L2 stage larvae were cultured on freshly prepared feeding RNAi plates on

day 1. The L4 larvae were then transferred to new feeding RNAi plates on day 2 and

imaged on day 3. For observing *let-502(RNAi)* AB cell, we performed L4 RNAi to avoid

abnormal AB spindle orientation. For *pod-1* and *mlc-4*, L4 larvae were cultured on

feeding RNAi plates and used for imaging on the next day.

Blastomere isolation

Blastomeres were isolated as described before [59], with some modification [60]. We cut the

gravid adult worms in egg salt buffer and treated them with hypochlorite solution [75%

Clorox (Clorox) and 2.5N KOH] for 50 s. After washing twice with Shelton's growth

medium [61], embryos were transferred to fresh Shelton's growth medium. Eggshell and

permeability barrier were removed by mouth pipetting with hand-drawn microcapillary

tubes (10 µL, Kimble Glass Inc.). The two-cell stage eggshell-free embryos were further

pipetted to remove the cell-cell contact.

Adhesive polystyrene bead preparation

The detailed method is described in our previous papers [1,60]. Approximately 10 mg

carboxyl-modified polystyrene beads with diameters of 30 µm (Kisker Biotech GmbH &

Co.), 20 µm, 10 µm, and 4.5 µm (Polysciences) were washed twice with 100 mM 2-(N-

morpholino) ethanesulfonic acid (MES) buffer (pH6.5) and incubated with 1 mL MES

buffer containing 10 mg 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) for

15 min at room temperature (22.5˚C). We washed the beads twice with phosphate-

buffered saline (PBS) and incubated them with 0.5 mL PBS containing 0.05 µg

Rhodamine Red-X succinimidyl ester (ThermoFisher Scientific) for 5 min. The

appropriate concentration was also determined by treating the beads with a series of

serially diluted Rhodamine Red-X solution [60]. The beads were washed twice with PBS

and stored in PBS at 4 ºC. Adhesiveness of the beads was confirmed by attaching them to

the isolated blastomere using the mouth pipette. If successful, the adhesion is firmly

established and cells do not dissociate spontaneously.

Live-imaging sample preparation

To obtain embryos, gravid adults were dissected on a coverslip, in a droplet of 10–12 µl

of refractive index-matching medium (30% iodixanol diluted in egg salt buffer,

supplemented with 30 µm diameter plain polystyrene beads) as described before [42]. After

placing the coverslip gently onto a slide glass, three edges of coverslip were sealed with

petroleum jelly (Vaseline), with one edge remaining open to the air. We realized that this

method improved the success rate of imaging for inexperienced users. Inexperienced

users may observe cell death due to the acute compression during the sample preparation

and require training using control strains. If all the processes were performed correctly,

the imaging condition does not have adverse effects on the embryonic viability, as judged

by normal cell division in the next cell cycle.

Microscopy

Intact embryos were imaged using a microscope Olympus IX83 (Olympus), equipped

with a spinning-disk confocal unit CSU-W1 (Yokogawa), a scientific CMOS camera

Prime 95B (Photometrics), a piezoelectric stage NANO-Z (Mad City Labs), a silicon

immersion objective UPLSAPO60XS2 (NA1.3, 60X; Olympus), and a beam splitter

Optosplit II (Cairn Research), which is controlled by Cellsense Dimension (Olympus). A

silicone immersion oil (Z81114; refractive index: 1.406 at 23 ºC; Olympus) was used as

an immersion medium. Samples were illuminated by diode-pumped lasers with 488 nm

and 561 nm wavelengths, and the simultaneous two color-imaging was performed with

150 ms camera exposure time, 1 µm Z-step size with a total of 31 slices per frame, 5.6-s

interval, and the duration of 15 min. Isolated blastomeres were imaged using a

microscope Leica DMi8 (Leica Microsystems), equipped with a spinning-disk confocal

unit CSU-W1 with Borealis (Andor Technology), dual EMCCD cameras iXon Ultra 897

(Andor Technology), and an oil-immersion PL APO objective lens (NA1.4, 63X; Leica),

and controlled by Metamorph (Molecular Devices). Data in Figure 7B and 7F were

imaged with 1.5 µm Z-step size and 15-s intervals. Data in Figure 7D were imaged with

1.5 µm Z-step size and 10-s intervals, with only the half volume closer to the objective

lens imaged.

Quantification of the P_0 contractile ring dynamics

The obtained 4D data were deconvoluted using a constrained iterative and advanced

maximum likelihood algorithm (iteration: 5), using Olympus Cellsens software

(Olympus, Inc). Each 4D tiff stack file was processed using Fiji [62] (Figure S1). The

deconvoluted images were processed using Gaussian blur (sigma = 2) and an image J

plug-in "attenuation correction" (opening =3, reference =15) [63]. A 10-µm W x 32 µm H

boxed region corresponding to the contractile ring was selected and adjusted for the

fluorescence intensity so that the signal would not be saturated in the next step, and

rotated by 3D projection (Brightest point, interpolation on). After selecting a plane of en

face ring view, the images were segmented and quantified using an Image J plug-in

Morpholib J [64]. The segmented contractile ring areas were measured for ring radius, ring

centroid, and ring angle. The radius of the segmented area was estimated using ellipsoid

fitting and derived by calculating the average of major and minor radii of the ellipsoid.

Data were aligned relative to the time point first exceeded 10% ring closure. The ring

trajectory images were obtained with an in-house image J macro using same data.

Quantification of the intact AB contractile ring dynamics

Since the AB cell undergoes rotation during its division, the precise quantification is

challenging. We first selected samples undergoing unilateral cleavage roughly in parallel

to the imaging plane, and corrected for cell rotation using Stackreg plug-in of Image J [65],

using chromosome signal (polar bodies were deleted using a brush tool to avoid abnormal

image registration) (Figure S5). The rotation-corrected images were then processed by

the same pipeline used for the P_0 cell.

3D visualization and quantification of contractile ring dynamics in isolated

blastomeres

The oblique view of cells in Figures 1A, 7A–B, and 7F were generated using an Image J

plug-in Clear Volume [66]. The analysis of the contractile ring dynamics in isolated

blastomeres is challenging since the cell is rotated along different axes, and not all the

planes were captured during imaging. Thus, we made en face ring view images for each

time point using Clear Volume, and estimated the cell and ring contours, centroids, and

diameters, by selecting more than four points along the cell and ring perimeter,

respectively, using an Image J macro "Smallest Enclosing Circle" [62]. The ring closure

rate and eccentricity were calculated using the method described above for other cells.

Measurements were performed three times per sample, and average values were used to

mitigate the relatively higher error rate compared to the automatic segmentation method

used for intact embryos.

Kymographs

Kymographs in Figure 4D were generated by stacking 1 μm H x 40 μm W rectangular

regions at a given time. Kymographs in Figure 4E were generated by stacking 11 μm W x

3.7 μm H rectangular regions corresponding to a myosin cluster. The timing is adjusted

so that the peak myosin cluster intensity comes fourth out of a total of eight frames, using

an in-house image J macro.

Quantification of myosin enrichment rate

We measured myosin enrichment using the ring en face view of NMY-2::GFP, as shown

in Figure 8B. Selection of the contractile ring region was performed using the

segmentation data described above. However, unlike the highly pre-processed data used

for segmentation (e.g., attenuation corrected), we used raw myosin data without

deconvolution and other processes. The only processes performed were 3D projection

and interpolation. After binarizing the segmentation data, we dilated the mask four times

(mask 1) and also created another mask with four times erosion (mask 2). In our

condition, mask1 covered most of the ring area, while mask 2 covered the area inside the

ring (cytoplasm). The myosin signal at each time point was then quantified by subtracting

the signal in mask 2 from the signal in mask 1. The timing of these time series data was

aligned relative to 10% ring closure.

Particle Image Velocimetry

A detailed image preprocessing pipeline is shown in Figure S3. Briefly, 4D data sets of

NMY-2::GFP and GFP::-SAS-7-expressing embryos were processed with Gaussian Blur

(sigma =1) and unsharp mask (radius =1, mask = 0.6) using Fiji. Centriole signals were

deleted by filling zero values. After intensity adjustment to avoid signal saturation, half

volume of image stack was filled with zero values to avoid projecting cortical myosin

from opposite cortical sides. The images obtained were projected using an Image J

function "3D projection." We rotated 3D projected data relative to angle of cleavage at

25% closure and generated leading and lagging cortex views. Note that not always both

lagging and leading cortices were clearly visible, so that in some embryos, either the

leading or lagging cortex was used. Using these flattened cell cortex images, Particle

Image Velocimetry was performed using MATLAB software PIV LAB (Algorithm: FFT

window deformation, Interrogation area: 5.8 μm, Step size: 2.9 μm, Sub-pixel estimator:

Gauss 2x3 point, Correlation robustness: Standard) [43]. We rejected vectors exceeding

0.25 μm/s to remove estimation errors (the setting was visually confirmed to reflect the

myosin foci movement). Obtained 3D matrix data (2D x time) were used for downstream

analyses. The PIV matrix data were aligned relative to 10% ring closure, centered relative

to the cell center corresponding to the furrow position, and resized to contain an entire

embryo using Numpy and Pandas [67,68].

Estimation of cortical convergence

Cortical convergence was derived using the PIV vector matrix and a NumPy gradient

function [67]. When we define A-P-axis and circumferential-axis cortical flow velocities in

an i x j matrix as $u_{i,j}$ and $v_{i,j}$, respectively, $u_{i+h,j}$, $u_{i-h,j}$, $v_{i,j+h}$, $v_{i,j-h}$ can be defined as

follows using Taylor series [69]:

577
$$u_{i+h,j} = u_{i,j} + h\frac{\partial u_{i,j}}{\partial i} + \frac{h^2}{2!}\frac{\partial^2 u_{i,j}}{\partial i^2} + \mathcal{O}(h^3) \quad (4)$$

578
$$u_{i-h,j} = u_{i,j} - h\frac{\partial u_{i,j}}{\partial i} + \frac{h^2}{2!}\frac{\partial^2 u_{i,j}}{\partial i^2} + \mathcal{O}(h^3) \quad (5)$$

579
$$v_{i,j+h} = v_{i,j} + h\frac{\partial v_{i,j}}{\partial j} + \frac{h^2}{2!}\frac{\partial^2 v_{i,j}}{\partial j^2} + \mathcal{O}(h^3) \quad (6)$$

580
$$v_{i,j-h} = v_{i,j} - h\frac{\partial v_{i,j}}{\partial j} + \frac{h^2}{2!}\frac{\partial^2 v_{i,j}}{\partial j^2} + \mathcal{O}(h^3) \quad (7)$$

581 And we will obtain the following by subtraction:

582
$$u_{i+h,j} - u_{i-h,j} = 2h\frac{\partial u_{i,j}}{\partial i} + \mathcal{O}(h^3) \qquad (8)$$

583
$$V_{i,j+h} - V_{i,j-h} = 2h\frac{\partial v_{i,j}}{\partial j} + \mathcal{O}(h^3) \qquad (9)$$

584 Thus, the cortical flow gradients at the interior points can be defined as follows:

585
$$\frac{\partial u_{i,j}}{\partial i} = \frac{u_{i+h,j} - u_{i-h,j}}{2h} + \mathcal{O}(h^2) \approx \frac{u_{i+h,j} - u_{i-h,j}}{2h} \qquad (10)$$

586
$$\frac{\partial V_{i,j}}{\partial j} = \frac{v_{i,j+h} - v_{i,j-h}}{2h} + \mathcal{O}(h^2) \approx \frac{v_{i,j+h} - v_{i,j-h}}{2h} \qquad (11)$$

587 At the boundary, first order one-sided differences were achieved as follows:

588
$$\frac{\partial u_{i,j}}{\partial i} = \frac{u_{i+h,j} - u_{i,j}}{h} + \mathcal{O}(h) \approx \frac{u_{i+h,j} - u_{i,j}}{h} \qquad (12)$$

589
$$\frac{\partial v_{i,j}}{\partial j} = \frac{v_{i,j+h} - v_{i,j}}{h} + \mathcal{O}(h) \approx \frac{v_{i,j+h} - v_{i,j}}{h} \qquad (13)$$

590 A-P-axis and circumferential-axis cortical convergence $p_{i,j}$, and $q_{i,j}$, respectively, were

591 calculated as follows, where a is the PIV step size:

592
$$p_{i,j} = -\frac{1}{a}\frac{\partial u_{i,j}}{\partial i} \approx -\frac{1}{a}\frac{\left(u_{i+1,j} - u_{i-1,j}\right)}{2} \quad (at\ the\ interior\ points)\ (14)$$

593
$$p_{i,j} = -\frac{1}{a}\frac{\partial u_{i,j}}{\partial i} \approx -\frac{1}{a}\left(u_{i\pm1,j} - u_{i,j}\right) \qquad (at\ the\ boundary) \quad (15)$$

594
$$q_{i,j} = -\frac{1}{a}\frac{\partial v_{i,j}}{\partial j} \approx -\frac{1}{a}\frac{\left(v_{i,j+1} - v_{i,j-1}\right)}{2} \quad (at\ the\ interior\ points)\ (16)$$

595
$$q_{i,j} = -\frac{1}{a}\frac{\partial v_{i,j}}{\partial j} \approx -\frac{1}{a}\left(v_{i,j\pm1} - v_{i,j}\right) \qquad (at\ the\ boundary) \quad (17)$$

To quantify leading and lagging cortex convergence at the equatorial region, we

calculated the mean convergence within a 6x3 grid (14.6 μm W x 5.9 μm H) around the

center of the furrow position.

Myosin foci tracking

Myosin foci were tracked using Manual Tracking plug-in of Image J.

In Figure 7D–E, cell position was registered using image J plug-in Stackreg, to eliminate

the movement of myosin foci caused by the movement of cell body.

Statistical analysis

For multiple comparisons, one-way ANOVA with the Holm–Sidak's method was used.

The comparison of two data was performed using the Welch's t-test. No statistical

method was used to predetermine the sample size. Error bars or bands correspond to the

95% confidence interval. The experiments were not randomized. The investigators were

not blinded to the study. Statistical analyses were performed using either SciPy or Prism

9 (Graphpad). Symbols such as "****," "***," "**," and "*" indicate $p < 0.0001$, $p <$

0.001, $p < 0.01$, and $p < 0.05$, respectively.

Data availability

The data used in this study is available in the Source Data file.

Code availability

The codes used in this study are available at

https://github.com/sugi01/unilateralcytokinesis.

Figure legends

Figure 1. Quantitative analysis of asymmetric contractile ring closure in the *C. elegans* zygote.

(**A**) Asymmetric contractile ring closure in *C. elegans* zygote. NMY-2::GFP (green, non-muscle myosin II), GFP::SAS-7 (green, centriole) and histone H2B::mCherry (magenta, chromosome) are shown. In this and subsequent figures, anterior and posterior are to the left and right, respectively. (**B**) Computationally reconstructed ring and segmentation results. The top left image shows cytokinesis onset, and the second and third rows highlight the advancement of the leading and lagging ring edges, respectively. See Fig S1 for method and full frame images. (**C**) Trajectory of ring constriction. Outlines of the contractile ring during cytokinesis are plotted based on the segmentation results. The color coding indicates time relative to cytokinesis onset. (**D**) Geometric analysis of the contractile ring closure. Eccentricity and ring edge advancement are the degree of ring off-centering and the distance between the ring edge and initial ring perimeter, respectively. (**E**) Mean ring closure and eccentricity. (**F**) Mean ring edge advancement. Lagging furrowing onset was defined as the timing when the $L_{lag}(t)$ exceeded 0.02 (2% relative to initial ring size). (**G**) Definitions of time lag and ring closure velocity (See text). Times are relative to cytokinesis onset. Error bands indicate 95% confidence intervals. Scale bars, 10 μm.

Figure 2. Ring closure velocity and a delay in lagging edge furrowing collectively determine the ring eccentricity.

(**A**) Relationship between peak eccentricity, ring closure velocity, and time lag in normal

cells. (**B**) Relationship between peak eccentricity and normalized time lag in normal cells.

(**C**) Ring trajectories of different RNAi and mutant backgrounds. (**D**) Peak eccentricity

and normalized time lag in different backgrounds. (**E**) Relationship between peak

eccentricity and normalized time lag in different backgrounds. (**F**) The relationship

between ring closure velocity and time lag in different backgrounds. (**G**) Time lag and

ring closure velocity. Times are relative to cytokinesis onset. Error bars indicate 95%

confidence intervals except for panel E and F, in which standard deviations were shown.

The value r represents Pearson's correlation coefficient. *P*-values were calculated using

one-way ANOVA followed by Holm–Sidak's multiple comparison test. Scale bars, 10

μm.

Figure 3. Reduced ring-directed cortical flow at the lagging cell cortex accompanies

circumferential-axis cortical flow.

(**A**) A cortical flow-dependent positive feedback model in the contractile ring assembly.

Ring-dependent cell cortex pulling generates ring-directed cortical flow and induces ring-

directed compression. Resulting myosin enrichment to the ring further facilitates ring

constriction. (**B**) Measurement of cortical flow. Leading and lagging cortex views were

generated by computationally rotating 4D timelapse data. Cortical flow vectors were

estimated using particle image velocimetry (yellow arrows). Convergence of cortical

flow vectors were calculated to estimate the influx of cortical flow within the equatorial

ROI (black boxes). (**C**) The relationship between ring edge advancement and ring-

directed cortical flow. Cortical flow in the area anterior to the ring is shown. (**D**) A-P axis

convergence and circumferential axis convergence in leading and lagging cell cortices.

(**E**) Myosin foci tracking during cytokinesis. Scale bars, 10 μm.

Figure 4. Cells undergoing asymmetric ring closure exhibit polarized mechanical

loading at the lagging cortex.

(**A**) Inferring cortical mechanics from cortical convergence data. Before furrowing onset,

cortical convergence is proportional to the cortical compression. After furrowing onset,

convergence is proportional to the sum of compression and invagination. (**B**) Cortical

compression at the lagging equatorial cell cortex. Mean cortical flow convergence before

the onset of lagging edge furrowing is shown. The transparent parts of graphs show the

convergence after the furrowing onset. Red and blue arrowheads indicate local maxima

and minima between cytokinesis onset (left dotted line) and lagging edge furrowing onset

(right dotted line) in each graph. (**C**) Reduction rate of ring-directed compression at the

lagging equatorial cortex. (**D**) Peak circumferential axis convergence observed at the

lagging equatorial cortex. (**E**) Relationship between peak circumferential axis

convergence and ring closure velocity. The value r represents Pearson's correlation

coefficient. (**F**) Summary of the cortical flow and cortical compression at the lagging

equatorial cell cortex. Ring-directed compression decreases as the circumferential

compression increases. P-values were calculated using one-way ANOVA followed by

Holm-Sidak's multiple comparison test. Times are relative to cytokinesis onset. Error

bands and bars indicate 95% confidence intervals. Scale bars, 10 μm.

Figure 5. Non-ring-directed cortical compression suppresses ring-directed cortical

flow.

(**A**) Kymographs of cortical myosin. Yellow boxes in the top images were used to

generate kymographs. (**B**) Ring-directed cortical flow in control zygotes. Leading edge

shows consistent flow while the lagging edge flow is preceded by the stabilization. (**C**)

Ring-directed cortical flow in *act-2* mutant zygotes. A myosin focus movement during

contraction and relaxation of the area behind the ring was shown. (**D**) Averaged ring-

directed flow in *act-2* mutant zygotes. (**E**) The mechanism of compression-dependent

suppression of the ring-directed cortical flow. While ring-directed compression promotes

ring assembly, non-ring-directed compression suppresses ring-directed cortical flow.

Times are relative to cytokinesis onset. Scale bars, 10 µm.

Figure 6. Non-ring-directed cortical compression delays ring edge advancement

without suppressing ring constriction.

(**A**) Contractile ring trajectory of a single *act-2(or295ts)* mutant (left panel) and the mean

values of ring closure and eccentricity (graph). (**B**) Planes shown in (C–E). Changes in

myosin intensity indicate contraction and relaxation. (**C**) NMY-2::GFP at the top surface

during cytokinesis. Yellow and light blue dotted circles indicate the area exhibiting

contraction and relaxation, respectively. (**D**) Ring en face view of the same embryo

shown in panel C. Dotted lines indicate top and bottom limits of the contractile ring.

Arrows indicate the movement of the ring limit. (**E**) Ring trajectories of the contractile

ring shown in (D). Top- and bottom-oriented movements are shown in yellow and blue,

respectively. (**F**) Relationship between top ring acceleration and myosin intensity

changes. Positive ring edge acceleration represents ring ingression toward the bottom

surface. (**G**) Top ring edge acceleration upon contraction and relaxation. (**H**) Top ring

edge acceleration measured 5.6 s post-contraction and relaxation. (**I**) Ring center

acceleration 5.6 s after contraction and relaxation. Error bars and bands indicate 95%

confidence intervals. Scale bars, 10 μm.

Figure 7. Artificial inhibition of ring-directed cortical flow is sufficient to induce

asymmetric ring closure.

(**A**, **B**) Contractile ring closure in the intact and isolated AB cell. NMY-2::GFP and

mCherry::histone H2B (left), ring trajectories (middle), and ring closure and eccentricity

curves (right) are shown. (**C**) Mean peak eccentricity of the contractile ring in the AB

cells. (**D**, **E**) Attachment of different sized adhesive beads to the isolated AB cell. Ring-

directed cortical flow were plotted for proximal and distal to the bead attachment site in

E. (**F**–**H**) Oblique view of contractile rings in the bead attached AB cell. Ring trajectory

and mean eccentricity are shown in G and H, respectively. Times are relative to

cytokinesis onset. Error bands and bars indicate 95% confidence intervals. Scale bars, 10

μm.

Figure 8. Anillin tunes contractile ring mechanosensitivity.

(**A**) Heatmaps of peak eccentricity and peak circumferential-axis convergence. *ani-*

1(RNAi) embryos exhibit defects in asymmetric ring closure in both P_0 and AB cells. (**B**,

C) Contractile ring myosin enrichment. Total myosin signal intensity in the ring (B) was

used to estimate myosin enrichment rate in (C). (**D-F**) Relationship among ring closure

velocity, peak ring-directed cortical flow, and myosin enrichment rate. (**G**) Proposed

model of contractile ring-dependent mechanosensation. (**H**) Proposed model of Anillin-

dependent tuning of contractile ring mechanosensitivity. Error bars indicate 95%

confidence intervals. Scale bars, 10 μm. p-values were calculated by one-way ANOVA

with Holm-Sidak's multiple comparison test.

Figure S1. Imaging and contractile ring analysis methods of P_0 zygotes.

(**A**) Imaging conditions and analytical flow charts. We used refractive index matching

medium and polystyrene beads to prevent light scattering and cellular compression. The

use of a refractive index matching medium reduced the required slice number from 34 to

31 and also improved image resolution, especially on the deeper side of the cell (focal

planes far from the objective lens). (**B**) Temporal color-coded image of 20 frames around

cytokinesis onset showing that compression-free embryos do not exhibit global cortical

rotation. (**C**) Ring en face view images of 3D projected data and segmentation results.

The first and last frames are the top left and bottom right in these figures, respectively.

(**D**) Contractile ring closure curve after alignment of time series data relative to 10 %

ring closure. Error bars indicate 95% confidence intervals. As we also measured myosin

signal intensity and cortical flow dynamics using the same data set, all the data were

aligned using the time relative to cytokinesis onset (or normalized time relative to

cytokinesis onset) unless otherwise specified.

Figure S2. Ring closure dynamics in RNAi and mutant backgrounds tested in this

study.

(**A-F**) Ring closure, Eccentricity, and Ring edge advancement curves were plotted against

time relative to cytokinesis onset. See Figure S3 for the data plotted against normalized

time. Lagging furrowing onset was defined as the time when the $L_{lag}(t)$ exceeded 0.02

(2% relative to initial ring size). (**G**) Relationship between peak eccentricity and time lag

(raw values without normalization).

Figure S3. Generation of cortical myosin data used for Particle Image Velocimetry

(PIV) analysis.

(**A**) An image preprocessing pipeline used to generate cortical myosin data for PIV

analyses. See also Methods section. During the quality control step, very noisy or dim

images were excluded from PIV analyses. These images are usually from image planes

furthest from the objective lens.

Figure S4. Cortical convergence in *let-502(RNAi)* and *pod-1(RNAi)*.

(**A**) A-P axis and circumferential axis convergece curves plotted against actual time (in

seconds)

Figure S5. Imaging and contractile ring analysis methods of the two-cell stage AB

cell.

(**A**) Imaging conditions and analytical flow charts. Intact AB cells were analyzed similar

to the P0 cells with some modifications to correct for cellular rotation. For isolated AB

blastomeres, we used normal growth medium and a different analytical pipeline to

estimate ring size and position due to the movement and rotation of the cells. Isolated AB

cells were measured three times, and average values were analyzed.

Movie S1. Dynamics of cortical myosin and contractile ring of a single embryo

Left top: maximum projection of cell surface NMY-2::GFP. Bottom left: ring en face

view of NMY-2::GFP. Bottom middle: segmented ring. Bottom right: reconstructed ring

trajectory. Right graph: Contractile ring dynamics of the same embryo. Note that the

same embryo was used to generate all data.

Movie S2. Cortical flow and flow vector map using Particle Image Velocimetry

The cell surface NMY-2::GFP of leading (left), lateral (middle), and lagging cortex views

(right). Yellow arrows are generated by Particle Image Velocimetry. Times are relative to

cytokinesis onset.

Movie S3. Dynamics of cortical compression and cortical ingression in *act-2(or295)*

Cell surface (top) and ring en face view (bottom left) of NMY-2::GFP. Ring outlines and

centroid trajectory were color-coded as in Figure 6E (yellow: movement towards the the

contraction site, blue: movement away from the relaxation site).

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Figure 1. Quantitative analysis of asymmetric contractile ring closure in the *C. elegans* zygote. (**A**) Asymmetric contractile ring closure in *C. elegans* zygote. NMY-2::GFP (green, non-muscle myosin II), GFP::SAS-7 (green, centriole) and histone H2B::mCherry (magenta, chromosome) are shown. In this and subsequent figures, anterior and posterior are to the left and right, respectively. (**B**) Computationally reconstructed ring and segmentation results. The top left image shows cytokinesis onset, and the second and third rows highlight the advancement of the leading and lagging edges, respectively. See Fig S1 for method and full frame images. (**C**) Trajectory of ring constriction. Outlines of the contractile ring during cytokinesis are plotted based on the segmentation results. The color coding indicates time relative to cytokinesis onset. (**D**) Geometric analysis of the contractile ring closure. Eccentricity and ring edge advancement are the degree of ring off-centering and the distance between the ring edge and initial ring perimeter, respectively. (**E**) Mean ring closure and eccentricity. (**F**) Mean ring edge advancement. Lagging furrowing onset was defined as the timing when the $L_{lag}(t)$ exceeded 0.02 (2% relative to initial ring size). (**G**) Definitions of time lag and ring closure velocity (See text). Times are relative to cytokinesis onset. Error bands indicate 95% confidence intervals. Scale bars, 10 μm.

Figure 2. Ring closure velocity and a delay in lagging edge furrowing collectively determine the ring eccentricity.

(**A**) Relationship among peak eccentricity, ring closure velocity, and time lag in normal cells. (B) Relationship between peak eccentricity and normalized time lag in normal cells. (C) Ring trajectories of different RNAi and mutant backgrounds. (D) Peak eccentricity and normalized time lag in different backgrounds. (E) Relationship between peak eccentricity and normalized time lag in different backgrounds. (F) The relationship between ring closure velocity and time lag in different backgrounds. (G) Time lag and ring closure velocity. Times are relative to cytokinesis onset. Error bars indicate 95% confidence intervals except for panel E and F, in which standard deviations were shown. The value r reperesents Pearson's correlation coeffiencient. P-values were calculated using one-way ANOVA followed by Holm-Sidak's multiple comparison test. Scale bars, 10 μm.

Figure 3. Reduced ring-directed cortical flow at the lagging cell cortex accompanies circumferential-axis cortical flow.
(**A**) A cortical flow-dependent positive feedback model in the contractile ring assembly. Ring-dependent cell cortex pulling generates ring-directed cortical flow and induces ring-directed compression. Resulted myosin enrichment to the ring further facilitates ring constriction. (**B**) Measurement of cortical flow. Leading and lagging cortex views were generated by computationally rotating 4D timelapse data. Cortical flow vectors were estimated using particle image velocimetry (yellow arrows). Convergence of cortical flow vectors were calculated to estimate the influx of cortical flow within the equatorial ROI (black boxes). (**C**) The relationship between ring edge advancement and ring-directed cortical flow. Cortical flow in the area anterior to the ring is shown. (**D**) A-P axis convergence and circumferential axis convergence in leading and lagging cell cortices. (**E**) Myosin foci tracking during cytokinesis. Scale bars, 10 μm.

Figure 4. Cells undergoing asymmetric ring closure exhibit polarized mechanical loading at the lagging cortex.

(**A**) Inferring cortical mechanics from cortical convergence data. Before furrowing onset, cortical convergence is proportional to the cortical compression. After furrowing onset, convergence is proportional to the sum of compression and invagination. (**B**) Cortical compression at the lagging equatorial cell cortex. Mean cortical flow convergence before lagging edge furrowing onset was shown. The transparent parts of graphs show the convergence after the furrowing onset. Red and blue arrowheads indicate local maxima and minima between cytokinesis onset (left dotted line) and lagging edge furrowing onset (right dotted line) in each graph. (**C**) Reduction rate of ring-directed compression at the lagging equatorial cortex. (**D**) Peak circumferential axis convergence at the lagging equatorial cortex. (**E**) Relationship between peak circumferential axis convergence and ring closure velocity. The value r represents Pearson's correlation coefficient. (**F**) Summary of the cortical flow and cortical compression at the lagging equatorial cell cortex. Ring-directed compression decreases as the circumferential compression increases. P-values were calculated using one-way ANOVA followed by Holm-Sidak's multiple comparison test. Times are relative to cytokinesis onset. Error bands and bars indicate 95% confidence intervals. Scale bars, 10 μm.

Figure 5. Non-ring-directed cortical compression suppresses ring-directed cortical flow.
(**A**) Kymographs of cortical myosin. Yellow boxes in the top images were used to generate kymographs. (**B**) Ring-directed cortical flow in control zygotes. Leading edge shows consistent flow while the lagging edge flow is preceded by the stabilization. (**C**) Ring-directed cortical flow in act-2 mutant zygotes. A myosin focus movement during contraction and relaxation of the area behind the ring was shown. (**D**) Averaged ring-directed flow in act-2 mutant zygotes. (**E**) The mechanism of compression-dependent suppression of the ring-directed cortical flow. While ring-directed compression promotes the ring assembly, non-ring-directed compression suppresses the ring-directed cortical flow. Times are relative to cytokinesis onset. Scale bars, 10 μm.

Figure 6. Non-ring-directed cortical compression delays ring edge advancement without suppressing ring constriction.
(**A**) Contractile ring trajectory of a single *act-2(or295ts)* mutant (left panel) and the mean values of ring closure and eccentricity (graph). (**B**) Planes shown in (C-E). Myosin intensity change indicates contraction and relaxation. (**C**) NMY-2::GFP at the top surface during cytokinesis. Yellow and light blue dotted circles indicate the area exhibiting contraction and relaxation, respectively. (**D**) Ring en face view of the same embryo shown in panel C. Dotted lines indicate top and bottom limits of the contractile ring. Arrows indicate the movement of the ring limit. (**E**) Ring trajectories of the contractile ring shown in (D). Top- and bottom-oriented movements are shown in yellow and blue, respectively. (**F**) Relationship between top ring acceleration and myosin intensity changes. Positive ring edge acceleration represents ring ingression toward the bottom surface. (**G**) Top ring edge acceleration upon contraction and relaxation. (**H**) Top ring edge acceleration 5.6 sec after contraction and relaxation. (**I**) Ring center acceleration 5.6 sec after contraction and relaxation. Error bars and bands indicate 95% confidence intervals. Scale bars, 10 μm.

Figure 7. Artificial inhibition of ring-directed cortical flow is sufficient to induce asymmetric ring closure.

(**A, B**) Eccentric ring closure in the intact and isolated AB cell. NMY-2::GFP and mCherry::histone H2B (left), ring trajectories (middle), and ring closure and eccentricity curves (right) are shown. (**C**) Mean peak eccentricity of the contractile ring in the AB cells. (**D, E**) Attachment of different sized adhesive beads to the isolated AB cell. Ring-directed cortical flow were plotted for proximal and distal to the bead attachment site in E. (**F-H**) Oblique view of contractile rings in the bead attached AB cell. Ring trajectory and mean eccentricity are shown in G and H, respectively. Times are relative to cytokinesis onset. Error bands and bars indicate 95% confidence intervals. Scale bars, 10 μm.

Figure 7. Anillin tunes contractile ring mechanosensitivity.
(**A**) Heatmaps of peak eccentricity and peak circumferential-axis convergence. *ani-1(RNAi)* embryos exhibit defects in asymmetric ring closure in both P0 and AB cells. (**B**, **C**) Contractile ring myosin enrichment. Total myosin signal intensity in the ring (B) was used to estimate myosin enrichment rate in (C). (**D**-**F**) Relationship among ring closure velocity, peak ring-directed cortical flow, and myosin enrichment rate. (**G**) Proposed model of contractile ring-dependent mechanosensation. (**H**) Proposed model of Anillin-dependent tuning of contractile ring mechanosensitivity. Error bars indicate 95% confidence intervals. Scale bars, 10 μm. p-values were calculated by one-way ANOVA with Holm-Sidak's multiple comparison test.