Article

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Simultaneous Regulation of Cytokinetic Furrow and Nucleus Positions by Cortical Tension Contributes to Proper DNA Segregation during Late Mitosis

Graphical Abstract

Highlights

- DNA segregation defects due to furrow mispositioning can be corrected
- Correction involves opposite nuclear and furrow movements
- Cortical relaxation and cytoplasmic flow due to blebbing reposition the furrow
- Microtubules and cytoplasmic flow both contribute to nuclear movement

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In Brief

Pacquelet et al. describe a mechanism that corrects DNA segregation defects due to furrow mispositioning during the first division of *C. elegans* embryos. Through their effect on cortical relaxation and cytoplasmic flow, cortical blebs play a key role in the regulation of furrow and nuclear positions, thereby ensuring proper DNA segregation.

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Simultaneous Regulation of Cytokinetic Furrow and Nucleus Positions by Cortical Tension Contributes to Proper DNA Segregation during Late Mitosis

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SUMMARY

Coordinating mitotic spindle and cytokinetic furrow positioning is essential to ensure proper DNA segregation. Here, we present a novel mechanism, which corrects DNA segregation defects due to cytokinetic furrow mispositioning during the first division of *C. elegans* embryos. Correction of DNA segregation defects due to an abnormally anterior cytokinetic furrow relies on the concomitant and opposite displacements of the furrow and of the anterior nucleus toward the posterior and anterior poles of the embryo, respectively. It also coincides with cortical blebbing and an anteriorly directed cytoplasmic flow. Although microtubules contribute to nuclear displacement, relaxation of an excessive tension at the anterior cortex plays a central role in the correction process and simultaneously regulates cytoplasmic flow as well as nuclear and furrow displacements. This work thus reveals the existence of a so-far uncharacterized correction mechanism, which is critical to correct DNA segregation defects due to cytokinetic furrow mispositioning.

INTRODUCTION

Ensuring equal DNA segregation is a critical feature of cell division. The correct assembly of the mitotic spindle at metaphase followed by the separation of sister chromatids at anaphase are essential steps in this process. Ingression of the cytokinetic furrow between the separated chromatids then ensures that the genetic material is equally inherited by the two daughter cells. It is therefore crucial that the positions of the cytokinetic furrow and the mitotic spindle are well coordinated. Two main signaling pathways emanating from the mitotic spindle control furrow position by restricting the localization of myosin contractile ring components to the vicinity of the central spindle [1, 2]. First, the centralspindlin complex, which localizes both at the central spindle and at the adjacent equatorial cortex, activates myosin contractility through the regulation of the small guanosine triphosphatase (GTPase) Rho [3–5]. Second, astral microtubules prevent myosin activity at the pole of dividing cells through mechanisms that remain to be precisely determined [6–10]. However, asymmetrically localized myosin also contributes to furrow localization [11–13]. During the first division of the one-cell *C. elegans* embryo, myosin transiently accumulates at the anterior cortex at early anaphase before being restricted to the equatorial cortex at the presumptive furrow ingression site [13]. This dynamic localization of myosin is tightly regulated. In particular, in embryos lacking both the kinase PIG-1 and the anillin ANI-1, myosin abnormally accumulates at the anterior cortex and leads to the displacement of the cytokinetic furrow, which position is thus no longer coordinated with the position of the mitotic spindle [13].

Mistakes occurring at any of the successive steps of mitosis may impair DNA segregation. However, several mechanisms, such as the spindle assembly [14] or the abscission checkpoints [15, 16], limit the occurrence of detrimental DNA segregation defects. In this study, we identify and characterize a new type of correction mechanism that prevents DNA segregation defects resulting from the lack of coordination between cytokinetic furrow and mitotic spindle positions.

RESULTS

DNA Segregation Defects due to Furrow Mispositioning Can Be Corrected

In *ani-1(RNAi);pig-1(gm344)* embryos (further referred to as *ani-1;pig-1*), excessive myosin accumulation at the anterior cortex displaces the furrow toward the anterior of the embryo. As a result, the two nuclei are often both located on the posterior side of the furrow (17/20 embryos; Figure 1A; t = 300 or 380 s; Video S1) [13]. However, most of those DNA segregation defects are corrected, with the anterior nucleus finally being located on the anterior side of the furrow (16/17 embryos; Figure 1A; t = 540 s; Video S1). After correction, furrow ingression completes in 13/16 embryos and persists until the beginning of the second division in 8/16 embryos. Moreover, the anterior nucleus contained the expected number (i.e., 12) of chromosomes (n = 10 embryos; Video S2). Hence, the correction process ensures that each daughter cell inherits the correct number of chromosomes.

As cytokinesis is delayed in *ani-1;pig-1* embryos (e.g., Figure 1A), we asked when the correction of DNA segregation

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Figure 1. DNA Segregation Defects Resulting from Furrow Mispositioning Are Corrected during Late Mitosis

(A) Furrow (arrow) and anterior nucleus (asterisk) positions during the division of control and *ani-1(RNAi);pig-1(gm344)* embryos. NMY-2::GFP labels non-muscle-myosin 2, and mCherry::HIS labels DNA.

(B) Nuclear envelope (labeled with EMR-1::mCherry) reformation in control and *ani-1(RNAi);pig-1(gm344)* embryos.

(C) Central spindle in control and *ani-1(RNAi);pig-1(gm344)* embryos. SPD-1::GFP labels the central spindle, centrosomes, and nuclei after division [17]; PH::GFP labels the membrane. Arrowhead indicates disassembled central spindle.

(D) *rga-3/4(RNAi)* embryos show DNA (asterisk) segregation defects (t = 180 s; 15/19 embryos), which are then corrected (t = 250 s; 13/15 embryos).

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Figure 2. Correction of DNA Segregation Defects Involves Both Nuclear and Furrow Displacement

(A and B) Average displacement of the anterior (A) and posterior (B) nuclei in control and *ani-1(RNAi);pig-1(gm344)* embryos.

(C) Average furrow tip position in control and *ani-1(RNAi);pig-1(gm344)* embryos.

(D) Anterior nucleus and furrow tip position in a representative *ani-1(RNAi);pig-1(gm344)* embryo. Error bars represent SD. See also Figure S1.

defects occurs relative to other cell cycle events. We first monitored nuclear envelope reformation. Both in control and *ani-1;pig-1* embryos, nuclear envelope reassembles during cytokinesis (Figure 1B; Video S3). In *ani-1;pig-1* embryos, this always precedes the correction of DNA segregation defects (n = 18/18). We next examined mitotic and central spindle disassembly. Although spindle disassembly coincides with the end of furrow ingression in control embryos, it precedes the correction of DNA segregation defects in *ani-1;pig-1* embryos (n = 11/12; Video S4). In control embryos, the central spindle starts to lose its compact structure at the end of furrow ingression and central spindle remnants associate with the ingressing furrow to form the midbody (Figure 1C; Video S5A). In *ani-1;pig-1* embryos, the central spindle starts to disassemble before the correction of DNA segregation defects (n = 21/22; Figure 1C; Video S5B). Some central spindle remnants associate with the closing cytokinetic ring (Video S5C and legend), but others become fainter and cannot be detected anymore at the end of cytokinesis (Video S5D). In summary, the correction of DNA segregation defects in *ani-1;pig-1* embryos occurs late during the cell cycle, when the nuclear envelope has already reformed and the mitotic and central spindle have disassembled.

Importantly, this correction mechanism is not specific to *ani-1;pig-1* embryos. Similarly, increased myosin activity following depletion of the Rho GAPs RGA-3/4 results in furrow displacement and DNA segregation defects, which are then corrected (Figure 1D and legend; Video S6). Laser ablation of anaphase centrosomes also leads to the relative mispositioning of the mitotic spindle and cytokinetic furrow. Notably, although DNA segregation defects resulting from anterior and posterior centrosome ablations occur in the opposite direction, both can be corrected (Figures 1E and 1F and legend; Video S7). Altogether, those results show that DNA segregation defects due to furrow and/or spindle mispositioning can be corrected in different contexts.

Correction of DNA Segregation Defects Involves Both Nuclear and Furrow Displacement

To elucidate the mechanisms underlying this correction process, we first tracked nuclei and cytokinetic furrow positions. In control embryos, the two nuclei are displaced toward the poles of the embryo during and after cytokinesis and furrow position remains stable during ring closure (Figures 1A and 2A–2C; Video S1). In *ani-1;pig-1* embryos, posterior nucleus displacement is similar to control embryos (Figure 2B), but the anterior nucleus moves faster and further (Figures 1A and 2A; Video S1). Furthermore, although the furrow initially moves toward the anterior, it is then displaced toward the posterior during the correction process (Figures 1A and 2C; Video S1). Notably, the opposite displacements of the anterior nucleus and the cytokinetic furrow occur concomitantly (Figure 2D). Similarly, opposite anterior nucleus and furrow movements occur when DNA segregation defects due to RGA-3/4 depletion or anterior centrosome ablations are corrected (Figures S1A–S1C and S1E; Videos S6 and S7). Reverse

(E and F) Anterior (E) and posterior (F) centrosome ablation leads to spindle and/or furrow mispositioning and DNA segregation defects (17/18 and 16/30 embryos). Defects are corrected in 15/17 and 15/16 embryos. Embryos express NMY-2::GFP (cortical and furrow signals), GFP::α-tubulin (labels the centrosome and to a lesser extent microtubules), and GFP::HIS (DNA). Dashed circles indicate ablated centrosomes. Arrowheads point to anterior (E) or posterior (F) DNA and arrows to the furrow before the correction. Asterisks indicate nuclei position after division.

In (A) and (C)–(F), t_0, anaphase onset. In (B), t_0 = nuclear envelope breakdown. Scale bars, 10 μm. See also Videos S1, S2, S3, S4, S5, S6, and S7.

Figure 3. Nuclear Microtubule Interactions Contribute to Nuclear Displacement

(A) ZYG-12 inactivation prevents nuclei/microtubule interactions. In control (n = 9) and *ani-1(RNAi);pig-1(gm344)* (n = 12) embryos, centrosomes are closely juxtaposed to the nuclei on average 435 s after anaphase onset. In *zyg-12(or577)* embryos (n = 9), centrosomes are never in close juxtaposition to the nuclei (embryos observed until the duplication of centrosomes preceding the second division). In *zyg-12(or577);ani-1(RNAi);pig-1(gm344)* embryos (n = 17), 14 embryos never showed juxtaposed centrosomes and nuclei, and juxtaposition occurred very late (>500 s after anaphase onset) in 3 embryos. In the embryos shown, posterior nuclei and most posterior centrosomes are out of focus. The second anterior centrosome is also out of focus in *ani-1;pig-1* and *zyg-12;ani-1;pig-1* embryos. t_0, anaphase onset. Scale bars, 10 μm.

(B and C) Average displacement of the anterior nucleus in control, *zyg-12(or577)*, *ani-1(RNAi);pig-1(gm344)*, and *zyg-12(or577);ani-1(RNAi);pig-1(gm344)* embryos. Error bars represent SD.

displacements are observed following posterior centrosome ablation (Figures S1D and S1E; Video S7). Opposite DNA and furrow movements thus correct DNA segregation defects due to furrow mispositioning.

Nuclear Microtubule Interactions Contribute to Nuclear Displacement

We next searched for the mechanisms involved in regulating nuclear displacement. We first tested the role of microtubules and used a thermosensitive allele (*zyg-12(or577)*) to inactivate the KASH protein ZYG-12 and prevent nuclei/microtubule interactions during the last steps of mitosis (Figure 3A and legend). This strongly inhibits anterior nuclear displacement in control embryos (Figure 3B) but only moderately affects anterior nuclear displacement in *ani-1;pig-1* embryos (Figure 3C). Moreover, it does not prevent DNA segregation defect correction (16/16 *ani-1;pig-1* and 13/14 *zyg-12;ani-1;pig-1* embryos with corrected DNA segregation defects). Hence, although nuclear/microtubule interactions are critical to move nuclei away from the cytokinetic furrow in control embryos, they are involved, but not essential for anterior nuclear displacement in *ani-1;pig-1* embryos.

Myosin Activity Regulates Both Nuclear and Furrow Displacement

We next looked for an additional mechanism that could participate in anterior nucleus displacement and tested the possible involvement of myosin by using a thermosensitive allele of non-muscle myosin-2 (*nmy-2(1490)*). *nmy-2;ani-1;pig-1* embryos were initially grown at permissive temperature to ensure that the increase in myosin activity due to the lack of PIG-1 and ANI-1 is sufficient to induce furrow mispositioning. They were then shifted at restrictive temperature 220 s after anaphase onset. In control embryos, myosin inactivation moderately reduces nuclear displacement (Figure 4A) and does not enhance the effect of ZYG-12 inactivation (Figure 4C). Thus, contrary to nuclear/microtubule interactions, myosin has a minor role in the regulation of nuclear position in control embryos. Myosin inactivation also moderately inhibits anterior nuclear displacement in *ani-1;pig-1* embryos (Figure 4B). By contrast, simultaneous inactivation of ZYG-12 and myosin severely reduces the efficiency of DNA segregation defect correction (correction in 12/12 *nmy-2;ani-1;pig-1* and 11/20 *nmy-2;zyg-12;ani-1;pig-1* embryos; Figure 4D). Furthermore, anterior nucleus displacement is strongly impaired, both in rescued and non-rescued

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Figure 5. Variations in Myosin Concentration Entail Different Levels of Cortical Tension

(A) Anterior (left) and posterior (right) cortical myosin levels in control and *ani-1(RNAi); pig-1(gm344)* embryos. Error bars represent SD.

(B) Laser-induced ablation performed during anaphase at the anterior cortex of an *ani-1(RNAi);pig-1(gm344)* embryo. Images of the anterior cortex 0.5 s before and 0.5 s and 1.5 s after ablation are shown. White bar indicates the area targeted for ablation. Measurement of the ellipse small axis allowed us to determine the cut opening kinetics (see STAR Methods). Scale bars, 5 μm.

(C) Initial opening velocities at the anterior and posterior cortex of control and *ani-1(RNAi); pig-1(gm344)* embryos. Boxplot central rectangles span the first to the third quartile of the data, middle lines correspond to median values, and lower and higher whiskers to the minimum and maximum value, respectively. p values, two-tailed non parametric Wilcoxon tests.

See also Video S9.

nmy-2;zyg-12;ani-1;pig-1 embryos (Figure 4D). Altogether, our results demonstrate that microtubules and myosin independently contribute to the positioning of the anterior nucleus during the correction of DNA segregation defects in *ani-1;pig-1* embryos.

Importantly, we also found that myosin inactivation prevents the furrow from moving back toward the posterior in *nmy-2; ani-1;pig-1* embryos (Figures 4E and 4F; Video S8). Myosin therefore plays a crucial role in the correction process by concomitantly regulating nuclear and furrow displacements.

Variations of Myosin Levels Entail Changes in Cortical Tension

Considering the importance of myosin in the correction process, we carefully examined myosin cortical levels. Anterior myosin cortical level briefly increases in control anaphase embryos whereas an excessive and long-lasting accumulation of myosin is observed in *ani-1;pig-1* embryos (Figure 5A, left) [13]. This accumulation slowly drops off during the correction process (Figure 5A, left). Those changes are specific to the anterior cortex, as myosin posterior cortical levels only weakly and briefly increase in control and *ani-1;pig-1* embryos (Figure 5A, right). Those observations prompted us to assess possible changes in cortical tension by performing laser-induced cortical ablation [18, 19] (Figure 5B; Video S9). In control embryos, cortical tension during anaphase is slightly higher at the anterior than at the posterior cortex (Figure 5C). Although posterior cortical tension does not change significantly in *ani-1;pig-1* embryos, anterior cortical tension increases during anaphase before declining at the end of the rescue process (Figure 5C). All these variations in cortical tension are consistent with the changes in myosin levels that we measured (Figure 5A).

Changes in Cortical Myosin Levels Contribute to the Repositioning of the Nascent Furrow

Cortical tension imbalance and asymmetric myosin-driven contraction of the cortex can alter daughter cell size in dividing HeLa cells [20]. Variations in myosin level and cortical

Figure 4. Myosin Activity Regulates Both Nuclear and Furrow Displacement

(A–D) Average displacement of the anterior nucleus in embryos of the indicated genotypes. In (B) and (D), only embryos with corrected DNA segregation defects were analyzed, except for *nmy-2(ne1490);zyg-12(or577);ani-1(RNAi);pig-1(gm344)*, for which embryos with and without correction are presented separately.

(E) Furrow position in *ani-1(RNAi);pig-1(gm344)* and *nmy-2(ne1490);ani-1(RNAi);pig-1(gm344)* embryos. Asterisk indicates DNA. White dashed line indicates the most anterior position reached by the furrow. Arrowhead points to a cortical bleb on the anterior side of the furrow. Scale bars, 10 μm.

(F) Average furrow tip position in *ani-1(RNAi);pig-1(gm344)* and *nmy-2(ne1490);ani-1(RNAi);pig-1(gm344)* embryos. Only embryos with corrected DNA segregation defects were analyzed. Embryos did not show furrow regression during furrow tracking.

Error bars represent SD. See also Video S8.

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tension could thus explain furrow movements in *ani-1;pig-1* embryos. Consistent with this hypothesis, movement of the nascent furrow (ring diameter > 15 μm) is correlated with cortical myosin levels: large differences of myosin levels between the anterior and posterior cortex (Δmyosin > 50 a.u.) are associated with the initial anterior displacement of the nascent furrow and weaker differences (Δmyosin < 30 a.u.) coincide with the furrow starting to move back toward the posterior pole (Figure 6A).

If this correlation results from the mechanical action of cortical myosin on the furrow, we reasoned that furrow speed must be affected by factors that influence the mechanical resistance to furrow movements. Forces resisting furrow displacement include the resistance of the cortex itself, F_{cort}, and the drag force exerted by the cytoplasm, F_{cyt} (Figure 6B). As the embryo is confined in its eggshell, the cytoplasm indeed needs to flow through the cytokinetic ring to allow furrow displacement in the opposite direction. This latter resisting force is expected to increase when the cytokinetic ring closes. If the difference of cortical myosin concentration between anterior and posterior cortices, ΔM, drives furrow motion, we expect the velocity of the furrow to scale as $v \sim \Delta M\, r^4/(\alpha r^4 + \beta\, R^2[R^2 - r^2])$, where R is the cell radius and r the cytokinetic ring radius (modeling details in STAR Methods). This model predicts that, for a given myosin difference, furrow speed depends on cytokinetic ring closure (Figure 6C, red continuous line [Δmyosin > 50 a.u.]). The effect of myosin concentration imbalance will become negligible as the furrow closes (Figure 6C, compare red [Δmyosin > 50 a.u.] and black [Δmyosin < 30 a.u.] continuous lines; Figure 6D, compare green [ring diameter > 15 μm] and purple [ring diameter < 15 μm] continuous lines). In agreement with this model, for a given myosin difference, furrow velocity strongly depends on cytokinetic ring opening (Figure 6C, red squares [Δmyosin > 50 a.u.]). Furthermore, although the speed of the nascent furrow is correlated with myosin levels (Figure 6D; ring diameter > 15 μm; green squares and green dotted regression line), this is not the case during furrow ingression (Figure 6D; ring diameter < 15 μm; purple dots and purple dotted regression line). Altogether, our data strongly suggest that changes in myosin cortical levels contribute to the displacement of the nascent cytokinetic furrow. However, as the cytokinetic ring closes, furrow movement is limited by the increasing cytoplasmic drag, suggesting the existence of a supplementary mechanism that contributes to further furrow displacement. Moreover, we observed a shift between the velocity of the furrow predicted by our model and our experimental data (Figures 6C and 6D). Although the model predicts that myosin influences the speed of the furrow movement toward the anterior cortex, it does not account for the reverse movement of the furrow toward the posterior (Figures 6C and 6D, continuous lines) and a constant drift velocity must be added to our model to fit experimental data (Figures 6C and 6D, dashed lines; see STAR Methods).

Myosin-Dependent Cortical Blebbing and Cytoplasmic Flow Are Associated with Furrow Displacement

We thus searched for additional mechanisms that could explain furrow repositioning. We first assessed the possible role of the central spindle. Inactivation of the central spindle protein SPD-1 during anaphase effectively disrupts the central spindle (Figure S2A) but does not inhibit either correction efficiency nor furrow displacement in *ani-1;pig-1* embryos (Figure S2B; Video S10A). Hence, the central spindle does not play an essential role in repositioning the furrow. This is consistent with our observations that the central spindle starts to disassemble before the correction process (Figure 1C; Video S5). We next tested whether nuclei could regulate cortical changes by controlling the localization of myosin regulators, similar to what was previously shown in *Drosophila* neuroblasts [21]. However, preventing nuclear reformation by depleting the nucleoporin NPP-8 (Figure S2C) does not prevent correction of DNA segregation defects or furrow displacement (Figure S2D and legend; Video S10B), indicating that nuclei do not control furrow repositioning through the sequestration of myosin regulators.

Notably, *ani-1;pig-1* embryos display strong cortical deformations, reminiscent of the blebs described in cultured cells [22, 23]. Consistent with previous observations [22, 24], cortical myosin level suddenly decreases at the site of bleb formation and then reappears at the edge of the bleb (Figure 7A; Video S11). Cortical blebs first arise at the posterior and anterior embryo poles (Figure 7A; t = 128 s; Video S11). They form early during anaphase, prior to or at the beginning of furrow ingression, when the furrow still moves toward the anterior pole of the embryo (Figures 7A and 7B; Video S11). About 250 s after anaphase onset, those posterior and anterior blebs cease and are replaced by blebs located on the anterior edge of the furrow (Figure 4E, left; t = 440 s; 10/10 embryos; Figure 7A; t = 300 s; Figure 7B; Videos S8 and S11). These latter blebs form during furrow ingression, appearing shortly prior to and then accompanying

Figure 6. Cortical Myosin Can Reposition the Nascent Furrow

(A) Displacement of nascent cytokinetic furrows (ring diameter > 15 μm) depends on ΔMyosin. Boxplot central rectangles span the first to the thirs quartile of the data, middle lines correspond to median values, and lower and higher whiskers to the minimum and maximum value, respectively. p value, two-tailed unpaired Student's t test.

(B) Model of the forces acting on furrow displacement. The stronger anterior cortical contractility exerts a pulling force on the nascent furrow (F ∼ΔM) while resistance forces from the cortex (F_{cort}) and the cytoplasm (F_{cyt}) oppose to furrow displacement. *R*, cell radius; *r*, cytokinetic ring radius.

(C) Furrow velocity as a function of ring opening and myosin concentration. Black boxplots and red squares measurements correspond to low and high ΔM, respectively (n = 89 for low ΔM and n = 59 for high ΔM). The predicted velocity for low and high ΔM is represented by the solid black and red lines, respectively. A constant drift velocity needs to be added to the model (black and red dashed lines) to fit the experimental data. Boxplot central rectangles span the first to the thirs quartile of the data, middle lines correspond to median values, and lower and higher whiskers to the minimum and maximum value, respectively.

(D) Furrow velocity as a function of myosin concentration and ring opening. Measured furrow velocity is correlated with ΔM in the case of nascent furrow (ring diameter > 15 μm; green squares and green dotted regression line), but not during ring closure (ring diameter < 15 μm; purple dots and purple dotted regression line). Predicted furrow velocity is represented without (solid lines) or with (dashed lines) addition of a constant drift, for a nascent furrow (ring diameter = 17 μm; green) or during ring closure (ring diameter = 12 μm; purple). In all panels, furrow velocity was measured between 300 s and 450 s after anaphase onset, and time points were collected from 15 different embryos.

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furrow posterior displacement (Figures 7A and 7B; Video S11). They do not form when myosin is inactivated (Figure 4E, right; 11/11 embryos; Video S8). This suggests that bleb formation is due to the increased cortical tension measured in *ani-1;pig-1* embryos (Figure 5C), similar to what was previously shown in cultured cells [23, 25]. Notably, blebs form in regions where the cortex is away from the eggshell and with the strongest cortical curvature (Figure 7A). Following the theory of bleb nucleation proposed in [23], we modeled the variation of free energy entailed by the nucleation of a bleb in the different regions of an embryo (modeling details in STAR Methods). In agreement with our observations, this model predicts a higher probability of forming a bleb where the cortex is strongly curved.

Creation of new cortex by cortical blebs may contribute to the displacement of the nascent furrow by increasing the surface area of the blebbing daughter cell and thus relaxing its cortex. Moreover, as the furrow moves back toward the posterior, blebbing mostly occurs on the anterior edge of the furrow (Figure 7B), where it has the direct effect of locally displacing the base of the furrow (Videos S8 and S11); this may contribute to the correction of the nascent furrow position and add up to the effect of myosin decrease (Figure 7E). However, similar to what we have described for the effect of myosin concentration (see above), we expect that cortical relaxation and displacement of the base of the furrow is not sufficient to overcome the cytoplasmic drag force and to drive overall furrow displacement during ring closure.

Blebs can transiently reduce intracellular pressure and have been described to be associated with the appearance of cytoplasmic flow [24, 25]. Interestingly, we observed local cytoplasmic flows directed toward cortical blebs in *ani-1;pig-1* embryos (Video S12). Thus, as blebs form on the anterior side of the furrow, the anterior daughter cell pressure decreases. When the cytokinetic ring narrows, we expect a comparatively slow pressure equilibration with the posterior daughter cell, driving in turn cytoplasmic flow. Indeed, posterior furrow displacement coincides with the appearance of a strong flow of cytoplasmic particles oriented toward the anterior pole of *ani-1;pig-1* embryos (Video S12). We used particle imaging velocimetry (PIV) to quantify this flow and found that 75% of the measured velocity vectors are oriented toward the anterior in *ani-1;pig-1* embryos (speed < -25 nm/s; n = 13 embryos; Figure 7C). Such a flow is not observed in control embryos (only 30% of the velocity vectors with speed < -25 nm/s; n = 17; Video S12; Figure 7C) or when myosin is inactivated (39% of the velocity vectors with speed < -25 nm/s; n = 11; Figure 7D).

Finally, we observed that correction of furrow mispositioning resulting from RGA-3/4 depletion or anterior centrosome ablation is also associated with the formation of cortical blebs on the anterior side of the furrow and the appearance of an anteriorly directed cytoplasmic flow (Videos S6 and S7). Reversely, the correction of defects due to posterior centrosome ablation is associated with the formation of cortical blebs on the posterior side of the furrow and with a cytoplasmic flow directed toward the posterior (Video S7).

Altogether, our results strongly suggest that formation of cortical blebs on the side of the furrow locally releases pressure and induces a cytoplasmic flow toward low pressure areas. Because the embryo is confined in its eggshell, the flow of cytoplasmic material must then be compensated through the repositioning of the furrow in the opposite direction. It is thus very likely that cortical blebbing and the subsequent cytoplasmic flow contribute to furrow repositioning during ring closure (Figure 7E).

DISCUSSION

Our work shows that DNA segregation defects due to cytokinetic furrow mispositioning in one-cell *C. elegans* embryos can be corrected during late mitosis. This so far uncharacterized correction mechanism relies on opposite cytokinetic furrow and nucleus displacements and is accompanied by cortical blebbing and cytoplasmic flow. This mechanism is not specific to *ani-1;pig-1* embryos and is, for instance, also observed when the Rho GAPs RGA-3/4 are depleted or when relative spindle/furrow mispositioning is induced upon centrosome ablation.

Myosin plays a crucial role in the regulation of the different facets of the correction process. First, the different contractility of the anterior and posterior cortices exerts a pulling force on the nascent cytokinetic ring, oriented toward the more contractile end. In control embryos, this differential contractility remains low and is therefore not sufficient to displace significantly the furrow from the location imposed by mitotic spindle signaling (Figure 7E). On the contrary, the strong increase of anterior myosin levels in anaphase *ani-1;pig-1* embryos explains the initial shift of the nascent furrow toward the anterior cortex. The subsequent decrease of anterior myosin then slows down this movement. It can, however, not explain the repositioning of the furrow toward the posterior. Moreover, as the ring closes, the moving furrow behaves like a mechanical piston, which

Figure 7. Furrow Repositioning Is Accompanied by Cortical Blebbing and Cytoplasmic Flow

(A) Formation of cortical blebs on the anterior and posterior poles (t = 128 s; arrows) and on the anterior side of the cytokinetic furrow (t = 300 s; arrowheads) in an *ani-1(RNAi);pig-1(gm344)* embryo. Scale bars, 10 μm.

(B) Presence of blebs at the posterior and anterior poles of the embryos precedes the formation of blebs close to the furrow. Time points were collected from 15 different embryos.

(C and D) Distribution of velocity vector intensities measured by PIV. Only embryos with corrected DNA segregation defects and not showing furrow regression during flow measurement were analyzed. Error bars represent SD.

(E) Model of the mechanisms driving furrow and nuclear positions in wild-type and *ani-1(RNAi);pig-1(gm344)* embryos. In wild-type embryos, the differential contractility between the anterior and posterior cortices is not sufficient to displace the furrow from the location imposed by mitotic spindle signaling. In *ani-1(RNAi);pig-1(gm344)* embryos, the strong increase of anterior myosin levels exerts a pulling force on the nascent cytokinetic ring, moving it toward the anterior cortex. The subsequent decrease of anterior myosin and cortical expansion due to blebbing reposition the nascent furrow. Once the furrow closes, cortical blebbing and the ensuing cytoplasmic flow drive the opposite displacement of the cytokinetic furrow and the anterior nucleus. Microtubules also contribute to the correction process by pulling on the anterior nucleus.

See also Figure S2 and Videos S10, S11, and S12.

needs to displace cytoplasm to allow its own motion. Our theoretical model indicates that this creates a drag force, which is too large to be overcome by cortical contractility. Cortical blebs provide additional mechanisms, which contribute to furrow displacement both before and during cytokinetic ring closure. Formation of cortical blebs on the anterior side of the furrow leads to cortical expansion and contributes to the repositioning of the nascent furrow both by relaxing the actomyosin cortex and displacing the base of the furrow. A "depressuring effect" of cortical blebs and the subsequent cytoplasmic flow next displace the furrow when the cytokinetic ring closes (Figure 7E).

Our findings are reminiscent of what has previously been described in unconfined cultured cells [20]. Indeed, we find that excessive polar myosin contractility favors the risk of aneuploidy by mispositioning the cytokinetic furrow, and cortical blebbing, which occurs in response to excessive cortical tension, contributes to the repositioning of the furrow, thereby helping to restore euploidy. In isolated cultured cells, variations in cortical tension induce changes in the Laplace force, which play a central role in cell shape regulation [20]. However, contrary to cultured cells, the one-cell *C. elegans* embryo is confined in a rigid eggshell. This restrains changes in cell curvature and creates a reaction force that buffers changes in the Laplace force. Confinement thus creates geometrical constraints that modulate the effects of cortical tension and the mechanisms involved in furrow repositioning. Moreover, our work highlights a new role for cortical blebs. Previous work suggested that blebs help providing more cortical surface and relaxing excessive pressure to stabilize cell shape during cell division [20]. Cortical expansion induced by cortical blebs also corrects spindle mispositioning in anaphase HeLa cells [24]. Those effects are similar to the effect of cortical blebs that we observe at the beginning of furrow ingression. However, we find that cortical blebs can also regulate the volume of each daughter cell through their depressuring effect when the dividing cell is in a confined environment. Intriguingly, cortical blebs also lead to the appearance of a cytoplasmic flow during correction of spindle mispositioning in anaphase HeLa cells [24]. Whether this flow is also involved in the correction of spindle mispositioning would require further investigation.

In addition to its role in furrow repositioning, we found that myosin also regulates nuclear displacement. Notably, the anteriorly directed movement of the nucleus and the flow of cytoplasmic particles occur simultaneously in *ani-1;pig-1* embryos. It is thus very likely that the cytoplasmic flow exerts a drag force on the anterior DNA to drive it through the cytokinetic ring. Remarkably, it was recently shown that regulation of myosin II activity is critical for proper nuclear movement during the cleavage divisions of *Drosophila* embryos. This effect of myosin also involves the regulation of cortical contractility and the appearance of cytoplasmic flows [26]. Finally, a supplementary and redundant mechanism based on nuclear/microtubule interactions also drives nuclear displacement—probably through the pulling of the nucleus by microtubules. The existence of these two mechanisms controlling nuclear displacement together with the coordination of furrow and nuclear displacements by the cytoplasmic flow is likely to increase the efficiency of DNA segregation defect correction (Figure 7E).

In conclusion, our work shows that dividing cells in a confined environment, as are most cells inside tissues, can benefit from a combination of rescue mechanisms, which prevents the polar actomyosin-driven destabilization of cytokinesis and can restore euploidy.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.09.013.

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AUTHOR CONTRIBUTIONS

A.P. designed experiments with input from G.M. A.P. and M.J. performed experiments. A.P., M.J., and J.E. analyzed the data. J.E. performed biophysical modeling. A.P., J.E., and G.M. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
E. coli: OP50	Caenorhabditis Genetics Center	RRID:WB-STRAIN:OP50
ani-1 RNAi bacterial clone	BioScience	Clone III-6E18
rga-3 RNAi bacterial clone	BioScience	Clone V-4K14
npp-8 RNAi bacterial clone	BioScience	Clone IV-8D03
Experimental Models: Organisms/Strains		
C. elegans strain: *pie-1p::GFP::TBA-2(ltIs25); pie-1p::GFP::H2B(oxIs279)*	Delattre's lab [17]	ANA058
C. elegans strain: *mex-5p::SPD-1::GFP(adeIs1); pie-1p::mCherry::HIS-58 (ltIs37); pie-1p::GFP::PH(PLC1delta1)(ltIs38)*	Delattre's lab [17]	ANA071
C. elegans strain: *pie-1p::mCherry::HIS-58 (ltIs37); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL160
C. elegans strain: *pig-1(gm344)*	This study	FL178
C. elegans strain: *TBA-2::YFP(ddIs15) nmy-2(ne1490)*	This study	FL185
C. elegans strain: *pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL188
C. elegans strain: *TBA-2::YFP(ddIs15) nmy-2(ne1490); pig-1(gm344)*	This study	FL191
C. elegans strain: *dhc-1(or195); pig-1(gm344) pie-1p::mCherry::HIS-58 (ltIs37); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL197
C. elegans strain: *emr-1p::EMR-1::mCherry(bqSi142); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL201
C. elegans strain: *emr-1p::EMR-1::mCherry(bqSi142); pig-1(gm344); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL202
C. elegans strain: *zyg-12(or577); pie-1p::mCherry::HIS-58(ltIs37); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL207
C. elegans strain: *zyg-12(or577); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL208
C. elegans strain: *spd-1(oj5); pie-1p::mCherry::HIS-58(ltIs37); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL209
C. elegans strain: *TBA-2::YFP(ddIs15); pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL212
C. elegans strain: *nmy-2(ne1490); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL213
C. elegans strain: *pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL214
C. elegans strain: *TBA-2::YFP(ddIs15); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL216
C. elegans strain: *TBA-2::YFP(ddIs15); zyg-12(or577); pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL217
C. elegans strain: *TBA-2::YFP(ddIs15); zyg-12(or577); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL218
C. elegans strain: *pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37); pie-1p::GFP::PH(PLC1delta1)(ltIs38)*	This study	FL220
C. elegans strain: *nmy-2(ne1490); pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL221
C. elegans strain: *spd-1(oj5); pig-1(gm344 pie-1p::mCherry::HIS-58 (ltIs37); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL223
C. elegans strain: *nmy-2(ne1490); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37); VIT-2::GFP(pwIs23)*	This study	FL225
C. elegans strain: *nmy-2(ne1490);); zyg-12(or577); pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL226

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
C. elegans strain: *pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37); VIT-2::GFP(pwIs23)*	This study	FL227
C. elegans strain: *pie-1p::mCherry::HIS-58(ltIs37); VIT-2::GFP(pwIs23)*	This study	FL228
C. elegans strain: *zyg-12(or577); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL229
C. elegans strain: *nmy-2(ne1490);zyg-12(or577); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL230
C. elegans strain: *zyg-12(or577); pie-1p::mCherry::HIS-58(ltIs37)*	This study	FL232
C. elegans strain: *nmy-2(ne1490); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37); pie-1p::GFP::PH(PLC1delta1)(ltIs38)*	This study	FL233
C. elegans strain: *pie-1p::GFP::TBA-2(ltIs25); pie-1p::GFP::H2B(oxIs279); nmy-2::NMY-2::GFP(zuIs45)*	This study	FL235
C. elegans strain: *mex-5p::SPD-1::GFP(adeIs1); pig-1(gm344) pie-1p::mCherry::HIS-58(ltIs37); pie-1p::GFP::PH(PLC1delta1)(ltIs38)*	This study	FL256
unc-119(od3); pie-1p::mCherry::HIS-58 (ltIs37)	Caenorhabditis Genetics Center	OD56
Recombinant DNA		
Plasmid L4440	Addgene	Addgene #1654
Software and Algorithms		
ImageJ (https://imagej.net/Welcome)	NIH Image	RRID:SCR_003070
Fiji (http://fiji.sc/)	NIH Image	RRID:SCR_002285
Zen Black	Zeiss	n/a
Imaris	Bitplane	RRID:SCR_007370
MATLAB (https://www.mathworks.com/products/matlab.html)	MathWorks	RRID:SCR_001622
Excel (https://www.microsoft.com/en-gb/)	Microsoft	RRID:SCR_016137
R Project for Statistical Computing (https://www.r-project.org/)	[27]	RRID:SCR_001905

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for *C. elegans* strains generated in this study should be directed to and will be fulfilled by the Lead Contact, Anne Pacquelet (anne.pacquelet@univ-rennes1.fr).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Worm strains and culture

C. elegans strains were grown on agar plates containing NGM growth media and seeded with *E. coli* (OP50 strain). Worms were maintained at 20°C, except strains carrying thermosensitive mutations, which were kept at 15°C. A list of the strains used in this study and conditions used for each experiment are detailed in the Key Resources Table and in Table S1.

METHOD DETAILS

RNAi conditions

RNAi was performed by feeding worms with RNAi clones from the Ahringer-Source Bioscience library [28] on 1 mM or 3 mM IPTG plates. L4440 was used as a control in all RNAi experiments. The clone used for *rga-3/4* RNAi is primarily directed against *rga-3* but also targets *rga-4* [29]. In the case of double RNAi experiments, RNAi cultures were initially grown separately; *ani-1* RNAi was then mixed either with control (L4440) or *npp-8* cultures (1:1) before seeding plates. Conditions used for each experiment are detailed in the Table S1.

Time-lapse recordings

Embryos were mounted on 2% agarose pads in a drop of M9 medium. In most experiments, temperature was controlled using a temperature-control platform, which enables temperature shifts within 10 s (CherryTemp, Cherry Biotech). Conditions used for each experiment are detailed in Table S1.

To analyze the behavior of the central spindle during the correction process, we first recorded the SPD-1::GFP signal during the whole division process. This experiment was used to quantify the number of embryos in which the central spindle already started to

disassemble at the time of correction. To further characterize what happens to central spindle remnants, we first imaged embryos with DIC during the first steps of the division and then recorded the SPD-1::GFP signal at the end of the correction process. This allowed us to reduce photobleaching and thereby to better observe and quantify central spindle remnant behavior when the cytokinetic ring closes.

Myosin inactivation was performed using a *nmy-2* thermosensitive allele. Previous work has shown that myosin inactivation leads to furrow regression and cytokinesis failure [30]. However, the effect of myosin inactivation is not immediate and the cytokinetic furrow can continue its ingression for several minutes before it starts regressing [30]. Based on these observations, we used temperature shift conditions (see Table S1) under which most embryos did not show furrow regression during the time course of experiments. One embryo showed furrow regression during cytoplasmic flow measurement and was excluded from our analysis (Figure 7D). Embryos in which furrow regression occurred at later time points than the ones used in our quantifications were kept for analysis.

Chromosome imaging

Chromosomes labeled with mCherry::HIS-58 were imaged with a LSM 880 microscope (Zeiss), using a 63x oil-objective and the Fast Airy Scan module to obtain superresolution images. 480x480 pixel images were obtained at zoom 5.7, and speed 6.5 (155 ms per image). Z stack were acquired with 300 nm steps.

Centrosome ablations

Centrosome laser ablations were performed on a LSM 880 microscope (Zeiss) with a 355 nm pulsed-UV laser (Rapp Optoelectronic, 40% laser + ND1 filter, 10 iterations / ablation) and a 40x water-objective. A circular area encompassing the anterior or posterior centrosome was defined for each ablation. Ablation was performed during early anaphase. Images were recorded every 2 s.

Cortical ablations

Cortical laser ablations were performed on a LSM 880 microscope (Zeiss) with a 800 nm biphoton laser (Mai Tai, 50% power, 10 iterations / ablation) and a 63x oil-objective. A 0.17 × 6 μm rectangle perpendicular to the antero-posterior axis was used to define the cut area. The cut area was positioned on the anterior or posterior cortex, approximately at equal distance from the pole and the cytokinetic furrow. Ablations were performed during anaphase (1 min 10 s to 1 min 50 s after anaphase onset) or at the end of the correction process (5 to 8 min after anaphase onset). Images were recorded every 0.5 s.

QUANTIFICATION AND STATISTICAL ANALYSIS

Image analysis and quantifications

Images were assembled for illustration using ImageJ and Fiji. All embryos are oriented with anterior pole to the left. Position of the nuclei and of the furrow tip during ingression was tracked manually in ImageJ. Furrow tip position was measured starting at furrow ingression onset. Position is expressed as a percentage of embryo length, 0 and 100 correspond to the anterior and posterior pole of the embryos, respectively. Nuclei position tracking was generally started 100 s after anaphase onset, when DNA separation due to anaphase spindle elongation is mostly finished. In Figure S2A, we started to measure anterior nucleus displacement at anaphase onset to be able to evaluate the effect of central spindle disassembly on DNA movement during anaphase. Nuclear displacement is expressed as a percentage of embryo length, positive and negative values correspond to a displacement toward the posterior and anterior pole of the embryos, respectively. Variations in experimental settings, in particular temperature shifts (see Table S1), explain the slight variations of nuclei or furrow dynamics observed between experiments for the same genotype.

Images of chromosomes obtained with the Fast Airy Scan module were processed to enhance resolution with the ZenBlack software. 3D reconstructions were then performed with the Imaris software.

Quantifications of myosin intensities (Figure 5A) were performed using ImageJ. A region corresponding to the most anterior or posterior 10%–15% of the embryo cortex was defined and NMY-2::GFP cortical intensity was measured in a central confocal plane from anaphase onset to cytokinesis ring closure.

Furrow speed (Figures 6 and 7B) was quantified based on furrow position by a LOESS regression over 4 time points. Time points were collected from 15 different *ani-1;pig-1* embryos with corrected DNA segregation defects. Positive and negative values of furrow velocity correspond to a displacement toward the posterior and anterior pole of the embryos, respectively. In Figure 6, analysis was restricted to a time window (300 s to 450 s after anaphase onset), during which both nascent (ring diameter > 15 μm) and closing (ring diameter < 15 μm) furrows coexist. All analyzed time points are shown on plots, t test and regression test are performed on subsamples for which velocities are calculated from disjoint time windows.

Cortical tension measurement

Analysis of cortical ablation experiments was performed by drawing an ellipse fitting the opening area (see Figure 5B) as previously described [19, 31]). Opening kinetics was analyzed by measuring the increase of the ellipse minimum axis length following ablation. Cortical tension has been shown to be proportional to the velocity of cortex displacement which immediately follows ablation [18]. Here, the initial opening velocity was calculated by measuring the increase of the ellipse minimum axis between the first (0.5 s) and third (1.5 s) images taken after laser ablation.

Flow measurement

Particle Imaging Velocimetry (PIV) was used to measure cytoplasmic flows in strains expressing VIT-2::GFP to label yolk particles. PIV analysis was performed with the MATLAB MPIV toolbox and the Minimum Quadric Differences algorithm. Images were analyzed using interrogation windows of 32*32 pixels with 50% overlap. Measurements were performed in the furrow region during the last 100 s of furrow closure. Frames in which nucleus displacement may have influenced the calculation of cytoplasmic flow were excluded from the analysis. Intensities of the velocity vectors along the embryo antero-posterior axis were calculated; positive and negative values correspond to a flow toward the posterior and anterior pole of the embryos, respectively. The distribution of the measured vector intensities was then determined for each embryo and finally averaged among all observed embryos.

Modeling forces acting on furrow position

Assuming that cortical myosin results in a force F acting on the furrow and proportional to the difference of myosin concentration between anterior and posterior cortices, ΔM, this force needs to be balanced with forces resisting furrow displacement (Figure 6B). Part of these forces are due to the resistance of the cortex itself (F_{cort}), this can be assumed proportional to furrow velocity v, and can be modeled as $-\alpha v$ (Figure 6B). Another resistance to furrow motion is the drag force exerted by the cytoplasm (F_{cyt}), which, due to confinement, needs to flow through the cytokinetic ring in order to allow displacement of the furrow in the opposite direction (Figure 6B). This drag force will be equal to the dynamic pressure difference on each side of the furrow times the section area of the furrow, R^2–r^2, where R is the cell radius and r the cytokinetic ring radius. Assuming a Poiseuille flow, the dynamic pressure drop will scale as vR^2/r^4. The cytoplasmic drag force can thus be modeled as $-\beta (R^2-r^2) vR^2/r^4$. Finally, the equilibrium between the force exerted by myosin and the forces resisting furrow displacement can be expressed as $F \sim \alpha v + \beta (R^2-r^2) vR^2/r^4$. Hence, the furrow velocity will scale as $v \sim \Delta M\, r^4 /(\alpha r^4 + \beta R^2(R^2-r^2))$, which we plot in Figures 6C and 6D. One aspect of the data not captured in this model is a drift velocity seemingly independent from both myosin and furrow opening, and which may be ascribed to another biological control of furrow positioning (e.g., local signaling of myosin recruitment and/or effect of blebs and cytoplasmic flow) adding up to the balance. When adding a constant drift velocity to the above model, adjusting the two parameters α and β allows to fit experimental datasets in the four curves shown in Figures 6C and 6D. In those conditions, we find that $\alpha/\beta = 0.03$, indicating that cytoplasmic drag dominates other friction sources.

Modeling bleb appearance probability

Following [23], we write the variations of free energy entailed by the nucleation of a bleb of height δ by the detachment from the cortex of a patch of membrane of radius a:

$$\Delta U = Ja^2 - \Delta p a^2 \delta + T\delta^2$$

where J is the membrane-cytoskeleton energy of adhesion, Δp the pressure jump across membrane, T the membrane tension; we have neglected bending energy.

In [23], the pressure jump is considered a uniform constant, since the cell is spherical and unconfined. In the present case, the confinement force adds up with the Laplace force and imposes a local curvature which is equal to the eggshell one (in locations where membrane is aposed to the eggshell) or larger than the eggshell one (at the furrow and at the poles when not in contact with eggshell). In these regions we have $\Delta p = 2\sigma/R$, where σ is the cortical tension and R the local characteristic radius of curvature. The lowest energy barrier to nucleate a bleb is at the saddle-point $\delta^* = JR/\sigma$, $a^* = (2TJ)^{1/2}R/\sigma$, and is $\Delta U = TJR/\sigma$. For a given cortical tension, the regions with smaller R (more curved) are thus more likely to nucleate blebs.

Statistical analysis

Statistical analysis and graphic representations were performed with Excel and RStudio softwares. n corresponds to the number of analyzed embryos except in Figure 6 where n corresponds to the number of time points used to measure furrow velocity (times points collected from 15 different embryos). Error bars represent standard deviation. Boxplots from Figures 5C and 6A: central rectangles span the first to the third quartile of the data, middle lines correspond to median values, lower and higher whiskers to the minimum and maximum value, respectively. Figure 5C: two-tailed non parametric Wilcoxon tests were performed (data did not pass the Shapiro normality test). Figure 6A: a two-tailed unpaired Student's t test was performed (data passed the Shapiro normality tests and sample variance was equal).

DATA AND CODE AVAILABILITY

This study did not generate any unique datasets or code.

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Supplemental Information

Simultaneous Regulation of Cytokinetic Furrow and Nucleus Positions by Cortical Tension Contributes to Proper DNA Segregation during Late Mitosis

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Figure S1. Correction of DNA segregation defects involves both nuclear and furrow displacement. Related to Figure 2. A. Average displacement of the anterior nucleus in control and *rga-3/4(RNAi)* embryos. **B.** Average furrow tip position in control and *rga-3/4(RNAi)* embryos. **C-D.** Average displacement of the anterior (C) and posterior (D) nuclei in control embryos and in embryos in which the anterior (C) or posterior (D) centrosome was ablated during early anaphase. **E.** Average furrow tip position in control embryos and in embryos in which the anterior or posterior centrosome was ablated during early anaphase. In A-E, only embryos with corrected DNA segregation defects were analysed. Error bars represent standard deviation.

Figure S2. Central spindle and nuclear envelop formation are not required for furrow displacement. Related to Figure 7. A. Average displacement of the anterior nucleus in control and *spd-1(oj5)* embryos. In *spd-1(oj5)* embryos, anterior nucleus displacement is accelerated upon temperature shift, indicating efficient central spindle disruption. **B.** Average furrow tip position in *ani-1(RNAi);pig-1(gm344)* and *spd-1(oj5);ani-1(RNAi);pig-1(gm344)* embryos. Only embryos with corrected DNA segregation defects were analysed. All displayed cortical blebbing on the anterior side of the furrow. Rescue efficiency was similar for *ani-1(RNAi);pig-1(gm344)* and *spd-1(oj5);ani-1(RNAi);pig-1(gm344)* embryos (75% and 73% rescue, respectively). **C.** *ani-1(RNAi);pig-1(gm344)* and *ani-1(RNAi);pig-1(gm344) npp-8(RNAi)* embryos expressing NMY-2::GFP (magenta) and EMR-1::mCherry (yellow). No nuclear envelope reformation is observed when *npp-8* is depleted. Scale bar: 10 µm. **D.** Average furrow tip position in *ani-1(RNAi);pig-1(gm344)* and *ani-1(RNAi);pig-1(gm344) npp-8(RNAi)* embryos. Only embryos with corrected DNA segregation defects were analysed. *ani-1(RNAi);pig-1(gm344)* and *ani-1(RNAi);pig-1(gm344) npp-8(RNAi)* embryos present similar frequencies of cortical blebbing on the anterior side of the furrow (90% and 83% of rescued embryos, respectively) and similar rescue efficiency (91% and 100% rescue, respectively). Error bars represent standard deviation. See also video S10.

Figure	Strains used	RNAi	Incubation Conditions	Recording Temperature
Figure 1A Video S1-2	FL160 FL188	1 mM IPTG, L4440/ani-1	20°C, 30-40h	23°C (Cherry Temp)
Figure 1B Video S3	FL201 FL202	1 mM IPTG, L4440/ani-1	20°C, 30-40h	23°C (Cherry Temp)
Video S4	FL212 FL216	1 mM IPTG, L4440/ani-1	15°C, 15-20h	15°C, shift at 24°C at anaphase onset (Cherry Temp)
Figure 1C Video S5	ANA071 FL256	1 mM IPTG, L4440/ani-1	20°C, 30-40h	23°C (Cherry Temp)
Figure 1D Video S6	FL160	3 mM IPTG, L4440/rga-3/4	25°C, 48h	23°C (Cherry Temp)
Figure 1E-F Video S7	FL235	OP50	20°C, 24h	20°C (Cherry Temp)
Figure 2A-D	FL160 FL188	1 mM IPTG, L4440/ani-1	20°C, 30-40h	23°C (Cherry Temp)
Figure 3A	FL212 FL216 FL217 FL218	1 mM IPTG, L4440/ani-1	15°C, 15-20h	15°C, shift at 24°C at anaphase onset (Cherry Temp)
Figure 3B	FL160 FL207	1 mM IPTG, L4440	15°C, 15-20h	15°C, shift at 24°C at anaphase onset (Cherry Temp)
Figure 3C	FL188 FL208	1 mM IPTG, ani-1	15°C, 15-20h	15°C, shift at 24°C at anaphase onset (Cherry Temp)
Figure 4A	OD56 FL221	1 mM IPTG, L4440	15°C, 20-25h	18°C, shift at 25.5°C 220 sec after anaphase onset (Cherry Temp)
Figure 4B	FL214 FL213	1 mM IPTG, ani-1	15°C, 20-25h	18°C, shift at 25.5°C 220 sec after anaphase onset (Cherry Temp)
Figure 4C	OD56 FL221 FL226 FL232	1 mM IPTG, L4440	15°C, 20-25h	18°C, shift at 25.5°C 220 sec after anaphase onset (Cherry Temp)
Figure 4D	FL214 FL213 FL229 FL230	1 mM IPTG, ani-1	15°C, 20-25h	18°C, shift at 25.5°C 220 sec after anaphase onset (Cherry Temp)
Figure 4E-F Video S8	FL220 FL233	1 mM IPTG, ani-1	15°C, 20-25h	18°C, shift at 25.5°C 220 sec after anaphase onset (Cherry Temp)
Figure 5A	FL160 FL188	1 mM IPTG, L4440/ani-1	20°C, 30-40h	23°C (Cherry Temp)
Figure 5B-C Video S9	FL160 FL188	1 mM IPTG, L4440/ani-1	20°C, 20-25h	Room temperature (~19-21°C)
Figure 6A,C-D	FL188	1 mM IPTG, ani-1	20°C, 30-40h	23°C (Cherry Temp)
Figure 7A-B Video S11	FL188	1 mM IPTG, ani-1	20°C, 30-40h	23°C (Cherry Temp)
Figure 7C, Video S12	FL227 FL228	1 mM IPTG, L4440/ani-1	20°C, 30-40h	23°C (Cherry Temp)
Figure 7D	FL227 FL225	1 mM IPTG, ani-1	15°C, 20-25h	18°C, shift at 25°C at anaphase onset (Cherry Temp)
Figure S1A-B	FL160	3 mM IPTG, L4440/rga-3/4	25°C, 48h	23°C (Cherry Temp)
Figure S1C-E	FL235	OP50	20°C, 24h	20°C (Cherry Temp)
Figure S2A	FL160 FL209	OP50	15°C, 24h	15°C, shift at 25°C 60 sec after anaphase onset (Cherry Temp)
Figure S2B Video S10A	FL188 FL223	1 mM IPTG, ani-1	15°C, 15-20h	15°C, shift at 25°C 60 sec after anaphase onset (Cherry Temp)
Figure S2C	FL202	1 mM IPTG, ani-1+L4440/ ani-1+npp-8	20°C, 30-40h	23°C (Cherry Temp)
Figure S2D Video S10B	FL188	1 mM IPTG, ani-1+L4440/ ani-1+npp-8	20°C, 30-40h	23°C (Cherry Temp)

Table S1. RNAi and temperature conditions used for each experiment. Related to STAR Methods