

1 Article

2 Uniaxial loading induces a scalable switch in 3 cortical actomyosin flow polarization and reveals 4 mechanosensitive regulation of cytokinesis

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14 **Abstract:** During animal development, it is crucial that cells can sense and adapt to mechanical
15 forces from their environment. Ultimately, these forces are transduced through the actomyosin
16 cortex. How the cortex can simultaneously respond to and create forces during cytokinesis is not
17 well understood. Here we show that under mechanical stress, cortical actomyosin flow switches its
18 polarization during cytokinesis in the *C. elegans* embryo. In unstressed embryos, longitudinal cortical
19 flows contribute to contractile ring formation, while rotational cortical flow is additionally induced in
20 uniaxially loaded embryos. Rotational cortical flow is required for the redistribution of the actomyosin
21 cortex in loaded embryos. Rupture of longitudinally aligned cortical fibers during cortex rotation
22 releases tension, initiates orthogonal longitudinal flow and thereby contributes to furrowing in loaded
23 embryos. A targeted screen for factors required for rotational flow revealed that actomyosin
24 regulators involved in RhoA regulation, cortical polarity and chirality are all required for rotational
25 flow and become essential for cytokinesis under mechanical stress. In sum, our findings extend the
26 current framework of mechanical stress response during cell division and show scaling of orthogonal
27 cortical flows to the amount of mechanical stress.

28 **Keywords:** actomyosin, cortical flow, embryo, cytokinesis, mechanosensitivity, *C. elegans*

29

30 1. Introduction

31 While cells remodel their actomyosin cortex during cell division, they have to simultaneously
32 integrate chemical and mechanical stimuli from the local environment to ensure successful
33 cytokinesis. In order for cytokinesis to be robust yet responsive to extrinsic stimuli, three fundamental

34 control principles have evolved, (a) redundancy [1], (b) mechanosensitivity [2], and (c)
35 positive/negative feedback [3]. Examples for these control principles are (a) partially redundant
36 molecular motors, actin cross-linkers, and membrane trafficking pathways, (b) molecular
37 mechanosensitivity of integral cytokinesis proteins such as non-muscle myosin II, α -actinin, and
38 filamin [4, 5], and (c) RhoA-dependent self-enhancing local assembly and contraction of actomyosin
39 as well as astral microtubule-based suppression of actomyosin contractility [6], which both are
40 required to generate cortical contractile actomyosin flow during cell division.

41 Work in the last decade has led to the identification of the main mechanosensory system that
42 operates during cell division. The core of this system is non-muscle myosin II, which can amplify
43 sensed forces through its lever arm [4], and which shows mechanosensitive accumulation through
44 cooperative binding to F-actin [7]. This results in a positive feedback on the assembly of non-muscle
45 myosin II bipolar thick filaments [2]. In addition, for other mechanosensitive proteins, two conserved
46 and distinct modes of force-dependent accumulation have been recently demonstrated, a rapid,
47 diffusion-based mode due to tensile forces increasing the lifetime of the F-actin bound state (catch
48 bonding), and a slower mode due to non-muscle myosin-II-dependent cortical flow [5]. The latter
49 serves as an additional biomechanical positive feedback, strongly suggesting that cytokinesis control
50 principles operate interdependently.

51 Among the control principles mentioned above, feedback during cytokinesis crucially depends
52 on spindle microtubules since they constitute key modulators of cortical contractility [3]. Wolpert's and
53 Rappaport's classical experiments have led to the astral relaxation model in which astral microtubules
54 soften the polar cortex (by suppressing actomyosin contractility) while the equatorial cortex stiffens
55 during division. Very recently, it was shown that polar clearing of contractile ring components requires
56 TPXL-1-dependent cortical activation of Aurora A [6].

57 Moreover, the ability of the actomyosin cortex to contract and to generate long-range flow not
58 only depends on the non-muscle myosin II motor protein but also on the spatial organization of actin
59 filaments (polarization, branching, bundling) and their connectivity (degree and density of crosslinks)
60 [8]. Recently, it has been shown that non-muscle myosin II-powered cortical actomyosin flow leads
61 to contractile ring formation by alignment of actin filaments in the *C. elegans* one cell embryo due to
62 compression of the gel-like cortex in the equatorial region [9]. This suggests that non-muscle myosin
63 II-dependent flow indeed re-organizes the cortical actin network during cytokinesis as has been
64 proposed previously [10]. It also suggests that self-enhancing feedback mechanisms are generally
65 involved in self-organization of the cytokinetic cortex and involved particularly in forming the
66 contractile ring. However, furrow formation due to coupling of cortical flow and actin alignment
67 apparently only enhances but is not required for cytokinetic ring formation [9]. Moreover, actomyosin
68 dynamics and architecture as well as cortical contractile actomyosin flows seem to variably contribute
69 to cytokinesis progression when comparing different systems [3, 9, 11-17].

70 Furthermore, cortical contractile actomyosin flows in the *C. elegans* embryo are strictly
71 dependent on RhoA activation and do not only cause translation of the cortex (like during
72 anteroposterior polarization) [18] but also its rotation immediately before division of the two-cell
73 embryo [19, 20] and during chiral symmetry breaking [21, 22]. Importantly, whole cell cortex rotation
74 occurs during cell division when cytokinetic actomyosin nodes have formed. This mesoscopic
75 rotational flow is most likely due to generation of torque at the molecular level: showed using in vitro
76 assays that during myosin-driven sliding of actin filaments, a torque component can be observed [23]
77 that induces a right-handed rotation of an actin filament around its long axis with one revolution per
78 sliding distance of approximately 1 μm [24]. Similar rotation or twirling of actin filaments have been
79 confirmed in more recent reports [25, 26]. Although the molecular origin of torque in actomyosin
80 dynamics is well understood, how torque leads to coordinated cortical rotational dynamics remains
81 unexplored.

82 The division of the one-cell *C. elegans* embryo represents a highly suitable model to
83 quantitatively dissect spatiotemporal dynamics of the cytokinetic actomyosin cortex and to uncover
84 underlying regulatory principles [9, 18, 27-33]. Previously, it has been shown through highly
85 informative ablation experiments of the contractile ring that it is able to repair requiring an increased
86 tension in the ring and reduced cortical tension in the vicinity [34]. This strongly suggests that global
87 cortical dynamics respond to mechanical stress during cytokinesis that might require differential
88 regulation of cytokinetic cortical flow. Here, we quantitatively describe the biomechanical responses
89 to a different type of stress, loading. For this, progressive uniaxial compression is used in the form of
90 the classical parallel plate assay [35-37]. With this mechanical manipulation, it is possible to
91 demonstrate that a recently uncovered type of polarizing cortical flow, rotational flow [19-21], is
92 mechanoresponsive, scales to the amount of load and contributes to successful division when cells
93 experience mechanical stress. Anisotropic mechanosensitive accumulation of non-muscle myosin II
94 suggests that cortical stress is similarly anisotropic in uniaxially loaded embryos as has been recently
95 shown for uniaxially loaded mammalian cells [38]. Importantly, rotational flow leads to a re-
96 arrangement of the anisotropically distributed of actomyosin in loaded embryos. Cortical rotation
97 requires a broad set of actomyosin regulators of which several only become essential for cytokinesis
98 under mechanical stress. Hence, our data suggests that the main biological role of cortical flow re-
99 polarization during cytokinesis lies in balancing spatial and tension anisotropies in the cortex and that
100 converging longitudinal flow is required for successful furrowing in mechanically stressed embryos.
101

102 **2. Materials and Methods**

103 *2.1 Worm Strains, maintenance and RNA interference*

104 Integrated *C. elegans* strains expressing lifeact-fusion proteins expressed from pie-1 promoters
105 have been described elsewhere [39, 40]. Strains JJ1473 (zuls45), LP162 (*nmy-2(cp13)*), and
106 RW10223 (itls37; stls10226) were provided by the Caenorhabditis Genetics Center (CGC), which is
107 funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Strains were maintained
108 under standard conditions [41]. RNAi was performed by feeding using clones from commercially
109 available libraries [42, 43].
110

111 *2.2 Microscopy and laser ablation*

112 Embryo preparation and mounting has been described elsewhere [39, 44]. Mounting was
113 modified by using differently sized polystyrene (15 μ m, 20 μ m, 25 μ m; Polysciences, Hirschberg,
114 Germany) and polymethylmethacrylate spheres (12 μ m and 13.5 μ m, PolyAn, Berlin, Germany).
115 Microscopy was performed with a VisiScope spinning disk confocal microscope system (Visitron
116 Systems, Puchheim, Germany) based on a Leica DMI6000B inverted microscope, a Yokogawa CSU
117 X1 scan head, and a Hamamatsu ImagEM EM-CCD. All acquisitions were performed at 21°C–23°C
118 using a Leica HC PL APO 63 \times /1.4-0.6 oil objective. Cell cortex ablations were performed using a
119 pulsed 355 nm UV laser mounted on the same microscope. One ablation cycle was performed per
120 acquisition with a residence time per pixel of 3.5 ms. Acquisitions pre-and post-ablation were
121 performed with 200 ms intervals.
122

123 *2.3 Particle image velocimetry (PIV)*

124 PIV analysis was performed on maximum intensity projected images using a custom version of
125 PIVlab developed for MATLAB [44, 45]. This customized software is available from the authors upon
126 request. Specifically, two pass interrogation windows of 64x64 pixels and 32x32 pixels with 50%

127 overlap were used to map consecutive frames acquired at 2 s intervals. To align the biological time
128 of flow across embryos, we choose foci formation as starting point. To calculate vector maps,
129 correlation between subsequent windows was computed using fast Fourier transformation (FFT).

130

131 2.4 Quantification and kymograph representation of flow profiles

132 The flow profile for each time point was projected on the long axis of the embryo by dividing the
133 whole vector profile of the embryo into 13 bins and taking a mean along the short axis. A time course
134 profile or kymograph was obtained by averaging bin velocities for 5 embryos in each condition. For
135 visualization, heat maps were generated after applying cubic interpolation using a custom MATLAB
136 script. Variability between embryos for each condition was estimated by calculating standard error of
137 mean.

138

139 2.5 Measurements

140 NMY-2 and TBB-2 signal intensities, NMY-2 node number and size, NMY-2 filament contraction
141 rate of linearly organized NMY-2, cortical residence times of NMY-2 and lifeact, NMY-2 outward flow
142 velocities, spindle microtubule angles, as well as furrow asymmetry and anterior-posterior domain
143 sizes were manually measured in ImageJ using the built-in toolset. Cortical residence times were
144 measured from traces in kymographs or by tracking cortical structures in sequential frames of high-
145 resolution time lapse series. Longitudinal flow range was measured in the anterior domain by
146 extracting continuous tracks from PIV data that show velocities higher than 0.5 $\mu\text{m}/\text{min}$ and
147 normalizing them to embryo length. Cleavage success was manually quantified by inspecting time-
148 lapse microscopy data.

149 For shape parameter quantification of embryos in utero (Figure 3A), the embryo perimeter was
150 segmented using a custom MATLAB script by applying a median filter and thresholding. Circularity
151 was defined as $4\pi(\text{area}/\text{perimeter}^2)$.

152 Calculation of curvature to quantify blebbing (Figure 3G) was performed by segmentation of cell
153 boundaries using a custom MATLAB script. For each time point, the boundary at the anterior end of
154 the embryo was divided into 400 equidistant points. A circle was fit for each boundary point using this
155 point and two boundary points that were four points away. The local curvature was defined as
156 reciprocal of the radius of this fitted circle.

157 To establish that the *C. elegans* embryo follows Laplace's law (Figure 2A), sideview projections
158 of embryos were obtained by using a custom MATLAB script. Projected images were denoised
159 (Wiener filter) and the embryo's boundary was segmented by adaptive thresholding. For each point
160 on the boundary, a circle was fitted on three points with a spacing of 30 points. Curvature was defined
161 as the inverse of the radius of the fitted circle. Contact angles were measured based on segmented
162 boundaries.

163

164 3. Results

165 3.1 Convergent longitudinal flow polarizes cortical NMY-2

166 In order to establish an unbiased readout for cortical dynamics during cytokinesis, we performed
167 time-lapse microscopy with high spatiotemporal resolution of the first division in wild type (wt) *C.*
168 *elegans* embryos expressing NMY-2::GFP (a CRISPR/Cas9 edited GFP-fusion of an essential non-
169 muscle myosin II gene) [46]. This data (Figure 1A) was then subjected to quantitative analysis by
170 particle image velocimetry (PIV). PIV tracking revealed longitudinal cortical NMY-2 flows with opposite
171 direction, from anterior ($6\pm 0.05 \mu\text{m}/\text{min}$, $n = 5$) and posterior poles ($6.5\pm 0.09 \mu\text{m}/\text{min}$, $n = 5$) towards
172 the cell equator (Figure 1B, top panel; Video 1). Convergence of these flows at the equator leads to

173 the transformation of cortical NMY-2 nodes ($1.8 \pm 0.1 \mu\text{m}$ in diameter, $n = 25$) into parallel, linearly
174 organized NMY-2 ($0.25\text{-}0.5 \mu\text{m}$ in width and $3.5 \pm 0.6 \mu\text{m}$ in length, $n = 20$; Figure 1A,C), which first
175 form a narrow stripe ($6.8 \pm 0.07 \mu\text{m}$; $n = 5$) that subsequently becomes part of the incipient contractile
176 ring by alignment and bundling (Figure 1D; Video 1).

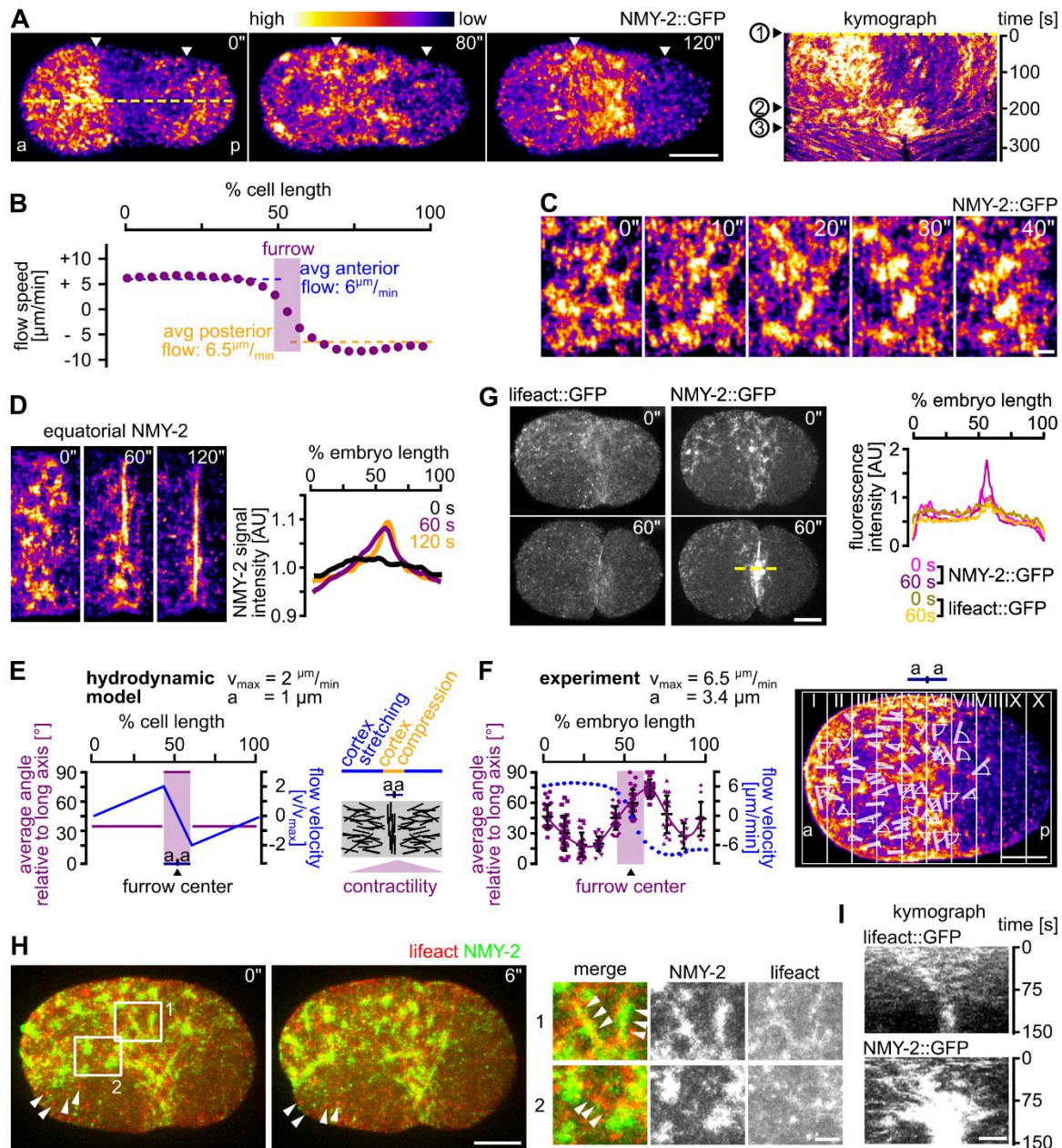
177 Previously, a physical model based on hydrodynamic active gel theory has explained formation
178 of the F-actin component of the contractile ring by cortical flow [9, 47]. In this model, opposing flows
179 that emerge at the poles and converge at the equator promote ordering of cortical actin filaments into
180 parallel bundles (Figure 1E). In agreement with the model, our analyses revealed similar flow velocity
181 profiles for NMY-2 and similar ordering during cortical flow (Figure 1F). Therefore, cortical flow not
182 only polarizes F-actin but also NMY-2, thereby promoting contractile ring formation from linearly
183 organized NMY-2 that undergoes bundling in the equatorial region (Figure 1E) [9]. Hence,
184 hydrodynamic active gel theory combined with PIV-based NMY-2 cortical flow analysis seem well
185 suited to investigate cytokinesis mechanics.

186 While analysis of NMY-2 foci dynamics during longitudinal flow revealed an average lifetime of
187 $29 \pm 2 \text{ s}$ ($n = 25$), analysis of F-actin (using lifeact::mCherry; [40]) shows that it does not concentrate
188 in cortical NMY-2 foci and forms much smaller, uniformly sized ($0.4 \pm 0.1 \mu\text{m}$; $n = 20$) and long-lived
189 ($124 \pm 48 \text{ s}$; $n = 20$) foci that do not undergo changes during cytokinesis (Figure 1G). Nevertheless,
190 NMY-2 decorates actin filaments shortly after onset of cytokinesis; while actin filaments disassemble
191 subsequently after around 15 s, linearly organized NMY-2 and NMY-2 foci have substantially longer
192 half-lives (Figure 1H). Consistent with F-actin showing faster cortical turnover, we also find that F-
193 actin shows slightly weaker longitudinal flow with a shorter range (0.3 ± 0.2 embryo lengths) when
194 compared to NMY-2 (0.6 ± 0.1 embryo lengths; Figure 1I). This difference is most likely due to long-
195 ranged flow requiring a certain degree of stable, filamentous network components. These kinetic
196 differences also seem to contribute to NMY-2 accumulating at the furrow while F-actin does not
197 accumulate at that site (Figure 1I). This is most striking during late cytokinesis where substantial
198 amounts of linearly organized NMY-2 still flow towards the future midbody while F-actin does not
199 show any recognizable flow at that stage (Figure 1F). These observations suggest - similar to what
200 has been recently found in mammalian tissue culture [48] - that non-muscle myosin II might also be
201 organized in aligned stacks in the *C. elegans* cortex that can span several micrometers and whose
202 turnover is independent of the turnover of actin filaments.

203

Figure 1

6 of 20



204

205 **Figure 1.** Longitudinal flow is organizes cortical NMY-2 during contractile ring formation. **(A)** Left:
 206 Maximum projected stills from time lapse microscopy of embryos expressing NMY-2::GFP. White
 207 arrow heads mark the boundaries of the anterior and posterior NMY-2 caps upon polarization. Right:
 208 Kymograph along the yellow dashed line in the leftmost panel. Numbers on the left refer to onset of
 209 cap formation (1), onset of NMY-2 cytokinetic foci formation (2), and start of furrow invagination (3).
 210 Scale bar = 10 μm . See also Video 1. **(B)** Average cortical NMY-2 flow velocity profile along the a-p
 211 axis generated from PIV data of 5 embryos over the time window of longitudinal flow (60 s). **(C)**
 212 Maximum projected stills from time lapse microscopy of the furrow region; scale bar = 2.5 μm . **(D)**
 213 Left: Stills from maximum projected embryos showing NMY-2 dynamics at the equatorial ring. Scale
 214 bar = 2.5 μm . Right: Normalized NMY-2::GFP signal intensities along the a-p axis in one-cell embryos.
 215 Intensity profiles at 0 s, 60 s and 120 s are represented by black, purple and yellow traces, respectively
 216 ($n = 5$ each). **(E)** Quantification of NMY-2 linear orientation. Left: Distribution of order parameter and
 217 flow velocity for a cylindrical system undergoing cytokinesis (see cartoon according to [47]). **(F)** Left:
 218 Measured angle and flow velocities along the a-p axis ($n = 5$). Right: Representative embryo with
 219 angles of linearly organized NMY-2 relative to the a-p-axis. **(G)** Left: Maximum projected stills from
 220 time lapse microscopy of representative wt embryos expressing either lifeact::mCherry or NMY-
 221 2::GFP. Scale bar = 10 μm . Right: Quantification of signal intensities from the embryos depicted in

222 the middle panel. **(H)** Left: Organization of NMY-2 and F-actin during onset of cytokinesis. Maximum
223 projected stills from time lapse microscopy of embryos expressing *lifeact::mCherry* and *NMY-2::GFP*.
224 White arrow heads mark persistent actin foci. Scale bar = 10 μm . Right: Enlarged cortical areas from
225 left panels showing localization of NMY-2 on actin filaments (1) and NMY-2 foci connected by actin
226 filaments (2). Scale bar = 2.5 μm . **(I)** Kymographs for *lifeact* (top) and NMY-2 (bottom) at the midbody
227 region (generated along the dashed yellow line in panel G). Scale bar = 2.5 μm .

228 3.2 Uniaxial loading counteracts longitudinal flows

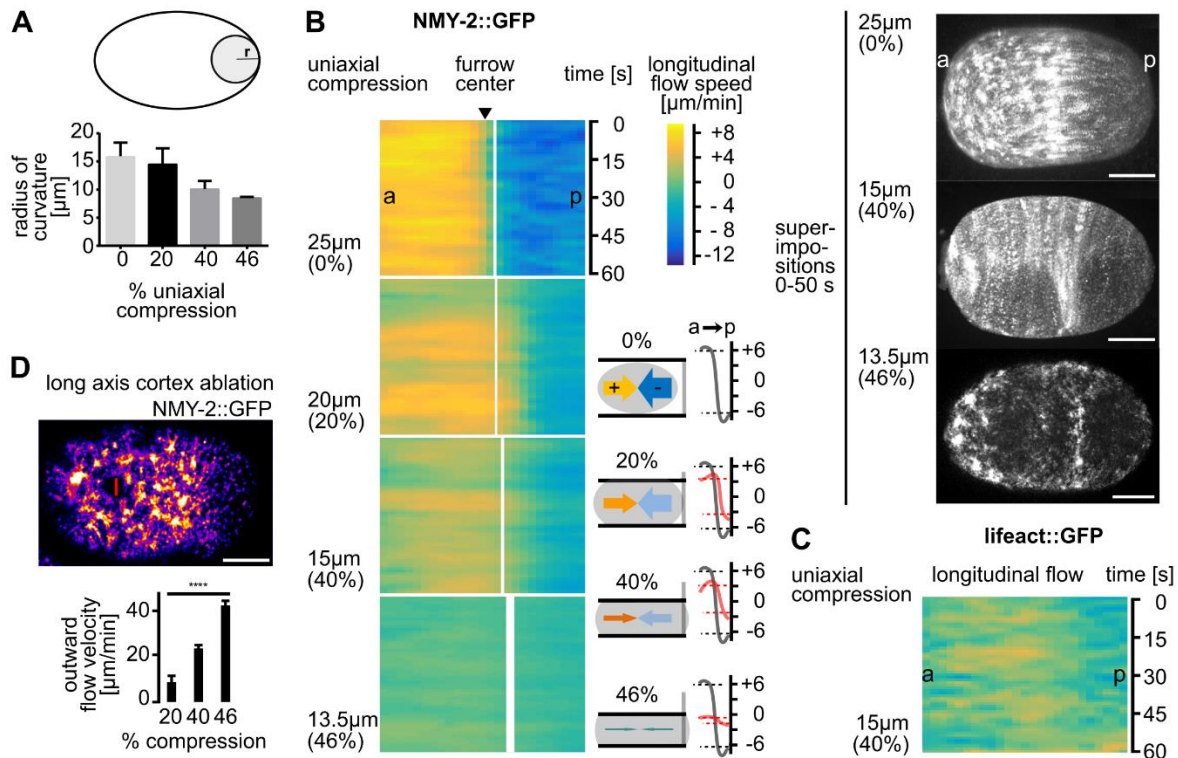
229 In order to probe cytokinesis mechanics, we used the well-established parallel plate assay [35-
230 37]. To achieve highly consistent uniaxial loading in the parallel plate assay, we employed
231 monodisperse, inert beads with diameters of 25, 20, 15, and 13.5 μm , (representing 0, 20, 40, and
232 46% uniaxial compression, respectively; Video 2). Uniaxial loading induces a shape anisotropy where
233 the surfaces contacting the plates become flat and the remaining surfaces start to bulge. Importantly,
234 it has been shown that uniaxial loading directly impinges on cortex mechanics since (a) the cell
235 boundary is governed by Laplace's law (Figure 2A) [37]; (b) external friction (friction between the
236 actomyosin cortex and the plasma membrane/vitelline membrane/egg shell) can be neglected [29,
237 49]; (c) the elastic cortical layer dominates cell mechanics in the system while the contribution of the
238 plasma membrane can be largely ignored [38, 49, 50]. Analyzing longitudinal NMY-2 cortical flow
239 prior to the onset of furrowing, we found that longitudinal flow velocities are highest in unloaded
240 embryos and decrease with increased loading (Figure 2B, left). Flow velocities were down to 3.5 and
241 3.4 $\mu\text{m}/\text{min}$ in anterior and posterior domains, respectively, in 20% compressed embryos and
242 decrease further to 1.8 and 2.8 $\mu\text{m}/\text{min}$ with 40% compression (Figure 2B; Figure S1A). Wt embryos
243 compressed by 46% reach only -0.7 and 1.8 $\mu\text{m}/\text{min}$ and fail to cleave (Video 2). The strong reduction
244 of longitudinal flow (flow along the a-p axis) is best apparent in superimpositions of consecutive
245 frames from time lapse recordings (Figure 2B, right). Interestingly, the reduction of longitudinal flow
246 scales to the amount of loading, suggesting that the cortex behaves like an elastic material (Figure
247 S1B).

248 Consistent with F-actin having faster cortical turnover, we find slightly weaker and less uniform
249 longitudinal flow for F-actin (*lifeact*) compared to NMY-2 (Figure 2C, Figure S1A). Since uniaxial
250 compression induces a shape anisotropy that leads to anisotropic stress in the cortex [38], this might
251 alter cortical tension and impinge on longitudinal cortical flows. To test this, we performed cortical
252 laser ablations [29] just prior to the onset of polarizing flow after fertilization parallel to the short axis
253 of the embryo (cuts of 23% embryo width; Figure 2D, Video 3). We chose this time point for ablations
254 since the cortex shows a highly similar architecture to the cortex just prior to cytokinesis [9] and the
255 measurements are not confounded by fast changing patterns of flows. We made sure that the cortical
256 wound induced by laser ablation did not vary in size under different degrees of compression (Figure
257 S1C). Measuring outward velocities of NMY-2 foci post ablation, we found that increased loading
258 generates increased outward flow velocities (11 ± 0.6 $\mu\text{m}/\text{min}$ at 20% compression, 23 ± 1 $\mu\text{m}/\text{min}$ at
259 40% compression, and 43 ± 2 $\mu\text{m}/\text{min}$ at 46% compression; Figure 2D). Notably, our ablation
260 experiments only allow measurements of changes in total mechanical stress but not the relative
261 contribution of passive and active stresses. Although our ablation experiments were performed before
262 onset of cytokinetic flows, they clearly demonstrate a response of the cortex that scales to loading
263 nevertheless. Thus, our observations are consistent with the interpretation that uniaxial compression
264 induces cortical stress which seems to counteract longitudinal flows (Figure 2B) and eventually
265 prevents successful furrowing.

266

Figure 2

8 of 20



267

268 **Figure 2.** Longitudinal NMY-2 flow is mechanosensitive. **(A)** Quantification of curvature increase due to compression. Smaller radii represent higher curvature (see cartoon and Methods). **(B)** Left: Heat map kymographs of cortical flow velocities obtained from PIV of NMY-2::GFP foci moving along the long axis of differently mounted one-cell *C. elegans* embryos. For statistical parameters of heat maps see Fig. S1A. Black arrow head points to the white line demarcating the future furrow. Thickness of the line represents standard deviation. Bottom middle: Paradigm of uniaxial compression and corresponding flow velocities. Bottom right: Averaged velocities (over 60 s) along the anterior-posterior (a-p) axis from the PIV analysis (right panels). Grey and red lines represent averaged velocities in uncompressed and compressed embryos, respectively ($n = 5$ each). Right: Superimpositions generated by overlaying stills from projected time lapse images. Scale bars = 10 μm . **(C)** Heat map kymographs generated by PIV of lifeact::mCherry for longitudinal flow. Embryos were imaged under 40% compression ($n = 5$). **(D)** Top: Representative still from NMY-2::GFP expressing embryo exhibiting a cortical wound inflicted by UV laser cutting along the short axis of the embryo. Left: Quantification of outward flow velocities following cortical wounding under increasing compression ($n = 5$ each). See also Video 3.

283 3.3 Rotational flow is induced upon uniaxial loading

284 Work from our lab and others has uncovered rotational flow of the cortex – which is orthogonal to longitudinal flow – in the one-cell *C. elegans* embryo directly before contractile ring formation
 285 (Figure 3A, top left) [19, 20]. This rotational flow also occurs *in utero* (Figure 3A, left) and is most likely
 286 due to deformations of embryos similar to 20-40% uniaxial loading when measuring circularity of
 287 embryos in utero and comparing this to contact angles measured *ex utero* for uncompressed embryos
 288 (Figure 3A, right). However, the questions whether this flow is an intrinsic property or whether it needs
 289 a trigger and how it contributes to cytokinesis itself have not been addressed so far. Utilizing the
 290 paradigm of the uniaxial loading by the parallel plate assay, we observed that while longitudinal NMY-
 291 2 flow velocities decrease, rotational cortical flow velocities increase concomitantly (Figure 3B, Figure
 292 S1D, Videos 2,4), from $0.8 \pm 0.02 \mu\text{m}/\text{min}$ in uncompressed to a maximum of $23 \pm 0.1 \mu\text{m}/\text{min}$ in 40%
 293 compressed embryos. Under very high loading, rotational flow is virtually absent due to accumulation
 294 of NMY-2, F-actin and activated RhoA on bulging surfaces (see below). Remarkably, this shows that
 295

296 rotational flow is strongly enhanced by mechanical stress. Again, consistent with F-actin showing
297 faster cortical turnover, we also find that F-actin shows a shorter range of rotational flow (Figure 3C,
298 Figure S1D). More importantly, the magnitude of rotational cortical flow scales to the amount of
299 loading (Figure S1E). Together with the scaling of longitudinal flows (Figure S1B), this strongly
300 suggests that the two phenomena are not simply occurring coincidentally but that they are most likely
301 interdependent.

302 Based on these findings we asked how stress created by uniaxial compression [38] contributes
303 to rotational cortical flow. Analyzing the distribution of NMY-2, F-actin and active RHO-1 (using a
304 RhoA sensor consisting of GFP fused to the AH-and PH-domains of ANI-1; [31]) we found cytokinetic
305 nodes assembling uniformly in uncompressed embryos. In contrast, in compressed embryos, NMY-
306 2, F-actin, and active RhoA are only found at the equator and on bulging surfaces (Figure 3D), for
307 which it has been shown that their cortex is more stressed [4, 7, 38]. This suggests that cell cycle-
308 dependent RhoA activation can be local and most likely in response to cortical deformation. Shortly
309 after their assembly, focally and linearly organized NMY-2 moves onto flattened surfaces through
310 rotational flow (Figure 3E; Video 5). Due to actomyosin being concentrated on bulging surfaces in
311 loaded embryos, its mobilization by rotational flow generates a flow front – the former boundary
312 between the bulged and flat cortex - that moves over the flattened surface until the front reaches the
313 bulged surface on the other side (Video 5).

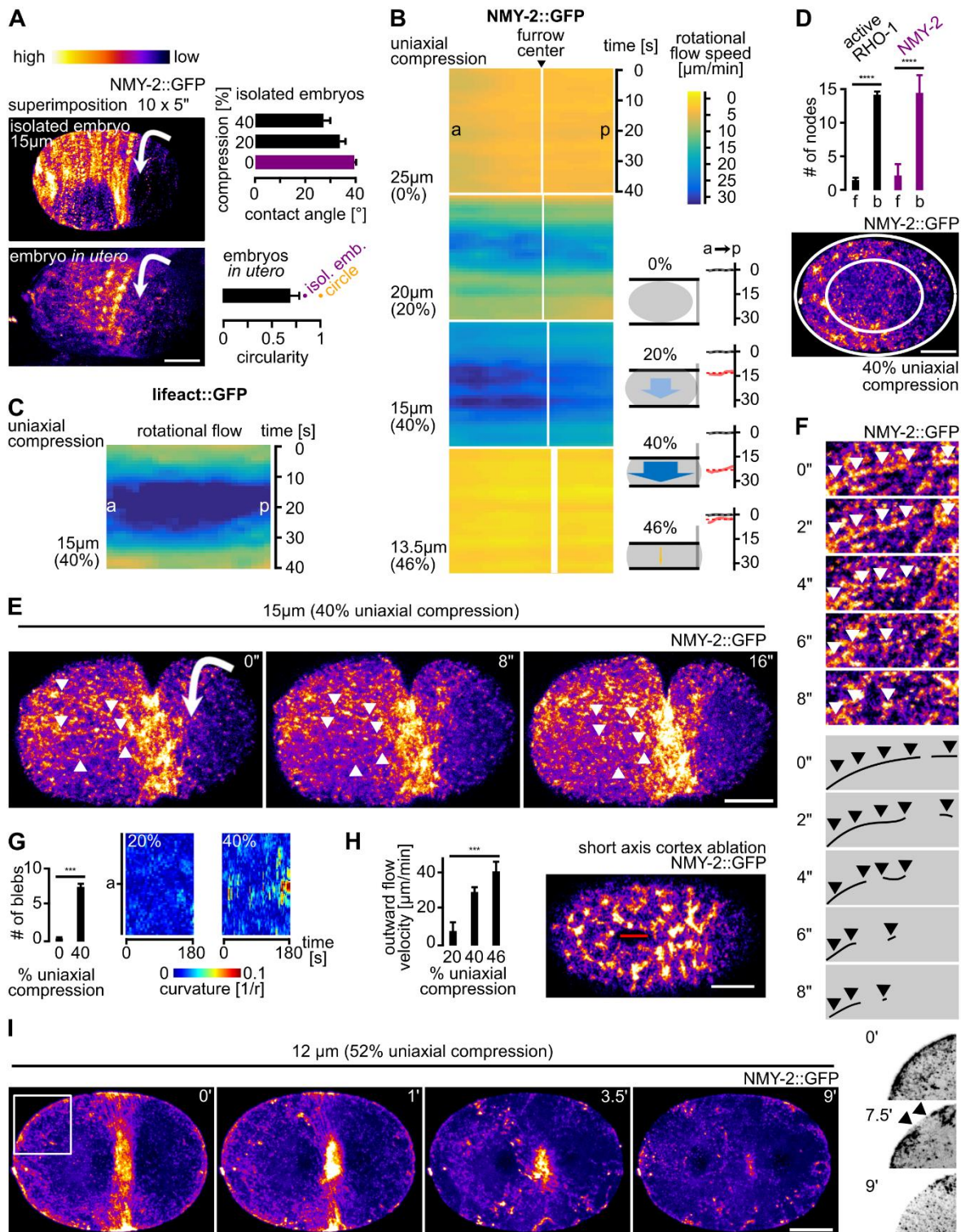
314 Moreover, while linearly organized NMY-2 connecting cytokinetic nodes in uncompressed
315 embryos constricts, it ruptures in compressed embryos (Figure 3E, 3F; Video 5). Rupture occurs
316 anisotropically in the direction of rotation, starting at the front of rotational flow (Video 5). Notably, this
317 anisotropy correlates with the asymmetric position of the midbody, midbodies always forming where
318 rotational flow emerged, opposite to the side where cortex filament rupture occurs at the rotational
319 flow front (Video 5). This always leads to asymmetric positioning of the midbody ($n>20$; data not
320 shown). Additionally, rupture leads to both flow towards the furrow (from the furrow-facing side of the
321 rupture) and flow towards the poles (from the pole-facing side of the rupture) (Video 5). Flows towards
322 the furrow have similar velocities as longitudinal flows in uncompressed embryos and can lead to
323 similar parallel alignment of cortical material in the equatorial zone (Figure 1C, 1E). Flows towards
324 the poles dissipate due to dissolution of nodes and lack of a barrier similar to the equatorial band of
325 focal and linear NMY-2 (Video 5). Furthermore, these flows occur at the same time as polar blebbing
326 is observed, which might additionally contribute to cortical relaxation of cortical tension caused by
327 pole-directed cortical flow (Figure 3G).

328 Since uniaxial compression leads to anisotropic cortex assembly at the onset of cytokinesis and
329 anisotropic disassembly during furrowing, we asked whether loading induces anisotropies in cortical
330 tension that could also contribute to rotational flow. To test this, we performed laser cutting of the
331 cortex (cuts of 16% embryo length; Figure 3H, Video 6) parallel to the long axis of the embryo just
332 prior to the onset of polarizing flow after fertilization and observed a loading-dependent increase in
333 initial outward flow velocities of NMY-2 particles at the site of the cortical wound (15 ± 0.5 $\mu\text{m}/\text{min}$ at
334 20% compression, 29 ± 2 $\mu\text{m}/\text{min}$ at 40% compression, and 32 ± 4 $\mu\text{m}/\text{min}$ at 46% compression; Figure
335 3H).

336 When measuring outward velocities 5 s after cortex ablation (as established previously; [29]), it
337 seems that tension increases along the short axis scales more linearly with loading (Figure 3H, $R^2 =$
338 0.94, Figure S1G) than along the long axis (Figure 2D; $R^2 = 0.83$, Figure S1G). Also consistent with
339 previous work [29], tension seems to be higher along the short axis under low loading. Given the
340 elegant theoretical framework of cortical mechanics that highlights the roles of effective viscosity and
341 local compression rate for the generation of polarizing cortical flow [29], the above measurements
342 suggest that besides cortical stress, viscosity and/or cortex compressibility might additionally
343 contribute to rotational flow.

344

Figure 3



345
346

347 **Figure 3.** Rotational cortical flow is required for furrowing under uniaxial compression. **(A)** Left:
348 Maximum projected stills from time lapse microscopy of a representative, isolated wt embryo (top)
349 and an embryo inside the uterus (bottom); scale bar = 10 µm. Direction of cortical rotation is indicated
350 by an arrow. Top right: Contact angles between coverslip and embryo. Bottom right: Circularity of
351 embryos *in utero* (n = 6), circularity for ellipsoidal, isolated embryos and a circle are also included. **(B)**
352 Heat map kymographs of cortical flow velocity values from NMY-2::GFP particle tracking along the
353 short axis of differently mounted embryos. For statistical parameters of heat maps see Fig. S1D. Black
354 arrow head points to the future furrow center. Bottom middle: Cartoon depictions of corresponding

355 rotational cortical flow velocities. Bottom right: Averaged velocities (over 60 s) along the a-p axis from
356 the PIV analysis (left panels). Grey and red lines represent averaged velocities in uncompressed and
357 compressed embryos, respectively (n = 5 each). **(C)** Heat map kymographs generated by PIV of
358 *lifeact::mCherry* for rotational flow. Embryos were imaged under 40% compression (n = 5). **(D)** Top:
359 Quantification of active RHO-1 (black) and NMY-2 (purple) nodes on flat (f) versus bulging (b) surfaces
360 in embryos under 40% compression (n = 5 each). Right: Representative projection of an embryo
361 illustrating the quantification for NMY-2::GFP (inner ellipse = flattened surface; see panel E for
362 fluorescence intensity color code). Scale bar = 10 μ m. **(E)** Projections from time-lapse data (see Video
363 5). Arrowheads point to linear cortical NMY-2 that undergoes rupture. Scale bar = 10 μ m. **(F)** Magnified
364 projection of the cortex showing rupture of linearly organized NMY-2. Scale bar = 2.5 μ m. **(G)** Left:
365 Quantification of the number of blebs in uncompressed and 40% compressed WT embryos over 60
366 s. Right: Quantification of curvature changes. Two representative curvature kymographs for a 20%
367 and a 40% compressed embryo are shown. See experimental procedures for details. **(H)** Left:
368 Quantification of outward flow velocities following cortical wounding under increasing loads (n = 5
369 each). Right: Representative still from a NMY-2::GFP expressing embryo exhibiting a cortical wound
370 inflicted along the long axis of the embryo by UV laser cutting. Scale bar = 10 μ m. See also Video 6.
371 **(I)** Cortex rupture for 52% compression. Representative projections from time-lapse microscopy are
372 shown; scale bar = 10 μ m. The right pictures show the boxed area of the leftmost still annotated with
373 arrowheads and inverted to illustrate cortex rupture. See also Video 8.

374 *3.4 Uniaxial loading and the limit of cytokinetic mechanostability*

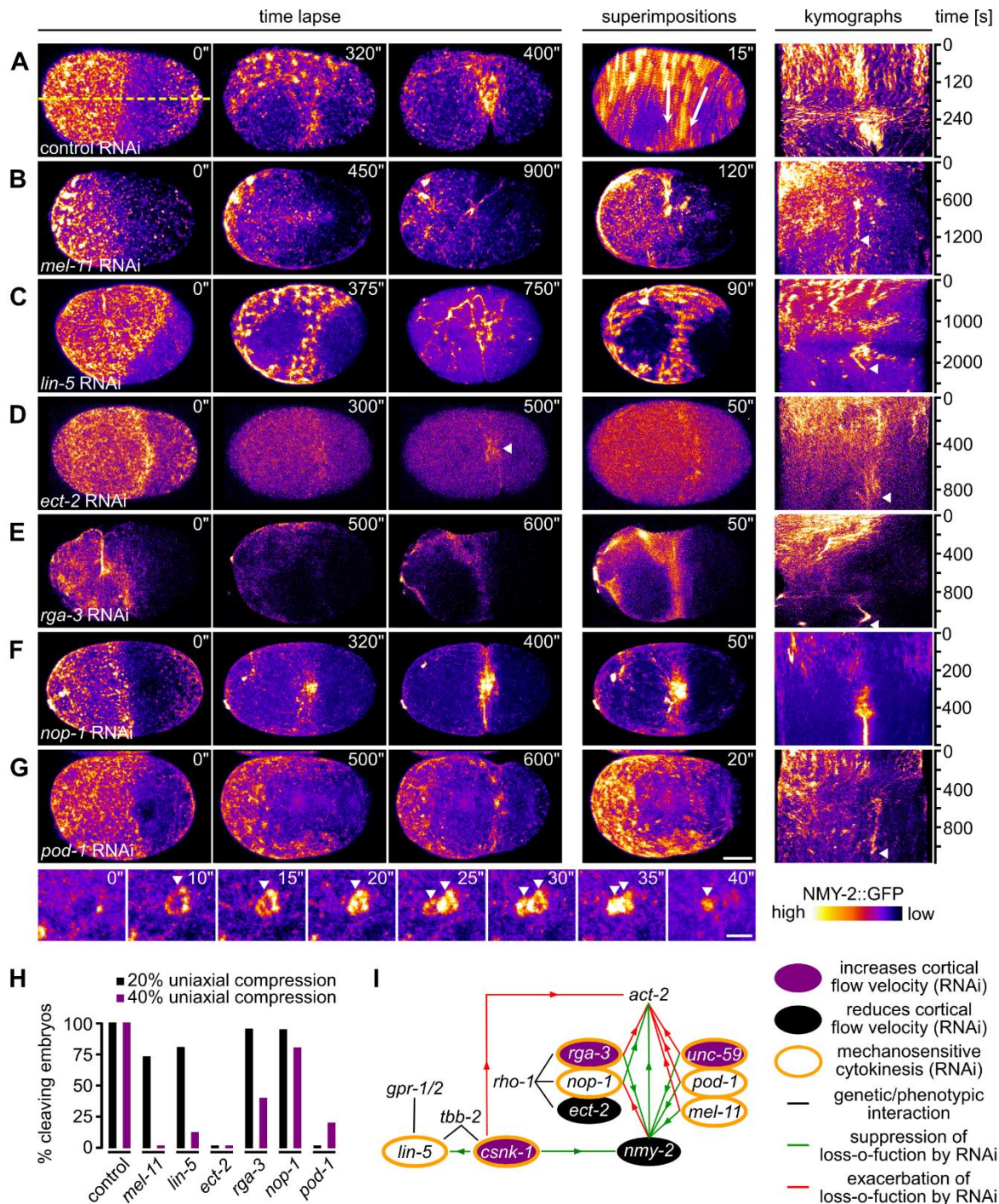
375 Next, we asked how rotational flow changes when we subject embryos to 46% compression, a
376 load where embryos do not divide (Video 7). Here, we found the same anisotropic distribution of
377 nodes as for 20% and 40% compression, however, nodes on bulged surfaces do not translocate by
378 rotational flow. Instead, streaming of linearly organized NMY-2 in the equatorial area is observed
379 (Video 7). Streaming does not lead to the bundling of linear NMY-2 at the equator and a contractile
380 ring is not formed (0% of embryos; n>15). Moreover, under 46% compression, actomyosin
381 recruitment to the equatorial zone by the central spindle pathway can still be observed, however,
382 equatorial actomyosin recruitment is insufficient for furrowing. Similar to human cells [37], we found
383 that the limit of cortex loading is reached at 52% (12 μ m beads; 50% for human cells). Due to
384 increased bulging, the cortex ruptures at these bulged sites and the equatorial NMY-2 band
385 disintegrates (Figure 3I; Video 8). This confirms that the cortex is bearing the load of compression
386 since we neither observed rupture of the plasma membrane nor of the eggshell. Moreover, it also
387 supports the idea that the cortex behaves like an elastic material that has a yield point at 52%
388 compression.

389 *3.5 Actin-myosin regulators required for mechanostable cytokinesis*

390 Since cortical tension along the short axis is under the control of the Rho GTPase cycle (Meyer
391 et al., 2010) and since NMY-2 cortical polarization is observed independently of the level of loading,
392 we performed a targeted screen to identify factors involved in cortical rotation and linear organization.
393 For the screen, we used 20% and 40% compression of embryos, where strong rotational flow is
394 observed in wild-type embryos (Figure 4A). This screen identified several factors including (1) MEL-
395 11, a myosin-associated phosphatase [51], required for both focal and linearly organized NMY-2
396 (Figure 4B; Video 9); (2) LIN-5, a factor known to regulate spindle positioning [52], which also
397 promotes the transition from focal to linear organization and seems to stabilize the latter (Figure 4C;
398 Video 10); (3) ECT-2, a cytokinesis regulatory RhoGEF [53], which is required for proper size and
399 density of focal and linear NMY-2 (Figure 4D; Video 11); (4) RGA-3, a cytokinesis regulatory RhoGAP
400 [54, 55], for which it has been previously shown that depletion leads to exaggerated rotational flow
401 [44], and which we find is also required for node formation and to suppress excess linear organization
402 (Figure 4E; Video 12); (5) NOP-1, a factor required in parallel with the RhoGAP CYK-4 to promote

403 RHO-1 activation and NMY-2 node formation during cytokinesis [31], which is also required for the
 404 transition to linearly organized NMY-2 (Figure 4F; Video 13); and (6) POD-1, a type III Coronin
 405 implicated in actin dynamics and crosslinking [56], which is as well required for this transition (Figure
 406 4G, top panels; Video 14). Moreover, *pod-1* RNAi leads to the formation of short-lived circular
 407 contractile NMY-2 structures, which suggests that Coronin-mediated actin crosslinking is required to
 408 coordinate formation of long-range NMY-2 linear organization to achieve pole-to-equator flow (Figure
 409 4G, bottom panels; Video 14).
 410

Figure 4



411

412 **Figure 4.** Antagonistic actin-myosin regulators are required for rotational flow and cytokinesis
 413 mechanostability. **(A)** Left: Maximum projected stills from time lapse microscopy of a representative
 414 wt embryo expressing NMY-2::GFP. Middle: Superimposition of frames from a 15 s time window.

415 White arrows indicate direction of rotational flow. Right: Kymograph generated along the dashed
416 yellow line in the leftmost panel. **(B-G)** Representations as in panel (A) but for embryos treated with
417 the indicated RNAi. Scale bar = 10 μm . See also Videos 9-14. Bottom of panel (G): Magnification of
418 projected stills showing formation of cortical circular structures (arrowheads) in *pod-1* RNAi embryos.
419 Scale bar = 2.5 μm . See also Video 14. **(H)** Quantification of successful first cell division for the
420 indicated RNAi treatments under 20% (black) and 40% (purple) compression ($n \geq 5$ each). **(I)** Genetic
421 network of factors controlling cytokinesis. Interactions are based on [21, 31] and data from panel H.

422
423 Although RNAi of these regulators gives rise to very distinct phenotypes, for all factors where
424 furrowing phenotypes were not known (MEL-11, LIN-5, RGA-3, NOP-1, and POD-1), we observed a
425 loading-dependent failure of cytokinesis completion (Figure 4H). With the exception of *pod-1* RNAi,
426 increased loading leads to an exacerbation of the phenotype. Remarkably, all regulators are known
427 to have opposing phenotypes in actin (*act-2*) and myosin (*nmy-2*) mutants [32] and are directly or
428 indirectly linked to the Rho GTPase cycle (Figure 4I). This network of factors is essential for
429 cytokinesis' mechanical robustness and by differentially regulating NMY-2 organization seems to
430 indirectly also affect cortical viscosity and compressibility.)

431 3.6 Persistent linearly polarized NMY-2 prevents cortical rotation

432 Previously it was shown that *rga-3* RNAi leads to exaggerated chiral flows during a-p polarization
433 of the one cell *C. elegans* embryo [21, 44]. However, the data above shows that *rga-3* RNAi embryos
434 do not divide under uniaxial compression. We therefore more closely investigated the origin of
435 exaggerated chiral flows in *rga-3* RNAi embryos and why this prevents cytokinesis under mechanical
436 stress. Although we observe the reported exaggerated chiral flow during a-p polarization under
437 uniaxial loading (Figure 5A), an important additional phenotype of *rga-3* RNAi embryos is persistent
438 and long range linearly organized cortical NMY-2, which can be observed both during a-p polarization
439 (Fig. 5A) and right after the onset of cytokinesis (Figure 5B). This organization is maintained during
440 cytokinesis and leads to peeling of the filaments towards the nascent midbody, lack of a proper
441 contractile ring (Figure 5B), and strongly reduced rotational flow under load (Figure 5C). Thus, unlike
442 in wt embryos, linearly organized cortical NMY-2 does not undergo remodelling or ruptures in *rga-3*
443 RNAi embryos. Considering the theory of cortical torques [21] it seems likely that a persistent linear
444 organization of NMY-2 can induce stronger and more long ranged torques than wt. We propose that
445 this leads to excessive chiral flow during polarization but later results in lack of cortical rotation and
446 failed cytokinesis long-range linear connections are not remodelled (Figure 5B).

447 3.7 Cortical chirality and polarity are required for rotational flow polarization

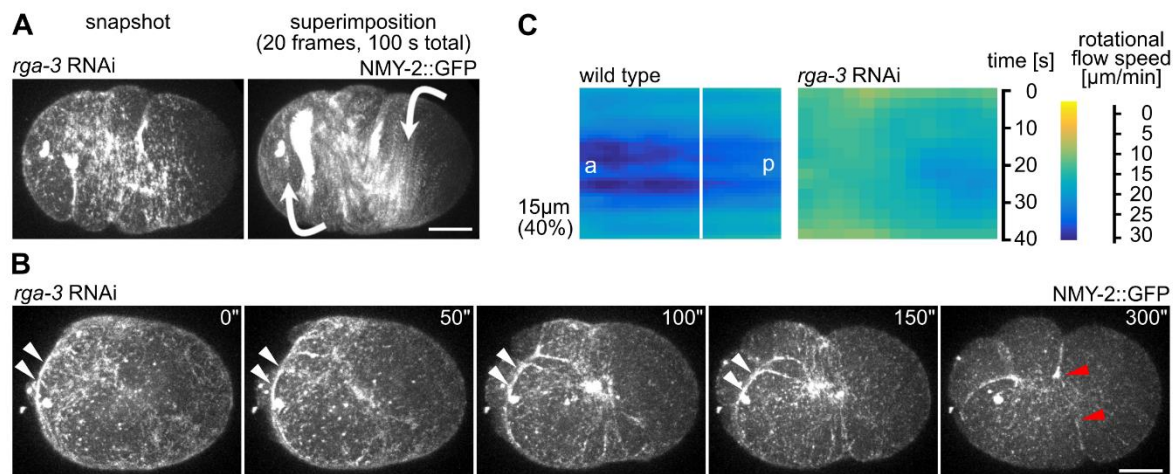
448 Based on previous findings demonstrating that the actomyosin cortex generates active chiral
449 torques with invariant handedness important for axial patterning [19-21], we reasoned that regulators
450 of cortical chirality will contribute to rotational cortical flow polarization. To do so, we used RNAi
451 inhibiting the expression of the casein kinase 1 γ , CSNK-1. In line with earlier observations [20], we
452 found that in *csnk-1* RNAi embryos, rotational cortical flows can switch their handedness across the
453 equator and, concomitantly, a strong reduction of compressive longitudinal flow occurs (Figure 6A,
454 left; Figure S2A). Importantly, the switch of rotational flow handedness generates shear flow in the
455 equatorial region, which leads to dissolution of the furrow under mechanical load (Figure 6A, right,
456 40% compression; Video 15). This phenotype is not restricted to *csnk-1* RNAi embryos, but also
457 occurs when components of the Wnt pathway that have been shown to be required for cortical torque
458 generation and chiral symmetry breaking are targeted by RNAi [21, 39], for instance *mom-2* (Figure
459 6B).

460 Since the contractile ring forms by alignment of linearly organized cortical material filaments
461 through RhoA-dependent flow, the whole system also needs to be polarized along the direction of
462 longitudinal compressive flow. Accordingly, we find that disruption of anterior-posterior polarity in *par-*
463 *2* or *tat-5* RNAi embryos phenocopies the *csnk-1* and *mom-2* RNAi, a lack of longitudinally polarized
464 compressive flow and shear flow in the equatorial region (Figure 5C, Figure S2B, S2C; Video 16).
465 Importantly, we also observed shear flow in wt embryos when they are compressed by 46% and do
466 not divide (Figure 6C; Video 7). This suggests that under these conditions uniform rotational cortical
467 polarization that is observed in wt embryos up to 40% compression fails after removing factors
468 responsible for cortical polarity and chirality or by excessive loading (Figure 6C, bottom right). Next,
469 we asked how furrowing itself is affected by uniaxial loading and we tested whether factors known to
470 be required for the intrinsic asymmetry of furrowing such as *unc-59* (encoding a septin; [27]) are also
471 involved (Figure 6B). Similar to the requirement of genes involved in cortical polarity and chirality, we
472 also found that *unc-59* RNAi embryos lack rotational cortical flow (data not shown) and fail to divide
473 under 40% compression (Figure 6B).

474 Taken together, although factors involved in cortex polarity, chirality and asymmetry have not
475 been found to be essential for cytokinesis in previous studies, they all become essential for
476 cytokinesis under mechanical stress (Figure 6B, 6C). Furthermore, since compression induces
477 rotational flow and all of the above RNAi embryos also show a loss of uniform polarized rotational
478 flow (Videos 15, 16), we measured the degree of asymmetric furrowing under increasing mechanical
479 load. In accordance with the above findings, we found that furrowing becomes increasingly
480 asymmetric with increased loading (Figure 6D, top). These results, although correlative, strongly
481 suggest that loading-induced rotational flows are involved in symmetry breaking during furrowing
482 (Figure 6D, bottom).

483
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Figure 5

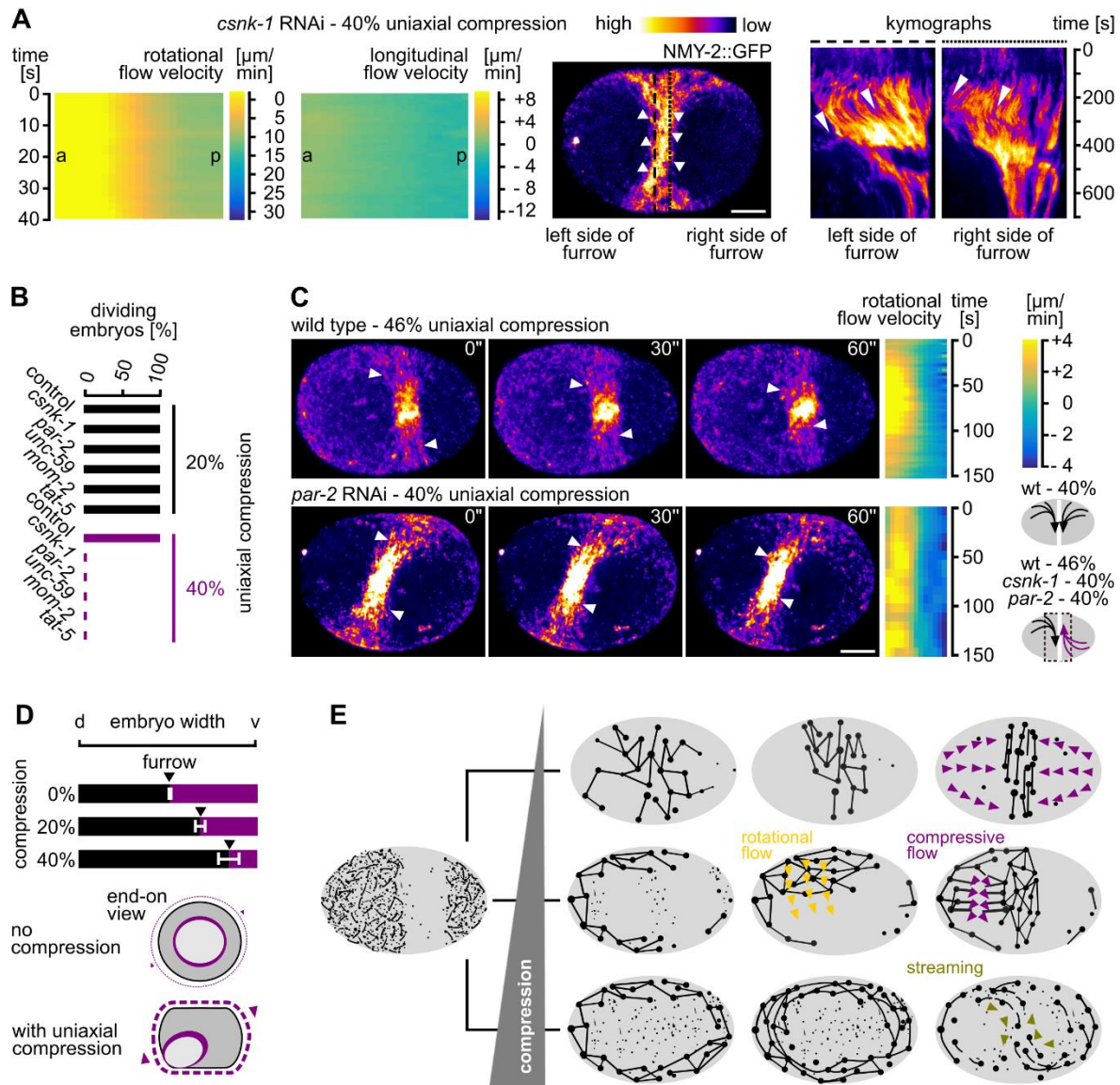


485

486 **Figure 5.** *rga-3* RNAi leads to increased linear organization of cortical NMY-2 and lack of rotational
487 flow under load. **(A)** Still and superimposed stills from time-lapse microscopy of a representative *rga-*
488 *3* RNAi embryo. Note the linear organization of NMY-2 and the almost exclusive rotational trajectories
489 of cortical NMY-2 in the superimposition. Direction of rotational trajectories (arrows) have opposite
490 polarity (anterior domain counterclockwise and posterior domain clockwise. Scale bar = 10 μm . **(B)**
491 Stills from a time-lapse series of a representative *rga-3* RNAi embryo during cytokinesis. White
492 arrowheads mark long linear cortical NMY-2 that peels from the sides towards the nascent midbody.
493 Red arrowheads mark the dissolving furrow. Scale bar = 10 μm . **(C)** Heat map kymographs of
494 rotational cortical flow velocity values from NMY-2::GFP particle tracking along the short axis of wt
495 and *rga-3* RNAi embryos mounted under 40% uniaxial compression (n =5 each).

Figure 6

15 of 20



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Figure 6. Cortical polarity and chirality are required for mechanostable cytokinesis. **(A)** Left: Heat map kymographs generated by PIV of NMY-2 particles along the short and the long axis of one-cell *C. elegans* *csnk-1* RNAi embryos. All embryos were imaged under 40% compression (n = 5 each). Middle: Maximum projected still from time lapse microscopy of a representative *csnk-1* RNAi embryo expressing NMY-2::GFP; white arrowheads indicate flow direction in the furrow region; scale bar = 10 μm . See also Video 15. Right: Kymographs generated along the dashed lines at the left and right boundary of the furrow. Opposite polarity of flow is indicated by arrowheads. Note the dissolution of the furrow after 400 s. **(B)** Quantification of successful first cell division for the indicated RNAi treatments under 20% (black bars) and 40% (purple bars) compression (n = 10 each). **(C)** Left: Maximum projected stills from time lapse microscopy of a representative wt (46% compressed) and *par-2* RNAi embryo (40% compressed) expressing NMY-2::GFP; scale bar = 10 μm . See also Video 16. Middle: Heat maps generated via PIV of NMY-2 particle flow in the furrow region along the short axis. Bottom right: Cartoons depicting rotational flow polarization in wt (top) and RNAi embryos (bottom). **(D)** Furrowing asymmetry quantified in wt embryos. Top: Average furrow position along the short axis is indicated by black arrowheads (n = 5 each). Bottom: Model how lack of rotational cortical flow influences furrow asymmetry. See text for details. **(E)** Model for linearly organized cortical myosin dynamics under different conditions. Left: Cortical NMY-2 distribution before the onset of cytokinesis. Top right: Linear and focal NMY-2 coalesce into an equatorial band in unstressed embryos through longitudinal pole-to-equator flow (purple arrowheads). Middle right: With increased loading, NMY-2

517 foci show an anisotropic distribution at the onset of cytokinesis, subsequently, focal and linear NMY-
518 2 show rotational flow (orange arrowheads) and linearly organized NMY-2 ruptures, thereby
519 generating longitudinal flow (purple arrowheads). Bottom right: With high load, anisotropically
520 distributed nodes transform into a linearly organized network that shows streaming, preferentially in
521 the equatorial region.

522

523 4. Discussion

524 Our data outline a poorly uncharacterized feature of cortical flow, its mechanosensitivity and –
525 up to a certain stress level – its mechanostability due to its ability to re-polarize from longitudinal to
526 rotational (Figure 6D). Moreover, we demonstrate that uniaxial compression is a straightforward
527 experimental paradigm to systematically investigate the mechanobiology of cortical flow during
528 asymmetric cell division. Importantly, this paradigm shows that the induction of rotational flow
529 depends on the magnitude of total mechanical stress. We also show that re-polarization of cortical
530 flow is followed by anisotropic cortex rupture (Figure 6D). Rupture can lead to equator-directed
531 cortical flows during cytokinesis which result in cortical compression around the cell equator and
532 furrowing. This seems to be one mechanism that can balance extrinsic and intrinsic forces during
533 cytokinesis (Figure 2B, Figure 3B). These results therefore extend previous work that identified
534 longitudinal flows as non-essential contributors to contractile ring formation [9, 17]. In addition, our
535 results reveal that besides polarization of actin filaments through flow-alignment coupling [9], cortical
536 non-muscle myosin II also shows flow-alignment coupling, however, by having much longer lifetimes,
537 cortical NMY-2 shows higher flow velocities than F-actin and accumulates at the equator and in the
538 midbody – unlike F-actin (Figure 1). The recent thorough characterization of long, linearly organized
539 non-muscle myosin II stacks whose lifetime is independent of the neighboring F-actin filaments [48]
540 together with our observation of different cortical flow profiles for NMY-2 and F-actin (lifeact) strongly
541 suggests that non-muscle myosin II has roles during cell division that are separable from those F-
542 actin, in particular during final stages of contractile ring constriction and midbody formation (Figure
543 1G, 1I). Moreover, the proposed attractive interactions between linearly organized non-muscle
544 myosin II [48] might also explain why we observe NMY-2 flows with longer duration and range than
545 F-actin flows.

546 Previously, it has been demonstrated that the actomyosin cortex of embryos can be viewed as
547 an excitable medium. In such a medium cortical flow in the form of waves is observed due to rapid
548 local auto-activation of RhoA at wave fronts and delayed F-actin-mediated RhoA inhibition at the back
549 of waves [57]. Treating the actomyosin cortex as an excitable material can therefore explain how the
550 spindle determines the site of cleavage during cytokinesis, namely by generating signals that tune
551 the auto-activation/inhibition cycle [57], it might, however, also explain the phenomena observed in
552 this study, namely local activation of RhoA that leads to locally restricted non-muscle myosin II
553 activation and stress-dependent flow polarization. In the framework of an excitable material, it seems
554 most likely that local RhoA activation in uniaxially loaded *C. elegans* embryos is due to cell cycle
555 state-dependent local auto-activation of RhoA on bulged areas of the cortex through a previously
556 characterized mechanosensitive positive feedback [2]. The spatially restricted RhoA activation at
557 these sites would then lead to the formation of a flow front during rotational flow (Video 5). Moreover,
558 it also seems likely that anisotropies in spindle organization and spindle-cortex contacts pattern local
559 auto-activation and thereby flow polarization. Thus, it is tempting to speculate that strong cortical
560 flows are restricted to cytokinesis since the cortex only shows sufficient excitability during this specific
561 cell cycle state and that only during cytokinesis the spindle or external mechanical forces can induce
562 patterned activation/inactivation of RhoA that will generate polarized flows.

563 In addition, we demonstrate that several pathways which all have specific, non-redundant
564 functions outside cytokinesis, fulfil essential roles for rotational cortical flow and furrow stability when
565 cells are mechanically stressed (Figures 4, 5, 6). These pathways include the PAR and the Wnt
566 pathway, which are known for their role in specifying the anteroposterior and the left/right body axes,
567 respectively. Only for the PAR pathway a connection to cortical dynamics during cytokinesis is known
568 (Jordan et al., 2016). Remarkably, interference with any of these pathways results in a similar
569 mechanical stress-dependent failure of cytokinesis, a loss of uniform rotational cortical flow
570 polarization, which leads to shear flow and dissolution of the contractile ring (Figure 6). This suggests
571 that proper anteroposterior cortical polarization (*csnk-1*, *par-2*, *tat-5*) and yet to be identified aspects
572 of cortical polarity that relate to left/right symmetry breaking or cortical torque generation (*rga-3*, *mom-*
573 *2*; [20, 21, 39]) become essential for furrowing under mechanical stress. Additionally, we find that
574 proper actomyosin regulation required for intrinsically asymmetric furrowing (*unc-59*) is also essential
575 for cytokinesis mechanostability. This data supports earlier findings based on which it was argued
576 that when the intrinsic asymmetry is disrupted, cytokinesis becomes sensitive to partial inhibition of
577 contractility [27]. It should be noted that not only *csnk-1*, *rga-3*, and *unc-59* but also *par-2* and *tat-5*
578 RNAi influence cortical cytoskeletal dynamics directly and further work involving super-resolution
579 microscopy will be required to identify the origin of cortical cytoskeleton polarization during
580 cytokinesis.

581 Although the data that we present here is correlative in many aspects, it nevertheless suggests
582 that cortical rotation and cytokinesis mechanostability are intricately linked and rely on factors
583 presumably required for symmetry breaking during cytokinesis, those that provide polarity information
584 parallel (*csnk-1*, *par-2*, *tat-5*) and orthogonal (*rga-3*, *mom-2*) to the contractile ring and factors that
585 potentially translate such polar bias into directional movement of actomyosin (*unc-59*). Moreover, our
586 data also suggests that generation of cortical torque seems to depend on linear organization of
587 cortical non-muscle myosin II (Figure 5). However, increased cortical torque alone is not sufficient for
588 cytokinesis to proceed normally under load. Under these conditions, the remodeling of linear cortical
589 structures seems crucial for the re-distribution of contractile cortical material towards the cleavage
590 furrow by longitudinal flow and assembly of a contractile equatorial ring. Taken together, our findings
591 show that Ray Rappaport's notion that the cytokinesis machinery is 'overbuilt, inefficient, never-failed,
592 and repaired by simple measures' [1] – in other words that cytokinesis is a robust process due to
593 redundant regulators – might only be appropriate for unstressed cells, however, apparently redundant
594 factors can become essential under mechanical stress.

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596 formal analysis, D.S., O.D., and C.P.; investigation, D.S., C.P.; writing—original draft preparation, C.P.; writing—
597 review and editing, C.P.; visualization, C.P.; supervision, C.P.; project administration, C.P.; funding acquisition,
598 C.P.

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602

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