Tension-oriented cell divisions limit anisotropic tissue tension in epithelial spreading during zebrafish epiboly

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Epithelial spreading is a common and fundamental aspect of various developmental and disease-related processes such as epithelial closure and wound healing. A key challenge for epithelial tissues undergoing spreading is to increase their surface area without disrupting epithelial integrity. Here we show that orienting cell divisions by tension constitutes an efficient mechanism by which the enveloping cell layer (EVL) releases anisotropic tension while undergoing spreading during zebrafish epiboly. The control of EVL cell-division orientation by tension involves cell elongation and requires myosin II activity to align the mitotic spindle with the main tension axis. We also found that in the absence of tension-oriented cell divisions and in the presence of increased tissue tension, EVL cells undergo ectopic fusions, suggesting that the reduction of tension anisotropy by oriented cell divisions is required to prevent EVL cells from fusing. We conclude that cell-division orientation by tension constitutes a key mechanism for limiting tension anisotropy and thus promoting tissue spreading during EVL epiboly.

In zebrafish gastrulation, the EVL is formed as a squamous epithelial cell layer at the animal pole of the embryo and spreads over the entire spherical yolk cell during the course of epiboly (4–10 h post fertilization (hpf)), thereby rapidly increasing its surface area. Actomyosin contraction within the yolk syncytial layer (YSL), to which the EVL is connected at its margin, is thought to drive EVL epiboly movements by pulling on the EVL margin in the direction of the vegetal pole. However, the mechanisms by which the epibolizing EVL rapidly increases its surface area and, at the same time, maintains its epithelial integrity during zebrafish epiboly remain unclear.

Studies in another teleost fish, Fundulus heteroclitus, have suggested that the EVL increases its surface area during epiboly by both passive cell spreading in response to pulling forces from the YSL, and active cellular rearrangement adjusting the shape of the EVL to the spherical geometry of the yolk cell on which it spreads. Consistent with the idea of EVL cells passively spreading as a result of the YSL pulling on the EVL margin are observations in zebrafish that marginal EVL cells become increasingly elongated along the axis of tissue spreading and in Fundulus of tension building up within the plane of the EVL during the course of epiboly. Cellular rearrangements have been noted to particularly occur at the margin of the EVL in both zebrafish and Fundulus with individual cells constricting at their leading edge and eventually being displaced from the EVL margin. However, whether EVL cell spreading and rearrangement constitute the sole mechanisms mediating EVL surface expansion during fish epiboly, or whether other mechanisms might also be involved, is still unclear.

Cell divisions have profound effects on epithelial tissue morphogenesis, and it is thus conceivable that they also play a critical role in EVL epiboly movements. In zebrafish, EVL cells have been shown to undergo divisions within the plane of the tissue during epiboly. Moreover, planar tension within the EVL has been speculated to function in maintaining EVL cell lineage by keeping the mitotic spindle of dividing cells oriented in the plane of the cell sheet. Here, we investigate how cell divisions contribute to EVL epiboly movements, and how tension within the EVL relates to the EVL cell-division rate and/or orientation.

RESULTS

Anisotropic tissue tension controls cell division orientation within the EVL

To obtain insight into the cellular processes underlying EVL tissue spreading during zebrafish epiboly, we first examined how the increase of total surface area of the EVL during epiboly correlates with changes in surface area, height and volume of individual EVL cells and with their division pattern (Fig. 1 and Supplementary Video 1). We found that EVL tissue spreading is accompanied by pronounced EVL cell

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flattening, as recognized by an increase in apical surface area and concomitant decrease in cell height along the apical–basal axis of individual EVL cells (Fig. 1b and Supplementary Fig. 1a,b). In contrast, EVL cell volume remained largely constant during flattening and was cut in half once these cells underwent division (Fig. 1c and Supplementary Fig. 1a, right). Cell divisions marked in yellow; \( t = 0 \) min corresponds to sphere stage (4 hpf).

EVL cell-shape changes and divisions. (Fig. 1d) Exemplary images of EVL cells as a function of time after sphere stage (4 hpf); plotted values, mean ± s.e.m. normalized to the average values at \( t = 120 \) min (area\(_{120}\) = 838 ± 9 \( \mu \)m\(^2\); height\(_{120}\) = 9 ± 0.29 \( \mu \)m); \( n \), number of cells per time point (for details, see Supplementary Table 1). (c) Volume of individual EVL cells both before and after cell division as a function of time after sphere stage (4 hpf). Vertical lines (dashed) indicate cell divisions; plotted values, mean ± s.e.m. normalized to the average values at \( t = 120 \) min (volume\(_{120}\) = 6,536 ± 600 \( \mu \)m\(^3\); \( n \), number of cells per time point (for details, see Supplementary Table 1). (d) Average percentage of EVL cells undergoing divisions as a function of their position along the animal–vegetal axis for sequential stages of early epiboly; note that EVL cell divisions become very rare after 55% epiboly stage\(^{25} \) (for 60% epiboly stage < 0.26% of all EVL cells, \( n = 10 \) embryos), and have therefore not been spatiotemporally analysed. For sphere and 30% epiboly stage embryos (4 and 4.66 hpf, respectively), the EVL was subdivided along its animal–vegetal axis into three bins (I, II and III), whereas for 55% epiboly stage embryos (5.66 hpf), the EVL was subdivided into five bins (I, II, III, IV and V); plotted values, mean ± s.e.m. normalized to the number of EVL cells per bin; see e for number of analysed cells and embryos. (e) The total number of EVL cells per stage was on average 496, 660 and 1,072 for sphere (4 hpf; \( n = 12 \) embryos), 30% (4.66 hpf; \( n = 10 \) embryos) and 55% (5.66 hpf; \( n = 10 \) embryos) epiboly stage, respectively; plotted values, mean ± s.e.m. (calculated by using embryo numbers). (f) Rose diagram of the cell-division axes at cytokinesis (yellow; \( n = 524 \) divisions, 6 embryos) for EVL cells dividing during the course of gastrulation; \( p \) (division orientation) = 0.0067 (calculated by using division numbers). A, animal; V, vegetal. Number of independent experiments: 6 (area) and 5 (height) (b), 5 (c), 1 (d,e), 6 (f).
division orientation (SDO) of EVL cells is consistent with a potential role of oriented cell divisions in EVL epiboly progression.

We next examined how SDO of EVL cells is controlled during EVL epiboly. Mechanical tension has been suggested to represent a powerful mechanism for orienting the divisions of isolated cells in culture11. Moreover, studies in F. heteroclitus have suggested that the epibolizing EVL is under tension both around the circumference and along the animal–vegetal axis of the embryo3,12. We therefore reasoned that anisotropic tension distribution within the EVL may control SDO of EVL cells. To test this hypothesis, we used a laser-cutting device to map tension within the EVL during the course of epiboly2,13. To detect tension along the animal–vegetal axis of the EVL, we cut the apical actomyosin cortex of the EVL along a 100-μm-long line parallel to the EVL margin and determined the recoil velocity of the cortex as a readout for animal–vegetal tension (Fig. 2a). Similarly, to detect EVL tissue tension perpendicular to the animal–vegetal axis, we determined the recoil velocity of the actomyosin cortex in cuts oriented perpendicular to the EVL margin (Fig. 2a and Supplementary Video 2). We found that tension along the animal–vegetal axis of the EVL is higher than along the circumference of the embryo, and that this anisotropic tension distribution is particularly pronounced at mid- to late-gastrulation stages (Fig. 2b). This global correlation between EVL tissue tension anisotropy and SDO of EVL cells is consistent with a function of tissue tension in orienting EVL cell divisions.

To determine whether there is a causal link between EVL tissue tension and cell-division orientation, we locally induced anisotropic tissue tension within the EVL and analysed resultant changes in cell-division orientation. We locally induced anisotropic tension within the EVL by simultaneously ablating two small groups of EVL cells (2–3 cells each) positioned close to each other (≈120 μm), which led to the extrusion of the ablated cells and, consequently, considerable stretching of the EVL tissue between the two ablation sites (Fig. 2c and Supplementary Video 3). We then analysed changes in spindle orientation of cells that were located between the two ablation sites and had their metaphase spindle axis oriented perpendicular to the axis of induced tension before tension was applied (Fig. 2c). We found that in these cells the mitotic spindle preferentially reoriented along the main axis of tension before cytokinesis (Fig. 2c), demonstrating that tissue tension anisotropy influences the cell-division orientation of EVL cells.

The orientation of EVL cell divisions by tension involves cell elongation and requires myosin II activity

We next examined how anisotropic tissue controls EVL cell-division orientation. Many cell types have been shown to preferentially orient their mitotic spindle along their longest axis14–17. Notably, EVL cells exhibit flat two-dimensional (2D) defined geometries and do not round up at their apical side during mitosis (Supplementary Video 4), and thus cell-shape anisotropies could influence metaphase spindle orientation through putative length-dependent astral microtubule forces14. Considering that EVL cells seem preferentially elongated along the main axis of tension shortly before undergoing cytokinesis (animal–vegetal axis; Fig. 3a), we therefore reasoned that anisotropic EVL tissue tension may control SDO of EVL cells by elongating cells along the main axis of tension. To assess the potential role of EVL cell elongation on division orientation, we made use of a computational model that predicts the preferred division orientation based on cell shape14. We found that EVL cell elongation is a reliable predictor for division orientation (Fig. 3b,c), supporting our hypothesis that EVL tissue tension controls cell-division orientation by cell elongation.

In addition to cell shape, external forces have also been proposed to control spindle orientation in a myosin II-dependent manner11. To determine whether myosin II is required for tension-controlled EVL cell-division orientation, we analysed division orientation in embryos with normal and reduced myosin II activity. To reduce myosin II activity, we exposed embryos to the myosin II inhibitor blebbistatin. We then compared the effect of cell elongation on division orientation between control and blebbistatin-treated cells and found that EVL cell shape less accurately predicted spindle orientation in cells with reduced myosin II activity (Fig. 3b,c). Moreover, the failure of the mitotic spindle to align with the longest axis of the cell and thus the main axis of tension in blebbistatin-treated embryos was often accompanied by increased spindle fluctuations (Fig. 3d and Supplementary Video 5). The effect of myosin II inhibition on spindle orientation could in principle be due to defects in cell elongation and/or spindle alignment with the longest cell axis. To distinguish between these two possibilities, we compared EVL cell elongation between control and blebbistatin-treated embryos and analysed how accurately our computational model can predict experimental spindle orientation on the basis of these measured cell shapes. Consistent with a critical function of actomyosin contraction in cell shape changes18, we found that EVL cell elongation was reduced in blebbistatin-treated embryos (Fig. 3e), suggesting that myosin II affects spindle orientation by cell elongation. However, even when taking these differences in cell shape into account, myosin II inhibition still increased the deviation of experimental spindle orientation in EVL cells from model predictions made on the basis of cell shape alone (Fig. 3f). This comparison of model predictions with experimental observations suggests that myosin II, in addition to controlling EVL cell elongation, might be required to align the mitotic spindle with the longest cell axis.

Tension-oriented EVL cell divisions can reduce tissue tension anisotropy and facilitate tissue spreading

Having shown that anisotropic tissue tension can reorient cell divisions along the main axis of tension, questions arise as to the function of these tension-oriented cell divisions for EVL epiboly movements. It has been suggested that oriented cell divisions facilitate tissue spreading by decreasing tissue tension along the axis of division10. We therefore first examined whether individual cell divisions affect EVL tissue tension. On the characteristic timescale of a single cell-division event, stresses due to individual cell deformations may have relaxed but deformations of the cellular junctional network imply persistent elastic stresses. When modelling the forces exerted by a dividing cell as a point force dipole in a continuous elastic medium, tensions and elastic deformations show that a cell division decreases elastic stresses in the surrounding tissue along the axis of division (Fig. 5a and Supplementary Note). To test this prediction for the EVL, we turned to our minimal tension assay where we locally induce anisotropic tension within the EVL by ablating two small groups of EVL cells positioned close to each other (Fig. 4a, left). We first compared the degree of EVL tension...
We then determined whether the reduction of tissue tension along the axis of cell division was significantly diminished in the presence of tension-oriented cell divisions. We injected with tau-GFP mRNA to mark spindle microtubules. Tension was induced orthogonally to the initial axis of the spindle by creating two constricting wounds in the EVL. The resulting spindle alignment (spindle axis) was determined by measuring the angle between the final spindle axis directly before cytokinesis and the induced tension axis. For controls, no wounds were induced and the endogenous rotation of the spindle from its initial axis was quantified in the same manner, that is, with the initial spindle axis orthogonal to the control axis.

To investigate the consequences of these findings made at the level of individual or small groups of cells for overall EVL spreading, we developed a model of the EVL that is based on our observations described so far, namely that external tensions can bias the orientation of the EVL cell-division axis, and that cell divisions release tissue tension and facilitate tissue spreading along the axis of division. We describe the EVL tissue as a continuous material on length scales that are large compared with that of individual cells, with a rheology that incorporates the effect of tension-oriented cell divisions in an effective shear viscosity. With these ingredients, our model predicts an anisotropic tension profile along the animal–vegetal axis of the EVL and, as a result of this, a global pattern of cell-division orientation within the EVL.
Figure 3 Effects of cell shape and myosin II on spindle orientation and positioning. (a) Rose diagram of the orientations of the longest cell axis of EVL cells undergoing division during the course of EVL epiboly \((n = 514\) cells, 6 embryos) 5 min before the onset of cytokinesis; \(P\) (longest axis) = 0.0369 (calculated by using cell numbers). A, animal; V, vegetal. (b) Alignment of the observed axis of cell-division orientation determined by the spindle axis (yellow) and the predicted axis of cell division (blue) given by cell shape in dividing EVL cells of \(Tg(\text{actb2:myl12.1-mCherry})\) embryos between 30–50% epiboly stage (4.66–5.25 hpf). Embryos were injected with \(\text{tau-mCherry}\) mRNA to mark spindle microtubules and treated with either the myosin II inhibitor blebbistatin or its inactive enantiomer (control); cell contour, white; scale bar, 20 \(\mu\)m. (c) Histograms show the frequency distributions of angles between predicted and observed spindle axis (Supplementary Methods) for both control (17 embryos) and blebbistatin-treated embryos (16 embryos); \(P = 0.0056; n\) = number of divisions. (d) Average maximum distance observed between experimentally determined spindle centre and the prediction by the shape model in myosin-II-inhibitor (blebbistatin)-treated (squares, 16 embryos) and control embryos (circles, 17 embryos); error bars, s.e.m.; \(P = 0.0330; n\) = number of divisions. (e) Minimum to maximum box-and-whisker plots of cell-shape anisotropy values (arbitrary units) computed using the shape model for EVL cells in myosin-II-inhibitor (blebbistatin)-treated (right, \(n = 32\) divisions, 16 embryos) and control embryos (left, \(n = 36\) divisions, 17 embryos); \(P = 0.0163\). Elongated cells have a higher value of shape anisotropy than rounder cells. (f) Energy penalty values (arbitrary units) for individual EVL cells computed using the shape model in myosin-II-inhibitor (blebbistatin)-treated (blue squares, \(n = 32\) divisions, 16 embryos) and control embryos (red circles, \(n = 36\) divisions, 17 embryos) plotted over the angles between predicted and observed spindle axis. The energy penalty quantifies the deviation between the observed angle of the spindle axis from that predicted by the shape model taking differences in shape anisotropy between cells into account. For instance, the same angular deviation between observed and predicted spindle orientation would result in a lower energy penalty for cells with a small degree of shape anisotropy compared with cells with a high degree of anisotropy. Thus, if the deviation between the observed and predicted spindle orientations in blebbistatin-treated embryos was due only to a lower degree of shape anisotropy in those cells (e), we would expect the energy penalty to level off with similar maximal values in control and blebbistatin-treated conditions. Instead, the energy penalties in these two conditions are significantly different \((P = 0.0396)\), suggesting that the effects of blebbistatin treatment on spindle orientation are not solely due to changes in cell-shape anisotropy. a.u., arbitrary units. Number of independent experiments: 6 (a), 13 (b–f).
predictions, we analysed EVL epiboly progression in embryos, in which we either reduced cell divisions during gastrulation by exposing them to the cell-division inhibitors aphidicolin and hydroxyurea\textsuperscript{19}, or interfered with cell-division orientation by injecting them with an α-dynein antibody\textsuperscript{20} (Supplementary Fig. 3a). Consistent with the model predictions, embryos with strongly reduced cell divisions showed slightly reduced EVL epiboly movements at late stages of epiboly (Fig. 5c). However, EVL tissue tension and flow seemed largely normal in embryos with no preferred cell-division orientation (Fig. 5d and Supplementary Fig. 3b), contrary to our model assumptions that orienting cell divisions by tension constitutes the main adaptive mechanism for limiting tension anisotropy within the spreading EVL. Instead, we found EVL cells fusing within the plane of the epithelium when cell divisions were reduced or misoriented (Fig. 6a,b and Supplementary Video 8). These fusions occurred with no preferred orientation, and fused cells remained integrated within the EVL and expanded their apical area at similar rates as their non-fused counterparts (Fig. 6c,d). Moreover, cell fusions were not the result of incomplete cell divisions, as fusions usually did not occur between sister cells (Supplementary Fig. 4). Importantly, EVL cell fusions were accompanied by a rapid extension of the collapsing junction directly before the fusion (Fig. 6e), indicative of tension release along this junction. These observations led us to reason that EVL cell fusions might be caused by augmented tissue tension in embryos...
Initial recoil velocity (μm min⁻¹)

Dynein antibody

Dynein antibody

WT

Perpendicular

Parallel

50–60%

0

2

4

n = 29

n = 25

n = 28

n = 11

n = 45

n = 41

n = 16

n = 28

n = 25

n = 45

n,

number of embryos; scale bar, 100 μm. (d) Initial recoil velocities for ultraviolet laser cuts perpendicular (red) and parallel (green) to the EVL margin for control and dynein antibody-injected embryos at 50–60% epiboly stage (5.25–6.5 hpf); error bars, s.e.m.; P(control versus α-dynein antibody-injected embryos, parallel cuts) = 0.19; P(control versus α-dynein antibody-injected embryos, perpendicular cuts) = 0.51. WT, wild type. Number of independent experiments: 3 (c), 5 (d).

Figure 5 Oriented cell divisions and EVL epiboly progression.

(a) Illustration of the main components of the theoretical model proposed to describe the role of oriented cell divisions for EVL tension and spreading during epiboly (Supplementary Note). A single cell division redistributes the stresses in the tissue on the timescale of the division (left) and is itself oriented by local stresses (middle). At the level of the whole tissue, different stress-relaxation mechanisms lead to an effective viscous behaviour, to which tension-oriented cell divisions contribute (right). (b) Schematic representation of the model predictions for tissue tensions and flow during epiboly. A reduction of tension-oriented cell divisions would be expected to increase shear viscosity, which in turn would lead to increased anisotropic tensions and a reduced tissue flow.

(c) Epiboly progression in embryos where cell division was either blocked with reduced or misoriented cell divisions, and that they might lead to a reduction of tissue tension anisotropy, partially compensating for the lack of tension-oriented cell divisions in this process. To experimentally address this hypothesis, we sought to subject the EVL to augmented tissue tension by deforming the embryos into a cylindrical shape and thereby expanding their surface area, assuming that the embryo volume is conserved (Fig. 7a). Consistent with our hypothesis of augmented tissue tension inducing cell fusions, we found EVL cell fusions to be strongly increased on surface expansion (Fig. 7b,c and Supplementary Video 9). Moreover, our previous observation that EVL epiboly movements seem largely unaffected in cylindrical embryos is compatible with the assumption that cell fusions, similar to tension-oriented cell divisions, promote EVL epiboly movements by releasing ectopic tissue tension.
Yet, questions remain as to the mechanisms by which tissue tension induces EVL cell fusions and, vice versa, EVL cell fusions release tissue tension. Although difficulties in predicting and/or inducing fusions of EVL cells did not allow us to directly address the reciprocal relationship between tension and cell fusion using our ectopic tension assay, our findings so far support the notion that globally elevated tissue tension can induce cell fusions, and that cell fusions can locally release tissue tension. Whether cell fusions can also reduce global tissue tension anisotropy is not yet entirely clear. However, considering that collapsing borders oriented along the main axis of tissue tension would be expected to release more tension than collapsing borders with other orientations, the combined effect of all EVL cell fusions without preferred orientation will probably reduce overall EVL tissue tension anisotropy.

DISCUSSION

Our study identifies the orientation of cell division by external tension as an efficient mechanism to release anisotropic tissue tension during EVL spreading. The degree of anisotropic tissue tension thus depends on the ability of oriented cell divisions to reduce tension anisotropy and, vice versa, the stereotypical orientation of cell divisions depends on the degree of anisotropic tension within the tissue. We expect that these reciprocal dependencies balance each other so that the EVL tissue generally exhibits a low degree of tension anisotropy, facilitating its...
spreading. Consistent with this, we observe low tension anisotropy at early stages of EVL spreading when the rate of cell divisions is high, and high tension anisotropy at later stages of epiboly when the rate of cell divisions is comparatively low (Figs 1d and 2b).

Tension has been proposed to control cell-division orientation through different mechanisms. One prime effector mechanism is cell elongation, as cell-shape anisotropies are thought to influence metaphase spindle orientation through putative length-dependent astral microtubule forces\textsuperscript{14}. Our finding that within the epibolizing EVL, spindle orientation, cell elongation and the main axis of tissue tension are all aligned to each other, suggests that tension controls EVL cell division orientation by cell elongation. However, tension has also been shown to orient the mitotic spindle in a myosin II-dependent manner independently of its effect on cell shape\textsuperscript{11}. Although we have no direct evidence for a tension-mediated polarization of myosin II to orient the division plane within the EVL, our observation that myosin II might be required for spindle orientation in EVL cells in addition to its function in cell elongation points to similar mechanisms for cell division orientation within the EVL. Yet, to prove this mechanism, further experiments will be needed to show how tension modulates myosin II activity and whether myosin II indeed mediates the function of tension in orienting the mitotic spindle in EVL cells.

Whereas there is increasing evidence for tissue tension controlling cell-division orientation\textsuperscript{16,21,22}, considerably less is known about the effect of tension-oriented cell divisions on tissue tension anisotropy. Importantly, the effect of oriented cell divisions on tension anisotropy that we observed is not a consequence of tissue growth along the division axis, as EVL cells do not increase their volume between divisions (Fig. 1c). Rather, tension-oriented cell divisions lead to cellular rearrangements releasing tension along this axis of division. Similarly, cell fusions are expected to release tissue tension, and thereby partially compensate for the lack of oriented cell divisions, by fusion-mediated cell rearrangements. However, as the degrees of cell rearrangements associated with division and fusion seem similar (Supplementary Videos 4 and 8) but the number of cell fusions compared with divisions is small (21 fusions versus 576 divisions per embryo from sphere to 55% epiboly stage; for details on how the total number of fusions and divisions per embryo was estimated see Supplementary Methods), cell fusions are unlikely to be sufficient to fully compensate for the function of oriented cell divisions in reducing tissue tension anisotropy. Observations in different teleost fish species of EVL cells undergoing junctional remodelling during epiboly\textsuperscript{4,6,23}, and in flies of anisotropic tension distribution within the spreading epidermis during dorsal closure being associated with cell elongation along the axis of main tension\textsuperscript{24}, point to junctional remodelling and cell elongation as additional mechanisms for tension release within the EVL. In agreement, we observe preferential junction disassembly within the EVL along the circumference of the embryo, which seems more pronounced when tension-oriented cell divisions are impaired (Supplementary Fig. 5). Thus, anisotropic tension release during EVL tissue spreading in epiboly relies on distinct cellular mechanisms that act in concert to limit tissue tension anisotropy and facilitate epiboly movements.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Note:** Supplementary Information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
P.C., M.B., J.R., T.R. and C-P.H. synergistically and equally developed the presented ideas and the experimental and theoretical approaches. P.C. performed the experiments; P.C. and M.B. did the data analysis; J.R. and T.R. developed the theory; M.B. contributed to the experimental work; N.M. contributed to the data analysis and interpretation.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.
METHODS

Embryo staging and maintenance. Fish maintenance and embryo collection were carried out as described previously. Embryos were raised in either E3 medium or Danieu’s buffer (for dechorionated embryos), kept at 28.5 °C and staged according to morphological criteria.

Fish lines. Wild-type TL, Tg(actb2:myl12.1–GFP/mCherry), Tg(actb2:lifeact–GFP) and Tg(actb2:GFP–utrCH) zebrafish lines were used in this study.

Plasmids. Bovine Tau was amplified from a Tau:eGFP vector and tagged using Gateway technology (Invitrogen). The following primers were used to flank the sequence with attb1 and attb2 overhangs: 5’-GGGACGACATTGTGACAAAAAGCGCCGCTTACCCGCCCACCTTGCC-3’ and 5’-GGGACGACATTGTGACAAAAAGCGCCGCTTACCCGCCCACCTTGCC-3’. The PCR product was recombined with pDONR221 (Lawson #208) and the resulting entry-clone was recombined with the middle-entry-clone vector, p3ENTR(R2-L3) (Lawson #441), and the destination vector, pDEST(R1-R3) (Lawson #444), to obtain the pCS-Tau–mCherry expression plasmid.

Antibody and mRNA injections. Injections into one-cell stage embryos were performed as described previously. To obtain mosaic expression for the cell volume and height measurements, messenger RNA was injected into single blastomeres of 32-cell stage embryos (1.75 hpf). Synthetic mRNA was produced using the SP6 mMessage mMachine Kit (Ambion). To visualize the mitotic spindle, plasma membrane, nucleus and/or filamentous actin, 100 pg of tau:eGFP/mCherry, 50 pg of GPI–RFP, 75 pg of h2a–Cherry and/or 50 pg of lifeact–GFP mRNA were injected in one-cell stage embryos.

Interference with EVL cell division rate and orientation. To block cell division during gastrulation, embryos were incubated in E3 medium containing 150 μM aphidicolin (A0781, Sigma) in dimethylsulphoxide and 30 μM hydroxyurea (H8627, Sigma) from sphere to bud stage (4–10 hpf). To randomize cell division orientation, a mouse anti-dynein intermediate chain monoclonal antibody (clone 70.1, D5167, Sigma) was injected at a 1:4 dilution into one-cell stage embryos. All embryos were fixed with 4% paraformaldehyde (PFA) once the respective control embryos (dimethylsulphoxide and ATP-supernatant/ascites) were at bud stage (10 hpf). F-actin was labelled using Alexa488-phalloidin (1:250, A12379, Invitrogen) and fluorescence micrographs were taken on a Leica M165 FC fluorescent stereo microscope. To determine EVL cell progression in cell division-inhibited embryos, the distance from the animal pole to the EVL margin was measured and divided by the distance from the animal pole to the vegetal pole. For determining EVL cell progression in the case of randomized cell division orientation, the uncovered blastopore area was measured as depicted in Fig. S5c. This quantification proved to be more sensitive for very weak reductions in epiboly progression than calculating the distance of the EVL margin to the animal pole. For consistency, the area measurement A was then transformed to percentage epiboly (Fig. S5c), assuming an average radius R for the spherical embryos of 350 μm (percentage of epiboly = A/(4πR²)).

Phalloidin, DAPI and phospho-histone H3 staining. Wild-type embryos at sphere and 30%, 55% and 60% epiboly stage (4, 4.66, 5.66 and 6.5 hpf, respectively) were fixed with 4% PFA. Rabbit anti-phospho-histone H3 antibody (1:300, 0.06–570, Upstate Biotech) was used in combination with goat anti-rabbit Cy3-conjugated antibody (1:500, 111-175-003, Jackson), F-actin and DNA were detected using Alexa488–phalloidin (1:250, A12379, Invitrogen) and 4,6-diamidino-2-phenylindole (DAPI, 1:300, D9564, Sigma), respectively. Image acquisition was performed on a Leica SP5 upright confocal microscope equipped with a Leica ×25/0.95 NA water-dipping lens for embryos at sphere and 30% epiboly stage (4 and 4.66 hpf, respectively). The embryos at 55% and 60% epiboly stage (5.66 and 6.5 hpf, respectively) were imaged using a Nikon ×16/0.8 NA water-dipping lens. Images were acquired using Leica TCS SP5 software and definitive images were obtained by deconvolution using the Leica LAS software. For consistency, the area measurement A was then transformed to percentage epiboly (Fig. S5c), assuming an average radius R for the spherical embryos of 350 μm (percentage of epiboly = A/(4πR²)).

EVL confocal imaging during epiboly progression. For EVL epiboly progression, embryos injected with GPI–RFP mRNA alone or in combination with tau–GFP mRNA were imaged using an inverted Zeiss LSM 510 confocal microscope equipped with a Zeiss ×20/0.8 NA air lens. Time-lapses typically lasted for 180 min, during which the sample was kept at a constant temperature of 28.5 ± 1 °C by using a stage incubation chamber. Consecutive z-stacks (up to 65 μm depth) were recorded every 2.5–3.4 min, from the start of epiboly (sphere stage; 4 hpf) until 70% (7 hpf). EVL cell division orientation, cell area and orientation of appearing/disappearing junctions were determined using the publicly available Paking Analyst software that allows semi-automatic cell segmentation on 2D images (Fig. 1a and Supplementary Video 1). To this end, the confocal time-lapses were first rendered in Velocity (Perkin Elmer) using the 3D opacity render function and then exported as an image sequence. EVL cell fusion counting and junction assembly rate measurements were performed manually using Fiji.

Note that in our time-lapse videos, we imaged only 1/5 of the embryo as estimated by counting the total number of EVL cells observed in the field of view in our videos at sphere and 55% epiboly stages (4 and 5.66 hpf, respectively), and then dividing those by the previously determined (fixed samples, see above) total number of EVL cells at the respective stages. Thereby, to determine the total number of cell divisions/fusions taking place in the whole EVL, we multiplied the number of fusions observed in our videos from sphere–55% epiboly stage (4–5.66 hpf) by the factor of 5 (21 fusions versus 576 divisions; the number of divisions was directly obtained from the fixed sample data).

For EVL cell volume and height measurements, embryos were injected at 32-cell stage (1.75 hpf, as described above) with 25 pg of GPI–RFP mRNA and 50 pg of DAPI and imaged using a Leica SP5 confocal microscope equipped with a Leica ×25/0.95 NA water-dipping lens. The temperature during imaging was kept constant at 28.5 ± 1 °C using a temperature chamber (Life Imaging Services). Consecutive z-stacks (up to 75 μm depth) were recorded every 10 min during a period of ~3–5 h starting either at sphere (4 hpf) or shield stage (6 hpf). Images were recorded using Imaris (Bitplane) software and EVL cells were manually segmented using the surfaces function.

To assess EVL tissue flows, Tg(actb2:lifeact–GFP) and Tg(actb2:GFP–utrCH) embryos were imaged on a Leica SP5 confocal microscope equipped with a Leica ×25/0.95 NA water-dipping lens. The temperature during imaging was kept constant at 28.5 ± 1 °C using a temperature chamber (Life Imaging Services). Consecutive z-stacks (up to 142 μm depth) were recorded every 2.5 min on time-lapses that typically lasted for 30 min and acquired throughout the course of epiboly (30–80% epiboly; 4.66–8.5 hpf). Flow velocities were quantified by particle image velocimetry (PIV) on the maximum intensity projections of the z-stacks using customized Matlab scripts, which corrected for the curvature of the embryo. Depicted distances are the arc length, and velocities are the tangential measure along the surface of the embryo. The resulting 2D flow vector fields were repositioned along the animal–vegetal axis according to their relative position to the detected EVL margin and averaged along the circumferential direction parallel to the EVL margin. Consistent with the radial symmetry of the EVL/YSL epiboly, the 2D flow vector fields were thereby reduced to 1D flow profiles along the animal–vegetal axis. Flow profiles of the consecutive acquisition loops throughout the duration of a video were subsequently averaged according to their position relative to the moving EVL margin.
EVL tissue tension measurements by cortical laser ablation. Cortical tension within the EVL tissue was assessed by conducting laser ablation on a previously described ultraviolet–laser ablation set-up6 equipped with a Zeiss ×40/1.2 NA water-immersion lens using Tg(actb2:myl12.1:eGFp) embryos injected with GPI-REM mRNA. EVL cells directly adjacent to a dividing (experimental) or non-dividing (control) cell were simultaneously ablated using a previously described ultraviolet–laser ablation set-up6. We then determined the change in distance between the edges of the ablated cells at 252 s after ablation (Fig. 4b and Supplementary Video 7). Experiments were performed at the animal pole of Tg(actb2:myl12.1:eGFp) embryos injected with both tau-mCherry and GPI-REM mRNAs between 30 and 50% epiboly stage (4.66–5.25 hpf). All experiments were performed at a constant temperature of 28.3 ± 1°C using both a custom-built objective heater and a stage incubation chamber.

Analysis of EVL spreading displacement in ectopic tension assay. To determine whether release of anisotropic tension by oriented cell division can facilitate epithelial spreading, we locally induced anisotropic tissue tension in either the presence or absence of an oriented cell division between the ablation sites. To locally induce anisotropic tension within the tissue, two EVL cells directly adjacent to a dividing (experimental) or non-dividing (control) cell were simultaneously ablated using a previously described ultraviolet–laser ablation set-up6. We then determined the change in distance between the edges of the ablated cells at 252 s after ablation (Fig. 4b and Supplementary Video 7). All experiments were performed at a constant temperature of 28.3 ± 1°C using both a custom-built objective heater and a stage incubation chamber.

Analysis of myosin II function in EVL cell-division orientation. Tg(actb2:myl12.1:mCherry) embryos between 30 and 50% epiboly stage (4.66–5.25 hpf) injected with tau-mCherry mRNA were incubated either in the presence or absence of 20 μM blebbistatin (active or inactive enantiomer), and imaged using a spinning-disc set-up (Andor Revolution Imaging System, Yokogawa CSU-X1) assembled onto an Axio Observer Z1 microscope (Zeiss) equipped with a Zeiss ×40/1.2 NA water-immersion lens. Images were acquired with an iXon DU-897-BV camera (Andor Technology), using exposure times of 300 ms and 15 s frame rates (20 z-stacks). Using custom-designed Matlab scripts, the spindle-pole position (yellow) and the outline of the cells (white) were manually tracked as depicted in Fig. 3b. On the basis of the cell outline, a previously described computational model14 allowed for predicting spindle orientation and position. As detailed in ref. 14, this shape model is based on astral-microtubule-length-dependent pulling forces that function to probe cellular space to position the spindle with respect to cell geometry. We chose for all conditions the same parameter settings as previously published (see Table S1 of ref. 14) except for the number of microtubules, where we chose 40 instead of 50 microtubules and the size of the theoretical spindle was adjusted to match the experimentally observed spindle size. To determine whether myosin II has an effect on cell-division orientation, we quantified the deviation of experimental spindle orientation from the prediction by the shape model in dependence of myosin II activity. The prediction of spindle orientation based on the shape model was averaged for all time points up to 2 min before cytokinesis. Time points close to cytokinesis were excluded to ensure that shape changes due to furrow ingression and cell elongation did not influence the prediction. The predicted spindle orientation was then compared with the final observed spindle orientation and position at cleavage furrow formation in the case of blebbistatin-treated and control embryos (Fig. 3c,d and Supplementary Video 5).

As detailed in ref. 14, more elongated cells were predicted to align spindles more closely with the long axis than less elongated cells. This dose-dependent effect can be quantified from computing the depth of the energy potential well in the model, and is referred to as the cell-shape anisotropy factor (Fig. 3e). In addition, the predicted energy profile can be used to compute the energy penalty, which is the difference between the experimental and theoretical potentials. This serves as a quantitative measure on how well the experimental spindle aligns along the predicted axis, independently of cell-shape anisotropy effects (Fig. 3f).

Embryo aspiration. Pre-gastrula stage Tg(actb2:GFP–utrCH) embryos (2.5 hpf) injected with GFP-REM and H2A–Cherry mRNAs were aspirated into agarose tubes made of 2% agarose with an inner diameter of 500 μm, which is smaller than that of the embryo diameter (~700 μm). Agarose tubes were produced by letting melted agarose (2%) solidify inside a glass capillary (Brand), in which a glass beam (~500 μm; Hilgenberg GmbH) had been inserted to set the inner diameter of the agarose tube. After solidification of the agarose and removal of the glass beam, embryos were manually aspirated into the glass capillary holding the agarose tube. After embryo aspiration, the agarose tube was transferred to a Petri dish and imaged using either a Leica SPS or a Zeiss LSM 700 upright confocal microscope equipped with a Leica ×25/0.95 NA or a Zeiss ×20/1.0 NA water-dipping lens, respectively. The temperature during imaging was kept constant at 28.3 ± 1°C using a temperature chamber (Life Imaging services). Consecutive z-stacks (up to 100 μm

Local induction of anisotropic tension (ectopic tension assay). To locally induce anisotropic tension within the EVL, two small groups of EVL cells directly adjacent to a dividing site were ablated using a previously described ultraviolet–laser ablation set-up6. To determine the outward velocity from the first two images after ablation, by averaging the component of the calculated PIV flow field that was orthogonal to the cut line over two adjacent rectangles (10.2 × 100 μm) placed at a distance of 6.8 μm away from the cut7. The resulting initial recoil velocity of each cut in a single embryo was averaged to yield the experimental recoil velocity for the depicted conditions. Under the experimental parameters used, we observed two different reactions when cutting embryos using cortical laser ablation: opening of the cortex in response to the ablation without affecting the cell–membrane integrity (~70% of all ablations; Supplementary Video 2) and cuts that elicited cortical opening in conjunction with cell-membrane rupturing (~30%), recognizable by loss of the GPI-REM labelling in the cut opening. For the analysis, we used only cuts that showed no membrane damage to ensure that leakage of cytoplasm through membrane opening did not influence the recoil velocity measurements8. Note that EVL cells on which the cortical laser ablation was performed exhibited apoptosis and were actively extruded from the epithelium.

METHODS

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depth) were recorded every 2.5 to 3.95 min during a period of 3–4 h starting at sphere (4 hpf) stage. Images were rendered using Imaris (Bitplane) software and EVL cell fusions were counted manually.

**Statistical analysis.** To determine whether a given observed distribution of angle orientations (cell division, cell longest axis and appearing/disappearing junctions) was different from a random distribution, we performed a $\chi^2$ test using Excel (Microsoft) to calculate $P$ values ($\alpha = 0.05$). For all of the other experimental results, unpaired Mann–Whitney $U$ tests were used to calculate two-tailed $P$ values ($\alpha = 0.05$) with Prism software (GraphPad). All $P$ values are mentioned within the figures legends. We used a non-parametric unpaired test because we assume that the data are not normally distributed and that in the compared groups the individual values were not paired or matched with one another.

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Supplementary Figure 1 EVL cell-shape changes. (a) Apical cell area of representative individual EVL cells (n = 30 cells, 6 embryos) as a function of time after sphere stage (4 hpf). (b,c) Apical-basal cell height (b) and volume (c) of individual EVL cells both before (full line) and after cell division (dashed line; n = 17 cells, 5 embryos) as a function of time after sphere stage (4 hpf).
Supplementary Figure 2 Cell-flow and anisotropic-tension profiles as predicted by the continuum theory. (a) EVL cell-flow velocity $v_{\theta}$ and (b) anisotropic tension $\tilde{t}_{\theta\theta} = (t_{\theta\theta} - t_{\phi\phi})/2$ plotted as a function of $\theta$ for different degrees of epiboly progression and different values of the ratio of shear to bulk viscosity $\eta/\zeta$. In order to minimize the number of potentially variable parameters, non-dimensionalized quantities are displayed; only simple global rescalings need to be performed to recover physical units. The cell-flow velocity is normalized by the reference velocity $v_{\theta} = R(t_{m} - t_{0})/\zeta$ and tensions are normalized by the reference tension $t_{m} - t_{0}$. For all plots shown, we assume a vanishing substrate friction for simplicity ($\gamma = 0$), consistent with previous work. The percentage of epiboly progression is simply related to the value of the opening angle $\theta$ by the relation: % e.p. = $ (1 - \cos \Theta )/2$. Note that an underlying assumption of the model description is that cell-division orientation is biased by tension anisotropy in the tissue, such that the anisotropic tension distribution shown here corresponds also to the predicted pattern of cell-division orientation in the tissue, up to a global proportionality factor.
Supplementary Figure 3 Randomized cell-division orientation. (a) Rose diagram of the orientation of cell-division axes at cytokinesis (yellow) for EVL cells dividing during the course of gastrulation in Dynin antibody-injected embryos (n = 291 divisions, 4 embryos); p = 0.37 (calculated by using division numbers). (b) Average tissue flow velocities within the EVL as a function of distance from the EVL margin for control (orange) and a-Dynein antibody-injected (blue) embryos at 30 – 50 %, 50 – 60 %, 60 – 70 % and 70 – 80 % epiboly stage; error bars, s.e.m.; n, number of embryos; control embryos for the a-Dynein antibody experiments were injected with the antibody supernatant/ascites. Number of independent experiments = 4 (a), 10 (b).
Lineage tracing reveals fusing cells are not sisters (n = 5/5)

Mid-body between fusing cells indicates cells are sisters (n = 1/16)

Mid-bodies with other neighbours indicate cells are not sisters (n = 3/16)

No mid-body between fusing cells indicates cells are not sisters (n = 12/16)

Supplementary Figure 4 Lineage relationship between fusing EVL cells. (a) Time course of an exemplary EVL cell-fusion event (arrowhead) in a cell-division inhibitor-treated embryo from sphere stage (t = 0 min) onwards for cases where the fusing cells could be manually back-tracked to resolve their lineage. Note that one of the fusing cells undergoes a division (arrow) prior to the fusion. Lineage tracing of all fusing cells where a previous division could be detected (n = 5 fusions), shows that this division was not giving rise to both fusing cells, demonstrating that the fusing cells are not sisters. (b) Time courses of exemplary EVL cell-fusion events (arrowheads) in cell-division inhibitor-treated embryos from sphere stage (t = 0 min) onwards for cases where lineage tracing of the fusing cells was not successful. To determine the relationship of fusing cells in such cases (n = 16 fusions), we analyzed whether these cells display mid-bodies (arrows) with neighboring cells, indicative of a previous division. Only in one fusion event (n = 1; upper panel), a mid-body was found between the fusing EVL cells, suggesting that these cells are sisters. In all other fusion events (n = 15 fusions), the fusing EVL cells either showed mid-bodies with other EVL cells (n = 3, middle panel) or no mid-bodies at all (n = 12, lower panel), suggesting that they are not sisters. Cell membrane and spindle microtubules marking the mid-body were visualized by GPI-RFP and Tau-GFP, respectively; n, number of fusions, 5 embryos; scale bar, 20 μm. Number of independent experiments = 5 (a,b).
Supplementary Figure 5  EVL junction remodeling. Rose diagrams of the orientations of appearing (green) and disappearing (red) junctions within the EVL of control (left, 6 embryos) and a-Dynein antibody-injected (right, 4 embryos) embryos during the course of gastrulation; n, number of junctions; p (control, appearing junctions) = 5.3E-12; p (control, disappearing junctions) = 1.4E-05; p (a-Dynein antibody-injection, appearing junctions) = 1.8E-09; p (a-Dynein antibody-injection, disappearing junctions) = 4.5E-09; p (a-Dynein antibody-injection vs. control, disappearing junctions) = 1.31E-06 (calculated by using junction numbers). Number of independent experiments = 10.
**Supplementary Table 1** EVL cell area, height and volume measurements. Number of cells and embryos analyzed for the measurements of apical cell area (Fig. 1b), apical-basal cell height (Fig. 1b), and cell volume of individual EVL cells (Fig. 1c) as a function of time after sphere stage (4 hpf). Note that cell area measurements were performed in 10 min time intervals (yellow table), while the cell volume and height measurements were performed in 20 min time intervals (green table).
**Supplementary Videos Legends**

**Supplementary Video 1** EVL cell divisions. Time-lapse of the EVL in a wild-type embryo expressing GPI-RFP to outline EVL cells and cell divisions marked in yellow; t = 0 min corresponds to sphere stage (4 hpf). Scale bar, 100 μm.

**Supplementary Video 2** UV laser cuts to map EVL tissue tension. Time-lapses of cortical laser cuts of the apical actomyosin cortex perpendicular (red) and parallel (green) to the EVL margin and at the animal pole (blue), in Tg(actb2:myl12.1-eGFP) embryos at 65% epiboly also expressing GPI-RFP to outline EVL cells. Red, green and blue lines (100 μm length) mark the position of the perpendicular, parallel and animal cuts, respectively. Scale bar, 20 μm.

**Supplementary Video 3** Ectopic EVL tissue tension re-orients the mitotic spindle. Time-lapse of the alignment of the cell division axis with the axis of induced tension in a Tg(actb2:myl12.1-mCherry) embryo at 40% epiboly also expressing Tau-GFP to mark spindle microtubules. Tension was induced orthogonally to the initial axis of the spindle (yellow) by creating two constricting wounds in the EVL. Scale bar, 20 μm.

**Supplementary Video 4** EVL cells do not round up during mitosis. Time-lapse of a typical EVL cell division (arrow) in a wild-type embryo at 50% epiboly expressing GPI-GFP to outline EVL cells. Scale bar, 20 μm.

**Supplementary Video 5** Myosin II activity is required for proper positioning of the mitotic spindle to the cell long axis. Time-lapse of dividing EVL cells in Tg(actb2:myl12.1-mCherry) embryos between 30-50% epiboly. Embryos also express Tau-mCherry to mark spindle microtubules and were treated with either the myosin II-inhibitor Blebbistatin (right) or its inactive enantiomer (left). Scale bar, 20 μm.

**Supplementary Video 6** Tension-oriented cell divisions release anisotropic tension within the EVL. Time-lapse of exemplary cortical laser cuts of the apical actomyosin cortex perpendicular (blue) or parallel (orange) to the axis of induced tension either in the presence (right) or absence (left) of an EVL cell division (white contour) oriented along the axis of tension. Blue and orange lines (50 μm length) indicate were the cuts will be performed. Tg(actb2:myl12.1-eGFP) embryos at 30-40% epiboly. Scale bar, 20 μm.

**Supplementary Video 7** Tension-oriented cell divisions facilitate EVL spreading. Time-lapse of the spreading displacement of an EVL cell (white cell contour) in Tg(actb2:myl12.1-eGFP) embryos at 30-40% epiboly upon induction of ectopic tension either in the presence (right) or absence (left) of a cell division oriented along the axis of tension. Red crosses mark the ablation sites where wounds were induced. Scale bar, 20 μm.

**Supplementary Video 8** EVL cells fuse when EVL cell divisions are inhibited. Time-lapse of a exemplary EVL cell fusion (arrowhead) in a cell division inhibitor-treated embryo, expressing both Tau-mCherry and GPI-RFP to mark the spindle microtubules and plasma membrane, respectively. Scale bar, 20 μm.

**Supplementary Video 9** EVL cells fuse in cylindrically deformed embryos. Time-lapse of an exemplary EVL cell fusion event (arrowheads) in a cylindrical Tg(actb2:GFP-utrCH) embryo from sphere stage (t = 0 min) onwards. Arrows point at a cell division, which gives rise to a daughter cell that subsequently fuses with another unrelated cell. Cell membrane and nuclei were marked by GPI-RFP and H2A-Cherry, respectively. Scale bar, 20 μm.
SUPPLEMENTARY NOTE

Tension-oriented cell divisions limit anisotropic tissue tension in epithelial spreading during zebrafish epiboly

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We propose a theoretical model for the mechanics of the enveloping cell layer (EVL) during zebrafish gastrulation in order to investigate the role of tension-oriented cell divisions during epiboly progression. Focusing on morphological processes on scales that are large compared with that of individual cells, we model the EVL as a continuous medium. The EVL epithelium is a cell monolayer thin compared to the yolk radius, and we make use of an effective two-dimensional description of the tissue. A similar description has been put forward to investigate the forces responsible for driving epiboly progression¹. We first investigate the role of a single cell-division event on the tension distribution in the surrounding tissue on short timescales (Sec. 1). Modeling the EVL epithelium as a continuous elastic medium and the cell division as a point force dipole, we show that in a prestressed tissue along a particular axis, a cell-division event oriented along that axis relaxes the pre-existing tension. We further investigate the origin of anisotropic tensions within the EVL, as well as the consequences of a reciprocal coupling between oriented cell divisions and anisotropic tensions, using a model of the whole epibolizing EVL that takes into account the aforementioned reciprocal coupling into effective parameters (Sec. 2). We assume for simplicity that the yolk cell underlying the EVL is a rigid spherical body, and the observed rotational symmetry around the animal-vegetal (AV) axis renders the problem effectively one-dimensional.
This allows us to derive an equation for the cell-flow field, which we can solve analyti-
cally (Sec. 3). Based on a previous work\(^2\) and our prediction at the single-cell level, we can
infer the influence of cell division on the parameters of the model and have predictions on
how impairing tension-oriented cell division should affect the tensions as well as the cell-flow
field during the course of epiboly (Sec. 4).

1. Tissue tensions associated with a single cell-division event

In general, the rheology of tissues is the result of a combination of several stress-relaxation
mechanisms operating at the cellular and tissue scales, such as cytoskeletal turnover and
junctional remodeling, which each occur on a characteristic timescale. On the timescale of
a single cell-division event, stresses due to individual cell deformations may have relaxed
but deformations of the cellular junctional network imply persistent elastic stresses. To
investigate the tensions associated with a single cell division in the surrounding tissue, we
describe how impairing tension-oriented cell division should affect the tensions as well as the cell-flow
field during the course of epiboly (Sec. 4).

In general, the rheology of tissues is the result of a combination of several stress-relaxation
mechanisms operating at the cellular and tissue scales, such as cytoskeletal turnover and
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a single cell-division event, stresses due to individual cell deformations may have relaxed
but deformations of the cellular junctional network imply persistent elastic stresses. To
investigate the tensions associated with a single cell division in the surrounding tissue, we
therefore model the two-dimensional EVL tissue as a continuous elastic medium with bulk
and shear elastic moduli \(\kappa\) and \(\mu\). The constitutive equation of such a tissue reads

\[
t = \kappa u 1 + 2\mu \tilde{u}.
\]  

Here, the tension tensor \(\mathbf{t}\) describes elastic forces in the tissue, and the displacement gradient
tensor \(\mathbf{u}\) that characterizes elastic deformations has been split into its isotropic and traceless
parts \(\mathbf{u}\) and \(\tilde{\mathbf{u}}\), respectively. Note that \(\mathbf{u} = u 1 + \tilde{\mathbf{u}}\), where \(u = \text{Tr}[\mathbf{u}]/2\) is half of the trace
of the tensor \(\mathbf{u}\) and \(1\) is the unity tensor. The tension tensor \(\mathbf{t}\) is the two-dimensional
equivalent of the stress tensor in three dimensions and has the physical dimension of a force
per unit length rather than a force per unit area. The displacement gradient tensor is given
by \(\mathbf{u} = \nabla \otimes \mathbf{u}\), where \(\mathbf{u}\) is the displacement field, \(\nabla\) denotes the spatial derivative operator
and \(\otimes\) the tensorial product operator.

Mechanics in the tissue is dictated by momentum balance, which, in the limit where
inertia plays a negligible role, reduces to force balance:

\[
\nabla \cdot \mathbf{t} = -\mathbf{f}^{\text{int}} - \mathbf{f}^{\text{ext}}. 
\]  

The two-dimensional force densities \(\mathbf{f}^{\text{int}}\) and \(\mathbf{f}^{\text{ext}}\) describe respectively internal forces in the
tissue due to cell-division and cell-death events, and external forces that account for the interaction of the EVL with its substrate. On short timescales, we focus on the changes in the distribution of tensions in the tissue due to a cell-division event prior to the subsequently induced tissue flows, and for the sake of simplicity we neglect the interaction between the EVL and its substrate, thus assuming \( f^{\text{ext}} = 0 \).

A cell-division event generates internal forces that can be described by a force dipole, such that the force density related to a division located at the origin reads

\[
f^{\text{int}} = -d \cdot \nabla \delta(r). \tag{3}
\]

Here, \( d \) is a symmetric tensor with the physical dimension of a force times a length, characterizing the strength of the forces generated by cell division. In the case where the force dipole does not introduce any additional torque, the tensor \( d \) reads

\[
d_{ij} = d_1 \delta_{ix} \delta_{jx} + d_2 \delta_{iy} \delta_{jy},
\]

where \( \delta_{ij} \) is the Kronecker symbol, \( i \) and \( j \) denote spatial-coordinate indices, and \( d_1 > 0 \) and \( d_2 > 0 \) characterize the amplitudes of the point force dipole along the \( x \)- and \( y \)-axes, respectively. Solving for the tension distribution in the tissue, one obtains the following components of the tension tensor induced by the cell-division force dipole along the \( x \)-axis and the \( y \)-axis:

\[
t_{xx}(x, 0) = \frac{-d_1(1 + \beta + \beta^2) + d_2(1 - \beta + \beta^2)}{2\pi(1 + \beta)} \frac{1}{x^2} \tag{4}
\]
on the \( x \)-axis, and

\[
t_{yy}(0, y) = \frac{d_1(1 - \beta + \beta^2) - d_2(1 + \beta + \beta^2)}{2\pi(1 + \beta)} \frac{1}{y^2} \tag{5}
\]
on the \( y \)-axis, where \( \beta = \kappa/\mu \) is the ratio between bulk and shear elastic moduli. In the experimental results presented in Fig. 4a of the main text, a cell-division event along the \( y \)-axis relaxes tension in both directions, but by a larger amount in the direction of the division. To recover this result here, it is necessary and sufficient to have positive values for the two force-dipole amplitudes \( d_1 \) and \( d_2 \), together with the condition

\[
1 < \frac{d_2}{d_1} < \frac{1 + \beta + \beta^2}{1 - \beta + \beta^2}, \tag{6}
\]

namely that a single cell division exerts forces mainly in the direction of the division, which
Figure A: Displacement field associated with a point force dipole in an elastic medium. Lengths are plotted in units of $\sqrt{d_1/\mu}$ such that the result depends on only two non-dimensional parameters, which take the values $d_2/d_1 = 2$ and $\beta = 2$. Each arrow represents the local displacement $u(x, y)$ and is centered at the point of coordinates $(x, y)$ where it is calculated.

is physically intuitive. In the left panel of Fig. 5a of the main text, we show the total tension distribution around a point force dipole in a prestressed elastic medium. Tensions are locally represented by ellipses, whose long and short axes are oriented along the two main axes of tension and whose lengths are proportional to the respective tension values along these axes. The main axes and respective tension values follow from the tension matrix $t_{ij}$ as given by the eigenvectors and corresponding eigenvalues of this matrix. Without the force dipole, tensions in the tissue are given by $t_{ij}^{(0)} = t_{1}^{(0)} \delta_{ix} \delta_{jx} + t_{2}^{(0)} \delta_{iy} \delta_{jy}$. The total tension distribution as modified by the force dipole is given by the sum of $t_{ij}^{(0)}$ and the contribution of the force dipole calculated above. Expressed in units of $\mu$, it is completely determined by the following non-dimensional parameters, which in Fig. 5a take the following values: $d_1/\mu = 1$, $d_2/\mu = 2$, $t_1^{(0)}/\mu = 1$, $t_2^{(0)}/\mu = 2$, and $\beta = 2$.

We can furthermore express the displacement field imposed by the force dipole, which reads

$$u(x, 0) = \frac{d_1 (1 + 2\beta) - d_2}{4\pi(1 + \beta)\mu} - \frac{1}{x} e_x$$

(7)

on the $x$-axis, and

$$u(0, y) = -\frac{d_1 + d_2 (1 + 2\beta)}{4\pi(1 + \beta)\mu} \frac{1}{y} e_y$$

(8)

on the $y$-axis. A full representation of the displacement field is presented in Fig. A. The fact that a cell division relaxes stresses and does so mainly in the direction of the division constitutes the main ingredient of the model presented in Section 2 below at the level of...
the whole epibolizing EVL, from which EVL flow and tension profiles are determined in a space- and time-dependent manner.

2. Tissue mechanics of the EVL

The two-dimensional EVL tissue is characterized in a continuum theory by a cell-number density $n$ and a cell-flow field $\mathbf{v}$. The cell-number density obeys the continuity equation

$$\partial_t n + \nabla \cdot (n \mathbf{v}) = n (k_d - k_a),$$

where $k = k_d - k_a$ is the effective cell-doubling rate and $k_d$ and $k_a$ denote the rates of cell division and cell death, respectively. Mechanics in the tissue is again given by force balance:

$$\nabla \cdot \mathbf{t} = -\mathbf{f}_{\text{ext}}.$$  \hspace{1cm} (10)

Here, the two-dimensional force density $\mathbf{f}_{\text{ext}}$ describes external forces and accounts for the interaction of the EVL with its substrate. We model this interaction by a friction force, which yields

$$\mathbf{f}_{\text{ext}} = -\gamma \mathbf{v},$$

where $\gamma$ is a friction coefficient per unit area. Internal forces due to cell divisions will be taken effectively into account in the constitutive equation of the tissue by means described below.

It has been shown that in a tissue that is purely elastic when cell division and cell death are absent, their introduction leads generically to a viscoelastic rheology, whose relaxation time is inversely related to a combination of the rates of cell division and cell death, the force dipoles generated by single cell-division and cell-death events, and the reciprocal coupling coefficient between the orientation of cell division and tension anisotropy in the tissue\textsuperscript{2,3}. If $\mu$ is the shear elastic modulus of the tissue on short timescales and $\tau_{\text{div}}$ this relaxation time, the effective shear viscosity on long timescales is obtained as $\eta_{\text{div}} = \tau_{\text{div}} \mu$. When shear tensions in the tissue can relax with a characteristic time $\tau_0$ by other means than cell renewal—such as cell-neighbor exchange or similar processes—the respective stress-relaxation rates add up.
For the effective shear viscosity, one then obtains
\[ \eta = \frac{\eta_0 \eta_{\text{div}}}{\eta_0 + \eta_{\text{div}}} , \]  
where \( \eta_0 = \tau_0 \mu \) is independent of cell division and apoptosis. Effective viscous rheologies on long timescales have been observed experimentally\(^4\text{-}^6\). They are illustrated for example by the existence of surface tension at tissue boundaries\(^7\text{-}^{10}\) as well as other phenomena analogous to liquid behaviors\(^{11}\). Theoretically, viscous descriptions have already been applied in other contexts of tissue mechanics\(^{11\text{-}13}\).

In line with the previous paragraph, we describe the EVL as a viscous fluid with two-dimensional shear and bulk viscosities \( \eta \) and \( \zeta \). Splitting the tension tensor into its traceless and isotropic parts \( \mathbf{t} = \mathbf{t}^\ast + \mathbf{t}^0 \), this yields the following constitutive equation for the traceless part of the stress tensor
\[ \mathbf{t}^\ast = 2 \eta \mathbf{\tilde{v}} \, , \]  
where \( \mathbf{\tilde{v}} \) is the traceless part of the velocity gradient tensor. It reads
\[ \mathbf{\tilde{v}} = \frac{1}{2} \left[ \nabla \otimes \mathbf{v} + (\nabla \otimes \mathbf{v})^T \right] - \frac{1}{2} (\nabla \cdot \mathbf{v}) \mathbf{1} , \]  
where \( (\nabla \otimes \mathbf{v})^T \) denotes the transposed tensor of \( \nabla \otimes \mathbf{v} \). The isotropic tension is given by
\[ \mathbf{t}^0 = \zeta \nabla \cdot \mathbf{v} + \mathbf{t}_0 \, , \]  
where \( \mathbf{t}_0 \) is a reference tension for vanishing cell movements. Such a reference tension is in general absent for a passive viscous fluid, but can exist in a tissue where internal forces can be actively generated. Active contractility of the EVL for example, potentially due to contractile apical actomyosin networks or actomyosin-enriched cell-cell junctions, would lead to a positive reference tension in the tissue.

3. Force balance, cell flow, and tensions in spherical geometry

We now discuss the two-dimensional force balance for a thin epithelial sheet spreading on the spherical yolk cell with rotational symmetry around a distinguished axis. The geometry is illustrated in Fig. B. The tissue partially covers the surface of the yolk cell up to the
opening angle $\Theta$. The tension tensor and the cell-flow field are defined on the surface and become functions of the spherical polar coordinates $\theta$ and $\phi$. Due to the rotational symmetry around the $z$-axis, the fields do not explicitly depend on $\phi$, and $\mathbf{v} = v_\theta(\theta) \, \mathbf{e}_\theta$. It follows that force balance projected along $\mathbf{e}_\phi$ is trivially satisfied. In addition, because we assume that the sphere is rigid with constant radius $R$, we do not need to consider force balance along the radial direction. The force-balance equation (10) thereby reduces to

$$\partial_\theta t_{\theta\theta} + \cot \theta \left( t_{\theta\theta} - t_{\phi\phi} \right) = R \gamma v_\theta ,$$

(16)

where $t_{\theta\theta} = t + \tilde{t}_{\theta\theta}$ and $t_{\phi\phi} = t + \tilde{t}_{\phi\phi}$ are given by the constitutive equations (13) and (15).

Due to the symmetry of the problem, the divergence of the cell-flow field and the traceless part of the velocity gradient are respectively given by

$$\nabla \cdot \mathbf{v} = \frac{1}{R} \left( \partial_\theta v_\theta + \cot \theta \, v_\theta \right) ,$$

(17a)

$$\tilde{v}_{\theta\theta} = -\tilde{v}_{\phi\phi} = \frac{1}{2R} \left( \partial_\theta v_\theta - \cot \theta \, v_\theta \right) .$$

(17b)

Plugging these expressions into the force-balance equation (16), we obtain a differential equation for $v_\theta$:

$$\sin^2 \theta \, \partial_\theta^2 v_\theta + \sin \theta \, \cos \theta \, \partial_\theta v_\theta + \left( \alpha \sin^2 \theta - 1 \right) v_\theta = 0 ,$$

(18)
where

\[
\alpha = \frac{2\eta - R^2\gamma}{\eta + \zeta}
\]  

(19)

is a dimensionless number that depends on the shear and bulk viscosities \(\eta\) and \(\zeta\), the yolk-cell radius \(R\) and the cell-substrate friction coefficient per unit area \(\gamma\).

The general solution to equation (18) can be written as

\[
v_\theta(\theta) = v_\theta \cdot f_{\alpha,\Theta}(\theta),
\]

(20)

where \(v_\theta = v_\theta(\Theta)\) is the EVL velocity at the margin and the shape of the flow profile is given by

\[
f_{\alpha,\Theta}(\theta) = \frac{P_1^1(\cos \theta)}{P_1^1(\cos \Theta)}.
\]

(21)

Here, \(P_1^1\) is the associated Legendre function of order one and degree \(\nu\), with \(\nu(\nu + 1) = \alpha\).

Using force balance at the margin, one can further express \(v_\theta = v_\theta(\Theta)\) as

\[
v_\theta = \frac{R(t_m - t_0)}{(\zeta + \eta) f'_{\alpha,\Theta}(\Theta) + (\zeta - \eta) \cot \Theta},
\]

(22)

where \(t_m\) is the tension exerted at the margin, and \(f'_{\alpha,\Theta}\) stands for the derivative of the function \(f_{\alpha,\Theta}(\theta)\) with respect to the variable \(\theta\), here expressed at \(\theta = \Theta\). Note that \(t_{\theta\theta}(\Theta) = t_m\) at the tissue margin, as required by force balance. The knowledge of the flow profile \(v_\theta(\theta)\) as given by equations (20) and (21) allows us to determine the profile of the anisotropic part of the tension \(\tilde{t}_{\theta\theta} = (t_{\theta\theta} - t_{\phi\phi})/2\) as well as that of the total tension \(t_{\theta\theta} = t + \tilde{t}_{\theta\theta}\) along \(\theta\) via the constitutive equations (13) and (15).

4. Discussion

We investigate the dependence of the cell-flow and tension profiles on the effective shear viscosity \(\eta\) in Supplementary Figure S2. In order to minimize the number of parameter dependencies, non-dimensionalized quantities are displayed. Simple global rescalings can be performed to recover physical units. Consistent with previous work\(^1\), we assume zero friction between the EVL and its substrate \((\gamma = 0)\), for which the non-dimensionalized quantities depend only on the ratio of shear to bulk viscosity \(\eta/\zeta\) and the opening angle \(\Theta\). We plot in Supplementary Figure S2 the cell-flow and anisotropic-tension profiles for three different
values of the ratio $\eta/\zeta$ and three different stages of epiboly progression.

In response to driving forces exerted at the margin, the EVL spreads over the yolk cell from the animal to the vegetal pole along the AV axis. The predicted profiles of the cell-flow velocity are shown in Supplementary Figure S2a. Both the shape of the velocity profile and its amplitude depend on the viscosity ratio $\eta/\zeta$; however, this effect is significant only for relatively late stages of epiboly progression at which the cell flow slows down with increasing shear viscosity $\eta$. Note that these statements remain valid when non-zero values of the friction coefficient $\gamma$ are considered (plots not shown).

Due to the spherical geometry of the underlying yolk cell, the EVL flow gives rise to tension anisotropy in the tissue. The profiles of the anisotropic tension $\tilde{t}_{\theta\theta} = (t_{\theta\theta} - t_{\phi\phi})/2$ are shown in Supplementary Figure S2b. For symmetry reasons, it vanishes at the animal pole, and anisotropy is most pronounced at the tissue margin. Our model predicts that the tension along the AV axis $t_{\theta\theta}$ outweighs the circumferential tension $t_{\phi\phi}$, as indicated by $\tilde{t}_{\theta\theta} \geq 0$. Tension anisotropy becomes more pronounced for increasing shear viscosity $\eta$, and that independently of the stage of epiboly progression. Here again, these statements remain valid for non-zero values of the friction coefficient $\gamma$ (plots not shown).

In the framework of the model presented here, the shear viscosity $\eta$ depends on the rates of cell division and cell death, the force dipoles generated by single cell-division and cell-death events, and the reciprocal coupling coefficient between the orientation of cell division and tension anisotropy in the tissue. The anisotropic tension profiles shown in Supplementary Figure S2b therefore suggest that cell divisions should be preferentially oriented along the AV axis in conditions where the coupling between cell-division orientation and anisotropic tension is not impaired. In addition, assuming a cell-division independent shear viscosity $\eta_0$, the effective tissue viscosity $\eta$ is smaller than $\eta_0$ when tension-oriented cell divisions are present, as can be seen from equation (12). In the limit of vanishing cell divisions or impaired coupling of cell-division orientation to tension anisotropy, however, $\eta_{\text{div}}$ becomes much larger than $\eta_0$, such that the effective viscosity $\eta$ takes the value $\eta \simeq \eta_0$. The model therefore predicts that such conditions lead to an increased tension anisotropy and a slower EVL spreading as compared to control conditions, as long as other tissue mechanical parameters are not affected (Fig. 5b of the main text and Supplementary Figure S2).

Taken together, our continuum description predicts (i) anisotropic tissue tensions in the EVL with higher values of tension along the AV axis than along its orthogonal axis, (ii) a
generic coupling of tension anisotropy to the orientation of cell division and thus stereotypical cell-division orientation along the AV axis during epiboly, and (iii) an increased tension anisotropy and a reduced EVL flow speed in conditions where such a coupling is impaired. Within this framework, the role of tension-oriented cell divisions can thus be understood as facilitating EVL spreading during epiboly via a reduced effective tissue viscosity that takes cell divisions into account.

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