



Initiation of rotational collective migration in *Drosophila* through tissue geometry and mechanochemical feedback

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Collective migration of epithelial cells drives diverse tissue remodeling processes. In many cases, a free tissue edge works alone or in combination with other external cues to align the cells for collective movement, but how edge-free or closed epithelia become polarized for directed migration without these cues is unclear. Here, we use the rotational migration of the follicular epithelial cells in the *Drosophila* egg chamber to explore how cells in an edgeless epithelium initiate rotational collective migration, and how the rotational axis is specified. By employing methods for long-term live imaging and delaying the onset of rotation, we show that symmetry breaking can occur at multiple developmental stages and that the atypical cadherin Fat2 promotes local motility at the basal epithelial surface before rotation begins. We then combine experiments with theoretical modeling to identify a positive feedback loop in which planar polarization of Fat2 aligns the front–rear axes of the individual cells in a common direction and the resulting tissue motion leads to the planar polarization of Fat2. This mechanosensitive feedback, coupled with rigid-body dynamics of the egg chamber, can break chiral symmetry and produce sustained rotation *in silico*. We further propose that mechanical constraints arising from intertissue interactions and tissue geometry ensure that rotation occurs around the anterior–posterior axis. Our findings suggest a biophysical mechanism—combining Fat2-mediated velocity–polarity alignment, rigid-body dynamics, and tissue geometry—by which a closed epithelial tissue can self-organize into persistent, large-scale rotational migration *in vivo*, expanding current flocking theories.

collective cell migration | epithelium | chiral symmetry breaking | self-organization | mathematical modeling

Collective migration of epithelial cells plays central roles in morphogenesis, intestinal turnover, wound repair, and metastasis (1–4). To migrate collectively, each epithelial cell must establish a front–rear axis with a protrusive leading edge. The front–rear axes of all the cells must also be globally polarized across the tissue plane so the entire epithelium can move in a directed manner (5). When an epithelium has a free edge, as in wound healing, this physical asymmetry can work alone or with other external cues to polarize the tissue (6–8). Epithelial migration can also occur without external cues. Epithelial cells plated on circular micropatterns spontaneously break chiral symmetry and rotate in either a clockwise or counterclockwise direction (9–13). Cultured epithelial spheres (14–21), epithelia grown on cylindrical surfaces (22), and the spherical alveoli of human mammary organoids also rotate persistently (23). These examples suggest that the ability to self-organize for rotational migration may be a fundamental property of epithelia that adopt distinct geometries (24), but how rotation initiates is unknown.

Here, we study a rotational migration that occurs in the *Drosophila* egg chamber. An egg chamber is an ovarian follicle that has a central cluster of germ cells, surrounded by an epithelium of follicle cells (25, 26). Egg chambers form in a stem cell compartment called the germarium and progress through 14 developmental stages on their way to becoming an egg (Fig. 1A). During early stages, the follicle cells collectively migrate along the basement membrane (BM) extracellular matrix that encapsulates the tissue (27, 28). This causes the egg chamber to rotate within the BM, which remains stationary (Fig. 1B) (28). The rotational motion, in turn, allows the egg chamber to lengthen along its anterior–posterior (AP) axis (29, 30). Rotation always occurs around the AP axis, but it can be either clockwise or counterclockwise for a given egg chamber. Once a direction is chosen, however, it is persistent for roughly two days until migration stops at stage 8 (27).

Unlike most epithelia that have a free apical surface, the apical surfaces of the follicle cells adhere to the germ cells. This causes the germ cells to rotate in concert with the

Significance

Collective cell migration shapes tissues during development, closes wounds, and drives cancer invasion. When collective migration occurs in sheets of cells, called epithelia, the individual cells within the sheet must become oriented in a common direction. Epithelia that migrate into open space, as in wound healing, have a free leading edge that can orient the cells. However, how cells in edgeless epithelia become oriented for collective migration is unknown. Here we combine experiments performed in fruit flies with theoretical modeling to explore how cells within the edgeless epithelium of the ovarian follicle initiate collective migration and specify a consistent migration direction. Our results suggest a biophysical mechanism that extends current theories for how cells coordinate their movements for collective migration.

The authors declare no competing interest.

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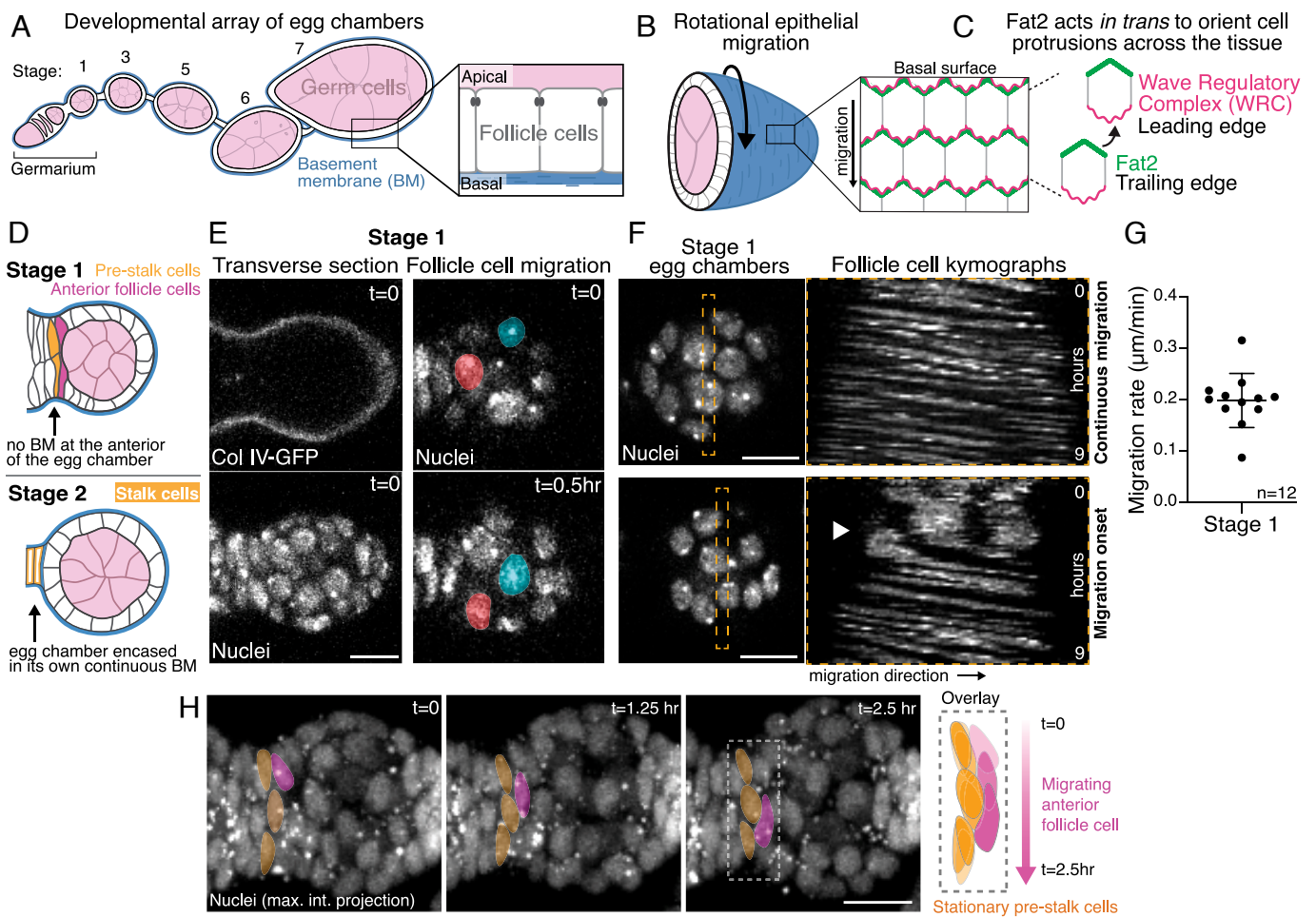


Fig. 1. Egg chamber rotation begins within the germarium. (A) Illustration of a transverse section through a developmental array of egg chambers. The box highlights the organization of the follicular epithelium. (B) Illustration of rotational epithelial migration (arrow), driven by follicle cell crawling along the basement membrane. (C) Illustration of the basal epithelial surface showing the activity of Fat2. (D) Illustrations of egg chambers, highlighting the differences between stages 1 and 2. (E) Movie stills of a transverse section through one egg chamber at stage 1 (Left). Movie stills focused on the follicular epithelium (Right), with two cells pseudocolored to show cell migration over 30 min (Movie S1). Nuclei are marked with SpyDNA. (F) Movie stills of stage 1 egg chambers with corresponding kymographs that show either constant migration or the onset of migration, which is indicated by an arrowhead (Movie S3). (G) Quantification of migration rates at stage 1. The bar represents mean $\pm$ SD. (H) Movie stills of maximum intensity projections of nuclei generated from a 2.5-h movie (Movie S4). Stationary prestalk cell nuclei are pseudocolored gold, migrating anterior follicle cell nuclei are pseudocolored magenta. The overlay shows the change in the cells' positions over time. (Scale bars, 10 μ m.)

follicle cells (27) and likely limits neighbor exchange between the follicle cells over short time scales (31). In this way, the egg chamber rotates as a rigid body where the primary forces driving rotation are confined to the basal epithelial surface. These forces are generated, in part, by lamellipodial protrusions that extend from each follicle cell along the BM (28, 32–34), and tissue-level alignment of the protrusions requires the atypical cadherin Fat2 (Fig. 1C). Fat2 localizes to the trailing edge of each cell (35), where it acts *in trans* to create a stable domain of protrusive activity at the leading edge of the cell behind (33, 34). Without Fat2, protrusions are unstable and unpolarized and rotation does not initiate (34).

Although we have learned a great deal about how Fat2 orients protrusions and other cellular features when rotation is occurring at steady state (32–38), we know very little about the symmetry-breaking mechanism that allows Fat2 to initiate rotation. Indeed, it has been notoriously difficult to capture the onset of rotation with live imaging, and the stage at which rotation begins is debated (28, 38). The mechanisms that ensure rotation always occurs around the egg chamber's AP axis are similarly unknown.

Here, we explore how Fat2 mediates chiral symmetry breaking in the follicular epithelium, and how the rotational axis is specified. By employing methods for long-term live imaging and delaying the onset of rotation, we show that symmetry breaking can occur at multiple developmental stages and reveal the dynamic behaviors of the follicle cells as rotation initiates. Through theoretical modeling, we then find that Fat2-based mechanochemical feedback and rigid-body dynamics of the egg chamber are sufficient to break chiral symmetry in the follicular epithelium and generate sustained rotation. We further propose that mechanical constraints from tissue geometry establish and maintain the rotational axis. These findings provide a general framework for understanding how epithelial cells initiate rotational migration in a developing tissue.

Results

Egg Chamber Rotation Begins in the Germarium. To understand how chiral symmetry is broken in the follicular epithelium, we need to know when rotation initiates under wild-type conditions. Rotation begins shortly after an egg chamber forms, but whether

it occurs at stage 1, when the egg chamber resides in the germarium and lacks a BM at its anterior end, or stage 2 when the egg chamber is fully encased in its own BM (Fig. 1D), has been difficult to discern. We embedded young egg chambers in a fibrinogen-thrombin clot (39), which allowed live imaging ex vivo for up to nine hours, and visualized the BM using an endogenous GFP tag on the $\alpha 2$ chain of collagen IV (Viking in *Drosophila*, Col IV-GFP) (40). In all 12 samples examined, rotation was already occurring at an average rate of 0.2 $\mu\text{m}/\text{min}$ at stage 1 and persisted into stage 2 (Fig. 1E–G and SI Appendix, Fig. S1A–C and Movie S1). Notably, the germ cells rotate in concert with the follicle cells, suggesting that rigid-body rotation also applies to stage 1 (Movie S2). In 2 of the 12 samples, we even captured the onset of rotation and found that it reaches a constant rate within minutes, indicating switch-like dynamics (Fig. 1F and SI Appendix, Fig. S1D and Movie S3). At stage 1, the anterior-most follicle cells contact the precursors of the stalk cells (prestalk) that will form short connections between egg chambers later in development (Fig. 1D) (41). Indeed, live imaging showed that the anterior follicle cells rotate with the rest of the egg chamber and slide past the prestalk cells, which remain stationary over the time scale of our movies (Fig. 1H and SI Appendix, Fig. S1E and Movie S4). These data show that rigid-body egg chamber rotation begins in the germarium and

that anterior follicle cells can remodel their contacts with prestalk cells to facilitate this motion.

Fat2 Can Mediate Symmetry Breaking at Multiple Developmental Stages. We next asked when Fat2 is competent to mediate symmetry breaking in the follicular epithelium. This activity could be restricted to stage 1, or Fat2 may be competent to initiate rotation at multiple stages. Prior work suggested the former (38); however, it was later shown that Fat2 is required to orient protrusions throughout rotation (36), and that rotation can initiate at later stages in some mutant conditions (42). To revisit this question, we asked if Fat2 can mediate symmetry breaking in older egg chambers.

We used two Gal4 drivers to deplete Fat2 with RNAi, *traffic jam-Gal4* (*tj-Gal4*) and *109-30-Gal4*, and assessed the level of depletion using an endogenous 3xGFP tag on Fat2 (Fat2-3xGFP) (33). *tj-Gal4* is expressed in the follicle cells throughout oogenesis (43) (SI Appendix, Fig. S2A). Using *tj-Gal4* to express *fat2-RNAi* reduces Fat2 to undetectable levels throughout the migratory period and blocks rotation (Fig. 2A–D). By contrast, *109-30-Gal4* is primarily expressed in follicle cells of the germarium and early egg chambers (44) (SI Appendix, Fig. S2A). When *109-30-Gal4* expresses *fat2-RNAi*, Fat2 is undetectable through stage 3, begins to be expressed in patches

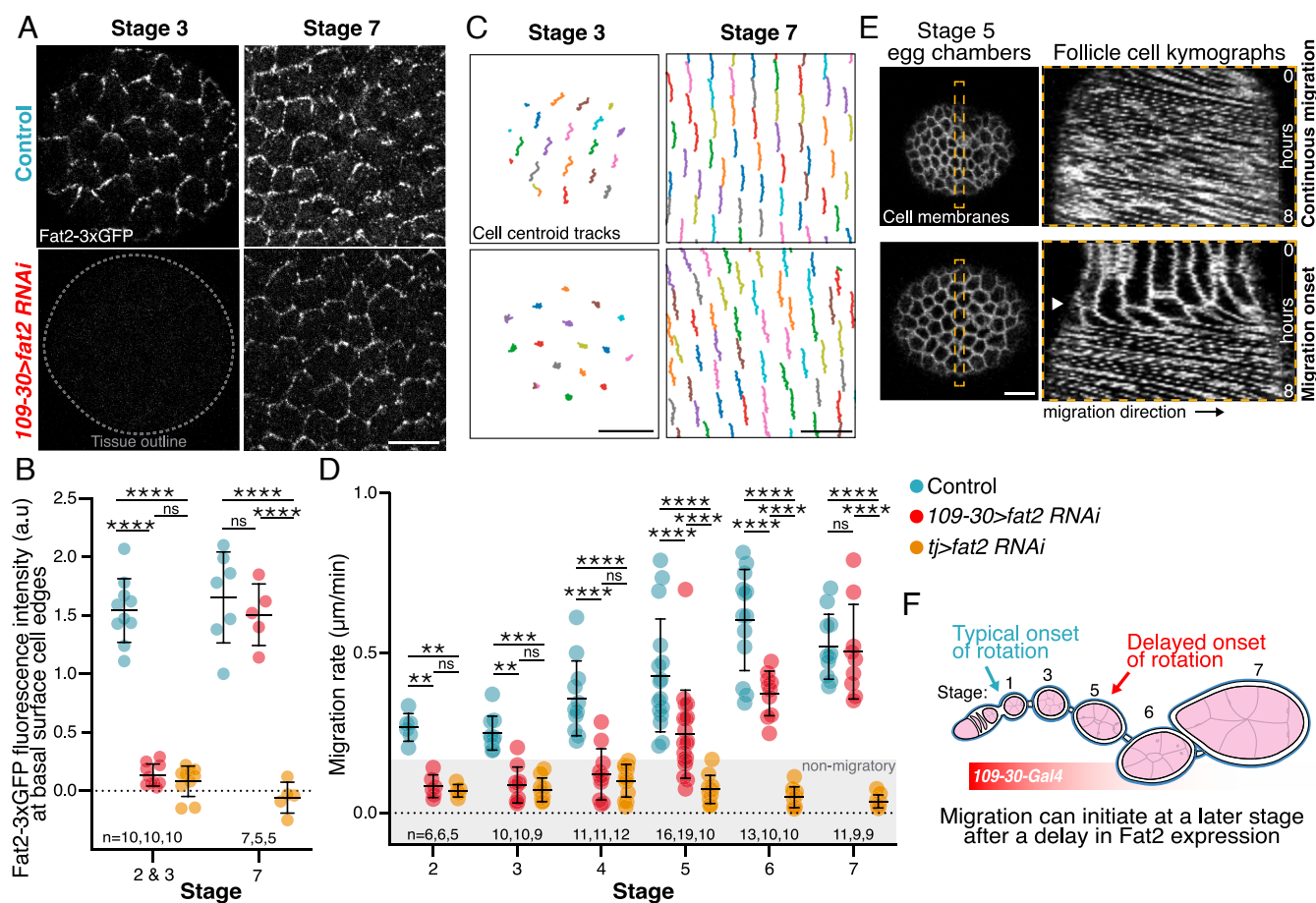


Fig. 2. Fat2 can mediate symmetry breaking at multiple developmental stages. (A) Representative images of Fat2-3xGFP at the basal epithelial surface. (B) Quantification of Fat2-3xGFP intensity at basal cell edges. *109-30-fat2-RNAi* depletes Fat2 at stages 2 and 3, but levels are equivalent to controls by stage 7. Each data point represents one egg chamber. (C) Representative centroid tracks of follicle cell movement over 10 min. (D) Quantification of migration rates by stage. *109-30-fat2-RNAi* blocks migration at early stages but migration is equivalent to controls by stage 7. Each data point represents one egg chamber. (E) Movie stills of stage 5 egg chambers with corresponding kymographs that show either constant migration or delayed migration (arrowhead) (Movie S6). (F) Illustration summarizing how *109-30-fat2-RNAi* delays the onset of migration. Panels (B and D), Two-way ANOVA with Tukey's multiple comparisons test; ns, $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. Bars represent mean $\pm$ SD. (Scale bars, 10 μm .)

of cells at stage 4 (SI Appendix, Fig. S2B), and reaches wild-type levels by stage 7 (Fig. 2A and B). Importantly, the pattern of rotation shows a similar temporal progression, with rotation blocked through stage 3, occurring slowly in some egg chambers at stages 4 to 5, and reaching normal speed by stage 7 (Fig. 2C and D and Movie S5). We then asked if early depletion of other proteins required for symmetry breaking can also delay the onset of rotation. Follicle cell protrusions form under the control of the WASP family verprolin homolog regulatory complex (WRC). Like Fat2, the WRC is required both to initiate and maintain follicle cell migration (28). We used *109-30-Gal4* to deplete the WRC component Abelson interacting protein (Abl) by RNAi and found that the onset of migration is delayed similarly to when Fat2 is depleted (SI Appendix, Fig. S2C). Finally, we asked if rotation initiation in these older egg chambers shows the same switch-like behavior seen at stage 1. We embedded ovarioles expressing *109-30>fat2-RNAi* in fibrinogen-thrombin clots and captured rotation initiation in multiple egg chambers that ranged from late stage 4 to early stage 6. This motion quickly reaches a constant rate, showing that initiation is a rapid process regardless of when it occurs (Fig. 2E and Movie S6). We conclude that Fat2 can mediate the symmetry-breaking event needed to initiate rotational migration at various stages (Fig. 2F), and that a later migratory start can likely be achieved

by delaying the expression of any component of the symmetry-breaking machinery.

Fat2 Promotes Local Motility at the Basal Surface Before Rotation Begins. The ability to delay the onset of rotation highlights the robustness of the symmetry-breaking process. It also provides a powerful method to visualize the dynamic movements of the follicle cells just before rotation begins and to determine how Fat2 influences these movements. We focused on stage 5, which is a ~5-h period (45) during which some *109-30>fat2-RNAi* epithelia are rotating, and some have not yet initiated rotation (prerotation) (Fig. 2D). For each epithelium, we took one 30-min movie near the apical surface and one at the basal surface (Fig. 3A and B), and tracked cell centroids at both locations over time (Movies S7 and S8). In rotating *109-30>fat2-RNAi* epithelia, the apical and basal centroids of each cell move processively and in concert with one another. By contrast, in the prerotation *109-30>fat2-RNAi* epithelia, the apical cell centroids are largely static, likely due to the adhesion to the germ cells, while the basal centroids are highly dynamic (Fig. 3A–C and SI Appendix, Fig. S3 A–C and Movie S8). Local movement of the basal surface appears to be generated by the same protrusive activity that will eventually drive rotation, with protrusions acting both cell-autonomously and nonautonomously to move the centroids of other cells (Movie S7). Notably, *fat2^{N103-2}* mutant epithelia, which never migrate, show little centroid movement at their basal

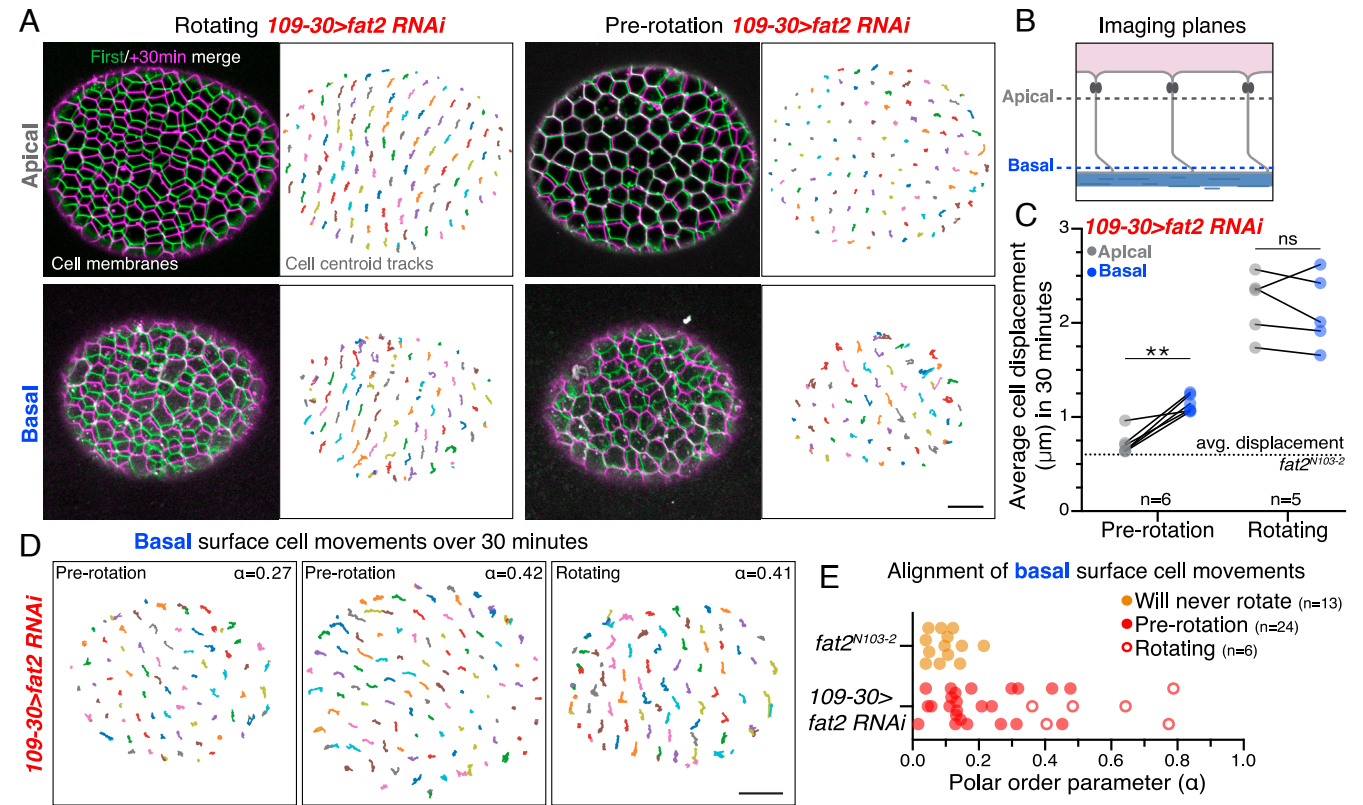


Fig. 3. Fat2 promotes local motility at the basal surface before rotation begins. (A) Representative images of a *109-30>fat2-RNAi* epithelium that is rotating, and one prerotation. Left panels show the overlay of the first (green) and last (magenta) frame of a 30-min movie (Movie S8). Right panels show cell centroid tracks from the same movie. (B) Diagram showing imaging planes. (C) Quantification of cell centroid displacement at apical vs. basal surfaces in *109-30>fat2-RNAi* epithelia prerotation or that are rotating. Local motility at the basal surface precedes rotation onset. Each data point represents the average of cell displacements in one epithelium. Solid lines connect measurements from the same egg chamber. The dotted line represents the average cell displacements from *fat2^{N103-2}* samples (SI Appendix, Fig. S3). Paired t test; ns, $P > 0.05$, and $^{**}P < 0.01$. (D) Representative cell centroid tracks taken at the basal surfaces of three epithelia. The polar order parameter increases from Left to Right. (E) Quantification of the polar order parameter for basal centroid tracks of *109-30>fat2-RNAi* epithelia that are either rotating or prerotation, compared to *fat2^{N103-2}* epithelia. Some prerotation epithelia have polar order parameters that exceed those of rotating epithelia, showing that follicle cells can align their basal surface movements before rotation begins. (Scale bars, 10 μm.)

surfaces (Fig. 3C and SI Appendix, Fig. S3A and B). Altogether, these data suggest that local, Fat2-dependent motility at the basal epithelial surface may be a key feature of how rotation initiates and that this process may also occur with rigid body dynamics.

We next investigated the extent to which the local movements of the follicle cells' basal surfaces become aligned before rotation begins by computing a polar order parameter α for each tissue, with $\alpha = 1$ reflecting perfectly aligned movements, $\alpha = 0$ reflecting isotropic movements (Materials and Methods). Prerotation $109-30 > fat2-RNAi$ epithelia showed a range of tissue-level order, with some epithelia being largely isotropic, like $fat2^{N103-2}$ epithelia, and others showing varying degrees of alignment (Fig. 3D and E and SI Appendix, Fig. S3D). Notably, in the $109-30 > fat2-RNAi$ condition, the cell alignment in three prerotation epithelia exceeded that of some epithelia that were rotating (Fig. 3D and E). We conclude that the follicle cells can align their basal surface movements before rotation begins. We further speculate that each $109-30 > fat2-RNAi$ epithelium represents a snapshot of the trajectory the tissue takes from a disordered to an ordered state as it transitions into rotation.

Fat2 Becomes Planar Polarized Concurrent with the Onset of Rotation. We next used our delayed migration assay to explore the planar polarization of Fat2 to each cell's trailing edge. We previously proposed that Fat2 operates in a positive feedback loop to promote rotational migration: Fat2 acts at the trailing edge of each cell to align the cells' protrusions in a common direction, and the resulting epithelial migration localizes Fat2 to each cell's trailing edge (33, 34). However, this proposal came from experiments in which the epithelium was either rotating at steady state or rotation was fully blocked (SI Appendix, Fig. S3E). Delaying the onset of migration now lets us ask if Fat2 becomes localized as rotation begins.

We used either $tj-Gal4$ or $109-30-Gal4$ to express $Abi-RNAi$ and assayed the planar polarization of Fat2-3xGFP by determining the ratio of fluorescence intensity along leading-trailing cell edges vs. side cell edges (Fig. 4A and B). When the epithelium is migrating, Fat2-3xGFP is enriched at leading-trailing edges at all stages examined. Conversely, blocking migration using $tj > Abi-RNAi$ causes Fat2-3xGFP to localize uniformly around all cell edges (33). When migration was instead delayed using $109-30 > Abi-RNAi$, we saw a dynamic temporal pattern of Fat2 localization, with Fat2-3xGFP uniform around cell edges at stages 3 to 4, weakly polarized to leading-trailing edges at stage 5 and robustly polarized by stage 6 (Fig. 4A and B). Hence, Fat2's tissue-level polarization is undetectable-to-weak during the time when migration normally initiates in these epithelia and robustly polarized only after migration is well underway, which provides further evidence that tissue motion feeds into Fat2's localization.

Rigid-Body Dynamics and the Mechanosensitive Behavior of Fat2 Can Initiate Rotation In Silico. Our results to this point show that the follicle cells can start migrating from an unpolarized state and that rotation appears to initiate with rigid-body dynamics. We further hypothesize that symmetry breaking depends on a mechanochemical feedback loop in which Fat2 acts at the trailing edge of each cell to align the cells' protrusions and the resulting tissue motion localizes Fat2 to the cells' trailing edges. Here, we develop a minimal theoretical model to elucidate whether these conditions can recapitulate rotation initiation. Throughout, we denote vectors and matrices in bold and scalars in regular font.

We modeled the egg chamber as an ellipsoidal rigid body (Fig. 4C). Crawling forces are generated by lamellipodial protrusions

at the basal epithelial surface, which we model as point forces $\mathbf{b}_i$ generated by each follicle cell $i \in \{1, 2, \dots, N\}$ at positions $\mathbf{r}_i$ (Fig. 4D). The BM provides the substrate for cell crawling but also generates resistance to motion. For motion tangential to the follicle cell-BM interface, we model this resistance as a viscous drag force $\mathbf{f}_i$ with viscous coefficient μ_b (Fig. 4D). For motion perpendicular to the egg chamber-BM interface, we model this resistance as an elastic restoring force. Intuitively, the egg chamber can be thought of as a rigid ellipsoid (aspect ratio $e > 1$) elastically confined by a surrounding BM. Given estimates of BM stiffness [≈ 50 kPa (30, 46)] and of traction stresses generated by other crawling cells in vivo [≈ 10 to 500 Pa (47)], we infer that deformation of the BM by the egg chamber generates a strong elastic restoring force/torque that ensures consistent rotation around the long axis (SI Appendix, section S1.1.1 for details). This allows us to reduce the full equation of motion for the egg chamber to a single-degree-of-freedom equation. If we assume negligible inertia, the nondimensional equation for rotation around the long axis (SI Appendix, section S1.1) is

$$\underbrace{\Pi(e)\omega_3}_{\text{drag torque}} = \underbrace{\left(\sum_i \mathbf{r}_i \times \mathbf{b}_i\right) \cdot \hat{\mathbf{d}}_3}_{\text{active torque from protrusions}}, \quad [1]$$

where $\Pi(e) = N\pi(40 + 26(e - 1))/15$, the angular velocity vector is $\boldsymbol{\omega} = \omega_3 \hat{\mathbf{d}}_3$ and $\hat{\mathbf{d}}_3$ is the unit vector pointing to the anterior pole along the AP axis (Fig. 4C).

Next, we modeled the interplay between the planar polarization of Fat2 and protrusions. We account for the Fat2 distribution as a concentration field $c_i(\beta, t)$, where β is the angular coordinate in the tangent (i.e. cell) plane at $\mathbf{r}_i$ (Fig. 4E). Although Fat2 acts *in trans* to orient protrusions, its function is restricted to the cell-cell interface where it resides (33). Given this local effect, we simplify the model by representing Fat2's function as being cell-autonomous. A minimal model of the cell protrusions' force dynamics is

$$\frac{\partial \mathbf{b}_i}{\partial t} = \underbrace{\boldsymbol{\omega} \times \mathbf{b}_i}_{\text{tissue rotation}} - \underbrace{\tau_1(\boldsymbol{\eta}_i(t) + \mathbf{b}_i)}_{\text{alignment with Fat2}}, \quad [2]$$

where $\tau_1 = t_c/\gamma$ is the ratio of the characteristic time scale of rotation initiation to the time scale over which the crawling force changes directions in response to a change in Fat2 distribution. The first term on the right-hand side accounts for force directional changes due to tissue rotation. The second term models the tendency of the protrusive force to align opposite to the direction of high Fat2 concentration $\boldsymbol{\eta}_i(t)$ inferred from the Fat2 distribution $c_i(\beta, t)$ (Fig. 4E and F and SI Appendix, sections S1.2 and S1.3).

Last, we modeled the mechanosensitive behavior of Fat2, which localizes to cell edges opposite to the local cell migration direction $\mathbf{v}_i(t)$ (Fig. 4E and F and SI Appendix, section S1.2.1):

$$\frac{\partial c_i(\beta)}{\partial t} = \underbrace{-\tau_2(\tau_3 \mathbf{v}_i(t) \cdot \mathbf{m}(\beta, \mathbf{r}_i))}_{\text{Fat2 redistributes opposite to } \mathbf{v}_i} + \underbrace{c_i(\beta)}_{\text{saturation}}, \quad [3]$$

where $\tau_2 = \epsilon_0 t_c c_c$, $\tau_3 = \epsilon_1 a/t_c c_c$, c_c is the characteristic concentration scale of Fat2, ϵ_0 is a characteristic rate of Fat2 dynamics, ϵ_1 sets the relative strength of the mechanosensitivity compared to saturation dynamics, a is the radius of the equatorial cross section of the egg chamber and $\mathbf{m}(\beta, \mathbf{r}_i)$ is a unit tangent

vector along the angular direction β at $\mathbf{r}_i$ (SI Appendix, section S1.2.1).

Consistent with observations of prerotation egg chambers in the delayed migration assay, we solve Eqs. 1–3 with initially

uniform distributions of Fat2 ($c_i(\beta, t_0) = 1/2\pi$) and cell crawling forces ($\mathbf{b}_i(t_0) = \mathbf{0}$) (See SI Appendix, section S1.4 for details). For parameter (τ_{1-3}) selection and sensitivity analysis, see SI Appendix, section S1.3. Using these initial conditions and

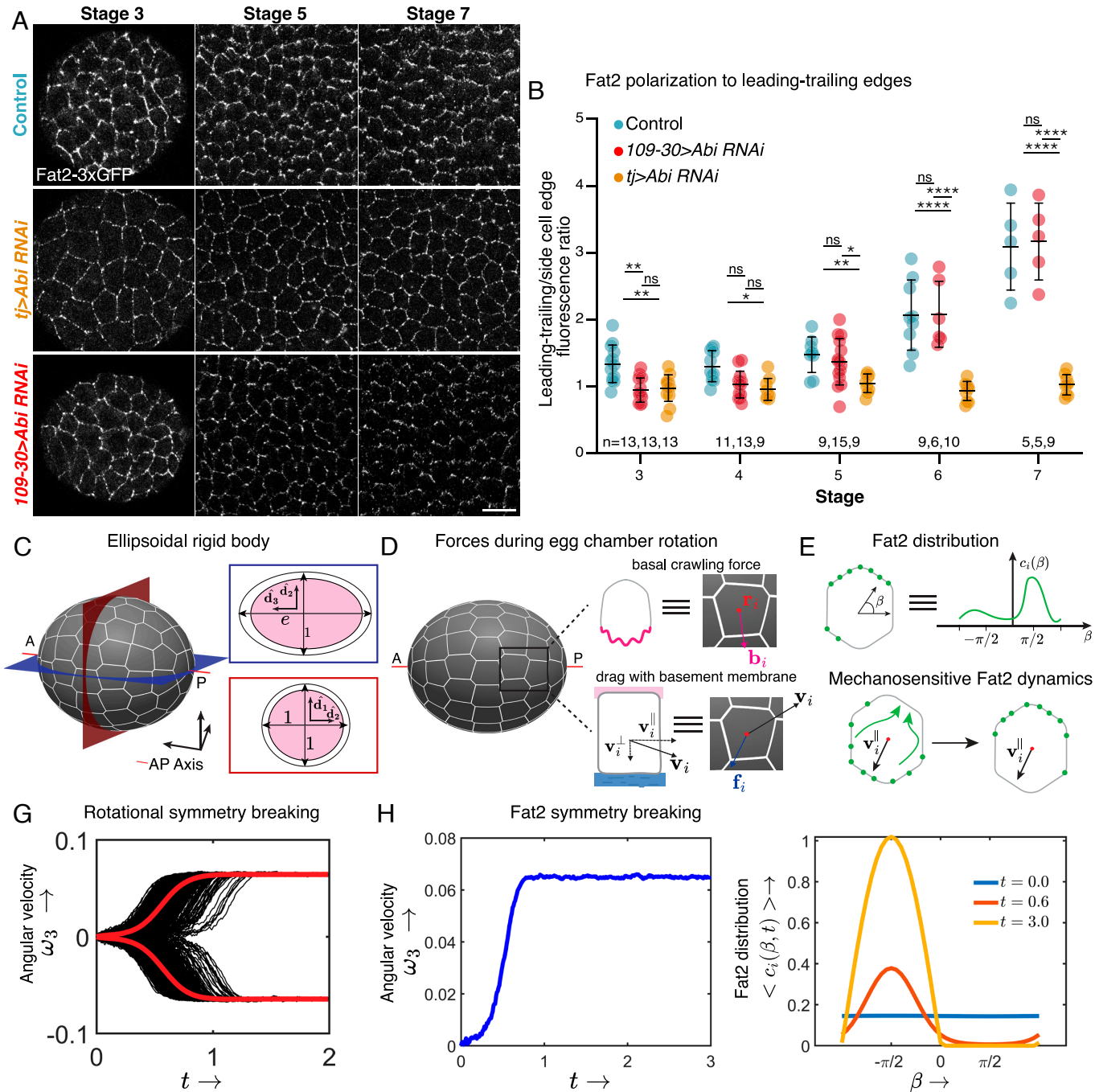


Fig. 4. Mechanosensitive behavior of Fat2 and rigid-body dynamics can initiate rotation. (A) Representative images of Fat2-3xGFP at the basal epithelial surface. (Scale bar, 10 μm .) (B) Quantification of Fat2 polarization to leading-trailing cell-cell interfaces. Each data point represents the ratio of Fat2-3xGFP brightness at leading-trailing (horizontal) vs. side (vertical) edges in one epithelium. Fat2 goes from unpolarized to polarized in 109-30>Abi-RNAi with roughly the same timing as the onset of rotation, suggesting that Fat2 becomes planar polarized concurrent with the onset of rotation. Two-way ANOVA with Tukey's multiple comparisons test; ns, $P > 0.05$, $*P < 0.05$, $**P < 0.01$, and $****P < 0.0001$. Bars represent mean $\pm$ SD. (C) To model the delayed migration assay, the egg chamber is represented as an ellipsoidal rigid body with aspect ratio e . The dimensions of the egg chamber are rescaled with the radius of the equatorial cross section. (D) The crawling force $\mathbf{b}_i$ models protrusive activity at the basal epithelial surface and the viscous drag $\mathbf{f}_i$ models the interaction between the epithelium and the BM for cell i . (E) The Fat2 distribution is modeled by $c_i(\beta, t)$ where β is the angular direction in the tangent (i.e. cell) plane. (F) Fat2 dynamics is mechanosensitive, whereby Fat2 is recruited to each cell's trailing edge opposite to the migration direction $\mathbf{v}_i$. (G) The model breaks rotational symmetry, generating either clockwise ($\omega_3 < 0$) or counterclockwise ($\omega_3 > 0$) rotation about the AP axis. $N = 1,000$ model trajectories starting from an isotropic initial condition ($c_i(\beta, 0) = 1/(2\pi)$, $\mathbf{b}_i = \mathbf{0}$). (H) Representative model trajectory with symmetry broken, resulting in sustained counterclockwise rotations and the corresponding evolution of the cell-averaged Fat2 distribution ($\langle c_i(\beta, t) \rangle$). The Fat2 distribution evolves from an isotropic to a polar configuration centered around $\beta = -\pi/2$. The time evolution of the quantities in (H) is given in Movie S9.

parameters, the model breaks symmetry and generates sustained rotations around the AP axis (Movie S9), with equal probability of clockwise ($\omega_3 < 0$) and counterclockwise ($\omega_3 > 0$) directions, consistent with experiments (Fig. 4G). The Fat2 and crawling force distributions transition from isotropic to polar centered around $\beta = \pi/2$ for clockwise and $\beta = -\pi/2$ for counterclockwise rotations. These observations reflect Fat2 being localized to the cells' trailing edges and the progressive alignment of cell protrusive activity as rotation initiates. Fig. 4H shows one simulation iteration that undergoes counterclockwise ($\omega_3 > 0$) rotational symmetry breaking. Our results are robust to large changes in parameters (SI Appendix, section S1.3).

Here, egg chamber geometry sets the rotation axis, rigid-body dynamics provides a global synchronizing mechanical cue for follicle cells, and the mechanosensitive behavior of Fat2 transduces this cue to align cell polarity. The transduction of the global rotational cue into the planar polarization of Fat2 further promotes crawling force in the direction of rotation, forming positive feedback between rotation, Fat2 distribution, and cell crawling force.

Mechanical Constraints Can Specify the Rotational Axis. Wild-type egg chambers rotate around their AP axis (27). This is also true when the onset of rotation is delayed. In our model, elongation of the tissue along its AP axis ($e > 1$) (egg chamber geometry), combined with elastic confinement from the BM, ensures that rotation occurs around the AP axis. However, egg chambers are thought to be spherical when rotation normally begins. Simulating the above model with a spherical tissue ($e = 1$), symmetry breaking occurs, but rotation is not restricted to any specific axis (SI Appendix, section S1.5 and Movie S10).

To ask how the rotational axis is specified under wild-type conditions, we looked more closely at the shape of stage 1 egg

chambers. Although these egg chambers are mostly round, the anterior pole that contacts the prestalk cells is flat (Fig. 5A). We hypothesized that this geometric cue might force rotation about an axis normal to the flattened interface which corresponds to the AP axis.

To test the hypothesis, we extended our model to account for the flattened interface between the egg chamber and the prestalk cells. The areal extent of this interface is quantified by the polar angle θ_s (Fig. 5B). The interaction is assumed to be elastic for motion normal to this interface. For motion tangential to the interface, we instead assume a viscous drag force (Fig. 5C). We use the same Fat2 and protrusion dynamics as in Eqs. 2 and 3. By numerically simulating this extended model (SI Appendix, section S2 for more details), the egg chamber breaks symmetry, generating sustained rotations about the AP axis (Fig. 5D and Movie S11). The rotational axis becomes normal to the flattened interface faster as the strength of the elastic interaction between the egg chamber and prestalk cells increases.

Therefore, the mechanochemical feedback loop that breaks symmetry, combined with mechanical interaction with the prestalk cells, can generate sustained rotation about the AP axis in stage 1 egg chambers. In this natural condition, the geometry of the prestalk/egg chamber interface sets the rotational axis as rotation initiates. At later stages, we propose that the elongated shape of the egg chamber ensures that the rotational axis remains stable over time.

Discussion

This work sheds light on how egg chamber rotation initiates in *Drosophila* and how the rotational axis is specified. We show that the follicle cells initiate rotation when they are still in

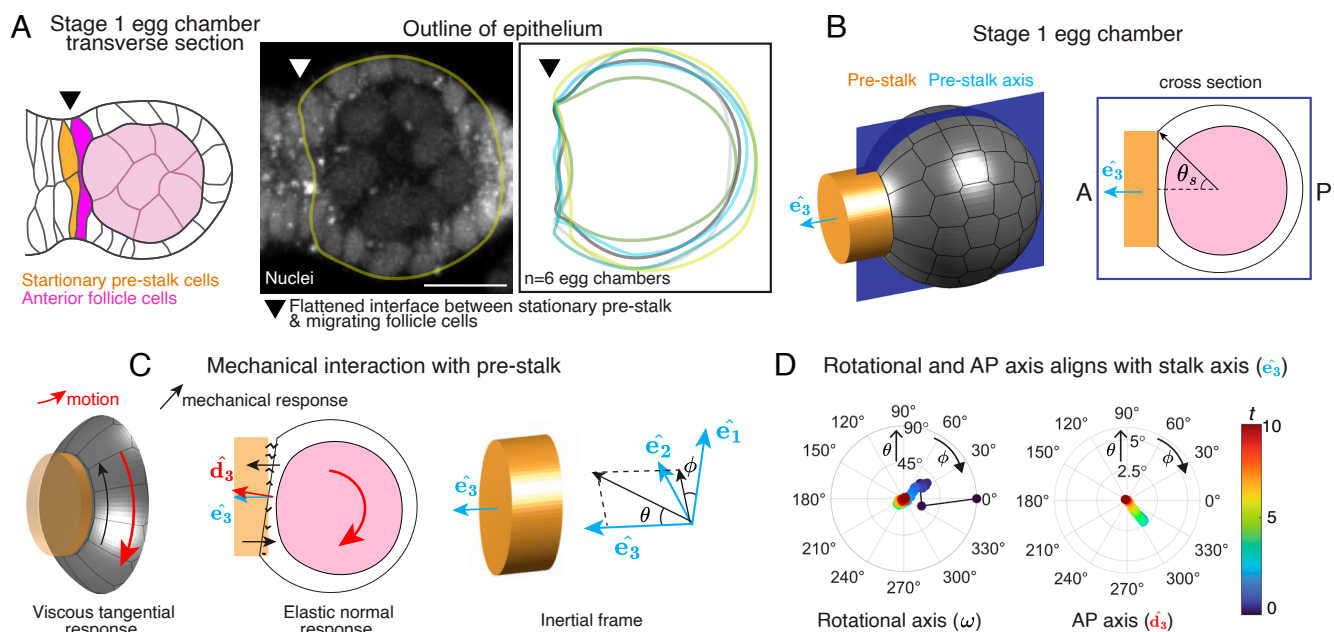


Fig. 5. Mechanical constraints specify the rotational axis at stage 1. (A) Illustration of a transverse section through a rotating egg chamber at stage 1, highlighting flattened interface between the prestalk cells and the anterior-most follicle cells (Left). Transverse section of one stage 1 egg chamber with an outline of the follicular epithelium (Middle). Outlines of epithelia from 5 additional stage 1 egg chambers, highlighting the flattened shape of the interface (Right). (Scale bars, 10 μm.) (B) The polar angle θ_s quantifies the extent of contact between the prestalk cells and the egg chamber. The unit vector $\hat{e}_3$ is along the fixed prestalk axis. (C) The motion of the egg chamber tangential (normal) to the prestalk/egg chamber interface generates viscous (elastic) resisting forces. Rotational motion of the egg chamber is described in the inertial (i.e. fixed) frame $E : \{\hat{e}_1, \hat{e}_2, \hat{e}_3\}$. The orientation of a vector is represented in spherical coordinates (θ, ϕ) . (D) The evolution of the orientation of the rotational axis ω and the AP axis $\hat{d}_3$ is described using the spherical coordinates (θ, ϕ) in the inertial frame E . The time evolution of the quantities (C and D) is given in Movie S11.

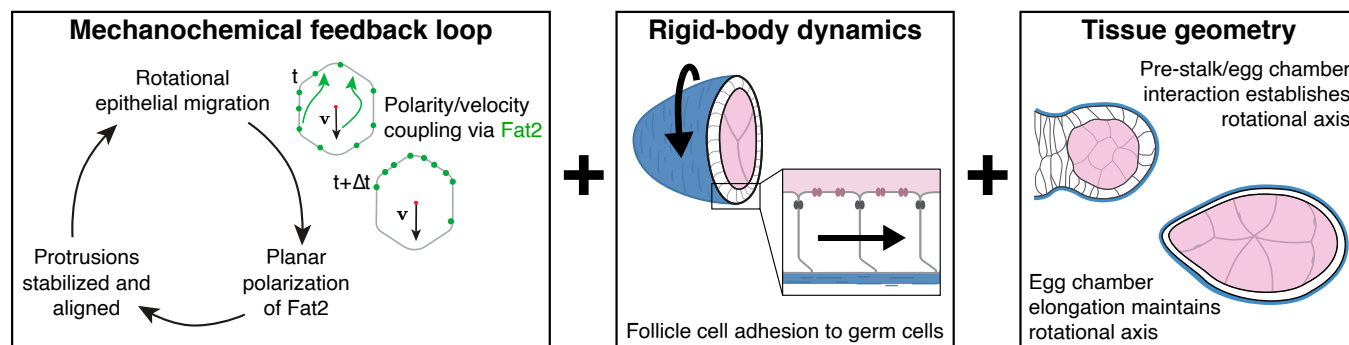


Fig. 6. Proposed biophysical model for egg chamber rotation. Fat2 operates in a mechanochemical feedback loop, in which cell motion polarizes Fat2 to cells' trailing edges, which in turn stabilizes and aligns individual cell crawling forces. Rigid-body dynamics of the egg chamber synchronize the individual movements of the follicle cells for collective migration. Tissue geometry provides a mechanical cue that ensures rotation around the AP axis. Synergy between the mechanosensitive behavior of Fat2, egg chamber rigid-body dynamics, and egg chamber geometry initiates and maintains the rotational migration of the follicle cells.

the germarium. Our data and modeling further suggest that Fat2-based mechanochemical feedback, combined with rigid-body motion of the egg chamber, can initiate and sustain rotation, and that rotation around the AP axis is specified by tissue geometry (Fig. 6). The central features of this proposed biophysical mechanism are detailed below.

We propose that Fat2 operates in a mechanochemical feedback loop to initiate and maintain rotational migration. We found that Fat2 can initiate rotation at multiple stages and that initiation can occur *ex vivo*. Moreover, because follicle cell number (48–50) and egg chamber volume (45) both increase ~ 10 -fold over the stages at which we have seen rotation initiate, symmetry breaking is robust to these factors. We also found that Fat2 becomes planar polarized concurrent with the onset of rotation. This observation, combined with prior data (33) and current modeling, suggests a positive feedback loop in which Fat2 aligns the crawling forces of individual cells and the resulting rotational motion polarizes Fat2 to the cells' trailing edges. We envision that Fat2 aligns crawling forces by globally orienting cell protrusions (33, 34), or possibly other migration machinery (32, 35, 37, 38). Together, these findings are consistent with symmetry breaking being a self-organized process, but we cannot yet rule out that other factors contribute to rotation initiation.

We further propose that rigid-body dynamics imposed by adhesion between the follicle cells and germ cells synchronizes follicle cell movements. Consistent with this mechanical constraint, we found that cell motility is restricted to the basal epithelial surface in prerotation egg chambers and that this local motility depends on Fat2. Local cell movements can also become aligned before rotation begins. Our model posits that rigid-body dynamics provide the global synchronizing cue for follicle cell alignment. Indeed, combining the rigid-body assumption with the mechanosensitive behavior of Fat2 (polarity-velocity coupling) (11, 51) is sufficient to break symmetry and generate sustained rotation *in silico*. Exploiting rigid-body dynamics, our model yields closed-form parametric expressions that relate tissue geometry (e.g., aspect ratio, prestalk interface size, and shape) to mechanical outputs, offering insight into how geometry influences tissue dynamics. Our biophysical mechanism extends prior models of symmetry breaking proposed for rotating epithelial spheroids, based on cell flocking through Vicsek-like polarity alignment (17, 18) or substrate curvature (22). We note, however, that whether follicle cell-germ cell adhesion is required for rotation initiation remains to be tested experimentally.

Another finding from this work is that tissue geometry may ensure that rotation occurs around the AP axis. At stage 1, the anterior-most follicle cells form a flattened interface with prestalk cells and can remodel these cell–cell contacts as rotation initiates. Our model suggests that an elastic interaction between these cells that is imposed by the flattened interface can specify the rotational axis at this stage. Starting at stage 4, progressive lengthening of the egg chamber, combined with elastic confinement from the BM, can then maintain the rotational axis over time (*SI Appendix, section S1.1.1*). It has been suggested that specialized follicle cells called polar cells define the rotational axis (52). Our findings are consistent with this assertion. At stage 1, it is the anterior polar cell precursors that form the flattened interface with the prestalk cells (41). Later, the polar cells control the early, rotation-independent phase of egg chamber elongation (31). In this way, the polar cells may, in fact, play two roles in specifying the rotational axis through distinct effects on tissue geometry.

Three features of our proposed mechanism for egg chamber rotation require interactions between the migrating follicle cells and other nonmigratory cell types—the germ cells, the prestalk cells, and the polar cells. This contrasts with many *in vitro* systems of epithelial rotation that involve uniform monolayers of migrating cells (10, 14–18, 22). One notable exception is human mammary organoids, which have rotating spherical acini attached to a central system of ducts (23). Unlike isolated epithelial spheres that do not maintain a coherent rotational axis, these acini rotate persistently around the ducts in a manner like that of a stage 1 egg chamber rotating around the prestalk cells. Thus, interactions with nonmigratory cell types may provide a general mechanism to constrain rotational epithelial migrations, even though the rotation, itself, is likely to be a spontaneous and self-organized process.

Materials and Methods

Fly Genetics, Sources, and Care. *Drosophila melanogaster* were fed cornmeal molasses agar food and raised at 25 °C. Experimental females were collected 0 to 3 d posteclosion and then aged with males and yeast before dissection. For all RNAi experiments, females were kept at 29 °C for 48 h prior to dissection, otherwise experimental females were kept at 25 °C. Detailed experimental genotypes are in *SI Appendix, Table S2*.

Egg Chamber Dissection. Ovaries were dissected into live imaging media [Schneider's medium + 1X Penicillin-Streptomycin + 15% fetal bovine serum + 200 $\mu\text{g}/\text{mL}$ insulin, (53)] in one well of a glass spot plate. Ovarioles

were carefully removed from the muscle sheath to not damage stages being analyzed. For standard live imaging, egg chambers older than the most mature egg chamber to be analyzed were trimmed from the ovariole by slicing through the stalk with a 27-gauge needle. For extended live imaging, egg chambers older than stage 9 were removed. For dissection movies see ref. 54.

Sample Preparation for Standard Live Imaging. Following dissection, the tissue was transferred to another well in the spot plate containing live imaging media with CellMask (1:500) for 8 min and then washed with fresh live imaging media. Stained ovarioles were transferred in 16 μ l of media to a slide, and glass spacer beads were added to the media to limit egg chamber compression by the coverslip. Bead sizes were 51/53 μ m for stage 6 to 8, 40 μ m for stage 5 to 6, 30 μ m for stage 4 to 5, 25 μ m for stage 3 to 4, and 15 μ m for germaria-stage 2. A coverslip was placed on top of the samples and sealed with petroleum jelly to prevent evaporation. Imaging was performed for up to 1 h.

Sample Preparation for Extended Live Imaging. For extended live imaging, we adapted a method from ref. 39. Following dissection, ovarioles were transferred to another well of the spot plate containing live imaging media with 1 $\times$ SpyDNA for 15 min. During incubation, 200 μ l of prewarmed (37 $^{\circ}$ C) live imaging media without insulin was added to an Eppendorf tube containing 0.002 g of fibrinogen (final concentration 10 mg/ml), vortexed briefly, then placed back at 37 $^{\circ}$ C for the rest of the staining period. After staining, the fibrinogen mixture was pipetted into an open well in the spot plate and stained ovarioles were transferred to the fibrinogen mixture, pipetting slowly up and down to ensure complete incorporation, then transferred in 10 μ l to an imaging dish (MatTek, 35 mm petri dish, 14 mm microwell). Thrombin (10U/ μ l) was added to the drop of fibrinogen in two 0.5 μ l increments. If the clot had not formed after 10 min, another 0.5 μ l of thrombin was added. The clot was determined to be formed if an eyelash could not break the surface tension of the clot-droplet, or polymerization was visible. The clot was then covered in 0.3 ml of live imaging media with insulin, a wet Kimwipe was added to the side of the dish to maintain humidity, and the lid was placed on the dish. The z-stack acquisition feature was used to capture at least half the volume of egg chambers imaged. Tissues were imaged for up to 13 h; however, egg chamber rotation often slowed after 8 to 9 h.

F-Actin Staining and Immunostaining of Fixed Tissues. All fixed tissues were stained for F-actin for egg chamber staging and N cadherin for cell segmentation. Dissected ovarioles were fixed in a 4% EM-grade formaldehyde in phosphate buffered saline (PBS) plus 0.1% Triton X-100 (PBT) for 15 min and then washed 3 $\times$ 5 min in PBT. Egg chambers were incubated with primary antibodies (anti-DN cadherin, 1:200) in PBT either overnight at 4 $^{\circ}$ C or for 2 h at room temperature while rocking, then washed 3 $\times$ 5 min in PBT. Ovarioles were then incubated in secondary antibodies (1:200) with either TRITC phalloidin (1:200) or Alexa Fluor 647 phalloidin (1:100) overnight at 4 $^{\circ}$ C or 2 h at room temperature while rocking. Ovarioles were then washed 3 $\times$ 5 min in PBT and mounted in 30 μ l SlowFade Diamond antifade on a slide using a 22 $\times$ 50 mm # 1.5 coverslip, sealed with nail polish and stored at 4 $^{\circ}$ C until imaged.

Microscopy. All imaging was performed at room temperature using laser scanning confocal microscopy. Extended live imaging used a Zeiss LSM 880 inverted microscope running Zen Black software with a 40 $\times$ PlanAPOCHROMAT 1.3NA oil immersion objective. All other imaging used a Zeiss LSM 800 upright microscope running Zen Blue software with a 63 $\times$ Plan-APOCHROMAT 1.4NA oil immersion objective. Images were collected such that the egg chambers' AP axes aligned with the horizontal (x) image axis, except in cases where alignment occurred in Fiji as described below.

Kymograph Generation. Movies were aligned in Fiji (imageJ) so that the egg chambers' AP axis corresponds with the horizontal (x) image axis. A maximum-intensity projection was generated to capture the height of follicular epithelium (3 to 5 μ m) for the duration of the timelapse. In Fiji, the straight line tool (1 pixel) was used to draw a vertical line and the KymoResliceWide plugin was used to create the kymograph.

Line-Scans of Col IV-GFP. Timelapse movies of a central optical plane through stage 1 egg chambers were rotated in Fiji so that the AP axes corresponded with the horizontal (x) image axis. In Fiji, straight lines of 0.28 μ m (1 pixel) thickness were drawn vertically through the anterior-most pole of the egg chamber where the anterior BM will form. Average Col IV-GFP intensities were measured at the start of the timelapse using Plot Profile in Fiji.

Cell Segmentation and Centroid Tracking of Live Tissues. Timelapse movies of CellMask-stained epithelia were segmented in each frame using the pretrained "cytoplasm" model in Cellpose (55) and tracked by linking regions of high overlap in consecutive frames. Napari was used to manually correct cell segmentation and tracking errors. To account for XY drift, Python scikit-image and scipy libraries were used to generate a mask of the tissue. Tissue masks were centered in each frame based on their centroid position, then movies were aligned to centered tissue masks. Scikit-image regionprop was then used to determine the individual cell centroid positions at each frame. For detailed protocols, see refs. 34 and 36 and *Data, Materials, and Software Availability* below.

Migration Rate Data Collection and Analysis. Ovarioles were stained with Orange or Deep Red CellMask (1:500) and mounted for standard live imaging. Timelapse movies were collected at a Z plane just below the apical epithelial surface every 30 s for 10 min. The multiple position acquisition feature was used to image many ovarioles in one session. Cells were segmented and tracked, and tissues were aligned to center, as described above. For stage 6 to 7, migration rate was calculated based on cell speeds at the central region of the egg chamber between the two poles. For stage 5 and under, all cells were used in the analysis. For stages 2 to 3, only egg chambers in which at least 3 cells could be tracked were included in the analysis. Migration rates were determined from centroid displacement (36). See also *Data, Materials, and Software Availability* below. For stage 1, we generated kymographs from timelapse movies by drawing single lines through the epithelium in the direction of migration. The migration rate was determined by measuring the slope of 3 kymograph lines and averaging the values (33).

Analysis of Relative Cell Movement at Basal vs. Apical Surfaces. For each egg chamber, a 30-min timelapse movie (15 s intervals) was acquired at the basal surface of the epithelium. After 30 min, the z-plane was shifted apically and another 30-min movie was collected. Cells were segmented and tracked, and centroid positions were obtained as described above. For analysis, cells present for less than 10 consecutive frames were discarded. First, we determined the difference between the first and last positions of each cell centroid in all tissues. To compare cell movement at basal vs. apical surfaces in one egg chamber, we calculated the average cell displacement by finding the total of all cell displacements at each z-plane divided by the number of cells analyzed in that plane.

Quantifying Polar Order from Cell Centroid Tracks. To further analyze the cell centroid tracks described above, centroid position was defined as $\mathbf{x}_i^j = [x_i^j, y_i^j]$ for each cell j at time $t = i$, where t is computed every minute for 30 min. We quantify the centroid movement for cell j at time i as $\mathbf{p}_i^j = \mathbf{x}_{i+1}^j - \mathbf{x}_i^j$. To quantify the coordination between all centroids in the field of view (FOV) over 30 min, we compute a polar order parameter α for an egg chamber. We calculate α from $\{\mathbf{p}_i^j\}$ by

$$\alpha = \frac{|\sum_{i=1}^{N_t} \sum_{j=1}^{N_c(i)} \mathbf{p}_i^j|}{\sum_{i=1}^{N_t} \sum_{j=1}^{N_c(i)} |\mathbf{p}_i^j|}, \quad [4]$$

where N_t is the total number of time points and $N_c(i)$ is the total number of follicle cell centroids in the FOV at both $t = i$ and $t = i + 1$. For perfectly ordered centroid movements where all the cells align in a single direction $\alpha = 1$. For random isotropic centroid movements $\alpha \rightarrow 0$. See also *Data, Materials, and Software Availability* below.

Fat2-3xGFP Fluorescence Intensity Quantification. To measure Fat2 brightness at the basal cell edges, an optical section was captured and the Fiji oval selection tool was used to measure the mean intensity of Fat2-3xGFP at the whole basal surface and three cell interiors at the basal surface of each sample. To control for variation between samples, the average of the basal surface cell interiors intensity measurement was subtracted from the total basal surface intensity measurement.

Fat2 Enrichment at Leading-Trailing Cell Edges. As a measure of Fat2 planar polarity at the basal surface, we determined the ratio of Fat2-3xGFP fluorescence intensity at horizontal (migratory leading/trailing) to vertical (lateral) cell edges. Cells were segmented based on N cadherin staining using the pretrained "cytoplasm" model in Cellpose (55). Next, we segmented the cell-cell edges between each pair of neighboring cells, and measured the angle of each cell-cell edge relative to the egg chamber's AP axis and Fat2 fluorescence intensity along those edges. Finally, we calculated the average fluorescence intensities of edges with angles between 0° and 10° (migratory leading/trailing) and between 80° and 90° (lateral) and determined the ratio between these values. A detailed protocol is in ref. 34. See also *Data, Materials, and Software Availability* below.

Reproducibility and Statistical Analysis. Egg chambers damaged during sample preparation were excluded from analysis. All experiments were performed at least twice. Each replicate included egg chambers pooled from multiple females. Experiments were not randomized, nor was data analysis performed blind. No statistical method was used to determine sample size. The number of biological replicates (n), statistical tests, and significance can be found in figures or figure legends. All statistical tests were performed in GraphPad Prism10. All data in which statistical tests were performed were tested for normality and found to follow an approximate normal distribution. Paired *t* tests were used for comparison of relative cell movements at basal vs. apical surfaces within epithelia (Fig. 3C). A two-way ANOVA with Tukey's multiple comparisons test was performed on datasets measuring migration rates or Fat2

planar polarity at multiple stages for multiple conditions (Figs. 2B and D and 4B and SI Appendix, Fig. S2C). An ordinary one-way ANOVA was performed when two or more conditions were compared within one stage (SI Appendix, Fig. S3B).

Movie Generation. Fiji was used to add labels and annotations and export timelapse videos as .avi, which were then converted to .mp4 using HandBrake. Simulations were generated using MATLAB R2023a.

Data, Materials, and Software Availability. The code used for the experimental analysis and model simulation is available in <https://github.com/SreejithSanthosh/egg-chamber-rotation> (56). Imaging data generated in this study is available at <https://doi.org/10.6084/m9.figshare.32562264> (57).

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- K. J. Cheung, S. Horne-Badovinac, Collective migration modes in development, tissue repair and cancer. *Nat. Rev. Mol. Cell Biol.* **26**, 741–758 (2025).
- E. Theveneau, R. Mayor, Collective cell migration of epithelial and mesenchymal cells. *Cell. Mol. Life Sci.* **70**, 3481–3492 (2013).
- T. J. Shaw, P. Martin, Wound repair: A showcase for cell plasticity and migration. *Curr. Opin. Cell Biol.* **42**, 29–37 (2016).
- D. Krdija et al., Active cell migration is critical for steady-state epithelial turnover in the gut. *Science* **365**, 705–710 (2019).
- R. Mayor, S. Etienne-Manneville, The front and rear of collective cell migration. *Nat. Rev. Mol. Cell Biol.* **17**, 97–109 (2016).
- M. Poujade et al., Collective migration of an epithelial monolayer in response to a model wound. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 15988–15993 (2007).
- S. Jain, B. Ladoux, R. M. Mège, Mechanical plasticity in collective cell migration. *Curr. Opin. Cell Biol.* **72**, 54–62 (2021).
- S. SenGupta, C. A. Parent, J. E. Bear, The principles of directed cell migration. *Nat. Rev. Mol. Cell Biol.* **22**, 529–547 (2021).
- S. Huang, C. Brangwynne, K. Parker, D. E. Ingber, Symmetry-breaking in mammalian cell cohort migration during tissue pattern formation: Role of random-walk persistence. *Cell Motil. Cytoskeleton* **61**, 201–213 (2005).
- K. Doxzen et al., Guidance of collective cell migration by substrate geometry. *Integr. Biol.* **5**, 1026–1035 (2013).
- S. Jain et al., The role of single-cell mechanical behaviour and polarity in driving collective cell migration. *Nat. Phys.* **16**, 802–809 (2020).
- S. Lo Vecchio, O. Pertz, M. Szopos, L. Navoret, D. Riveline, Spontaneous rotations in epithelia as an interplay between cell polarity and boundaries. *Nat. Phys.* **20**, 322–331 (2024).
- F. J. Segerer, F. Thüroff, A. Piera Alberola, E. Frey, J. O. Rädler, Emergence and persistence of collective cell migration on small circular micropatterns. *Phys. Rev. Lett.* **114**, 228102 (2015).
- K. Tanner, H. Mori, R. Mroue, A. Bruni-Cardoso, M. J. Bissell, Coherent angular motion in the establishment of multicellular architecture of glandular tissues. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 1973–1978 (2012).
- H. Wang, S. Lacoche, L. Huang, B. Xue, S. K. Muthuswamy, Rotational motion during three-dimensional morphogenesis of mammary epithelial acini relates to laminin matrix assembly. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 163–168 (2013).
- A. S. Chin et al., Epithelial cell chirality revealed by three-dimensional spontaneous rotation. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 12188–12193 (2018).
- T. H. Tan et al., Emergent chirality in active solid rotation of pancreas spheres. *PRX Life* **2**, 033006 (2024).
- T. Brandstätter et al., Curvature induces active velocity waves in rotating spherical tissues. *Nat. Commun.* **14**, 1643 (2023).
- G. Xie et al., Rotational migration in human pancreatic ductal organoids depends on actin and myosin activity. *Commun. Biol.* **8**, 1749 (2025).
- P. Ender et al., Spatiotemporal control of ERK pulse frequency coordinates fate decisions during mammary acinar morphogenesis. *Dev. Cell* **57**, 2153–2167.e6 (2022).
- E. Hirata, T. Ichikawa, S.-I. Horike, E. Kiyokawa, Active K-RAS induces the coherent rotation of epithelial cells: A model for collective cell invasion in vitro. *Cancer Sci.* **109**, 4045–4055 (2018).
- A. Glentis et al., The emergence of spontaneous coordinated epithelial rotation on cylindrical curved surfaces. *Sci. Adv.* **8**, eabn5406 (2022).
- P. A. Fernández et al., Surface-tension-induced budding drives alveologenesis in human mammary gland organoids. *Nat. Phys.* **17**, 1130–1136 (2021).
- P. Rørth, Fellow travellers: Emergent properties of collective cell migration. *EMBO Rep.* **13**, 984–991 (2012).
- S. Horne-Badovinac, D. Bilder, Mass transit: Epithelial morphogenesis in the *Drosophila* egg chamber. *Dev. Dyn.* **232**, 559–574 (2005).
- C. Berg, M. Sieber, J. Sun, Finishing the egg. *Genetics* **226**, iyad183 (2024).
- S. L. Haigo, D. Bilder, Global tissue revolutions in a morphogenetic movement controlling elongation. *Science* **331**, 1071–1074 (2011).
- M. Cetera et al., Epithelial rotation promotes the global alignment of contractile actin bundles during *Drosophila* egg chamber elongation. *Nat. Commun.* **5**, 5511 (2014).
- A. J. Isabella, S. Horne-Badovinac, Rab10-mediated secretion synergizes with tissue movement to build a polarized basement membrane architecture for organ morphogenesis. *Dev. Cell* **38**, 47–60 (2016).
- J. Crest, A. Diz-Muñoz, D. Y. Chen, D. A. Fletcher, D. Bilder, Organ sculpting by patterned extracellular matrix stiffness. *eLife* **6**, e24958 (2017).
- H. Alégot, P. Pouchin, O. Bardot, V. Mirouse, Jak-Stat pathway induces *Drosophila* follicle elongation by a gradient of apical contractility. *eLife* **7**, e32943 (2018).
- A. J. Squarr et al., Fat2 acts through the WAVE regulatory complex to drive collective cell migration during tissue rotation. *J. Cell Biol.* **212**, 591–603 (2016).
- K. Barlan, M. Cetera, S. Horne-Badovinac, Fat2 and lar define a basally localized planar signaling system controlling collective cell migration. *Dev. Cell* **40**, 467–477 (2017).
- A. M. Williams, S. Donoughe, E. Munro, S. Horne-Badovinac, Fat2 polarizes the wave complex in trans to align cell protrusions for collective migration. *eLife* **11**, e8343 (2022).
- I. Viktorinová, C. Dahmann, Microtubule polarity predicts direction of egg chamber rotation in *Drosophila*. *Curr. Biol.* **23**, 1472–1477 (2013).
- A. M. Williams, S. Horne-Badovinac, Fat2 polarizes Lar and Sema5c to coordinate the motility of collectively migrating epithelial cells. *J. Cell Sci.* **137**, jcs261173 (2023).
- I. Viktorinová, I. Henry, P. Tomanek, Epithelial rotation is preceded by planar symmetry breaking of actomyosin and protects epithelial tissue from cell deformations. *PLoS Genet.* **13**, e1007107 (2017).
- D. Y. Chen, K. R. Lipari, Y. Dehghan, S. J. Streichan, D. Bilder, Symmetry breaking in an edgeless epithelium by fat2-regulated microtubule polarity. *Cell Rep.* **15**, 1125–1133 (2016).

39. S. G. Wilcockson, H. L. Ashe, Live imaging of the *Drosophila* ovarian germline stem cell niche. *STAR Protoc.* **2**, 100371 (2021).
40. M. Buszczak *et al.*, The Carnegie protein trap library: A versatile tool for *Drosophila* developmental studies. *Genetics* **175**, 1505–1531 (2007).
41. E. Assa-Kunik, I. L. Torres, E. D. Schejter, D. S. Johnston, B. Z. Shilo, *Drosophila* follicle cells are patterned by multiple levels of Notch signaling and antagonism between the Notch and JAK/STAT pathways. *Development* **134**, 1161–1169 (2007).
42. C. G. Stedden *et al.*, Planar-polarized semaphorin-5c and plexin a promote the collective migration of epithelial cells in *Drosophila*. *Curr. Biol.* **29**, 908–920 (2019).
43. M. A. Li, J. D. Alls, R. M. Avancini, K. Koo, D. Godt, The large Maf factor Traffic Jam controls gonad morphogenesis in *Drosophila*. *Nat. Cell Biol.* **5**, 994–1000 (2003).
44. T. R. Hartman *et al.*, *Drosophila* Bci limits Hedgehog levels to suppress follicle stem cell proliferation. *J. Cell Biol.* **191**, 943–952 (2010).
45. A. C. Spradling, "Developmental genetics of oogenesis" in *The Development of Drosophila melanogaster*, M. Bate, A. Martinez Arias, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993), vol. I, chap. 1.
46. U. Töpfer, K. Y. Guerra Santillán, E. Fischer-Friedrich, C. Dahmann, Distinct contributions of ECM proteins to basement membrane mechanical properties in *Drosophila*. *Development* **149**, dev200456 (2022).
47. N. Yamaguchi *et al.*, Rear traction forces drive adherent tissue migration in vivo. *Nat. Cell Biol.* **24**, 194–204 (2022).
48. L. Fasano, S. Kerridge, Monitoring positional information during oogenesis in adult *Drosophila*. *Development* **104**, 245–253 (1988).
49. L. X. Morris, A. C. Spradling, Long-term live imaging provides new insight into stem cell regulation and germline-soma coordination in the *Drosophila* ovary. *Development* **138**, 2207–2215 (2011).
50. D. Y. Chen, J. Crest, S. J. Streichan, D. Bilder, Extracellular matrix stiffness cues junctional remodeling for 3d tissue elongation. *Nat. Commun.* **10**, 3339 (2019).
51. R. Alert, X. Trepast, Physical models of collective cell migration. *Annu. Rev. Condens. Matter Phys.* **11**, 77–101 (2020).
52. M. C. Díaz *et al.*, Laminin levels regulate tissue migration and anterior-posterior polarity during egg morphogenesis in *Drosophila*. *Cell Rep.* **20**, 211–223 (2017).
53. M. Prasad, A. C. C. Jang, M. Starz-Gaiano, M. Melani, D. J. Montell, A protocol for culturing *Drosophila melanogaster* stage 9 egg chambers for live imaging. *Nat. Protoc.* **2**, 2467–2473 (2007).
54. M. Cetera, L. Lewellyn, S. Horne-Badovinac, Cultivation and live imaging of *Drosophila* ovaries. *Methods Mol. Biol.* **1478**, 215–226 (2016).
55. C. Stringer, T. Wang, M. Michaelos, M. Pachitariu, Cellpose: A generalist algorithm for cellular segmentation. *Nat. Methods* **18**, 100–106 (2021).
56. S. Santhosh, Code and data from "Initiation of rotational collective migration in *Drosophila* through tissue geometry and mechanochemical feedback." Github. <https://github.com/SreejithSanthosh/> egg-chamber-rotation. Deposited 5 September 2025.
57. S. Schwabach, Data from "Initiation of rotational collective migration in *Drosophila* through tissue geometry and mechanochemical feedback." Figshare. <https://doi.org/10.6084/m9.figshare.32562264.v1>. Deposited 2 June 2026.

Supplementary information for Initiation of rotational collective migration in *Drosophila* through tissue geometry and mechanochemical feedback

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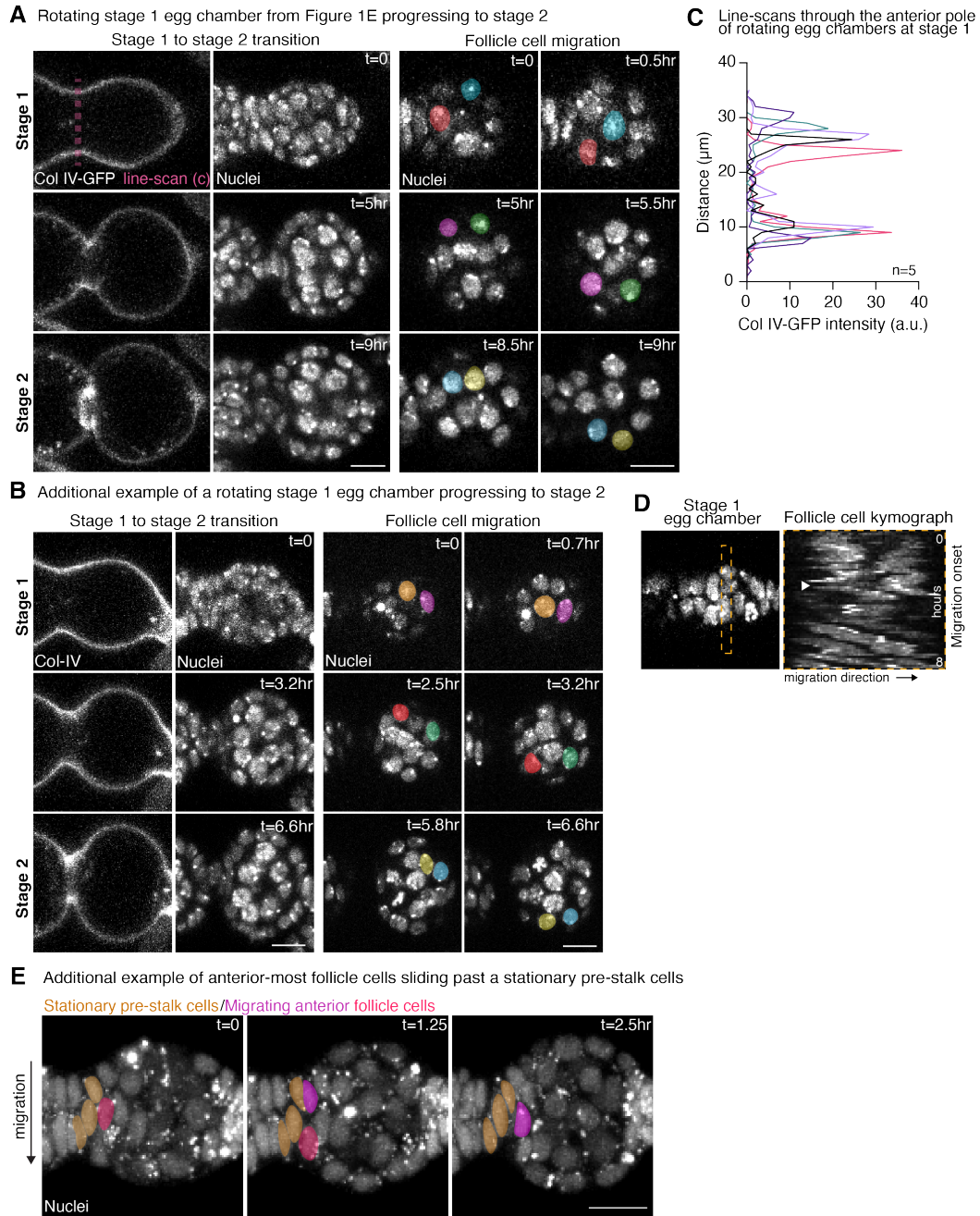


Figure S1: Additional characterization of the onset of migration at stage 1, related to Figure 1. A) Movie stills of a transverse section through one egg chamber that capture the transition from stage 1 (shown in Figure 1) to 2 (left). Pink dashed line shows the location of the line-scans used to generate the plot in C. Movie stills focused on the follicular epithelium of the same egg chamber (right). Two different cells are pseudocolored in each row of images to show cell movement over 30 minutes at each of the three time points. **B)** Additional example of stage 1 to stage 2 transition. Movie stills of a transverse section (left) and stills of the follicular epithelium (right) showcase continuous follicle cell migration as the egg chamber progresses to stage 2. **C)** Line-scans of Col IV-GFP through the anterior-most pole of the follicular epithelium for 5 rotating egg chambers at stage 1. The bimodal distribution indicates that the BM has not yet formed in this location. **D)** Additional example of migration onset (arrowhead) at stage 1. Nuclei marked with SpyDNA. **E)** Movie stills of maximum intensity projections of nuclei generated from a 2.5 hour movie. Stationary pre-stalk cell nuclei are pseudocolored gold, rotating pre-polar and follicle cell nuclei are pseudocolored magenta and blue, respectively. Scale bars, 10 μm .

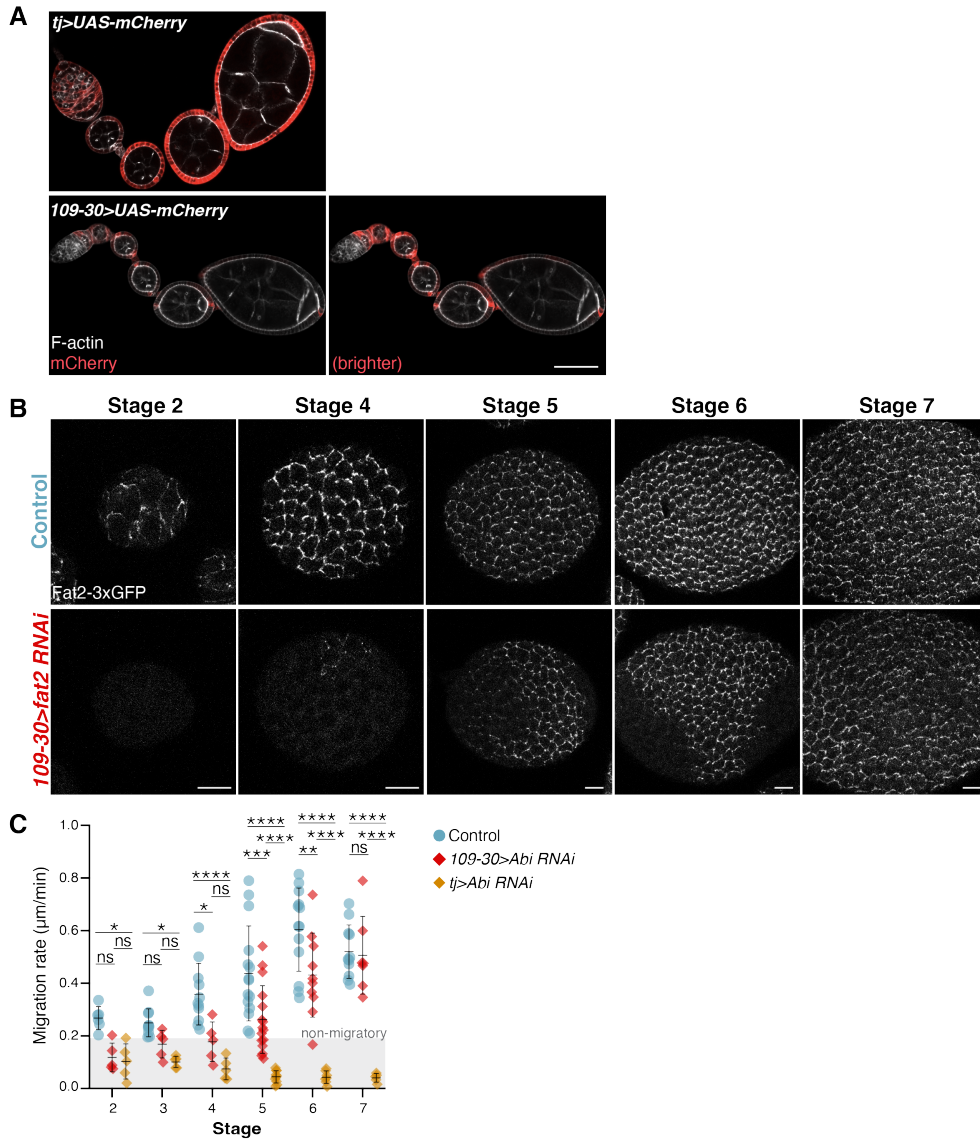


Figure S2: Additional characterization of the delayed-migration assay, related to Figure 2. A) Representative images showing the two Gal4 drivers used in this study driving *UAS-mCherry*. The *tj-Gal4* driver is expressed in the follicle cells throughout the stages when the egg chamber rotates, whereas the *109-30-Gal4* driver is primarily expressed in the earliest rotation stages. The two images on the left were taken with the same microscope settings. The fluorescence signal in the right image has been enhanced to better show the expression pattern. Scale bar, 50μm. **B)** Representative images of Fat2-3xGFP at the basal epithelial surface. *109-30>fat2-RNAi* eliminates Fat2 expression through stage 2, but Fat2 gradually reaches full expression by stage 7 as the RNAi stops being expressed. Scale bar, 10μm. **C)** Quantification of follicle cell migration rates over developmental time. *109-30>Abi-RNAi* blocks migration at early stages but migration is indistinguishable from controls by stage 7. Each data point represents one egg chamber. Control values are repeated from Figure 2D. Two-way ANOVA with Tukey's multiple comparisons test; ns, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. In order on graph, $n = 6, 5, 5, 10, 5, 5, 11, 5, 5, 15, 16, 10, 13, 10, 7, 11, 7, 5$. Bars represent mean $\pm$ SD.

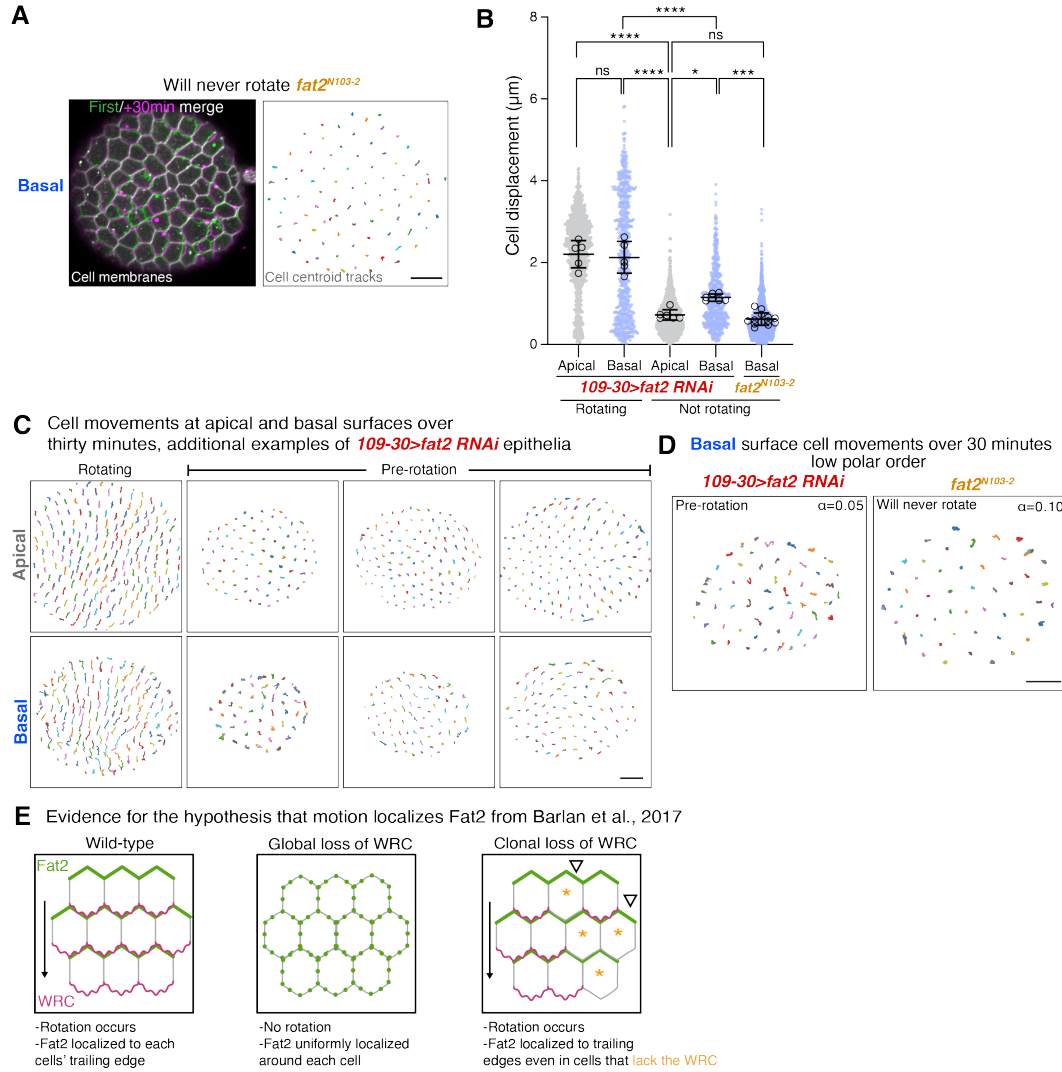


Figure S3: **Additional characterization of cell movement at apical and basal surface planes, related to Figure 3, and the relationship between Fat2 polarity and global rotation, related to Figure 4.** **A)** Representative image of a *fat2^{N103-2}* epithelium that will never rotate. Panels on the left show the overlay of the first (green) and last (magenta) frame of a 30-min movie (**Movie 8**). Panels on the right show cell centroid tracks from the same movie. **B)** Quantification of cell displacement at different imaging planes. Closed dots represent individual cells across multiple egg chambers, black open circles represent the average value for each egg chamber. For egg chamber averages: Ordinary one-way ANOVA with Tukey's multiple comparisons test; ns, $p>0.05$, $*p<0.05$, $***p<0.001$, $****p<0.0001$. In order on graph, $n=5,5,6,13$. Bars represent mean $\pm$ SD. **C)** Additional examples of cell centroid tracks over 30 minutes at apical and basal surface planes in rotating or not-rotating *109-30>fat2-RNAi* egg chambers. Scale bars, $10\mu\text{m}$ **D)** Representative cell centroid tracks taken at the basal surfaces of two epithelia, showing low polar order. **E)** Illustration of the experiment from Barlan et al., 2017 that first suggested that the localization of Fat2 to the trailing edge of each follicle cells depends on tissue motion.

S1 Mechanochemical model of delayed migration egg chamber

S1.1 Mechanics of the egg chamber

We model the egg chamber as a rigid body, as there are negligible neighbor exchanges between follicle cells, and these cells tightly adhere to the germ cells. Egg chambers have an ellipsoidal geometry and are slightly elongated along the AP axis at stages 5-6, when the delayed onset of rotation occurs. We parametrize the egg chamber (i.e. a surface $\mathcal{M}$, Fig S4A) as

$$\mathbf{r}(\theta, \phi) = \{a \cos \phi \sin \theta, a \sin \phi \sin \theta, ae \cos \theta\}; \quad \theta \in [0, \pi] \text{ and } \phi \in [0, 2\pi), \quad (\text{S1})$$

where a is the radius of the equatorial cross-section and e is the aspect ratio (Fig. S4B). At each point on the egg chamber $\mathbf{r}(\theta, \phi)$, the tangent vector basis (${}_1\boldsymbol{\zeta}, {}_2\boldsymbol{\zeta}$) and the surface element $d\mathbf{A}$ (Fig. S4A) are given by

$$\begin{cases} {}_1\boldsymbol{\zeta} = \left\{ \frac{\cos \theta \cos \phi}{\sqrt{\cos^2 \theta + e^2 \sin^2 \theta}}, \frac{\cos \theta \sin \phi}{\sqrt{\cos^2 \theta + e^2 \sin^2 \theta}}, -\frac{e \sin \theta}{\sqrt{\cos^2 \theta + e^2 \sin^2 \theta}} \right\}, & {}_2\boldsymbol{\zeta} = \{-\sin \phi, \cos \phi, 0\} \\ d\mathbf{A} = g(\theta, \phi) \hat{\mathbf{n}}(\theta, \phi) d\theta d\phi, & g(\theta, \phi) = a^2 \sqrt{\frac{1+e^2 - (-1+e^2) \cos 2\theta}{2}} \sin \theta, \end{cases} \quad (\text{S2})$$

where $\hat{\mathbf{n}}(\theta, \phi)$ is the outward normal vector. To describe the dynamics, we define an inertial (i.e. fixed) frame $\mathcal{E} : \{\hat{\mathbf{e}}_1, \hat{\mathbf{e}}_2, \hat{\mathbf{e}}_3\}$ and a body-frame $\mathcal{B} : \{\hat{\mathbf{d}}_1, \hat{\mathbf{d}}_2, \hat{\mathbf{d}}_3\}$ that rotates with the egg chamber and moves with its center of mass, with $\hat{\mathbf{d}}_3$ oriented along the long axis (Fig. S4B). The motion of a rigid body in 3D can be described by its center of mass velocity $\mathbf{v}$ and its angular velocity $\boldsymbol{\omega}$. The dynamics of the egg chamber given external forces and torques obeys linear momentum and angular momentum principles in the over-damped limit, which in the body-frame $\mathcal{B}$ [1], read

$$\mathbf{F} = \mathbf{0}, \boldsymbol{\Gamma} = \mathbf{0}, \quad (\text{S3})$$

where $\mathbf{F}, \boldsymbol{\Gamma}$ are the total applied force and torque, which we model below.

Tangential drag force at the basement membrane. The basement membrane encases the egg chamber and resists its relative tangential motion via viscous drag due to remodeling of integrin-based adhesion complexes (Fig S4B). The basement membrane responds elastically to motion normal to the surface, which we model and discuss the consequences in Sec. S1.1.1. We model the tangential surface drag force density as

$$\mathbf{f}(\theta, \phi) = -\mu_b \mathbf{v}_{\parallel}(\theta, \phi), \quad (\text{S4})$$

where μ_b is the viscous drag coefficient and $\mathbf{v}_{\parallel}$ is the component of the velocity tangential to the surface at (θ, ϕ) . The velocity $\mathbf{v}(\theta, \phi)$ of a surface element is given by

$$\mathbf{v}(\theta, \phi) = \mathbf{v} + \boldsymbol{\omega} \times \mathbf{r}(\theta, \phi), \quad (\text{S5})$$

where $\mathbf{r}(\theta, \phi)$ is the position vector of the surface element with respect to the center of mass of the egg chamber. The total force and torque generated by the local viscous drag forces given in Eq. (S4) are obtained by integrating over the entire surface of the egg chamber (Eq. (S1)). Given that $e \approx 1.2$ for a stage 5 egg chamber we compute the total viscous friction force $\mathbf{F}_f$ and torque $\boldsymbol{\Gamma}_f$ (expressed in $\mathcal{B}$) by expanding about $e = 1$ in linear order, which gives

$$\begin{cases} \mathbf{F}_f = \int_{\mathcal{M}} \mathbf{f}(\theta, \phi) g(\theta, \phi) d\theta d\phi = -\frac{8}{3} a^2 \pi \mu_b \mathbf{v} - \frac{4}{15} a^2 \pi \mu_b (e - 1) \begin{bmatrix} 2v_1 \\ 2v_2 \\ 11v_3 \end{bmatrix}, \\ \boldsymbol{\Gamma}_f = \int_{\mathcal{M}} \mathbf{r}(\theta, \phi) \times \mathbf{f}(\theta, \phi) g(\theta, \phi) d\theta d\phi = -\frac{8}{3} \mu_b a^4 \pi \boldsymbol{\omega} - \frac{2}{15} \mu_b a^4 \pi (e - 1) \begin{bmatrix} 31\omega_1 \\ 31\omega_2 \\ 13\omega_3 \end{bmatrix}, \end{cases} \quad (\text{S6})$$

where $\mathbf{v} = [v_1, v_2, v_3]$ and $\boldsymbol{\omega} = [\omega_1, \omega_2, \omega_3]$ are expressed in the body-frame $\mathcal{B}$.

Protrusive crawling force. The follicle cells use cryptic lamellipodia at their basal surfaces to crawl on the basement membrane [2]. We model these active point forces $\mathbf{b}_i$ acting at the cell-centroid $\mathbf{r}_i \in \mathcal{M}$ (Fig S4C) on the

basal surface of cell i . Therefore, the total crawling (or active) force $\mathbf{F}_a$ and torque $\mathbf{\Gamma}_a$ exerted by the basal surface protrusions are given in the body frame by

$$\mathbf{F}_a = \sum_{i=1}^N \mathbf{b}_i, \quad \mathbf{\Gamma}_a = \sum_{i=1}^N \mathbf{r}_i \times \mathbf{b}_i. \quad (\text{S7})$$

S1.1.1 Elastic confinement due to basement membrane

The basement membrane elastically resists motion perpendicular to the egg chamber-basement membrane interface. The elastic surface force density is given by

$$\mathbf{f}(\theta, \phi) = -K_B(\delta r_\perp(\theta, \phi)/h)\hat{\mathbf{n}}(\theta, \phi) \quad (\text{S8})$$

where K_B, h are the stiffness and thickness of the basement membrane, and $\delta r_\perp(\theta, \phi)$ is the displacement of the area element normal to the interface. For an egg chamber with the center of mass displaced by $\delta \mathbf{r}^C = [\delta r_1^C, \delta r_2^C, \delta r_3^C]$ in the body-frame $\mathcal{B}$ and body-frame orientation given by rotation matrix $\mathbf{A}$,

$$\delta r_\perp(\theta, \phi) = (\delta \mathbf{r}^C + (\mathbf{A} - \mathbb{I})\mathbf{r}(\theta, \phi)) \cdot \hat{\mathbf{n}}(\theta, \phi). \quad (\text{S9})$$

The rotation matrix $\mathbf{A}$ gives the orientation of the body-frame $\mathcal{B}$ with respect to the inertial-frame $\mathcal{E}$. The orientational rest-configuration of the egg chamber is assumed to be $\mathbf{A} = \mathbb{I}$ and we analyze small deviations $\delta\theta$ of the egg chamber AP axis away from $\hat{\mathbf{e}}_3$ (Fig S4D-E). Integrating over the egg chamber, we obtain the total force and torque to linear order in e represented in the $\mathcal{B}$ frame:

$$\left\{ \begin{array}{l} \mathbf{F}_e = \int_{\mathcal{M}} \mathbf{f}(\theta, \phi) g(\theta, \phi) d\theta d\phi = -\frac{4}{3h} a^2 \pi K_B \delta \mathbf{r}^C - \frac{8}{15h} a^2 \pi K_B (e-1) \begin{bmatrix} 3\delta r_1^C \\ 3\delta r_2^C \\ \delta r_3^C \end{bmatrix}, \\ \mathbf{\Gamma}_e = \int_{\mathcal{M}} \mathbf{r}(\theta, \phi) \times \mathbf{f}(\theta, \phi) g(\theta, \phi) d\theta d\phi = -\frac{8}{15h} K_B a^4 \pi (e-1) \begin{bmatrix} A_{23} + A_{32} \\ A_{13} + A_{31} \\ 0 \end{bmatrix}. \end{array} \right. \quad (\text{S10})$$

Active crawling forces drive the rigid rotation. An order of magnitude estimate of the active force $\mathbf{F}_a$ and torque $\mathbf{\Gamma}_a$ is given by

$$|\mathbf{F}_a| = N b_c, |\mathbf{\Gamma}_a| = N b_c a, \quad (\text{S11})$$

where b_c is the characteristic crawling force of one cell. $b_c = \sigma_a A_c$, where σ_a is the mean traction stress generated by a crawling cell and A_c is the cell area. There are currently no available experimental measurements of σ_a in the *Drosophila* egg chamber. Instead, we use an estimate of traction stresses ≈ 10 Pa measured from collectively migrating epithelial cells in another *in vivo* system [3]. Equating the magnitude of active crawling and elastic response force (torque), we can estimate the theoretical maximum translation (rotational motion) of the egg chamber. Using the values of $K_B \approx 50\text{kPa}$, $h \approx 90\text{nm}$, $a \approx 25\mu\text{m}$, $e \approx 1.2$ [4-7] we get,

$$|\delta \mathbf{r}^C| \approx 5.4 \times 10^{-2} \text{ nm}, A_{23} + A_{32} \text{ and } A_{31} + A_{13} \approx 2.7 \times 10^{-5}. \quad (\text{S12})$$

Therefore, since the motion of the center of mass is significantly smaller than the dimensions of the egg chamber ($|\delta \mathbf{r}^C| \ll a$), we assume that the center of mass remains stationary for all our simulations. The entries $A_{23}, A_{32}, A_{31}, A_{13}$ of the orientation matrix $\mathbf{A}$ quantifies the deviation of the AP axis ($\hat{\mathbf{d}}_3$) from the rest orientation ($\hat{\mathbf{e}}_3$). Since Eq. (S12) holds for any rotation of the egg chamber about an axis in the $\{\hat{\mathbf{d}}_1, \hat{\mathbf{d}}_2\}$ plane, we can independently infer that $A_{32}, A_{23}, A_{31}, A_{13} \approx 2.7 \times 10^{-5}$. This demonstrates that rotation of the egg chamber is confined to its long axis, consistent with experimental observations [8]. This constraint on rotational axis does not hold for spherical egg chambers ($e \rightarrow 1$), which can rotate along any axis (See Sec. S1.5).

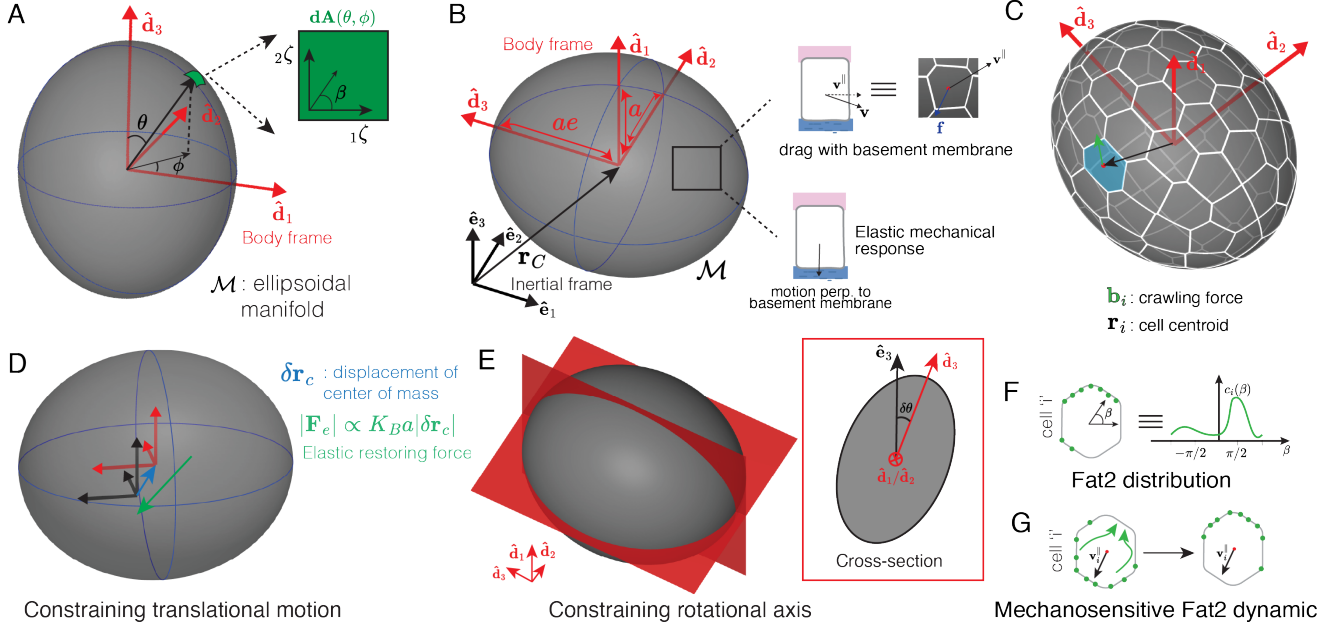


Figure S4: **Biophysical model of egg chambers during delayed migration onset.** (a) Egg chamber geometry is described using an ellipsoidal manifold $\mathcal{M}$ parameterized using angles (θ, ϕ) in the body frame $\mathcal{B} : \{\hat{\mathbf{d}}_1, \hat{\mathbf{d}}_2, \hat{\mathbf{d}}_3\}$. The area element $d\mathbf{A}$ at (θ, ϕ) has tangent bases vectors $\{1\zeta, 2\zeta\}$ and β is the angular coordinate on the tangent plane. (b) The dynamics can be described both in the inertial frame $\mathcal{E}$ and the body frame $\mathcal{B}$. The basement membrane provides viscous (elastic) resistance force to motion tangential (perpendicular) to it. (c) Follicle cell positions described in the body frame. (d-e) Elastic response of the basement membrane to motion of the egg chamber normal to the interface constrains translational motion (d) and rotational axis (e). (f) The Fat2 concentration for cell i is described using $c_i(\beta)$. (g) The Fat2 concentration is mechanosensitive.

S1.2 Crawling force and cell polarity dynamics

S1.2.1 Fat2 polarity dynamics

We model the Fat2 distribution for cell i as $c_i(\beta, t)$, where β is the angular coordinate in the tangent space of $\mathcal{M}$ at the cell-centroid position $\mathbf{r}_i$ (Fig. S4F). Previous work [9] has demonstrated that Fat2 localizes to cell's trailing edges, and also suggests that tissue rotation may play a role in localizing Fat2. Therefore, we construct a minimal model of mechanosensitive Fat2 dynamics as

$$\frac{\partial c_i(\beta)}{\partial t} = -\epsilon_0(\epsilon_1 \mathbf{v}_i \cdot \mathbf{m}(\beta, \mathbf{r}_i) + c_i(\beta))c_i(\beta), \quad \mathbf{m}(\beta, \mathbf{r}_i) = \cos(\beta(t))_1 \zeta(\mathbf{r}_i) + \sin(\beta(t))_2 \zeta(\mathbf{r}_i), \quad (\text{S13})$$

where $\mathbf{v}_i = \mathbf{v} + \boldsymbol{\omega} \times \mathbf{r}_i$ is velocity of cell i , ϵ_0 is a characteristic rate constant for the dynamics of Fat2 polarity and ϵ_1 sets the strength of coupling between migration and Fat2 polarity. Intuitively, Eq. (S13) models an increase in Fat2 polarization to the cell edge in the direction opposite of motion (Fig. S4G).

S1.2.2 Crawling force dynamics

Fat2 acts at cells' trailing edges to create a stable protrusive domain in the leading edge of the neighboring cell behind [9]. Thus we assume the direction of the crawling force $\mathbf{b}_i$ to be influenced by Fat2 polarity. For simplicity, we model the activity of Fat2 as cell-autonomous and the crawling force $\mathbf{b}_i$ as

$$\partial_t \mathbf{b}_i = \boldsymbol{\omega} \times \mathbf{b}_i - \frac{1}{\gamma}(\zeta_i(t) + \mathbf{b}_i), \quad \zeta(t) = b_c(\cos(\beta_i(t))_1 \zeta(\mathbf{r}_i) + \sin(\beta_i(t))_2 \zeta(\mathbf{r}_i)), \quad (\text{S14})$$

where $\beta_i(t)$ is sampled from the probability distribution $P_i(\beta, t) \propto c_i(\beta, t)$, b_c is the characteristic scale of the force generated by cell protrusions and γ is the timescale taken by the protrusion to respond to changes in Fat2 polarity

encoded in $\zeta_i(t)$. The term $\omega \times \mathbf{b}_i$ arises from differentiation in rotating frames and maintains the consistency of the protrusion direction during rigid rotation of the egg chamber.

S1.3 Nondimensional equations of motion and parameter analysis

Equations (S6-S7, S13-S14) constitute a mechanochemical model of the egg chamber. We nondimensionalize the variables using the transformations $t \rightarrow t_c t$, $\omega \rightarrow \omega_c \omega$, $\mathbf{r}_i \rightarrow a \mathbf{r}_i$, $\mathbf{v} \rightarrow \omega_c a \mathbf{v}$, $\mathbf{b}_i \rightarrow b_c \mathbf{b}_i$, $c_i \rightarrow c_c c_i$ and $\zeta_i \rightarrow b_c \eta_i$; where t_c , ω_c , b_c , c_c are the characteristic time-scale of rotation initiation, angular-speed of egg chamber, force scale of protrusions and characteristic concentration scale of Fat2. Rotation initiation occurs over a time-scale $t_c \approx 1 \text{ hour}$. The radius of the egg chamber equatorial cross-section $a \approx 25 \mu\text{m}$ during rotation initiation. The characteristic angular velocity $\omega_c \approx t_c^{-1}$ for an egg chamber during delayed rotation initiation. We further use the fact that after symmetry breaking, the egg chamber rotates in a single direction with an angular velocity $\omega \approx \omega_c$. In this regime, torque from crawling forces must balance torque from drag with the basement membrane, which, using the angular momentum balance, gives $b_c a N \approx \mu_b a^4 \omega_c$ (order of magnitude calculations), leading to $b_c \approx \frac{1}{N} \mu_b a^3 \omega_c$. Due to the strong elastic confinement from the basement membrane, the center of mass remains stationary and rotations are restricted to the long axis (See Sec. S1.1.1). Therefore, we obtain the non-dimensional equations of motion

$$\begin{cases} \Pi_\omega(e) \omega_3 = (\sum_{i=1}^N \mathbf{r}_i \times \mathbf{b}_i) \cdot \hat{\mathbf{d}}_3 \text{ for } e > 1 \\ \partial_t \mathbf{b}_i = \omega \times \mathbf{b}_i - \tau_1 (\eta_i(t) + \mathbf{b}_i) \\ \partial_t c_i = -\tau_2 (\tau_3 \mathbf{v}_i \cdot \mathbf{m}(\beta, \mathbf{r}_i) + c_i) c_i \end{cases}, \quad \begin{cases} \Pi_\omega(e) = N\pi(40 + 26(e-1))/15 \\ \eta_i(t) = \cos(\beta_i(t))_1 \zeta(\mathbf{r}_i) + \sin(\beta_i(t))_2 \zeta(\mathbf{r}_i) \\ \tau_1 = t_c/\gamma, \tau_2 = \epsilon_0 t_c c_c, \tau_3 = \epsilon_1 a/t_c c_c \end{cases} \quad (\text{S15})$$

For simulations of the delayed onset of rotation, we use $e = 1.2$ and $N = 300$ consistent with experimental observations [7]. To compute the cell centroid locations $\mathbf{r}_i$ for a given number of follicle cells N , we initialized it by placing N equi-distant points on the surface of an ellipsoid.

S1.3.1 Parameters τ_1, τ_2 and τ_3

Rotation initiation occurs over a time-scale $t_c \approx 1 \text{ hour}$, and the time-scale over which protrusions respond to changes in Fat2 distribution is $\gamma \approx 3 \text{ min}$ [10]. Therefore, we set $\tau_1 = 20$. There is currently no reliable experimental estimate for (τ_2, τ_3) . Therefore, we numerically explore the parameter space and observe that the egg chamber undergoes symmetry breaking for all positive values, but the time taken to break symmetry changes. We quantify this difference by estimating the time taken to reach 95 percent of the ensemble average of the steady state angular velocity $|\omega_3|^s$ after symmetry breaking (See SI Fig S5 A) for a parameter set. Increasing (τ_2, τ_3) decreases the time taken for the egg chamber to symmetry break (See SI Fig S5 B-C).

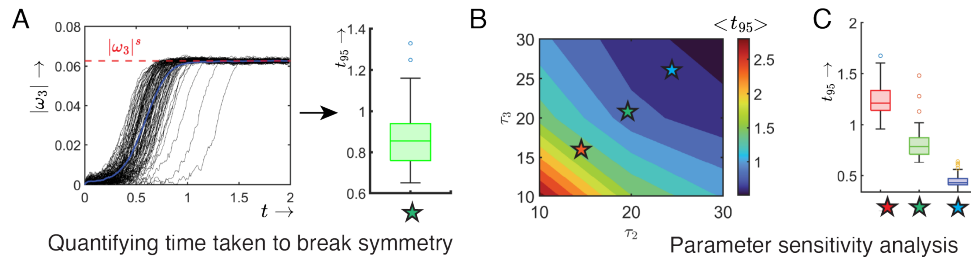


Figure S5: **Sensitivity analysis to changes in (τ_2, τ_3) .** (a) $N = 100$ runs of the theoretical model of a delayed egg chamber for the parameter values $(\tau_2, \tau_3) = (20, 20)$. (b-c) The ensemble average time taken $\langle t_{95} \rangle$ to reach 95 percent of the steady-state symmetry broken angular velocity $|\omega_3|^s$ for different parameter sets.

S1.4 Numerical implementation

We simulate an egg chamber undergoing delayed rotation initiation by solving Eqns. (S15) using forward Euler integration method with time-step $dt = 10^{-2}$ in MATLAB. The evolution of the body frame is tracked using a

rotation matrix $\mathbf{A}(t)$ which relates vectors in the inertial-frame $\mathcal{E}$ and body-frame $\mathcal{B}$ as

$$\mathbf{r}_E = \mathbf{A}(t)\mathbf{r}_B, \quad (\text{S16})$$

where $\mathbf{r}_E, \mathbf{r}_B$ are the coordinates of the same vector in the inertial and body frames. The rotation matrix $\mathbf{A}(t)$ evolves using the relation

$$\frac{d}{dt}\mathbf{A}(t) = \boldsymbol{\Omega}\mathbf{A}, \quad \boldsymbol{\Omega} = \begin{pmatrix} 0 & -\omega_3 & \omega_2 \\ \omega_3 & 0 & -\omega_1 \\ -\omega_2 & \omega_1 & 0 \end{pmatrix}, \quad (\text{S17})$$

where $\boldsymbol{\omega} = [\omega_1, \omega_2, \omega_3]$ is the angular velocity of the egg chamber represented in the inertial frame. For an ellipsoidal ($e > 1$) egg chamber $\omega_2, \omega_1 = 0$ due to the strong confinement from the basement membrane (Sec S1.1.1).

S1.5 Spherical egg chamber

In contrast to an elongated egg chamber, the elastic confinement from the basement membrane does not constrain the rotational axis in a spherical egg chamber (Sec S1.1.1 for details). Therefore, using the same non-dimensionalization scheme as introduced in Sec. S1.3 the non-dimensional equation of motion for a spherical egg chamber is given by

$$\Pi_\omega(1)\boldsymbol{\omega} = \sum_{i=1}^N \mathbf{r}_i \times \mathbf{b}_i. \quad (\text{S18})$$

To simulate rotation initiation in a spherical egg chamber, we use Eq. (S18) for the rotational dynamics and the same equations for Fat2 concentration and crawling force as in the elongated egg chamber (Eq. (S15)). We simulate these equations with the same parameters τ_{1-3} as the delayed migration egg chamber, but with $N = 100$, as quantified for the stage 1 egg chamber. Starting from an isotropic initial condition ($c_i(\beta, 0) = 1/2\pi, \mathbf{b}_i(0) = \mathbf{0}$), the spherical egg chamber undergoes symmetry breaking (Fig S6A-C) but with a randomly oriented rotational axis (Fig S6D).

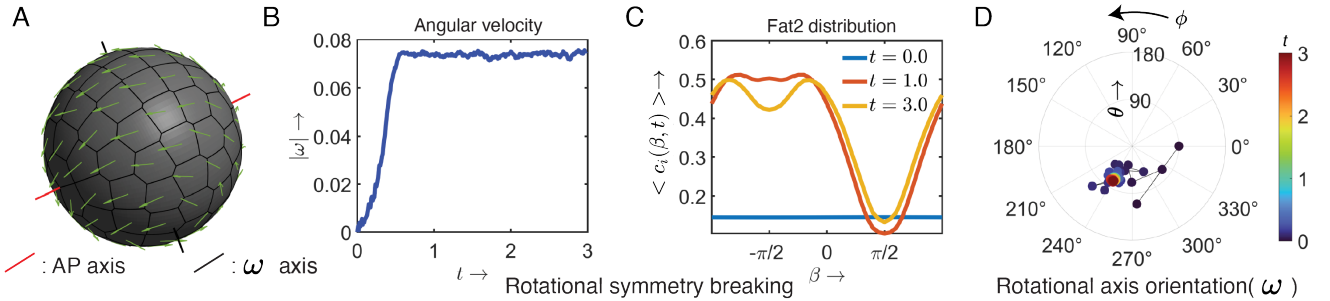


Figure S6: **Random rotational axis in spherical egg chambers.** (a) Rotation of a spherical egg chamber. (b-c) The egg chamber undergoes symmetry breaking, generating sustained rotations. (d) The orientation of the angular velocity $\boldsymbol{\omega}$ described in the inertial frame $\mathcal{E}$ is quantified using spherical coordinates (θ, ϕ) . The time-evolution of the quantities in a-d is given in Movie 10. We mark the future AP axis (red) for reference.

S2 Mechanochemical model of stage 1 egg chamber

S2.1 Mechanics of stage 1 egg chambers

The mechanics of stage 1 egg chambers is distinct from the later stages due to the contact of follicle cells with the pre-stalk cells (Fig. S7A). We model the mechanical interaction with the stationary pre-stalk cells to elucidate how the stage 1 egg chamber undergoes symmetry breaking. The stage 1 egg chamber is spherical ($e = 1$), but with a flat contact with the pre-stalk cells. The spatial extent of the contact region is quantified using the polar angle θ_s . We use a similar framework to the model introduced in Sec. S1.1, but accounting for the interaction with the

pre-stalk cells. The strong confinement from the basement membrane, discussed in Sec. S1.1.1, holds even for the stage 1 egg chamber; therefore, the center of mass is fixed. We next discuss the changes in the external torques of this system compared to the delayed migration model.

Tangential drag force at the basement membrane. The tangential drag force is the same as for the egg chamber at the time of delayed-rotation onset Eq. (S4), but the surface of integration is given by $\mathcal{M}_b$ (Fig. S7B):

$$\mathbf{r}(\theta, \phi) = \{a \cos \phi \sin \theta, a \sin \phi \sin \theta, a \cos \theta\}; \quad \theta \in [\theta_s, \pi] \text{ and } \phi \in [0, 2\pi). \quad (\text{S19})$$

The total (friction) torque due to the drag with the basement membrane is given by

$$\mathbf{\Gamma}_f = \int_{\mathcal{M}_b} \mathbf{r}(\theta, \phi) \times \mathbf{f}(\theta, \phi) g(\theta, \phi) d\theta d\phi = -\frac{1}{12} \mu_b a^4 \pi \begin{bmatrix} (16 + 15 \cos \theta_s + \cos 3\theta_s) \omega_1 \\ (16 + 15 \cos \theta_s + \cos 3\theta_s) \omega_2 \\ 32 \cos(\theta_s/2)^4 (2 - \cos \theta_s) \omega_3 \end{bmatrix}. \quad (\text{S20})$$

Tangential drag force at the pre-stalk interface. We assume a viscous drag between the stationary pre-stalk and the egg chamber for motion tangential to the interface. The surface drag force density is given by

$$\mathbf{f}(\eta, \rho) = -\mu_s \mathbf{v}_{\parallel}(\eta, \rho), \quad \mathbf{v}_{\parallel}(\eta, \rho) = \mathbf{v}(\eta, \rho) - (\mathbf{v}(\eta, \rho) \cdot \hat{\mathbf{d}}_3) \hat{\mathbf{d}}_3, \quad (\text{S21})$$

where (η, ρ) is the polar parameterization of the pre-stalk interface (See Fig S7B), μ_s is the drag coefficient for the viscous interaction between the egg chamber and the pre-stalk, and $\mathbf{v}(\eta, \rho)$ is the velocity of the area element. The torque exerted by the viscous force given in Eq. (S21) is given by

$$\boldsymbol{\tau}(\eta, \rho) = -\mathbf{r}(\eta, \rho) \times \mu_s \mathbf{v}_{\parallel}(\eta, \rho), \quad (\text{S22})$$

where $\mathbf{r}(\eta, \rho)$ is the position vector of the area element at (η, ρ) . Integrating Eq. (S22) over the egg chamber/pre-stalk interface $\mathcal{M}_s$, we get the corresponding total torque exerted by this viscous force:

$$\mathbf{\Gamma}_s = \int_{\mathcal{M}_s} \boldsymbol{\tau}(\eta, \rho) dA = -\frac{1}{2} \mu_s a^4 \sin(\theta_s)^2 \pi \begin{bmatrix} 2 \cos(\theta_s)^2 \omega_1 \\ 2 \cos(\theta_s)^2 \omega_2 \\ \sin(\theta_s)^2 \omega_3 \end{bmatrix}. \quad (\text{S23})$$

Elastic confinement at the pre-stalk interface. Motion of an area element $d\mathbf{A}$ at (η, ρ) on the stalk-egg chamber interface normal to it generates an elastic resisting force. The elastic surface force density is given by

$$\mathbf{f}_e(\eta, \rho) = -k_s r_{\perp}(\eta, \rho) \hat{\mathbf{e}}_3, \quad r_{\perp}(\eta, \rho) = (\mathbf{A}\mathbf{r}(\eta, \rho)) \cdot \hat{\mathbf{e}}_3 - a \cos \theta_s, \quad (\text{S24})$$

where k_s is the stiffness of the pre-stalk cells and $r_{\perp}(\eta, \rho)$ is the displacement of the area element normal to the interface (See Fig. S7C). Similar to the tangential drag force given above, we compute the torque and integrate over the interface $\mathcal{M}_s$ to get the total elastic torque

$$\mathbf{\Gamma}_e = \frac{1}{8} k_s \pi a^4 \sin(\theta_s)^2 \begin{bmatrix} A_{32}(-4 + 3A_{33} + (-4 + 5A_{33}) \cos 2\theta_s) \\ A_{31}(4 - 3A_{33} + (4 - 5A_{33}) \cos 2\theta_s) \\ 0 \end{bmatrix}, \quad (\text{S25})$$

where A_{ij} are the components of the rotation matrix that transforms vectors from the body frame $\mathcal{B}$ to $\mathcal{E}$.

S2.1.1 Non-dimensional equation of motion and parameter selection

We use the same non-dimensionalization scheme in Sec. S1.3. The Fat2 polarity and protrusion dynamics are as above, while the angular momentum balance is different, given by

$$\begin{aligned} & \overbrace{(N\pi/12) \begin{bmatrix} (16 + 15 \cos \theta_s + \cos 3\theta_s) \omega_1 \\ (16 + 15 \cos \theta_s + \cos 3\theta_s) \omega_2 \\ 32 \cos(\theta_s/2)^4 (2 - \cos \theta_s) \omega_3 \end{bmatrix}}^{\text{drag with basement membrane}} + \overbrace{(N\mu \sin(\theta_s)^2 \pi/2) \begin{bmatrix} 2 \cos(\theta_s)^2 \omega_1 \\ 2 \cos(\theta_s)^2 \omega_2 \\ \sin(\theta_s)^2 \omega_3 \end{bmatrix}}^{\text{drag with pre-stalk}} = \\ & \underbrace{\sum_{i=1}^N \mathbf{r}_i \times \mathbf{b}_i}_{\text{active crawling}} + \underbrace{(NK\pi \sin(\theta_s)^2/8) \begin{bmatrix} A_{32}(-4 + 3A_{33} + (-4 + 5A_{33}) \cos 2\theta_s) \\ A_{31}(4 - 3A_{33} + (4 - 5A_{33}) \cos 2\theta_s) \\ 0 \end{bmatrix}}_{\text{elastic response of pre-stalk}}, \quad (\text{S26}) \end{aligned}$$

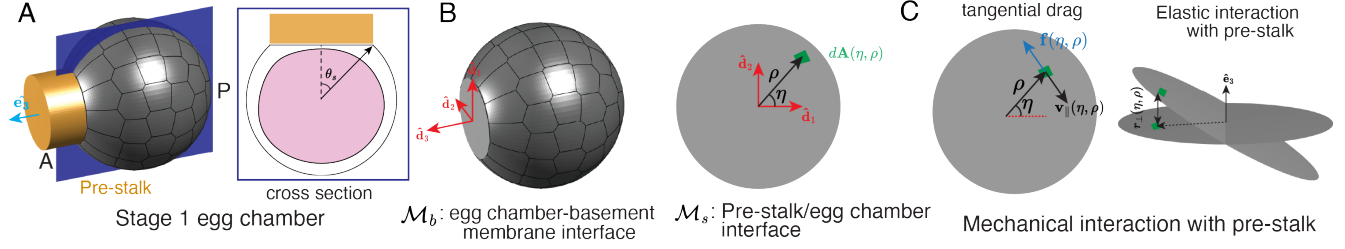


Figure S7: **Model of mechanical interactions in a stage 1 egg chamber.** (a) Geometry of a stage 1 egg chamber. (b) The pre-stalk/egg chamber interface $\mathcal{M}_s$ is parameterized by the polar coordinate (η, ρ) . (c) Motion tangential (normal) to the interface is assumed to have a viscous (elastic) resisting force.

where $\mu = \mu_s/\mu_b$ and $K = k_s/\mu_b\omega_c$. To simulate these equations, we use the same parameter values for τ_{1-3} as the delayed migration egg chamber, but with $N = 100$ corresponding to a stage 1 egg chamber. There are no experimental measurements of either μ or K ; therefore, we set $\mu = 1$ and $K = 20$. We observe no significant change in the qualitative behavior of the model even for significant changes in these parameter values, consistently generating egg chamber rotations along the pre-stalk axis at steady state.

References

1. Taylor, J. R. *Classical mechanics* (University science book, 2005).
2. Cetera, M. *et al.* Epithelial rotation promotes the global alignment of contractile actin bundles during *Drosophila* egg chamber elongation. *Nature Communications* **5**, 5511 (2014).
3. Yamaguchi, N. *et al.* Rear traction forces drive adherent tissue migration in vivo. *Nature Cell Biology* **24**, 194–204 (2022).
4. Crest, J., Diz-Muñoz, A., Chen, D.-Y., Fletcher, D. A. & Bilder, D. Organ sculpting by patterned extracellular matrix stiffness. *eLife* **6**, e24958 (2017).
5. Töpfer, U., Guerra Santillán, K. Y., Fischer-Friedrich, E. & Dahmann, C. Distinct contributions of ECM proteins to basement membrane mechanical properties in *Drosophila*. *Development* **149**, dev200456 (2022).
6. Chlasta, J. *et al.* Variations in basement membrane mechanics are linked to epithelial morphogenesis. *Development* **144**, 4350–4362 (2017).
7. Chen, D.-Y., Crest, J., Streichan, S. J. & Bilder, D. Extracellular matrix stiffness cues junctional remodeling for 3D tissue elongation. *Nature Communications* **10**, 3339 (2019).
8. Haigo, S. L. & Bilder, D. Global tissue revolutions in a morphogenetic movement controlling elongation. *Science* **331**, 1071–1074 (2011).
9. Barlan, K., Cetera, M. & Horne-Badovinac, S. Fat2 and Lar Define a Basally Localized Planar Signaling System Controlling Collective Cell Migration. *Developmental Cell* **40**, 467–477.e5 (2017).
10. Williams, A. M., Donoughe, S., Munro, E. & Horne-Badovinac, S. Fat2 polarizes the WAVE complex in trans to align cell protrusions for collective migration. *eLife* **11**, e78343 (2022).

Movie legends

Movie 1. Egg chamber rotation occurs during stage 1. Related to Figure 1. An example egg chamber transitioning from stage 1 to stage 2, which is signified by the basement membrane (Col-IV-GFP) becoming continuous over the anterior portion of the egg chamber. This egg chamber undergoes sustained rotations for the length of the movie. This sample was used to generate Fig. 1E and S1A. Nuclei were visualized using SpyDNA, which labels all follicle cell and germ cell nuclei. Time stamp shows hours:minutes.

Movie 2. Rigid body egg chamber rotation at stages 7 and 1. Related to Figure 1. A maximum intensity projection (left), and a single orthogonal slice (right) of an egg chamber rotating at steady state, either at stage 7 or stage 1. For the stage 7 egg chamber nuclei were visualized using SpyDNA and for the stage 1 egg chamber nuclei were visualized using ubi-nls-mRFP, both markers label all follicle cells and germ cell nuclei. Movies reveal germ cells rotate with the follicle cells, indicating rigid body rotation.

Movie 3. Egg chamber rotation initiates during stage 1. Related to Figure 1. The follicular epithelium of two different stage 1 egg chambers undergoing either sustained rotation (left) or initiating rotation (right). The yellow arrow appears at the onset of rotation. These egg chambers were used to generate the kymographs in Fig. 1F. Nuclei were visualized using SpyDNA, which labels all follicle cell and germ cell nuclei. Time stamp shows hours:minutes.

Movie 4. Anterior-most follicle cells migrate past pre-stalk cells in stage 1 egg chambers. Related to Figure 1. Timelapse of a stage 1 egg chamber with pre-stalk cells highlighted in orange and anterior-most follicle cell in magenta. Over time, the anterior-most follicle cells slide past the pre-stalk cells which remain stationary. This sample was used to generate Fig. 1H. Nuclei visualized with ubi-mRFP-NLS. Time stamp shows hours:minutes.

Movie 5. $109-30>fat2$ RNAi delays the onset of migration. Related to Figure 2. A control ovariole (left) and $109-30>fat2$ RNAi ovariole (right) each containing a stage 3 and stage 6 egg chamber, focused on the follicular epithelium. Temporal knock down of Fat2 using the $109-30-Gal4$ driver (right) blocks migration specifically in the stage 3 egg chamber, while migration occurs normally in the adjacent stage 6 egg chamber. Follicle cells in both stage 3 and 6 egg chambers migrate in the control ovariole (left). Cell membranes were visualized using CellMask.

Movie 6. Rotation can initiate *ex vivo*, after a delay in *fat2* expression. Related to Figure 2. The follicular epithelium of (left) two control egg chambers undergoing continuous rotation *ex vivo*, and (right) four examples of $109-30>fat2$ RNAi egg chambers undergoing delayed onset of rotation *ex vivo*. All examples are of stage 5 egg chambers. Indy-GFP labels follicle cell membranes. Time stamp shows hours:minutes.

Movie 7. Tracking of cell centroid positions. Related to Figure 3. The basal surface of a $109-30>fat2$ RNAi epithelium in a stage 5 egg chamber pre-rotation to demonstrate how cell centroid positions are used to track cell movement. Cell membranes were visualized using CellMask.

Movie 8. Follicle cells' basal surfaces are highly dynamic before rotation begins. Related to Figure 3. Example of a $109-30>fat2$ RNAi epithelium that is rotating, a $109-30>fat2$ RNAi epithelium pre-rotation, and a $fat2^{N103-2}$ epithelium that will never rotate. In rotating egg chambers, the apical and basal surfaces move in concert, while motion in pre-rotating egg chambers is restricted to the basal epithelial surface. This local motility is dependent on Fat2 activity. All examples are of stage 5 egg chambers. Samples were used to generate Fig. 3A. Cell membranes were visualized using CellMask.

Movie 9. Simulation of the delayed onset of egg chamber rotation. Related to Figure 4, panel H. The time evolution of the biophysical model of a delayed migration egg chamber with an ellipsoidal geometry.

Movie 10. Simulation of spherical egg chamber rotation. Related to figure S6. The time evolution of the biophysical model of a delayed migration egg chamber with a spherical geometry.

Movie 11. Simulation of the onset of rotation at stage 1. Related to Figure 5, panels C and D. The time evolution of the biophysical model of a stage 1 egg chamber.

Table 1: Key Resources Table

Reagent (species) or re-source	type	Designation	Source	Identifiers
antibody		anti-DN Cadherin	Developmental Studies Hybridoma Bank	Antibody Registry ID: AB_528121
antibody		Alexa Fluor 647, goat anti-rat secondary	Thermo Fisher Scientific	Cat# A21450
chemical compound, drug		CellMask™ Deep Red Membrane Stain	Thermo Fisher Scientific	Cat# C10046
chemical compound, drug		SPY650-DNA	Cytoskeleton, Inc.	Cat# CY-SC501
chemical compound, drug		Alexa Fluor™ 647 phalloidin	Thermo Fisher Scientific	Cat# A22287
chemical compound, drug		Invitrogen™ Rhodamine Phalloidin	Thermo Fisher Scientific	Cat# R415
chemical compound, drug		Formaldehyde, 16%, methanol free, Ultra Pure	Polysciences, Inc.	Cat# NC1040701
chemical compound, drug		Recombinant Human Insulin	Millipore Sigma	Cat# 12643
chemical compound, drug		Schneider's Drosophila Medium	Thermo Fisher Scientific	Cat# 21720024
chemical compound, drug		Fetal Bovine Serum	Gibco™	Cat# A5670701
chemical compound, drug		Soda Lime Solid Glass Microspheres	Cospheric	S-SLGMS-2.5 (various sizes, see methods)
chemical compound, drug		Fibrinogen, Bovine Plasma	Millipore Sigma	Cat# 341573
chemical compound, drug		Thrombin Protease	Cytiva	Cat# 27084601
chemical compound, drug		SlowFade Antifade Kit	Thermo Fisher Scientific	Cat# S36963
chemical compound, drug		PBS, Phosphate Buffered Saline, 10X Solution, Fisher BioReagents™	Thermo Fisher Scientific	Cat# BP399-1
genetic reagent (<i>Drosophila melanogaster</i>)		<i>w[1118]</i>	Bloomington Drosophila Stock Center	BDSC: 3605; FlyBase ID: FBst0003605
genetic reagent (<i>Drosophila melanogaster</i>)		<i>traffic jam-Gal4 (tj-Gal4)</i>	Kyoto Stock Center	DGRC: 104055; FlyBase ID: FBst0302922
genetic reagent (<i>Drosophila melanogaster</i>)		<i>109-30-Gal4</i>	Bloomington Drosophila Stock Center	BDSC: 7023; FlyBase ID: FBti0027548
genetic reagent (<i>Drosophila melanogaster</i>)		<i>UAS-fat2-RNAi</i>	Bloomington Drosophila Stock Center	BDSC: 40888; FlyBase ID: FBst0040888

Reagent type (species) or re-source	Designation	Source	Identifiers
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-Abi-RNAi</i>	National Institute of Genetics, Japan	NIG: 9749R-3; FlyBase ID: FBtp0079430
genetic reagent (<i>Drosophila melanogaster</i>)	<i>20XUAS-6XmCherry-HA</i>	Bloomington Drosophila Stock Center	BDSC: 97863
genetic reagent (<i>Drosophila melanogaster</i>)	<i>Fat2-3xeGFP, FRT80B</i>	Lab of S. Horne-Badovinac; PMID:28292425	FlyBase ID: FBal0326664
genetic reagent (<i>Drosophila melanogaster</i>)	<i>viking-gfp (vkg-GFP)</i>	Buszczak et al.; PMID:17194782; Morin et al.; PMID:11742088	FlyBase ID: FBal0211825
genetic reagent (<i>Drosophila melanogaster</i>)	<i>indy-GFP</i>	Bloomington Drosophila Stock Center	BDSC: 50860; FlyBase ID: FBal0212048
genetic reagent (<i>Drosophila melanogaster</i>)	<i>yw; ubi-nls-mRFP, vkg-GFP, FRT40</i>	Lab of David Bilder; PMID: 21212324	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>fat2^{N103-2}, FRT80B</i>	Lab of S. Horne-Badovinac; PMID:22413091	FlyBase ID: FBal0267777
software, algorithm	Fiji (ImageJ)		PMID:22743772; 26153368
software, algorithm	Zen Blue	Zeiss	
software, algorithm	Zen Black	Zeiss	
software, algorithm	Prism9 GraphPad	GraphPad Software	
software, algorithm	Python3	Python Software Foundation	https://www.python.org
software, algorithm	imageio	imageio contributors	
software, algorithm	napari		https://napari.org/stable/
software, algorithm	numpy		https://numpy.org/
software, algorithm	scipy		https://scipy.org/
software, algorithm	scikit-image		https://scikit-image.org/
software, algorithm	pandas		https://pandas.pydata.org/
software, algorithm	cellpose	Carson Stringer and Marius Pachitariu; PMID: 33318659	https://www.cellpose.org/
software, algorithm	Numbers spread-sheets	Apple Inc.	
software, algorithm	Adobe Illustrator	Adobe	
software, algorithm	MATLAB R2023a	MathWorks	

Table 2: Experimental genotypes

Figure	Panel	Name or label	Genotype
Figure 1	E-G	Col IV-GFP	<i>w; vkg-GFP/+</i>
Figure 1	G,H	Nuclei	<i>yw; ubi-nls-mRFP, vkg-GFP,FRT40/+</i>
Figure 2	A,B	Control	<i>w; 109-30-Gal4/+; Fat2-3xeGFP,FRT80B</i>
Figure 2	A,B	<i>109-30>fat2-RNAi</i>	<i>w; 109-30-Gal4/fat2-RNAi; Fat2-3xeGFP,FRT80B</i>
Figure 2	A,B	<i>tj>fat2-RNAi</i>	<i>w; tj-Gal4/UAS-fat2-RNAi; Fat2-3xeGFP,FRT80B</i>
Figure 2	C,D	Control	<i>w; 109-30-Gal4/+</i>
Figure 2	C,D	<i>109-30>fat2-RNAi</i>	<i>w; 109-30-Gal4/UAS-fat2-RNAi</i>
Figure 2	C,D	<i>tj>fat2-RNAi</i>	<i>w; tj-Gal4/UAS-fat2-RNAi</i>
Figure 2	E	Control, cell membranes	<i>w;; indy-GFP</i>
Figure 2	E	<i>109-30>fat2-RNAi</i> , cell membranes	<i>w; 109-30-Gal4/UAS-fat2-RNAi; indy-GFP</i>
Figure 3	A,C-E	<i>109-30>fat2-RNAi</i> (Rotating and pre-rotation)	<i>w; 109-30-Gal4/UAS-fat2-RNAi; Fat2-3xeGFP,FRT80B</i>
Figure 3	A,C-E	<i>fat2^{N103-2}</i> (Will never rotate)	<i>w;; fat2^{N103-2},FRT80B</i>
Figure 4	A-B	Control, Fat2-3xGFP	<i>w; 109-30-Gal4/+; Fat2-3xeGFP,FRT80B</i>
Figure 4	A-B	<i>109-30>Abi-RNAi</i> , <i>Fat2-3xGFP</i>	<i>w; 109-30-Gal4/+; UAS-Abi-RNAi, Fat2-3xeGFP,FRT80B/Fat2-3xeGFP,FRT80B</i>
Figure 4	A-B	<i>tj>Abi-RNAi</i> , <i>Fat2-3xGFP</i>	<i>w; tj-Gal4/+; UAS-Abi-RNAi, Fat2-3xeGFP,FRT80/Fat2-3xeGFP,FRT80</i>
Figure 5	B	Nuclei	<i>yw; ubi-nls-mRFP, vkg-GFP,FRT40/+</i>
S1	A-D	Col-IV-GFP	<i>w; vkg-GFP/+</i>
S1	E		<i>yw; ubi-nls-mRFP, vkg-GFP,FRT40/+</i>
S2	A	<i>tj>UAS-mCherry</i>	<i>tj-Gal4/+; 20xUAS-6xmCherry-HA</i>
S2		<i>109-30>UAS-mCherry</i>	<i>109-30-Gal4/+; 20xUAS-6xmCherry-HA</i>
S2	B	Control, Fat2-3xGFP	<i>w; 109-30-Gal4/+; Fat2-3xeGFP,FRT80B</i>
S2	B	<i>109-30>fat2-RNAi</i> , <i>Fat2-3xGFP</i>	<i>w; 109-30-Gal4/UAS-fat2-RNAi; Fat2-3xeGFP,FRT80B</i>
S2	C	Control	<i>w; 109-30-Gal4/+</i>
S2	C	<i>109-30>Abi-RNAi</i>	<i>w; 109-30-Gal4/+; UAS-Abi-RNAi/+</i>
S2	C	<i>tj>Abi-RNAi</i>	<i>w; tj-Gal4/+; UAS-Abi-RNAi/+</i>
S3	A-D	<i>109-30>fat2-RNAi</i> (Rotating and pre-rotation)	<i>w;109-30-Gal4/UAS-fat2-RNAi; Fat2-3xeGFP,FRT80B</i>
S3	A-D	<i>fat2^{N103-2}</i> (Will never rotate)	<i>w;;fat2^{N103-2},FRT80B</i>