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Modeling cell elongation during germ band retraction: cell autonomy versus applied anisotropic stress

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Abstract
The morphogenetic process of germ band retraction in Drosophila embryos involves coordinated movements of two epithelial tissues—germ band and amnioserosa. The germ band shortens along its rostral–caudal or head-to-tail axis, widens along its perpendicular dorsal-ventral axis, and uncurls from an initial ‘U’ shape. The amnioserosa mechanically assists this process by pulling on the crook of the U-shaped germ band. The amnioserosa may also provide biochemical signals that drive germ band cells to change shape in a mechanically autonomous fashion. Here, we use a finite-element model to investigate how these two contributions reshape the germ band. We do so by modeling the response to laser-induced wounds in each of the germ band’s spatially distinct segments (T1–T3, A1–A9) during the middle of retraction when segments T1–A3 form the ventral arm of the ‘U’, A4–A7 form its crook, and A8–A9 complete the dorsal arm. We explore these responses under a range of externally applied stresses and internal anisotropy of cell edge tensions—akin to a planar cell polarity that can drive elongation of cells in a direction parallel to the

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minimum edge tension—and identify regions of parameter space (edge-tension anisotropy versus stress anisotropy) that best match previous experiments for each germ band segment. All but three germ band segments are best fit when the applied stress anisotropy and the edge-tension anisotropy work against one another—i.e., when the isolated effects would elongate cells in perpendicular directions. Segments in the crook of the germ band (A4–A7) have cells that elongate in the direction of maximum external stress, i.e., external stress anisotropy is dominant. In most other segments, the dominant factor is internal edge-tension anisotropy. These results are consistent with models in which the amnioserosa pulls on the crook of the germ band to mechanically assist retraction. In addition, they suggest a mechanical cue for edge-tension anisotropy whereby cells do not globally orient their internal elongation axis towards the amnioserosa, but instead orient this axis perpendicular to the local principal stress direction.

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Keywords: finite element model, germ band retraction, amnioserosa, Drosophila, morphogenesis, embryogenesis, laser microsurgery

1. Introduction

Germ band retraction is a critical stage of Drosophila embryogenesis in which two epithelial tissues—germ band and amnioserosa—undergo coordinated cell and tissue shape changes [1]. Figure 1 shows one side of a roughly ellipsoidal and bilaterally symmetric Drosophila embryo. At the start of retraction (figure 1(a)), the germ band is wrapped around the posterior end of the embryo in a ‘U’ shape. One arm of the ‘U’ covers the ventral surface of the embryo (bottom in figure 1(a)) and the other covers most of the dorsal surface (top in figure 1(a)). At this point in early retraction, the ventral arm is composed of the rostral or head-most end of the germ band (segments T1–A2) and the dorsal arm is composed of its caudal or tail-most end (A6–A9) [2]. The surface between the two arms of the U-shaped germ band is occupied by amnioserosa cells, which also extend over the dorsal surface and partially overlap segment A9 to connect to a bilaterally symmetric region of amnioserosa on the other side of the embryo [3]. During retraction, the germ band shortens along its long rostral–caudal (RC) axis, widens in the perpendicular dorsal–ventral direction and uncurls from its previous U-shape. The amnioserosa undergoes complementary changes—a lateral and then dorsoventral shortening—to eventually cover most of the embryo’s dorsal surface. Figure 1(b) shows an embryo during the late stages of this process. This tissue reshaping is accompanied by parallel changes in individual cells. Those in the germ band elongate orthogonally to the tissue’s RC axis [1, 2]. Those in the amnioserosa begin germ band retraction with highly elongated shapes (aspect ratios > 7) that are aligned with the tissue’s long axis, but finish retraction with nearly isodiametric shapes [1]. Throughout these coordinated cell and tissue shape changes, the amnioserosa and germ band maintain epithelial integrity and remain tightly connected to one another.
Figure 1. Schematic of a fly embryo near the (a) start and (b) end of germ band retraction. Germ band segments are labeled T1–T3 and A1–A9. Amnioserosa is labeled AS. The dashed line marks the RC axis of the germ band. The large crosshairs between the two schematics denotes the global dorsal (D), ventral (V), anterior (A) and posterior (P) directions. The smaller crosshairs in segment A6 denote the local, segment-specific As- and RC-directions, with the positive RC-direction always rostral. Prior laser wounding experiments are summarized for each segment in (b) by two markers representing the mean aspect ratios of fully expanded wounds: one for wound expansion following 15-μm long linear incisions in the local RC-direction; the other for similar incisions in the local As-direction. The long axis of each rectangular marker parallels the incision direction. The three dashed lines denote wound aspect ratios of 1, 2 and 3 (as noted in segment T1) and the error bars for each marker represent ± one standard deviation ($N = 5$ to 18 for each direction in each segment). The scale bar is 20 μm and applies to (a) and (b). Example images from laser wounding experiments are shown in (c) for segment A2 and (d) for segment A5. These are confocal images of embryos expressing GFP-E-cadherin to label cell–cell junctions. The large cell-free elliptical areas are expanded wounds (marked by dashed outlines); the dark marks in the middle of each wound are damage to the overlying vitelline membrane, which serves as a visual indication of the initial incision direction. Images have inverted grayscales for clarity and have been rotated so that the As-direction is up. The scale bar in (d) is 20 μm and applies to all four images in (c) and (d).
Previous laser-microsurgery experiments found that cell shape changes in the germ band were driven in part by anisotropic applied stress and in part by mechanically autonomous cell elongation [4]. The anisotropic applied stress comes from the amnioserosa physically pulling on at least some germ band segments. An intact amnioserosa is required for germ band retraction [1, 5–7], and these laser-microsurgery experiments showed that part of the amnioserosa’s role is mechanical [4]. The evidence for mechanically autonomous cell elongation comes from two observations. First, isolated patches of germ band cells maintain their slightly elongated shape, indicating at least autonomous shape maintenance. Second, cells in some segments still undergo near normal elongation even after ablation of an entire lateral flank of the amnioserosa—an ablation that halts germ band retraction [4]. This cell elongation is only mechanically autonomous; it may be triggered by paracrine and/or juxtacrine signals from the amnioserosa [6]. Here, we use computational modeling to determine exactly how anisotropic stress and mechanically autonomous cell elongation coordinate to reshape the germ band.

We do so by matching models to previous experimental results on the opening of 15-μm long laser incisions cut into each segment of the germ band [4]. These experiments were all conducted during the middle stages of retraction (% retraction of 36 ± 30%) and their results are summarized in figure 1(b). For most segments in the ventral and dorsal arms of the U-shaped germ band, linear wounds along either the segment-specific RC-axis or the orthogonal As-axis opened in similar manners. Even at maximum extent, wound shapes for both incision directions retained a small amount of anisotropy due to the initial cut direction (mean wound aspect ratios of 1.2 to 1.8, see figure 1(c) for an example). On the other hand, for segments found in the crook of the germ band during the middle stages of retraction (segments A4–A7), incisions along these orthogonal directions yielded quite different wound shapes. Incisions along the local RC-axis gaped open, usually leading to nearly isotropic wound shapes at maximum extent (mean aspect ratios ~1), but occasionally leading to anisotropic wound shapes with the wound’s long axis orthogonal to the initial incision direction (aspect ratios denoted as <1). Incisions along the local As-axis opened in a very different manner and retained much more of the anisotropy imposed by the incision direction (mean aspect ratios >1.9). Example incisions are shown for segment A5 in figure 1(d). Even without complementary modeling, these experimental results strongly suggested that segments A4–A7 were subject to anisotropic tensile stress.

Here, we present the needed complementary modeling. We use a well-established cell-level finite-element method [8–14] that has been previously used to model the opening of laser-induced wounds [15]. We model each germ band segment as a two-dimensional patch of polygonal cells with viscous and incompressible cytoplasm and edge tensions that potentially vary with edge orientation. Internal edge-tension anisotropy is modeled using an orientation dependence that works to autonomously elongate cells. Non-autonomous elongation is modeled by applying anisotropic stress to the external boundary of the simulated cell sheet. We use such models to explore variations in wound expansion under different combinations of internal edge-tension anisotropy and anisotropic external stress.

We find that the finite-element model reasonably reproduces both cell elongation and wound expansion. For most segments, the models match experiments only when the external anisotropic stress works against mechanically autonomous cell elongation. For segments in the curve of the germ band, cells elongate in the direction of greatest external force—consistent with the amnioserosa pulling on these segments—but are better fit when the edge-tension anisotropy would tend to drive elongation in an orthogonal rather than parallel direction. For
most other segments, cells elongate in the direction dictated by their edge-tension anisotropy. This difference means that cellular edge-tension anisotropy is not aligned in a common direction for all segments, neither towards the amnioserosa or dorsoventrally.

2. Computational model

2.1. Length and time scaling

To facilitate comparison of simulations and experiments, we simulate cell sheets with the same cell size and density as found experimentally. Specifically, we match the spatial scale factor $\rho = (\text{total edge length})/(\text{total sheet area})$ [10], which allows direct comparison of distances and areas between experiments and simulations. To do so, we simulate $45 \times 100 \mu m$ patches of 300 cells. Patches are rectangular to conserve computational resources. For each set of conditions, we run simulations on seven cell sheets with the above characteristics, but different cell geometries. These geometries are obtained from non-wounding simulations that initialize cell shapes based on Voronoi tessellations of randomly chosen seed points, but then allow the cells to reach a stable equilibrium configuration under the given conditions.

Each modeled sheet represents a single germ band segment; however, these sheets are purposely wider than actual germ band segments ($\sim 40 \mu m$), the extra width being used to reduce boundary effects, especially under conditions with highly elongated cells. To evaluate whether $45 \times 100 \mu m$ patches were sufficiently large, we ran multiple simulations of wound expansion under conditions that yielded highly elongated cells in patches initialized at sizes ranging from $45 \times 56 \mu m$ to $113 \times 140 \mu m$. Simulated linear incisions led to wounds that did become slightly more isotropic as patch width increased; however, the differences across this range of patch sizes were less than the standard deviation among results at a single patch size. Complete results for this patch size evaluation can be found in supplementary figure S1 (available from stacks.iop.org/NJP/16/055003/mmedia).

We cannot as readily equate experimental and simulated time scales. Instead, we run simulations at a unit viscosity and later match each simulation time scale to experiments using two identifiable events: the time of ablation and the time at which an expanding wound reaches 90% of its maximum size. The time of ablation is easily identified in simulations and to a lesser extent in experiments. In the simulations, $t_{\text{sim}} = 0$ is easy to define because wounding is instantaneous. In experiments, ablation of a 15-\(\mu m\) line takes $\sim 2 s$. Since tissue starts to move right after the first ablation pulse, we assign $t_{\text{expt}} = 0 s$ to the image just prior to ablation, even though this is not precisely when wounding occurs. The point at which a wound reaches 90% of its maximum expansion ($t_{\text{expt,90}}$) is a straightforward calculation for experiments in which wounds reach a maximum area and then shrink as they begin to heal [4]. Our simulations do not include a wound healing response, so each wound expands asymptotically towards its true maximum. We therefore calculate an effective maximum area based on the time at which the relative change in area between successive unit time steps $(1/A)(dA/dt)$ is first less than $1 \times 10^{-4}$. The corresponding area is taken as an effective maximum area and used to find the simulation’s $t_{\text{sim,90}}$. We then scale the simulated times to match the experiments using the factor $t_{\text{expt,90}} / t_{\text{sim,90}}$. 
2.2. Model elements

The finite-element models used here are modifications of $\gamma-\mu$ models in which a cell sheet is an assembly of tightly packed polygonal cells, and each cell has a line tension $\gamma$ along each of its boundary segments and an effective cytoplasmic viscosity $\mu$ that scales the strength of an internal network of dashpots [8, 9]. This combination provides viscoelastic resistance to cell shape deformations. At each step in the simulation, there may be small changes in area for individual cells due to approximations in the numerical solutions. Since cells in the germ band do not undergo large changes in size during retraction, the model also includes an area constraint for each cell, attempting to reverse any changes in cell area over five time steps. This constraint is implemented as a Lagrange multiplier and physically represents an isotropic cell-internal stress or pressure. In addition, since neighbor exchanges are uncommon during germ band retraction [1], such rearrangements are not allowed in the model.

Previous work has demonstrated that tissues in Drosophila embryos act largely as continuous sheets [16], i.e., tensions along cell–cell interfaces bear less than $\sim 30\%$ of the tissue-level stress. We thus fix the average external stress $\bar{\sigma}$ to $3\times$ the amount carried by cell-edge tensions. Anisotropic external stress is applied by setting $\sigma_1 = \bar{\sigma} + \Delta$ and $\sigma_2 = \bar{\sigma} - \Delta$, with the degree of anisotropy given by

$$\frac{\Delta}{\bar{\sigma}} = \frac{\sigma_1 - \sigma_2}{\sigma_1 + \sigma_2}. \quad (1)$$

Strong anisotropy in the external stress can cause even isotropic cells to elongate in the direction of maximum tensile stress.

To promote autonomous cell elongation, we allow the cell edge tensions, $\gamma$, to vary with edge orientation according to

$$\gamma(\theta) = \bar{\gamma}\left(1 - \frac{f}{2}\cos 2\theta\right), \quad (2)$$

where $\bar{\gamma}$ is the mean edge tension, $f$ is the edge-tension anisotropy factor (0 to 2) and $\theta$ is the angle of each cell edge relative to the cell’s autonomous elongation axis. Note that equation (2) only defines a nematic anisotropy of the edge tensions, i.e., a preferred axis, but not a preferred ‘head’ or ‘tail’ direction along that axis. The functional form is the simplest smooth function defining such an anisotropy. With this assumed functional dependence, the autonomous elongation axis of each cell is parallel to its smallest edge tension, $\gamma_{\text{min}} = \bar{\gamma}(1 - f/2)$. One could also define a principal edge-tension axis as parallel to the largest edge tension. Cells would tend to autonomously elongate perpendicular to this principal edge-tension axis. In this paper, we primarily refer to each cell’s autonomous elongation axis; however, with either descriptor, one would expect edge-tension anisotropy to correlate with different protein distributions and/or dynamics along edges parallel and perpendicular to the chosen reference axis. There is clear evidence in germ band cells for anisotropic distributions of myosin II and the bazooka/Par-3 complex during the preceding morphogenetic process of germ band elongation [17], and for anisotropic recycling of junction proteins during the subsequent morphogenetic process of dorsal closure [18]. Future observations of anisotropic protein distributions and/or dynamics during germ band retraction will be a key experimental test of the model presented here.

In addition to the line tension $\gamma$, each cell edge also carries a viscoelastic truss element. In strictly $\gamma-\mu$ models, wounds are unstable [15]. The additional truss elements increase tension
along the wound border as it expands and thus prevent wounds from expanding indefinitely. Each truss is represented as a Kelvin–Voigt element—a linear spring and linear dashpot in parallel—because this is the simplest element that yields wound behavior similar to experiments [15]. To determine appropriate parameters for the spring and dashpot, we matched simulations of wound expansion under isotropic external stress to experiments in which wound expansion did not depend on the incision’s orientation. These parameters are used for all truss elements in all subsequent simulations.

With the above elements, we simulate a wound by releasing the area constraint on any wounded cells (equivalent to setting cell-internal pressure or stress to zero), by setting the cell edge tensions between wounded cells to zero, and by removing the viscoelastic truss elements from any wounded edges. To mitigate boundary effects, we did not wound cells within three cells of a boundary. Note that a 15-μm linear incision could cut a variable number of cells, both due to differences in cell shape for different parameters and the fact that cells are not typically packed end-to-end. The same variability occurred in experiments.

2.3. Characterization of cell and wound shape

We tracked cell and wound shapes using area moment of inertia tensors, $J$ [19]. The rotations that diagonalize this tensor (to $J'$) yield the directions of a shape’s principal axes (equivalent to the axes of its best-fit ellipse). We take $\alpha$ as the direction of the longest principal axis and track cell elongation or wound anisotropy through a ratio of the two principal moments of inertia

$$\kappa = \frac{\sqrt{J_{11}}}{\sqrt{J_{22}}}.$$  

(3)

If $J'_{11}$ is the largest diagonal entry in the rotated $J'$ tensor and $J'_{22}$ is the smallest diagonal entry, then $\kappa$ is equal to the aspect ratio of a shape’s best-fit ellipse.

To characterize wound shapes, we choose a diagonalizing rotation that places the first principal axis closest to the incision direction. If the long axis of a wound remains parallel to its initial incision, then $\kappa$ is greater than one and equal to the aspect ratio of its best-fit ellipse. On the other hand, if the long axis of a wound is orthogonal to its initial incision, then $\kappa<1$ and is equal to one over this aspect ratio.

To characterize cell shapes, we average the $J$ tensors of multiple cells and then calculate $\alpha$ and $\kappa$ for an equivalent composite cell. Averaging at the tensor level then includes information on the uniformity of cell elongation. For example, if all cells in a simulated patch each had $\kappa=2$ and were all aligned in the same direction, then the composite $\kappa$ would also equal two; however, if the cells were randomly oriented, then the composite $\kappa$ would be close to one. This latter situation can be differentiated from a collection of isodiametric cells by also calculating the mean of the cell’s individual $\kappa$ values. If cells are elongated but randomly aligned, the composite $\kappa$ will be substantially less than the mean $\kappa$. Finally, to differentiate composite alignments in the $As$- versus $RC$-directions, we choose a diagonalizing rotation that places the first principle axis of the averaged $J$ tensor closest to the $As$-direction. Thus, simulations with cells generally aligned in the $RC$-direction would yield $\kappa<1$ and equal to one over the composite cell’s aspect ratio. Such simulations do not match the experimental results, and should therefore be carefully distinguished from those with cells generally aligned in the $As$-direction (which do have $\kappa>1$ and equal to the composite cell’s aspect ratio).
2.4. Software

Finite-element models and their analysis were run on custom software developed for $\gamma-\mu$ models by Brodland et al [8, 9, 11, 13]. Statistical analysis and plotting were performed in Mathematica (Wolfram Research, Champaign, IL).

3. Results and discussion

3.1. Parameter space

In our simulations, we explore a four-quadrant parameter space of internal edge-tension anisotropy and external stress anisotropy, defining a parameter as positive if it would independently drive cell elongation along the local segment-specific As-axis defined in figure 1 (we represent this axis vertically in all simulated cell patches). Figure 2 illustrates the relationship between internal edge-tension anisotropy and external stress in various regions of our model’s parameter space. In the first and third quadrants, the autonomous elongation axis aligns with the principal stress direction; in the second and fourth, they are orthogonal. Figure 2 shows sample patches from quadrants II and IV that both have cell elongations similar to experiments, but in which these elongations are driven either by internal edge-tension anisotropy (against external stress) or by external stress (against internal edge-tension anisotropy).

For each explored point in parameter space, we ran 10 to 14 simulations: two linear wounds each—one 15 $\mu$m long in the As-direction, the other in the RC-direction—for $N=5$ to 7 different simulated patches. We used multiple simulations to insure results that were independent of the specific geometry of any single cell patch. Figure 3 shows the expansion of such wounds under four conditions: anisotropic external stress only, edge-tension anisotropy only, anisotropic stress dominant over edge-tension anisotropy, and vice versa. We tracked cell elongation through the measurement $\kappa_{\text{cells}}$, which represents the aspect ratio (or 1/aspect ratio) of the best-fit ellipse to a composite cell. As detailed in section 2.3, $\kappa_{\text{cells}}$ is determined by the average cellular area moment of inertia tensor. Similarly, we track maximally expanded wound shapes through $\kappa_{w,\text{RC}}$ and $\kappa_{w,\text{As}}$, the aspect ratios of the best-fit ellipse to wounds made in the RC- and As-directions, respectively.

Even though each patch in figure 3 has a similar $\kappa_{\text{cells}}$, the wounds vary in smoothness and shape anisotropy. We therefore compare simulations and experiments via our aspect ratio measurements for the cells and wounds: $\kappa_{\text{cells}}$, $\kappa_{w,\text{RC}}$ and $\kappa_{w,\text{As}}$. Experimentally, every germ band segment has $\kappa_{\text{cells}} \approx 1.33$, but $\kappa_{w,\text{RC}}$ and $\kappa_{w,\text{As}}$ vary strongly by segment. They range between 1.18 to 1.72 for $\kappa_{w,\text{RC}}$ and 1.24 to 2.29 for $\kappa_{w,\text{As}}$. The simulation values for $\kappa_{\text{cells}}$, $\kappa_{w,\text{As}}$, and $\kappa_{w,\text{RC}}$ vary through parameter space as illustrated in figure 4. The unshaded regions in figure 4 are those for which simulated cell patches failed to reach a stable equilibrium (even before wounding). This instability occurs in large regions of quadrants I and III where the external stress anisotropy and edge tension anisotropy work to elongate cells in the same direction. It is unclear if this instability would occur for any choice of function for the anisotropies or whether it is specific to the simulations as run here. Nonetheless, the simulated regimes that match the experimentally observed values all cover slightly different regions that lie mostly within quadrants II and IV. The limited regions of quadrants I and III that are stable and match any of the experimental ranges are small and near the origin. Below, we will look at
the matching parameter space for each germ band segment individually, but these overall trends already suggest that the best fits are likely to lie in regions where edge-tension anisotropy and stress anisotropy work against one another (quadrants II and IV).

3.2. Germ band segment fits

To determine each germ band segment’s fit region and best-fit point, we use a $\chi^2$ test that considered how well all three cell and wound shape measurements were matched to experiments at each point in parameter space (figure 5(a) and supplementary figure S2). Using this test, most germ band segments have an edge-tension anisotropy that works against the principal stress direction. The best fit points for half of the segments (T1, A1–A4, and A7) fall in quadrant II, where cells elongate along their autonomous elongation axis and perpendicular to the principal stress direction. The best fit points for another three (A5, A6, and A9) fall in

Figure 2. Edge-tension anisotropy and anisotropic stress in simulated cell sheets. (a) A simulated cell sheet in which cell elongation is driven by internal anisotropy of each cell’s edge tensions (greater tension for horizontally aligned edges, which drives vertical elongation). This elongation was opposed by the external stress anisotropy. Color denotes edge tension and arrow lengths indicate the relative external stress applied to each boundary of the cell sheet. (b) A similar cell sheet in which cell elongation is driven by stress anisotropy and opposed by internal edge-tension anisotropy. Both cell sheets have a composite $\kappa_{\text{cells}} = 1.31$, which is close to the value found experimentally for germ band cells during the middle of germ band retraction. The anisotropy parameters used in these example simulations are noted below each cell sheet. The inset in upper right shows a division of the model parameter space into quadrants. The long direction of each ellipse represents the direction in which cells would elongate due only to their internal edge-tension anisotropy. The ellipses’ major and minor axes are proportional to $1 + f/2$ and $1 - f/2$, respectively. The long direction of the crosshairs indicates the direction of maximum external anisotropic stress. The length of each crosshairs is proportional to applied stress in each direction. The cell sheet in (a) represents quadrant II. The one in (b) represents quadrant IV.
Figure 3. Expansion of simulated line cuts. Time series of simulated wounds under each of four marked patch conditions and two wound directions. For each patch, $\kappa_{\text{cells}}$ is close to the experimental mid-retraction value of 1.33. First four columns are times when the wound reaches certain changes in area, $\Delta A = 0, 1/3 \Delta A_{\text{max}}, 2/3 \Delta A_{\text{max}},$ or $\Delta A_{\text{max}}.$ The local segment-specific $RC$-direction is horizontal (i.e., the amnioserosa would lie above each of these patches). To the right of each set of images is an indication of the principal stress direction, the autonomous elongation axis, and where each patch falls in parameter space. Individual edge tensions are color-coded according to the color scale at bottom.
Figure 4. Cell and wound shape parameters throughout the explored parameter space: composite cell elongation, $\kappa_{\text{cells}}$; aspect ratio of maximally expanded wounds when the incision was along the local $A_s$-direction, $\kappa_{w,A_s}$; and same for incisions along the local
quadrant IV, where cells elongate along the principal stress direction and perpendicular to their autonomous elongation axis. Among those segments with a best-fit point in quadrant II, three actually have a larger region of nearly equally good fits in quadrant IV (A1, A4, A7). The only segments whose best fit is not in quadrant II or IV are T3, which is best fit by isotropic edge tensions and T2, A8, which are best fit by isotropic external stress. The fit region for only three segments (T2, A2, and A8) includes any part of quadrant I, where edge-tension anisotropy and the principal external stress synergistically drive cell elongation.

The only cell-shape parameter used to fit the simulations to experiments was the global composite $\kappa_{\text{cells}}$ value of 1.33 found during the middle stages of germ band retraction. We can thus further test the model by comparing the distributions of cell shapes and alignments on a per segment basis. To date, the most closely matching experimental data available in the literature is for segments A2 and A5 during initiation and near completion of germ band retraction [4]. Cells are not yet elongated during the earliest stages of retraction, so only the late stage data is useful for comparison. Although this data is not an exact match to the staging used to fit the model (% retraction of 92 and 87% versus $36 \pm 30\%$), the simulations produce cell shape and alignment distributions that are still quite similar to experiments, as shown in figures 6(a) and (b). The major difference between the simulated and experimental shape distributions is the slightly greater elongation and alignment in the experiments, which is not surprising given that the experimental data are from later stages of retraction. There is not extant experimental data in the literature to compare to simulated cell shape distributions for other segments. We thus use the best-matching simulations for each segment (based on $\kappa_{\text{cells}}$, $\kappa_{w,\text{RC}}$ and $\kappa_{w,\text{As}}$) and present their cell shape and alignment distributions in figure 6(c) as model predictions testable by future experimental image analysis.

To elucidate what the simulated fits imply with regards to the mechanism of germ band retraction, we first consider the implications of fits in quadrant II versus quadrant IV. The fits in quadrant II are consistent with autonomous cell elongation towards the amnioserosa (positive edge-tension anisotropy), which occurs despite an external tensile stress along the germ band’s RC axis, i.e., from each segment’s connections to neighboring segments (negative stress anisotropy). The fits in quadrant IV are consistent with elongation driven by a pulling force from the amnioserosa (positive stress anisotropy). In fact, the six segments with either their best fit or a larger region of good fits in quadrant IV (A1, A4–A7, A9) all correspond to experimental results with a noticeably higher wound aspect ratio for cuts made in the As-direction, previously taken as evidence that a pulling force from the amnioserosa was generating a local stress anisotropy [4]. With these implications, one can then consider the spatial distribution of the best fits by dividing them into three categories (figure 5(b)): large fit regions in quadrant II and IV.
Figure 5. Matching simulations to experiments. (a) Combinations of internal edge-tension anisotropy and external force anisotropy for which simulations best match experiments in selected germ band segments (A2, A4 and A5). The match of simulations and experiments considers $\kappa_{\text{cells}}$, $\kappa_{w,\text{RC}}$, and $\kappa_{w,\text{As}}$. The best fit for each segment is labeled in red and surrounded by a rectangle. Black segment labels indicate additional points in the tested parameter space where $\chi^2$ indicates a good fit ($p \leq 0.05$). Dashes indicate tested points that were not a good fit. Similar plots for other germ band segments can be found in supplementary figure S2. The best-fit region for segment A2 is similar to that for segments T1, T2, A3 and A8; that for A4 is similar to that for
segments A1 and A7; and that for A5 is similar to that for segments A6 and A9. (b) Image of an embryo during mid-germ band retraction. Ellipses and crosshairs denote each segment’s best fit, where the ellipses’ major and minor axes are proportional to $1 + f/2$ and $1 - f/2$, respectively and crosshairs are proportional to the applied stress in each direction. Colors denote where the segments in each category fall on the embryo: (blue) large good-fit regions in quadrant II and IV; (magenta) large good-fit region in quadrant IV, but best fit in quadrant II; and (red) best fit in quadrant IV. T3 remains uncolored since it has an atypical fit region (see supplementary figure S2). Scale bar indicates 20 $\mu$m.

The implied external stress distribution aligns well with the results from previous experiments [4]; however internal edge-tension anisotropy behaves in surprising ways. It is not always aligned with the local $A_s$-direction and thus is not simply directed towards the amnioserosa. The principal direction of this edge-tension anisotropy is also not aligned along any global axis across all segments (e.g., dorsal–ventral or anterior–posterior) nor along the $RC$-direction of the germ band’s curved RC axis.

We thus propose a hypothesis that the direction of internal edge-tension anisotropy is determined by a mechanical cue—aligning this anisotropy to work against the local principal stress. Certainly, our simulated cell patches are most stable under such opposing conditions and the best fitting models for the expansion of linear wounds in each segment follow this pattern. Nonetheless, this hypothesis will require further validation using probes of internal edge-tension anisotropy and conditions that controllably change the direction of the largest local principal stress.

4. Conclusions

The finite-element method reasonably reproduces the differences in the opening of linear incisions made in different directions in different segments of the germ band. For most segments, the match between experiments and simulations requires the autonomous elongation axis to lie perpendicular to the principal external stress direction. These two processes then compete to determine the direction in which cells elongate. External anisotropic stress dominates in only a handful of segments, most along the curve of the germ band. Internal edge-tension anisotropy dominates in the rest.

Such competition may be a necessity for stability in sheets of epithelial cells. Certainly, with the specific model we have constructed, cell sheets are unstable in most regions of parameter space where the external stress anisotropy and edge-tension anisotropy work to elongate cells in the same direction. The only exceptions are regions near the origin where both anisotropies are small. The universality of this instability is unclear; it may be possible to find functional forms for the two anisotropies that eliminate the instability and allow the two processes to cooperatively drive extreme cell elongations. Nonetheless, the instability does occur with at least one reasonable pair of choices and may play an important role in how
epithelial cells are shaped. Given this instability and the fact that most germ band segments had best-fitting models when the two anisotropies opposed one another, our results are consistent with a mechanical cue for edge-tension anisotropy, one where the direction of autonomous cell elongation aligns orthogonally to the greatest mechanical stress.

Figure 6. Comparison of cell elongation and alignment during germ band retraction from experimental images and finite-element simulations. Each rose diagram compiles data from N = 5 to 9 embryos or simulations with an angle of 90° representing alignment of cells along the As-axis. The length and shading of each sector wedge are respectively proportional to the mean aspect ratio and the fraction of cells aligned within that sector. The dashed semicircle denotes a mean aspect ratio of 1.5 and shading follows the grayscale bar at right. The longest sector wedge in each diagram is also labeled with that sector’s mean aspect ratio. (a) and (b) compare previous experimental results from [4] with the best matching simulations for segments A2 (a) and A5 (b). Note that the experimental data is from late germ band retraction (with % completion noted as $<R> = 92$ and 87% respectively). (c) presents cell shape distributions for the simulations that best matched each of the other germ band segments. Comparable experimental data for these segments has not been analyzed and presented in the published literature. Rose diagrams for simulations are labeled with values used for stress anisotropy, $\Delta/\sigma$, and edge-tension anisotropy, $f$. 

(a) A2

(b) A5

(c) Simulation: T1,A3
$\Delta/\sigma = -0.15$, $f = 0.8$

Simulation: T2,A8
$\Delta/\sigma = 0.0$, $f = 0.3$

Simulation: T3
$\Delta/\sigma = 0.05$, $f = 0.0$

Simulation: A1,A3,A4,A7
$\Delta/\sigma = -0.05$, $f = 0.5$

Simulation: A6
$\Delta/\sigma = 0.35$, $f = -1.1$
Figure 7. Conclusions regarding the mechanics of germ band retraction. Only forces on segments T1, A5, and A8 are illustrated. Blue arrows indicate forces from neighboring segments. These are always tensile and along the overall long RC axis of the germ band. Red arrows indicate forces from the amnioserosa. These are largest at the posterior end where amnioserosa cells (filled gray shapes) tend to align perpendicular to the amnioserosa-germ band boundary. Each panel shows two representative amnioserosa cells, one from posterior and one from anterior regions. Green arrows indicate forces internal to each segment, i.e., those due to anisotropy of the cell edge tensions. Many germ band segments undergo cell and segment elongation in the direction dictated by their edge-tension anisotropy—indeed the amnioserosa (e.g. T1 and A8). Others, especially those in the curve of the germ band, elongate specifically in the direction of the amnioserosa’s pull. This pull induces an edge-tension anisotropy that opposes the applied stress and prevents cells in these segments from over-elongating relative to neighboring segments. This combination of amnioserosa pull and induced edge-tension anisotropy assists retraction by helping segments successfully travel around the bend in the tissue.

Figure 7 presents our current model of germ band retraction, combining the insights from both our simulations and previous experiments [4]. The amount of tension applied on a germ band segment by the adjacent amnioserosa depends on how the segment/amnioserosa boundary is oriented with respect to the long axis of the amnioserosa—the same direction in which amnioserosa cells are aligned. Thus tension from the amnioserosa is greatest on those segments
in the curve of the germ band. Each germ band segment is also subject to tensions applied by neighboring germ band segments (along the RC-axis). For segments in the curve of the germ band, tension from the amnioserosa is larger than that from adjacent segments. These germ band cells respond with an induced edge-tension anisotropy in which the maximum edge tensions are parallel to the largest principal stress. This induced cell-internal anisotropy is insufficient to match the anisotropic stress, so these cells elongate towards the amnioserosa. In other segments, the tensile stress applied by the amnioserosa is less than that from adjacent segments, resulting in a weak stress anisotropy with greatest stress along the RC axis. The autonomous elongation axis is again perpendicular to this stress, but now dominates. Cells in these segments thus also elongate towards the amnioserosa, but for a different reason—anisotropy of their cell edge tensions. In this model, the local mechanical stress determines the (perpendicular) direction of the cells’ autonomous elongation axis, but stress anisotropy does not determine the magnitude of edge-tension anisotropy. We hypothesize that the amnioserosa assists in uncurling the germ band by creating this dynamic, segment-specific pattern. Further insights as to how this mechanical assist works will require embryo-scale models of the entire amnioserosa-germ band system in three-dimensions.

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