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PERSPECTIVE

In preprints: buckling under pressure during gastrulation

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Early in embryonic development, gastrulation produces the three primordial germ layers – endoderm, mesoderm and ectoderm – and shapes them into a rudimentary body plan. This generally involves internalization of the mesoderm and endoderm layers. In the fruit fly *Drosophila melanogaster*, mesoderm invagination occurs via formation of the ventral furrow (VF). During this event, future mesoderm cells along the ventral midline undergo apical constriction-driven wedging, causing the epithelial sheet to bend inward and invaginate (Sweeton et al., 1991; Leptin and Grunewald, 1990; Martin et al., 2009). A crucial role for mechanical forces has been recognized during gastrulation in *Drosophila* and many other species. For example, in *Drosophila*, deformation of one tissue layer is necessary for proper gene expression and morphogenesis within a neighboring layer (Desprat et al., 2008; Farge, 2003; Butler et al., 2009). This is also true of vertebrates, in which circumferential hoop stress is a major driver of blastopore closure in *Xenopus* embryos (Keller and Shook, 2008), and solid-fluid phase transitions are important for epiboly and axial extension in zebrafish (Mongera et al., 2018; Petridou et al., 2019). These and many other findings highlight a role for mechanical forces as 'morphogenic machines' that function across the whole embryo (Shook and Keller, 2008). Three recent preprints further examine the influence of tissue mechanics on cell- and tissue-scale behaviors during *Drosophila* gastrulation.

How mechanical forces drive tissue-scale behaviors

Guo and colleagues (Guo et al., 2021 preprint) and Fierling and colleagues (Fierling et al., 2021 preprint) ask the question: is apical constriction necessary and/or sufficient for VF formation (VFF) during *Drosophila* gastrulation? Using distinct experimental approaches, the authors of each study block apical constriction at the site of VFF. Fierling et al. use laser ablation to disrupt apical actomyosin networks, whereas Guo et al. use an optogenetic dominant-negative (DN) Rho to inhibit myosin contractility acutely in the ventrolateral region of the embryo. By blocking apical constriction prior to VFF, Fierling et al. demonstrate that cell apical constriction is necessary for VFF. Guo et al. similarly observe that activation of DN-Rho blocks apical constriction, myosin recruitment, and tissue tension at the ventral midline, leading to failed VFF. In addition, Guo et al. identify a two-stage response to Rho inactivation: inhibition prior to or shortly after VFF onset prevents tissue furrowing or reverses it, respectively, whereas inhibition beyond a certain point allows VFF to proceed almost normally. This 'point of no return' is indicative of mechanical bistability within the future mesoderm.

Both studies then create mathematical models of the forces within the *Drosophila* gastrula. Fierling et al. model the *Drosophila* embryo as a 3D, thin, elastic shell without individual 'cells'. They then apply an isotropic 'pre-strain' on its ventral surface, reflecting the accumulation of contractile myosin. Owing to the geometric asymmetry of the ventral tissue (which is approximately three times longer than it is wide), even an isotropic force yields an anisotropic response: the shell is preferentially strained (deformed) along the shorter dorsoventral (DV) axis and preferentially stressed along the longer anterior-posterior (AP) axis. They further show that cell torque (apical-basal tension difference), and thus cell wedging, is dispensable for furrow formation. By contrast, AP tension at the apical surface of the mesoderm (working like a 'cheese-cutter wire') is sufficient to drive VFF and furrow propagation from the mid- to the anterior and posterior poles.

In contrast, Guo et al. model the embryo in cross-section as a 2D vertex model in which 'cells' are subject to apical constriction ventrally and compressive stresses laterally. This model recapitulates the bistability observed experimentally, but only when both apical constriction and lateral compression are applied. The authors further propose that the source of this compressive stress is apicobasal cell shortening of the lateral ectoderm, which they show occurs independently of apical constriction or invagination.

Despite the differences in their experimental and modeling approaches, both studies come to a similar conclusion: that in-plane compressive stress causes the ventral side of the embryo to 'buckle', resulting in VFF. Guo et al. describe this as a result of lateral stress from ectodermal cell shortening, whereas Fierling et al. identify embryo-scale surface tension as the cause of buckling. Together, these studies support a model in which compression of the embryo's ventral surface from the sides causes it to buckle and 'snap through', resulting in the tissue-scale deformation that forms the VF. Notably, the model proposed by Fierling et al. does not require the 3D geometrical shape change of individual cells (e.g. cell wedging), as demonstrated by the acellular model presented in this study and as highlighted experimentally in studies of *Drosophila* mutants that fail to cellularize but still form a VF (Fierling et al., 2021 preprint; He et al., 2014).

How tissue-scale mechanical forces influence morphogenetic cell behaviors

These findings highlight the importance of tissue-scale mechanical forces in morphogenesis independent of individual cell behaviors. However, tissue-scale forces can in turn influence cell behaviors that drive further tissue- and embryo-scale shape changes. A third preprint by Camuglia and colleagues (Camuglia et al., 2022 preprint) similarly describes how mechanical forces produced by mesoderm invagination ultimately determine the orientation of cell divisions independently of planar cell polarity cues.

The *Drosophila* gastrula contains several mitotic domains, clusters of cells that divide in stereotyped spatial and temporal patterns. Among these, domains 1, 3 and 5 exhibit divisions in which the mitotic spindle is oriented along the AP axis, but the

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underlying mechanisms of this orientation were unknown. Camuglia et al. find that the cell polarity protein Pins exhibits planar polarized distribution with mitotic domain 1, 3, and 5 cells, and that this polarized distribution precedes spindle orientation in these cells. Overexpression of a membrane targeted Pins not only disrupts Pins localization, but also randomizes spindle orientation, demonstrating that Pins localization dictates the polarity of cell divisions. The authors find that Pins localization does not require planar cell polarity signaling or the positional code of Toll-like receptors that drive planar polarized cell intercalations in the germ band (Paré et al., 2014). Instead, they determine that mechanical isolation of the mitotic domains blocks Pins localization and disrupts oriented divisions. Using *snail* knockdown, they show that both Pins localization and oriented cell divisions are disrupted when VFF and mesoderm invagination are blocked. Together, these results indicate that mechanical forces produced during mesoderm invagination serve as a polarity cue to promote localization of the Pins polarity protein and, ultimately, the orientation of cell divisions.

Outlook and broader implications

These three studies contribute to a substantial body of evidence suggesting that mechanical forces regulate gastrulation morphogenesis and that this role is highly conserved among invertebrate and vertebrate embryos. Indeed, two additional recent preprints report that primitive streak formation and the associated 'polonaise movements' in chick gastrulae result from a balance of tissue contraction in the posterior region and tension in the anterior region of the embryo (Serra et al., 2021 preprint; Caldarelli et al., 2021 preprint). Even clusters of pluripotent cells, both in two- and three-dimensional culture, activate Wnt/β-catenin signaling and ultimately mesoderm differentiation at sites of increased tissue tension (Muncie et al., 2020; Sagy et al., 2019). It is increasingly clear that a more complete understanding of early embryonic development will require further study of the integration of chemical signaling, gene expression and tissue mechanics.

Note added in proof

Guo et al., 2021 has now been published as: Guo, H. Swan, M. and He, B. (2022). Optogenetic inhibition of actomyosin reveals mechanical bistability of the mesoderm epithelium during *Drosophila* mesoderm invagination. eLife 11, e69082. doi:10.7554/eLife.69082.

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