GUT DEVELOPMENT

Asquash and a squeeze

Advanced imaging techniques reveal details of the interactions between the two layers of the embryonic midgut that influence its ultimate shape.

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The gastrointestinal tract of most animal species is far longer than the body in which it is housed. The human gut, for example, is approximately 20 feet long and must fold, loop and twist to fit inside the body (Helander and Fändriks, 2014). Remarkably, contortions of the gut tube are highly stereotyped and species-specific, indicating that the formation of the folds is genetically controlled (Savin et al., 2011). However, it remains unclear how the instructions encoded within the genome lead to such precise and reproducible changes of shape.

To get to the bottom of this, developmental biologists seek to describe the movements of individual cells and connect these to a change in the shape of the whole organ. This remains a challenge, especially for internal organs, which develop deep within embryos and whose shape is determined by the interactions between multiple layers of tissue. Recent advances in live imaging using 3D light-sheet microscopy have allowed biologists to visualize morphological change on the surface of whole embryos, but internal organs like the gut have remained largely out of reach (Wan et al., 2019).

Now, in eLife, Sebastian Streichan of the University of California Santa Barbara and colleagues – including Noah Mitchell as first author – report a new method that combines deep-tissue light-sheet microscopy with a framework to analyze shape changes between tissue layers in the gut of fruit flies (Mitchell et al., 2022).

The midgut of fruit flies begins as a simple tube consisting of an inner epithelial layer ensheathed by smooth muscle. The gut tube then constricts at three precise positions, which subdivides the tube into four chambers as it changes shape and gets longer (Figure 1). To visualize this folding and elongation, Mitchell et al. expressed fluorescent markers selectively in cells of the midgut and used genetically modified, transparent embryos to reduce light scatter. Using confocal multiview light-sheet microscopy, the researchers generated time-lapse movies of full, 3D volumes of the developing midgut (de Medeiros et al., 2015). By measuring the geometry of whole organs, they found that the length of the gut tube triples during folding, while maintaining a near constant volume. This occurs in the absence of cell divisions, suggesting that changes in the shape of cells may be responsible for the elongation.

To find out how the behavior of individual cells drives the constriction and elongation of the gut, Mitchell et al. developed an image analysis package aptly named TubULAR. This programme combines machine learning and computer vision techniques that link cell movements to changes in the shape of the whole organ. In many epithelia, cell intercalations – a process during which neighboring cells switch places – drive tissue convergence and extension (Paré and Zallen, 2020; Sutherland et al., 2020). In the gut tube, however, constriction and elongation correlated with patterned changes in the epithelial cell shape (Figure 2A and B).

Modeling the gut epithelium as an incompressible material, they found that localized changes
in the shape of cells in the gut folds accounted entirely for both folding and extending of the organ. In other words, gut constrictions simultaneously converge the tissue circumferentially and extend the tissue longitudinally. Mitchell et al. term this new morphological mechanism “convergent extension via constriction”.

Based on prior work, the researchers hypothesized that localized muscle contractions by the outer layer of the gut could provide the force necessary for the gut to constrict (Bilder and Scott, 1995; Wolfstetter et al., 2009). To test this idea, they employed optogenetic tools to either inhibit or stimulate muscle contractions

Figure 1. Shaping of the developing midgut of fruit flies. Top: Automatic segmentation tools enable layer-specific imaging of the muscle (yellow) and endoderm (blue) to generate a 3D shape. Bottom: The midgut initially consists of muscle cells (yellow) and a layer of endodermal cells (blue), which interact to mold the gut into shape. The gut tube constricts at three precise positions, which subdivide it into four chambers before it starts to coil.

Figure 2. Changes in the shape of endodermal cells are linked to a change in the shape of the whole organ. (A) Top: Layer-specific imaging of the developing gut (early stages to the left, more developed ones to the right). Endodermal cells are initially elongated along the circumferential direction, but they change their shape during organ folding. (B) Three-dimensional representation of cells near the anterior fold. The aspect ratio of the endodermal cells (a/b, where a and b are the lengths of the cells in the circumferential and longitudinal directions) changes from greater than two to about one. (C) Hox genes regulate calcium signaling, which mediates muscle contraction (yellow cells), thus linking hox genes to organ shape through tissue mechanics. The resulting muscle contractions are mechanically coupled to the endoderm (blue), which places strain on the tissue and ultimately influences the shape of the organ.

Image credit: Adapted from Mitchell et al., 2022 (CC BY 4.0).
Insight

Tissue layers may mechanically interact. A vast variety of internal organs. Moreover, they analysis open new possibilities for in toto imaging of advances in deep-tissue imaging and image analysis. Shyer et al., 2013; mammalian lung (Mitchell et al., 2019). The importance of smooth muscle as a sculptor of the growing body of research emphasizing the stereotyped contortions. These findings add to our understanding of morphogenesis of the Drosophila embryonic midgut. Genetics 141:1087–1100. DOI: https://doi.org/10.1093/genetics/141.3.1087, PMID: 8582615 de Medeiros G, Norlin N, Gunther S, Albert M, Panavaitė L, Fiuzza U-M, Peri F, Hiragi T, Krizc U, Hufnagel L. 2015. Confocal multiview light-sheet microscopy. Nature Communications 6:8881. DOI: https://doi.org/10.1038/ncomms9881, PMID: 26602977 Helander HF, Fändriks L. 2014. Surface area of the digestive tract - revisited. Scandinavian Journal of Gastroenterology 49:681–689. DOI: https://doi.org/10.3109/00365521.2014.989326, PMID: 24694282 Huycke TR, Miller BM, Gill HK, Nerurkar NL, Sprinzak D, Mahadevan L, Tabin CJ. 2019. Genetic and mechanical regulation of intestinal smooth muscle development. Cell 179:90–105. DOI: https://doi.org/10.1016/j.cell.2019.08.041, PMID: 31539501 Jaslove JM, Nelson CM. 2018. Smooth muscle: a stiff sculptor of epithelial shapes. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 373:20170318. DOI: https://doi.org/10.1098/rstb.2017.0318, PMID: 30249770 Mitchell NP, Cicso DJ, Shankar S, Lin Y, Shraimian BI, Streichan SJ. 2022. Visceral organ morphogenesis via calcium-patterned muscle constrictions. eLife 11:e77355. DOI: https://doi.org/10.7554/eLife.77355, PMID: 35593701 Paré AC, Zallen JA. 2020. Cellular, molecular, and biophysical control of epithelial cell intercalation. Current Topics in Developmental Biology 136:167–193. DOI: https://doi.org/10.1016/bs.ctdb.2019.11.014, PMID: 31959287 Savin T, Kurpios NA, Shyer AE, Floresco P, Liang H, Mahadevan L, Tabin CJ. 2011. On the growth and form of the gut. Nature 476:57–62. DOI: https://doi.org/10.1038/nature10277, PMID: 21814276 Shyer AE, Tallinen T, Nerurkar NL, Wei Z, Gil ES, Kaplan DL, Tabin CJ, Mahadevan L. 2013. Villification: how the gut gets its villi. Science 342:212–218. DOI: https://doi.org/10.1126/science.1238842, PMID: 23989955 Sutherland A, Keller R, Lesko A. 2020. Convergent extension in mammalian morphogenesis. Seminars in Cell & Developmental Biology 100:199–211. DOI: https://doi.org/10.1016/j.semcdb.2019.11.002, PMID: 31734039 Tremml G, Bienz M. 1989. Homeotic gene expression in the visceral mesoderm of Drosophila embryos. The EMBO Journal 8:2677–2685. DOI: https://doi.org/10.1002/j.1460-2075.1989.tb04808.x, PMID: 2573526 Wan Y, McDole K, Keller PJ. 2019. Light-sheet microscopy and its potential for understanding developmental processes. Annual Review of Cell and Developmental Biology 35:655–681. DOI: https://doi.org/10.1146/annurev-cellbio-100818-125311, PMID: 31299171 Wolfstetter G, Shrinian M, Stute C, Grabbe C, Hummel T, Baumgartner S, Palmer RH, Holz A. 2009. Fusion of circular and longitudinal muscles in Drosophila is independent of the endoderm but further visceral muscle differentiation requires a close contact between mesoderm and endoderm. Mechanisms of Development 126:721–736. DOI: https://doi.org/10.1016/j.mod.2009.05.001, PMID: 19463947