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Morphogenesis: Shaping Tissues through Extracellular Force Gradients

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Organ sculpting requires directed physical force generation. Force imbalances are primarily thought to arise from within cells. A new study, however, demonstrates that an extracellular-matrix-based stiffness gradient in the *Drosophila* egg chamber instructs tissue elongation.

The form of an organ, such as the convoluted tubules of kidneys, the branching buds of lungs, and the iconic shape of the heart, is important in carrying out its specialized function. Organs are sculpted during their formation through the action of asymmetrical physical forces. Intracellular mechanical imbalances or anisotropies that drive shape change are well characterized. For example, both planar cell polarized cortical myosin contractility and actin-based protrusions direct cellular movements and rearrangements that facilitate tissue elongation [1–3]. Comparatively little, however, is known about extracellular mediators of tissue shaping. Basement membrane (BM) is a thin, dense, sheet-like extracellular matrix that surrounds most organs and regulates many cellular and tissue functions, including mechanical support, polarization, differentiation, and proliferation [4]. BM composition and localization also appear to regulate tissue shaping. For example, alterations in the levels of type IV collagen, a major structural component of BM, are thought to constrict the shape of *Drosophila* wing imaginal discs and the *Caenorhabditis elegans* gonad [5–7]. A collagen-rich BM accumulates along the bud ducts of mammalian mammary and salivary glands, where it might narrow these structures during their formation [8–10]. Direct measurements of BM mechanical properties, however, have been a technical hurdle in definitively implicating biophysical features of BM in organ sculpting. In a new study published in *eLife*, Bilder and colleagues [11] directly measure and perturb the mechanical properties of BM surrounding elongating *Drosophila* egg chambers and elegantly demonstrate that a BM force gradient mediates changes in organ shape.

The *Drosophila* egg chamber is a simple tube-like organ made up of an inner germ cell cluster surrounded by a somatic epithelium of follicle cells that is encased in a planar, sheet-like BM (Figure 1A). The egg chamber develops in 14 stages to form a mature egg. It is initially spherical and expands isotropically for the first 4 stages. At stage 5, it elongates along the anterior–posterior (A–P) axis to form an ellipsoidal shape (Figure 1A). During rapid elongation (stages 5–9), the follicle cells collectively migrate, causing the egg chamber to rotate within the BM. As the chamber rotates, the follicular epithelium deposits BM fibrils into the planar BM that orient perpendicular to the A–P axis (Figure 1A). These fibrils have been proposed to act as a molecular corset that resists expansion along the dorso–ventral (D–V) axis, thus directing egg chamber elongation along the A–P axis [12]. However, prior to the study of Bilder and colleagues, the mechanical properties of the egg chamber BM had not been directly examined to determine whether the fibrils alter BM stiffening and whether other mechanical changes occur to the BM during egg chamber lengthening.

To directly study the stiffness of the egg chamber, the authors used an atomic force microscope (AFM) probe as a picoindenter and calculated the Young's modulus from its deflection. The external location of the BM, the lack of interstitial matrix, and the ability to culture egg chambers *in vitro* allowed unobstructed access to the BM. Fortuitously, the underlying epithelium does not appear to contribute to egg chamber stiffness; thus,

AFM measurements likely reflect only BM properties. The authors found that the egg chamber BM becomes stiffer as it develops, progressing from ~30 kPa (stage 3) to ~70 kPa (stage 7), consistent with the idea that circumferential (D–V) BM fibrils deposited from the spinning epithelium increase BM stiffness. Unexpectedly, however, a gradient of stiffness is present along the A–P axis, with the poles having only half the stiffness of the central region of the egg chamber. Importantly, this stiffness asymmetry originates prior to BM fibril formation and is proportionally maintained during egg chamber elongation. Thus, in addition to an increase in overall stiffness that correlates with fibril formation, a BM stiffness gradient along the A–P axis exists prior to and during egg chamber elongation (Figure 1A).

To determine how the mechanical gradient is generated, the authors performed quantitative image analysis of BM components. Laminin, perlecan, and type IV collagen were found to vary in levels along the A–P axis of the BM. Most notably, type IV collagen levels are uniquely elevated in the central regions of the egg chamber, and taper towards the poles, matching the stiffness gradient in the BM. This collagen distribution is controlled by a gradient of the cytokine ligand Unpaired, which is secreted at both poles and activates JAK/STAT signaling in a graded manner along the A–P axis of the follicular epithelium. Loss of JAK/STAT signaling eliminates the differential distribution of collagen, abolishes the stiffness gradient, and leads to the formation of round egg chambers.

Direct confirmation of collagen's role in establishing the BM stiffness gradient along the A–P axis was provided by manipulating collagen levels. The egg chamber BM is produced locally by the underlying follicular epithelium, allowing global and region-specific alterations in BM using different *Drosophila* GAL4 drivers to specifically overexpress or reduce type IV collagen. Follicle-wide depletion of type IV collagen led to homogenously soft BM and defective elongation. In contrast, follicle-wide overexpression of EHBP1, which upregulates collagen IV fibril deposition, increases the stiffness of the central BM region, resulting in hyperelongated egg

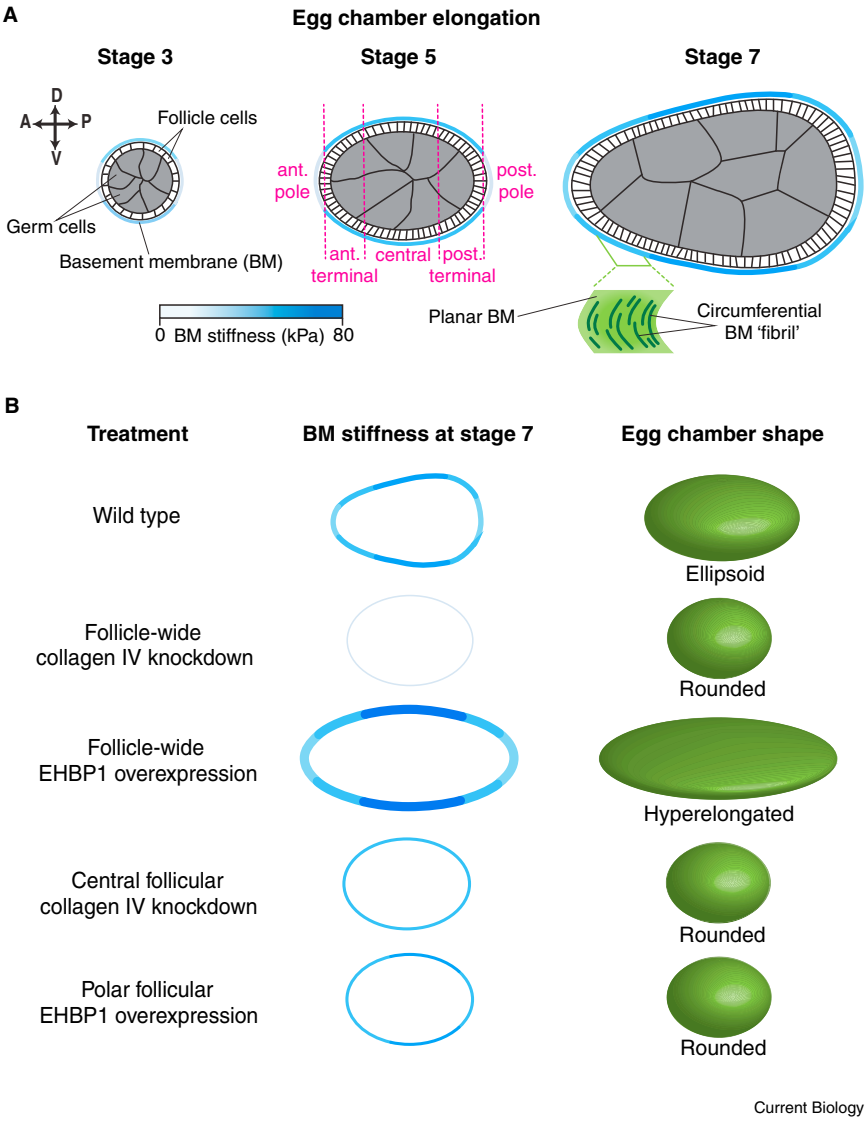

Figure 1. A basement membrane (BM) stiffness gradient shapes the *Drosophila* egg chamber.
(A) The *Drosophila* egg chamber is an initially spherical organ that expands isotropically (stages 1–4), and then elongates rapidly along the A–P axis (stages 5 through 14; the oocyte/egg is the most posterior germ cell and it grows dramatically between stages 8 and 14). Prior to and during elongation, a force gradient exists in the BM surrounding the egg chamber along the A–P axis, where the central BM regions are stiffer than the poles (illustrated by a spectral heatmap). (B) Manipulations of BM type IV collagen levels alter the A–P mechanical gradient, causing defects in egg chamber shape. Follicle-wide reduction of collagen results in a uniformly soft and rounded egg chamber. Follicle-wide increase in collagen deposition steepens the stiffness gradient, causing central BM regions to become much stiffer, resulting in hyperelongated egg chambers. Both central follicular collagen knockdown and polar follicular increase in collagen deposition equilibrate the stiffness gradient, causing egg chamber rounding.

chambers (Figure 1B). More restricted manipulations revealed that depletion of collagen specifically in central follicle cells eliminates the stiffness gradient and leads to rounder egg chambers. In addition, overexpression of EHBP1 in follicle cells at the poles equilibrates stiffness and disrupts egg chamber elongation (Figure 1B). Importantly, the changes in stiffness correlated with the ability of the BM to resist follicle swelling following culture in deionized water. Together, these observations suggest that morphogen signaling through the JAK/STAT pathway leads to differential accumulation of type IV collagen along the A–P axis, thereby translating a chemical gradient into a mechanical

gradient that restricts tissue expansion and drives egg chamber elongation.

Previous studies have implicated the importance of the circumferential BM fibrils in acting as a molecular corset that instructs egg chamber lengthening. The current study, however, challenges this ascribed role for these fibers and suggests instead that it is the A–P stiffness gradient that is instructive. Particularly, loss of JAK/STAT signaling eliminates the collagen gradient and leads to rounded egg chambers, but does not appear to alter the deposition of fibrils. Countering this view, earlier work showed that a modest increase in BM fibril formation at the expense of planar BM leads to egg chamber elongation without altering overall collagen levels (and presumably stiffness) [13]. This discrepancy remains to be resolved. To clearly determine the function of BM fibrils in egg chamber elongation, it will be important for future studies to develop approaches to specifically eliminate these fibrils without altering the planar BM. Bilder and colleagues [11] suggest a potentially parsimonious model for how the circumferential BM fibers and the A–P stiffness gradient work together to promote egg elongation. The fibrils may form successive circumferential corsets spanning the A–P axis, with each corset section providing a distinct constrictive force that reflects the A–P stress gradient. Thus, the circumferential BM fibrils may propagate the A–P force gradient on a tissue-wide scale to elongate the egg chamber. Given that circumferential fibrils are found in other elongating structures, such as the apical extracellular matrix of the *C. elegans* embryo [14], and chitin filaments surrounding *Drosophila* tracheal tubes [15], it is likely that the BM fibril molecular corset has a vital function during egg chamber elongation.

One of the most important discoveries from this work is the demonstration that type IV collagen stiffens BM and that modulating this stiffness can instructively shape tissues. Thus, collagen within the BM of the *Drosophila* wing disc [5], *C. elegans* gonad [6,7], and around the neck of glandular ducts during branching events in mammals [8–10] likely stiffens BMs to shape these tissues. In addition, the finding that a gradient of type IV collagen within the plane of a BM can act as a mechanical morphogen has potentially profound implications that extend beyond this study. This observation suggests that BM proteins such as collagen may function analogously to morphogens secreted during development, including Wnt, Hedgehog, and transforming growth factor β (TGFβ) ligands, whose levels along a gradient are instructive [16]. Furthering this analogy, perlecan, a matrix protein shown by the authors and others to oppose collagen function [5], might act similarly to inhibitors of developmental morphogens that sharpen morphogen gradients. BM components bind growth factors, directly signal to matrix receptors, and release fragments through proteolysis that signal to cells [17]. Thus, gradients and varying levels of matrix components within sheets of BM might have fundamental and unforeseen roles in instructively patterning numerous cellular functions, including differentiation, growth, and cell death. Understanding how these gradients of matrix components are created within BMs and their interplay with cellular and tissue-level functions will be exciting areas of future research.

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