



The extracellular matrix in tissue morphogenesis: No longer a backseat driver

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ABSTRACT

The forces driving tissue morphogenesis are thought to originate from cellular activities. While it is appreciated that extracellular matrix (ECM) may also be involved, ECM function is assumed to be simply instructive in modulating the cellular behaviors that drive changes to tissue shape. However, there is increasing evidence that the ECM may not be the passive player portrayed in developmental biology textbooks. In this review we highlight examples of embryonic ECM dynamics that suggest cell-independent activity, along with developmental processes during which localized ECM alterations and ECM-autonomous forces are directing changes to tissue shape. Additionally, we discuss experimental approaches to unveil active ECM roles during tissue morphogenesis. We propose that it may be time to rethink our general definition of morphogenesis as a cellular-driven phenomenon and incorporate an underappreciated, and surprisingly dynamic ECM.

1. Introduction

"Morphogenesis is brought about through a limited repertoire of variations in cellular processes"

Developmental Biology, Scott Gilbert

(Barresi and Gilbert, 2020)

"Change in form in animal embryos are brought about by cellular forces"

Principles of Development, Lewis Wolpert

(Wolpert, 2019)

The textbook view of tissue morphogenesis assumes that the forces that shape a developing embryo are solely attributed to cellular activities (e.g., migration, contraction, division, cell death) (Barresi and Gilbert, 2020; Wolpert, 2019). If one wants additional proof of the current primacy of the cell in developmental biology, then look no further than the title of this journal. However, embryos are not just a collection of cells. There is also a significant amount of extracellular matrix (ECM), which by the end of gestation likely contributes just as much if not more mass to the animal than the cells themselves. Due to the perceived view of the ECM as a static, rigid structure, along with our textbook description of cellular-driven morphogenesis, it is presumed that the ECM plays a passive role during animal development, where it influences cellular behavior through mechanochemical signaling events

that simply modify processes like cell adhesion, migration, and differentiation (Barresi and Gilbert, 2020; Walma and Yamada, 2020). Additionally, when the ECM is directly observed to change shape or dynamics during a morphogenetic process, the assumption is primarily that this occurs through cellular force production. Is this always a safe assumption?

The ECM was not always viewed as a passive structure. Pre-1950, early studies of skin wound repair led to the idea that wound contraction was driven by a sudden rise in collagen concentration and the spontaneous shortening of connective tissue fibrils (Carrel, 1910; Carrel and Hartmann, 1916; Loeb, 1920; Watts et al., 1958). This idea persisted for decades until Michael Abercrombie proposed a role for fibroblast contraction (Abercrombie et al., 1956), rather than the shrinking of collagen fibrils, which his colleagues considered an "audacious and highly original conception" (Medawar, 1980). More than 50 years later the opposite is now likely the case: it is probably an audacious suggestion to many cell and developmental biologists that the ECM may actively cause tissue movements independent of cellular contractile activity.

On what rationale could anyone have invoked an active role for collagen fibril formation in driving wound contraction independent of the cells themselves? While we now think that fibroblast movements and contractions drive wound closure (Desmouliere and Hinz, 2021), a contractile ECM hypothesis was not based on pure fantasy (and the jury

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is still out as to whether this idea is entirely incorrect). First, nearly all polymer networks, including the ECM, involve a significant amount of self-assembly. Anyone who has polymerized Matrigel or collagen in a tissue culture dish appreciates that cells are not essential to create a stable ECM network. While cellular forces can clearly modulate interactions between ECM components (Kubow et al., 2015), we often neglect cell-independent, ECM-autonomous mechanisms of network assembly. Non-covalent interactions between self-assembling ECM components are thought to drive the formation of all types of matrix networks, from fibrillar, interstitial matrices to basement membranes (BM) (Hohenester and Yurchenco, 2013; Revell et al., 2023; Revell et al., 2021; Yurchenco and Furthmayr, 1984; Yurchenco et al., 1986). In vitro reconstitution experiments reveal that the mechanical properties of polymerizing ECM components are progressively altered during maturation and that simply altering the chemistry of the polymerization process can affect ECM network mechanics (Christiansen et al., 2000; Danielsen, 1981). Additionally, alterations in ionic concentrations or osmotic pressure can induce tensile stresses through remodeling of collagen networks – independent of cellular activity (Masic et al., 2015; Sussman and Katchalsky, 1970). Second, and related to the previous point, nearly all polymers will spontaneously alter their organization during self-assembly leading to some amount of stress generation. Case in point are tooth filling composites that must be precisely formulated to dissipate stresses that build up during the curing process, which could lead to cracks in the filling or the composite pulling away from the tooth margin (Malhotra et al., 2010). Another example, which is important for precision manufacturing, are thin film polymer assemblies that also have a propensity to generate stresses and defects during their formation (Freund and Suresh, 2009). These forces are generated simply due to the polymerization process itself – no actomyosin-like motors required. Therefore, while cells are clearly important for producing and secreting ECM components, once outside of the cell the ECM network likely has a life of its own.

Here we will highlight how surprisingly dynamic the ECM can be during development and discuss examples of processes for which the ECM has an active role in shaping tissues. For the sake of this review, we will ignore processes involving more subtle ECM modulatory functions during which alterations in ECM mechanical properties and signaling to surrounding cells leads to changes in cell fate or dynamics. We will also leave out examples of gross cellular remodeling of the ECM that occurs during morphogenesis. These passive, ‘backseat driver’ functions of the ECM during development are well appreciated and discussed in numerous reviews (Bonnans et al., 2014; Brown, 2011; Daley and Yamada, 2013; Green, 2022; Rozario and DeSimone, 2010; Walma and Yamada, 2020) and textbooks (Barresi and Gilbert, 2020) [although in one major developmental biology textbook there is not even enough information on ECM function to garner a listing in the index (Wolpert, 2019)]. However, recent work, along with a smattering of studies over the years, have suggested that the ECM can play a far more prominent role in driving tissue morphogenesis, with biophysical properties and dynamics that breaks the mold of how we normally discuss ECM behavior. In some examples, it is simply ambiguous which is the active agent – cells or matrix – highlighting that our textbook list of cellular behaviors hypothesized to drive morphogenesis are overly simplistic and underappreciates the role of the ECM. In other cases, it is clearer that the ECM is indeed contributing actively to drive alterations in tissue morphology. Along the way we will also highlight approaches to elucidate active ECM behaviors during embryogenesis as it will be important to determine how widespread these phenomena are during animal development.

2. Coherent ECM motion suggests ECM-autonomous dynamics during embryogenesis

Live imaging of several developmental processes has revealed long-range, coherent flow of cells and their surrounding extracellular

environment during embryogenesis, which has been described as ‘convective’ (Czirok et al., 2006; Loganathan et al., 2016). One of the first descriptions of large-scale cell/ECM flow within a tissue during development was in chick embryos along neural crest migratory pathways in the trunk. Injection and subsequent tracking of latex beads revealed a coherent movement of beads ventrally within the embryo, which mimicked the motion of the neural crest, leading to speculation that neural crest dispersal may be influenced by unknown forces from their surrounding extracellular environment (Bronner-Fraser, 1982). Live imaging of the embryonic quail vasculature and heart also showed a significant amount of coherent and coordinated drift in the developing ECM network, which appeared independent of movement of the cells themselves (Aleksandrova et al., 2012; Rupp et al., 2004). Direct labelling of ECM components also revealed coherent movement of the quail primitive streak (Zamir et al., 2008) and mesoderm (Czirok et al., 2004). And when both cells and ECM were simultaneously imaged within the quail mesoderm it became apparent that some of the cellular motion may be directly arising from convective movements within the surrounding ECM environment (Zamir et al., 2006). The thing to note about this convective motion is that it is spatially coherent and persistent in time. While others have suggested that “the most parsimonious explanation for these observations is that migratory cells and tissue carry their ECM with them” (Rozario and DeSimone, 2010), it is difficult – in our opinion – to invoke cellular movement as the explanation in some of these examples. Cells do not normally move individually with such a high degree of persistence in a way that could lead to such convective motion. Nor are cells that are surrounded by an ECM network obviously coordinated in a way that could lead to coherent movement. What else could therefore be driving this tissue-scale flow?

One possible explanation for viscous flows observed in embryonic ECM is that there are instabilities in the developing polymer network. As mentioned in the introduction, assembling polymer networks will often generate intrinsic stresses, which can lead to bulk movement. This is not just theoretical with regards to the ECM. In vitro, a mixture of two non-uniform networks of polymerizing Collagen-I and Fibronectin can induce long-range motion of cells and polystyrene beads (Newman et al., 1987; Newman et al., 1985). While the biophysical explanation of this ECM motion is unclear, it was speculated that the movement was the result of percolation of collagen fibers and wetting forces between the two networks (Forgacs et al., 1989; Newman et al., 1997). This is not to suggest that all of the convective tissue movements observed in embryos are driven by such a biophysical phenomenon; it is simply to highlight that complex dynamics can be autonomously induced in ECM networks, independent of cellular contractile forces, when the ECM is out of equilibrium. And ECM networks in developing organisms are likely far from steady state: the embryo of most animals starts off primarily – if not entirely – cellular in nature, but by the end of development the ECM is predominant. Therefore, nonequilibrium phenomena leading to stress development and viscous flows in assembling ECM networks may be more widespread than currently appreciated.

3. Spatiotemporally controlled ECM alterations modulating cellular forces to drive morphogenesis

There are now several examples of morphogenetic processes that require either a dynamic or anisotropically arranged ECM network to drive changes in tissue shape. In these selected examples the cells may be providing forces, but without a temporally controlled alteration in the ECM network, the morphogenetic change in shape would be severely affected or never have been initiated. Importantly, the limited repertoire of cellular behaviors thought to be driving morphogenesis are insufficient – on their own – to mechanistically explain these developmental events.

3.1. Alterations in tissue shape through localized changes in BM mechanical properties

The *Drosophila* egg chamber consists of 15 nurse cells and one oocyte surrounded by a simple epithelium. The egg chamber progressively increases in size and simultaneously changes morphology going from a round to an elliptical shape (Fig. 1). The change in shape of the egg chamber is caused by an anisotropic expansion of the chamber towards the anterior and posterior poles. The force driving this expansion is thought to come from the increase in volume of the nurse cells and oocyte within the chamber. However, the growth of these cells is presumed to be isotropic; therefore, how does the chamber change from a circular to an elliptical morphology? While the actomyosin network of follicular epithelial cells plays some role in the elongation process (Qin et al., 2017), a critical component driving anisotropic egg chamber growth is the surrounding BM, which envelops the egg chamber (Haigo and Bilder, 2011). Concomitant with expansion of the egg chamber, the follicular epithelial cells collectively migrate using the BM as a substrate and deposit BM fibrils oriented perpendicularly to the antero-posterior axis. Additionally, there are spatial variations in BM fibril properties (i.e., mechanical anisotropy) across the egg chamber such that the BM at the poles is softer than in the center (Crest et al., 2017; Isabella and Horne-Badovinac, 2016; Jayadev and Sherwood, 2016; Topfer et al., 2022). The result is that the BM acts like a corset which biases egg expansion to the poles thus creating an elliptically shaped egg chamber. What is currently unknown are the molecular mechanisms leading to BM mechanical anisotropy around the growing egg chamber. An increase in Collagen-IV levels is observed within the BM in the center of the egg chamber compared to the polar regions (Crest et al., 2017), but this appears to be unrelated to local differences in collagen transcription (Crest et al., 2017; Van de Bor et al., 2015). There are many non-mutually exclusive mechanisms that could explain this gradient in BM mechanical properties, which have yet to be revealed, such as anisotropic ECM deposition, component turnover, or crosslinking. Recent work also suggests that the mechanics of the developing BM surrounding the egg chamber is under complex spatial control: specialized cells at the poles of the egg chamber, the polar cells, sense local and distant BM properties to precisely alter the structure and mechanics of the ECM (Ku et al., 2023). This feedback between cells and ECM likely allows for fine

tuning of the morphogenetic process. What is clear from all this work is that the forces from the internal cellular expansion are not driving the asymmetric change in organ shape; it is the spatiotemporally controlled alterations in the surrounding ECM network that is the morphoregulator of the process, which sets the final shape of the egg chamber.

Anisotropic ECM properties have also been observed in the *Drosophila* cuticle (an apical ECM that forms the fly exoskeleton), which are thought to be important in regulating larval shape alterations during development. The larval cuticle appears to grow predominantly along an antero-posterior axis, allowing the cuticle to act as a corset (analogous to the egg chamber BM) that restricts circumferential growth, and loss of two specific cuticle proteins (Cuticular protein 11A and Tubby) perturbs this anisotropy and leads to lateral larval expansion (Tajiri et al., 2021). In contrast, during the larval/pupal transition an oriented cuticle shape change leads to contraction along the antero-posterior axis and expansion in the lateral orientation, and loss of a different cuticle protein (Obstructor-E) leads to circumferentially constricted pupae (Tajiri et al., 2017). While it cannot be completely ruled out that the loss of cuticle proteins leads to alterations in the properties of internal tissues, such as the underlying epithelium, the current speculation is that developmentally regulated mechanical anisotropy of the apical ECM may be an active contributor to *Drosophila* body shape. It will be interesting to determine how/why the complex cuticle network exhibits anisotropic mechanical properties and growth, and how this is dynamically altered throughout *Drosophila* development.

3.2. Tissue bending through localized BM growth or dissolution

The developing larval wing disc of the fly is composed of two continuous, interconnected layers of epithelial cells. On the top is the peripodial epithelium (PPE), which is squamous, while the bottom disc proper epithelium (DPE) is pseudostratified. Surrounding both epithelial layers on the outside of the wing disc is a continuous BM. Late in larval development in the pouch region, the DPE layer bends concavely with respect to the PPE, forming a dome-like tissue structure (Harmansa et al., 2023) (Fig. 2). Many cell-autonomous processes have been proposed to explain how narrow folds develop within an epithelium, but these cannot explain the formation of large domes, hundreds of cells in diameter. One hypothetical explanation for such large epithelial

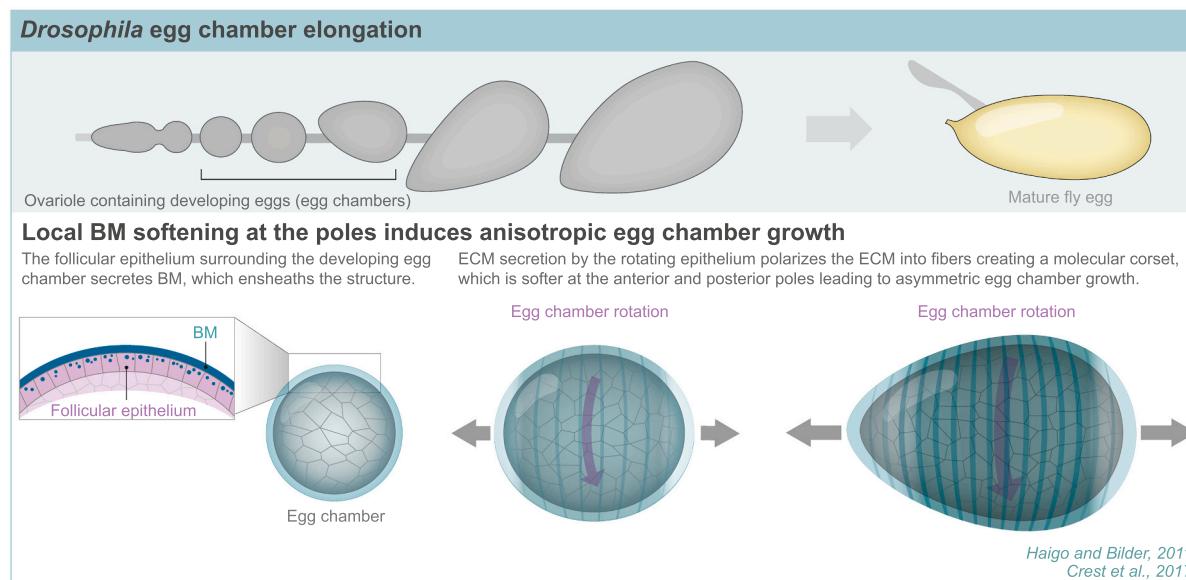


Fig. 1. Alterations in tissue shape through localized changes in BM mechanical properties. *Drosophila* egg chamber elongation involves polarized growth of the tissue towards polar regions. Anisotropic arrangement of BM fibrils perpendicular to the axis of tissue elongation along with BM softening at the poles modulates isotropic forces within the chamber to polarize growth.

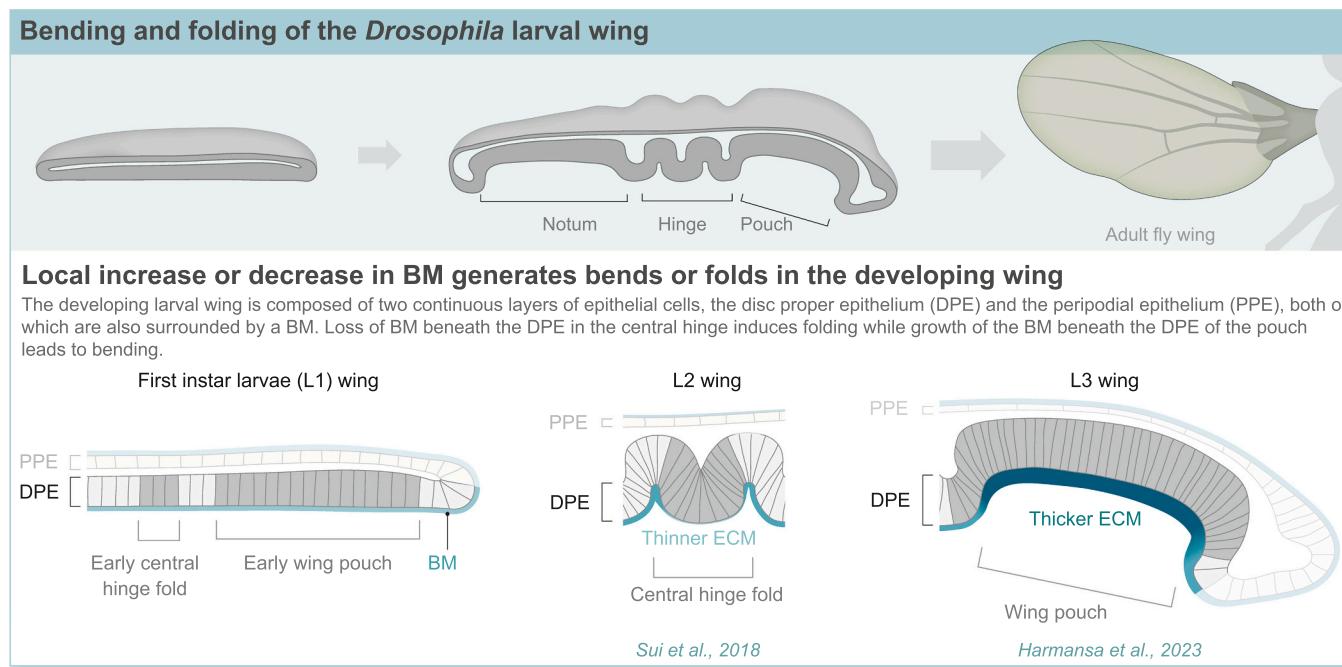


Fig. 2. Tissue bending through localized BM growth or dissolution. The *Drosophila* wing develops several folds during larval development. Localized BM reduction in the hinge or growth in the pouch modulates epithelial stresses to induce fold formation.

bending could be differential growth of the PPE and DPE layers. However, experimental analysis and finite element modelling ruled this out. Imaging of fluorescently-tagged Collagen-IV revealed that the BM specifically beneath the DPE of the wing disc pouch increases in thickness. Finite element modelling subsequently highlighted that bending could be explained by a mismatch in the production of the basal ECM versus proliferation of the DPE. Interestingly, localized growth of interstitial matrices has also been proposed to regulate morphogenesis in vertebrates. The leftward tilt of the primitive chick gut tube is thought to be partly driven by left/right alterations in growth of a hyaluronic acid (HA)-rich ECM network within the developing mesenchyme (Kurpios et al., 2008; Sivakumar et al., 2018); additionally, left-sided accumulation of HA between the myocardium and endocardium of the developing zebrafish heart is correlated with initiation of left/right asymmetry of the tissue (Derrick et al., 2022) [see also Section 4.2 on HA-driven hydrostatic pressure and tissue expansion].

While the force controlling the bending of the fly wing disc is predicted to come from the expansion of the epithelial cell layer of the disc proper, it is the spatiotemporally regulated growth of the underlying BM that drives the alteration in the shape of the tissue. The pertinent question therefore is not related to the mechanisms controlling the cellular behavior involved in disc bending but how local ECM dynamics are controlled. The epithelial cells of the DPE are simply expanding as developing epithelial populations tend to do. But what is driving the specific ‘growth’ of the BM of the DPE in the pouch? Similar to the anisotropic changes of the egg chamber BM during egg elongation, the unique growth of the BM underlying the DPE, compared to the PPE and other regions of the wing, could also be driven by a number of non-mutually exclusive possibilities. What is interesting about the ECM of the wing disc is that most of the BM components are thought to come from a distant source of production. The larval fat body secretes BM components into the hemolymph, which subsequently diffuse throughout the animal to assemble around most of the tissues of the developing organism, including the wing disc (Pastor-Pareja and Xu, 2011). Therefore, a localized increase in ECM production cannot explain the specific growth of the BM underlying the DPE. Other possible mechanisms are increased ECM component assembly due to integrin

receptor activity, reduced local matrix turnover, or increased local BM component crosslinking.

Localized BM alterations are also essential for a second *Drosophila* wing-related morphogenetic process, which occurs slightly earlier in larval development. Proximal to the wing disc pouch region (discussed above) are the wing notum and hinge, which form stereotyped creases in their epithelia through precise folding. One of these folds forms in the center of the hinge, and the formation of this crease involves an increase in basal area of the epithelial cells (Sui et al., 2018) (Fig. 2). Live imaging revealed that the increase in basal area correlates with a 20% reduction in Collagen-IV within the BM along with a basal decrease in cell membrane tension. Furthermore, ectopic local expression of a surface-bound matrix metalloprotease (MMP) was sufficient to locally reduce basal tension and generate an ectopic fold, as predicted by a 3D vertex model accounting for the tension along apical, basal and lateral cell membranes. This suggests that the loss of tension due to BM degradation and subsequent increase in basal cell area can cause buckling of an epithelium. The questions remaining are, how is the BM locally disrupted and precisely why does BM depletion lead to a reduction in basal cell tension? One possible explanation for the latter is that the epithelial actomyosin network within the basal region associated with the BM requires integrin adhesion for force generation. However, another intriguing possibility is that the BM itself maintains some amount of autonomous tension due to intrinsic ECM stress (see Section 4.3) with BM degradation leading to release of these stresses. Previous examination of BM components in the *Drosophila* wing revealed that Collagen-IV is involved in generating constrictive forces around the tissue (Pastor-Pareja and Xu, 2011) and a recent preprint suggests that the BM of the wing disc may indeed be a long-term store of elastic stresses to control fold formation (Santillan et al., 2022).

Related to these bending phenomena are studies showing that strain mismatches between adjacent tissue layers lead to the formation of wrinkles, bends, or folds depending on the precise nature of the mechanical instability (Nelson, 2016). For example, condensation of the ECM within a mesenchyme underlying an epithelial layer is thought to lead to patterned folding of a variety of tissues (Hughes et al., 2018; Sakar and Baker, 2018). While contraction of cells within the

mesenchyme is the predominant mechanism invoked for ECM compaction and the generation of the instability, it seems plausible that ECM-specific alterations (e.g., deposition, crosslinking, and turnover) could also be playing a role.

3.3. Tissue folding through localized accumulation and degradation of an apical ECM

While the buckling of a tissue due to a mechanical instability as described in the previous section can indeed lead to the development of tissue folds (Nelson, 2016), the process is somewhat stochastic, resulting in folds that are difficult to control. Recent work, again in the developing *Drosophila* wing, reveals that localized epithelial attachments to an apical ECM when combined with a buckling instability can define fold position (Tsuboi et al., 2023). During pupal development, the wing initiates another set of precisely controlled folds: 4 develop along longitudinal veins ("vein folds" L2-L5) while another develops along the margin of the wing blade ("marginal fold") (Tsuboi et al., 2023) (Fig. 3). The assumption was that these wing folds may develop as a result of buckling caused by uniform expansion of the wing epithelia within a confined apical cuticle network surrounding the entire wing. However, these folds are all highly stereotyped, which is difficult to explain through buckling alone. L2 and L4 vein folds bend ventrally and L3 and L5 bend dorsally, while the marginal fold is formed by bending of the entire wing in a posterior direction forming a fold perpendicular to the margin of the wing blade. Inhibiting actomyosin contraction within the

epithelium led to a minimal effect on fold pattern or position, suggesting that localized cellular forces were not the major driver. Imaging of a GFP-tagged apical ECM component, Dumpy (Dpy), which is known to link the wing epithelia to the *Drosophila* cuticle (Ray et al., 2015), revealed a spatiotemporally localized pattern of expression. A fibrous Dpy network was observed to accumulate on the wing margin and specifically on the epithelial surface of veins L3 and L5, which connects to an outer cuticle network also containing the Dpy protein (Etournay et al., 2015; Ray et al., 2015; Tsuboi et al., 2023). Dpy is also patterned dorso-ventrally on the surface of the wing; on the distal half of the wing, Dpy is largely absent on the ventral side of the epithelium, suggesting that L3 and L5 veins may be more strongly anchored to the cuticle dorsally. Indeed, experimental removal of Dpy on the dorsal epithelial surface randomized the pattern of vein folds revealing that localized pinning of the epithelium to the dorsal cuticle can aid in stereotyping the buckle-induced fold pattern. What is currently unclear is how Dpy-controlled epithelial anchors are regulated. Localized overexpression of Dpy in the wing did not affect either epithelial anchorage or fold formation, which suggests that vein anchorage and Dpy network formation must be defined by molecular or mechanical alterations controlled by the vein cells. What about the marginal fold? Live imaging of Dpy revealed that it is temporally disassembled from the wing surface starting in a distal region that correlates with the position of the marginal fold. Depletion of two enzymes known to be involved in Dpy degradation during wing morphogenesis (Díaz-de-la-Loza et al., 2020; Díaz-de-la-Loza et al., 2018), Stubble or Notopleural, prevented loss of

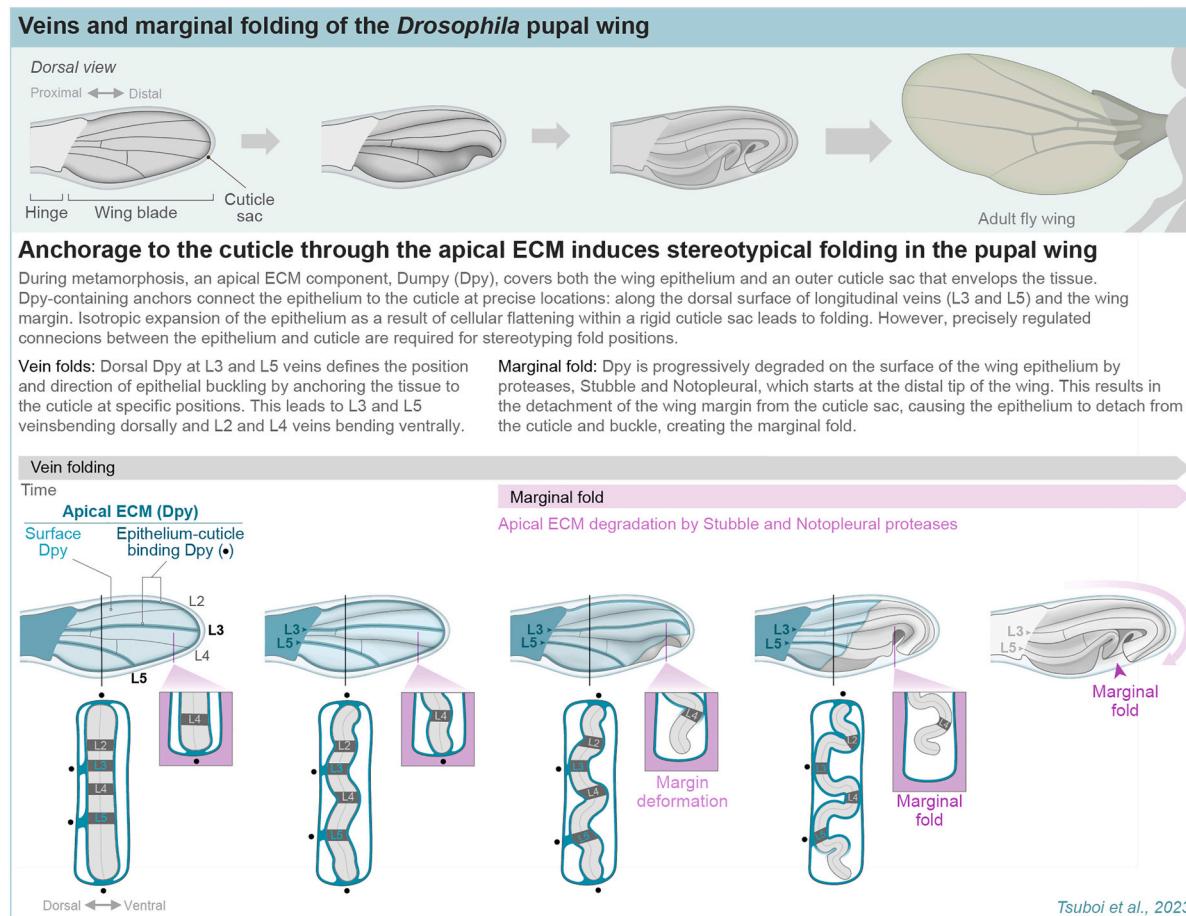


Fig. 3. Tissue folding through localized accumulation and degradation of an apical ECM. The *Drosophila* pupal wing develops 4 longitudinal vein folds (L2-L5) and a marginal fold at the distal tip of the wing. Localized pinning of the epithelia of veins L3 and L5 to the dorsal cuticle coupled with a buckling instability as a result of epithelial expansion within a confined cuticle network defines vein fold formation. In contrast, localized loss of epithelial-cuticle anchorage within the marginal region drives epithelial buckling and marginal fold formation.

the surface epithelial Dpy network and marginal folding – but not vein folds. This suggests that epithelial detachment from the cuticle is responsible for buckling of the tissue in this region. The pertinent question remaining for marginal fold initiation is what controls the activation of the enzymes responsible for Dpy degradation (e.g., localized expression or localized catalytic activation of the inactive zymogen). However, another intriguing possibility is that the physical properties of the Dpy network may alter its sensitivity to proteolytic cleavage. The Dpy network is observed to undergo a transformation into filaments of increased tensile strength in response to tension (Chu and Hayashi, 2021), which could alter its sensitivity to degradation or even the extent of network assembly. As there is a patterning of epithelial stresses during wing morphogenesis (Aigouy et al., 2010; Etournay et al., 2015; Iyer et al., 2019), it will be interesting to determine whether there is concomitant patterning of Dpy network organization that alters the mechanochemical properties of this apical ECM, which ultimately shapes fold development within the tissue.

3.4. Asymmetric tissue expansion through anisotropic BM remodeling

The early mouse embryo consists of an egg cylinder containing the internal epiblast and outer visceral endoderm separated by a BM. These pre-gastrulation stage embryos first expand asymmetrically along a proximo-distal axis. Subsequently, on the posterior side of the embryo, the primitive streak, which is a localized thickening of ectoderm cells, extends distally and undergoes the process of gastrulation. Recent work, once again, highlights a role for anisotropic BM alterations driving these processes (Kyprianou et al., 2020) (Fig. 4). Within the egg cylinder, spatially oriented, MMP-induced BM perforations first surround the epiblast. The orientation of these elliptically shaped BM holes aligns with the embryo's growth axis, which is hypothesized to be a consequence of the predominant direction of embryo growth along the proximo-distal axis causing anisotropic stresses to the weakened BM. However, enzymatic removal of the BM with collagenase altered this growth axis leading to rounder embryos suggesting that mechanical

asymmetries in the BM network may also be directly aiding asymmetric embryo growth. Interestingly, BM micro-perforations and an increase in ECM fluidity is observed during branching of lungs, mammary glands, and salivary glands suggesting that localized BM remodeling may be a common mechanism allowing for anisotropic tissue expansion (Harunaga et al., 2014; Spurlin et al., 2019). Slightly later in development, BM perforations are directed to the posterior side of the embryo as a result of Nodal-induced MMP inhibition anteriorly. MMP-induced BM perforation coupled with tension associated with tissue growth leads to BM weakening on the posterior side of the embryo, which is subsequently thought to aid anterior extension of the primitive streak and facilitate gastrulation. While some of the upstream signals inducing asymmetric BM dissolution in pre-gastrulation stage embryos are understood (e.g., Nodal), it is interesting to speculate that there may be feedback mechanisms, analogous to the fly egg chamber, that allow the developing embryo to precisely tune BM properties as too much or too little degradation would clearly be detrimental. Similar to the previous examples, while the forces driving asymmetric embryo expansion and branching morphogenesis are thought to arise from the internal pressure of growing cells, it is the patterned, localized changes to the ECM that instigate the anisotropic alterations in tissue shape.

3.5. Branching morphogenesis driven by localized deposition of interstitial matrix

Branching morphogenesis is a process whereby individual cells (e.g., neurons) or organized epithelial tubes (e.g., lung airways, vasculature) progressively bud and grow to develop tree-like structures. While at first glance these networks may appear haphazard, there is order to these branching patterns and it is currently unclear how local signals drive global patterning of the tree. Recent work reveals that a patterned interstitial ECM at the site of bifurcations can behave as one of these local cues (Nerger et al., 2021) (Fig. 5). The murine mammary gland consists of epithelial tubes, which are embedded in an adipose-rich stroma called the fat pad. The gland undergoes postnatal development

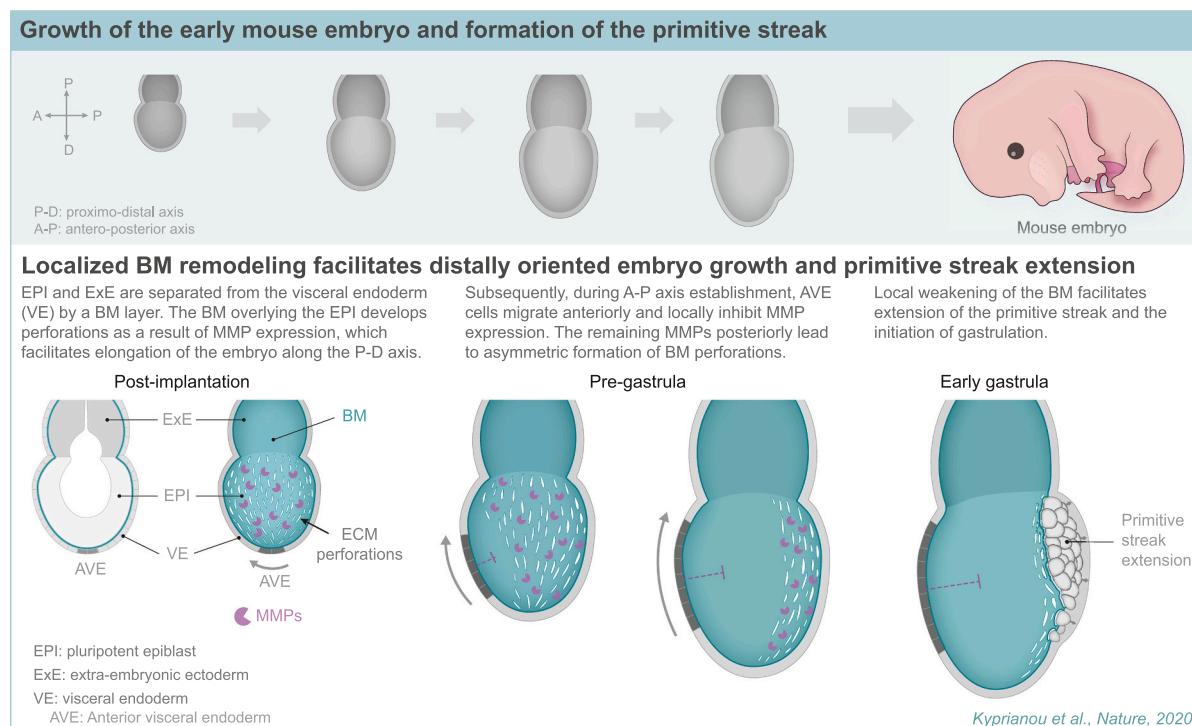


Fig. 4. Asymmetric tissue expansion through anisotropic BM remodeling. The early mouse embryo elongates asymmetrically, and the primitive streak subsequently extends distally. Localized BM dissolution as a result of directed MMP expression modulates the stresses associated with tissue growth to drive both processes.

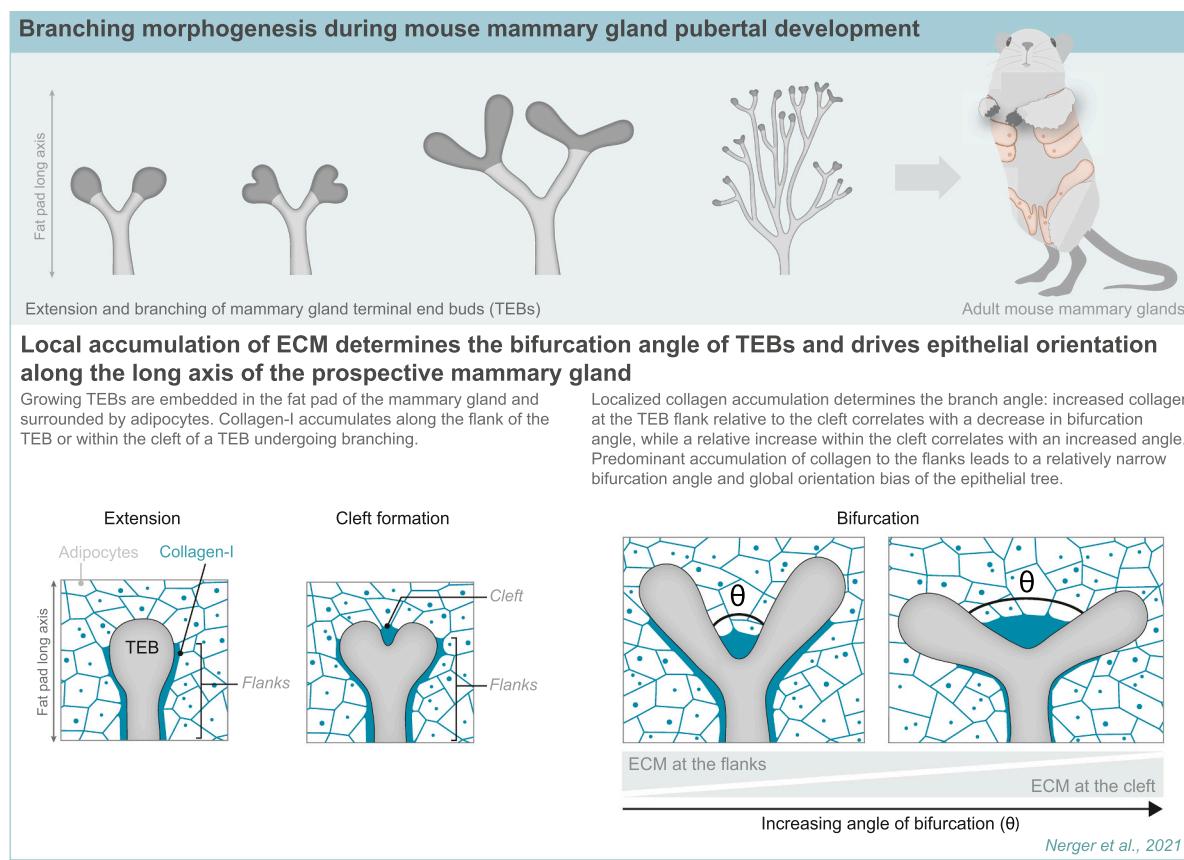


Fig. 5. Branching morphogenesis driven by localized deposition of interstitial matrix. The developing mouse mammary gland undergoes biased growth along the long axis of the fat pad. Localized accumulation of Collagen-I to the flank or cleft of the extending terminal end bud (TEB) of the developing mammary gland biases branch bifurcation, which globally orients growth of the epithelial tree.

upon puberty whereby the epithelial terminal end buds (TEB) extend and progressively branch to eventually fill the fat pad. While the final branched structure is thought to have a random geometry, the developing tree has a global orientation bias that is aligned with the long axis of the fat pad, which is hypothesized to be essential to efficiently fill the available space within the tissue (Brownfield et al., 2013). However, what factors bias the growth of the tree along the long axis of the fat pad? Quantification of the bifurcation angles of the growing TEBs during mammary gland development revealed that the end buds consistently orient within 20° of the long axis of the fat pad, driving tree extension predominantly in this direction (Nerger et al., 2021). Previous work suggested that a preexisting aligned collagen fiber network may regulate this orientation (Brownfield et al., 2013), but high resolution imaging of the TEBs showed that aligned collagen fibers are absent from the fat pad and that the TEBs are instead in close association with adipocytes (Nerger et al., 2021). Nevertheless, there is interstitial ECM within the fat pad and on closer inspection it became apparent that Collagen-I accumulates locally at the sides of the TEB and within the cleft of each branch. Further analysis showed that there is a relationship between the branch angle and the patterned accumulation of collagen either at the sides of the TEB or at the cleft; an increase in collagen at the sides of the TEB relative to the cleft correlates with a decrease in bifurcation angle, while relative increase in collagen within the cleft correlates with an increased angle. Additionally, a finite element model taking into account previously measured stiffness differences between the collagen and adipose components, which both directly interact with the TEB, revealed that localized accumulation of a stiff ECM is indeed sufficient to bias the overall branching pattern. Similar to the previous examples of ECM controlled morphogenesis, the forces driving mammary gland branching are from the growth of the epithelial bud. However, it is the

patterned accumulation of interstitial ECM (as opposed to the patterned degradation of previous examples), and associated mechanical asymmetries experienced by the TEB, that drives the shape of each branch and regulates the final global orientation of the tree. Questions remain regarding the dynamics of this collagen accumulation in relation to TEB branching and how local ECM deposition is regulated. Collagen is produced by cells within the stroma suggesting that there may be epithelial-stromal crosstalk driving localized ECM accumulation to TEB sides versus clefts to control progressive branching of the gland.

4. ECM-autonomous force production driving morphogenesis

The previous examples highlighted processes during which spatio-temporally regulated, anisotropic alterations in the ECM drive global changes in tissue shape, with forces provided by isotropic cellular behaviors. However, there is recent work suggesting that the ECM can generate forces directly. Here we will highlight a few examples along with speculative processes that may involve similar mechanisms.

4.1. Tissue elongation through anisotropic expansion of interstitial matrix

Growth of long bones occurs at the growth plate, which is a cartilaginous structure that progressively expands along a proximo-distal axis prior to ossification. Embedded within the developing growth plate cartilage are chondrocytes, which deposit an interstitial ECM into the intercellular space. Live imaging of the developing embryonic chick growth plate within a metacarpal bone in which the chondrocytes were retrovirally tagged with GFP allowed for the characterization of the dynamics of growth plate expansion (Li et al., 2015) (Fig. 6). This revealed that all of the cellular displacements were along a proximo-

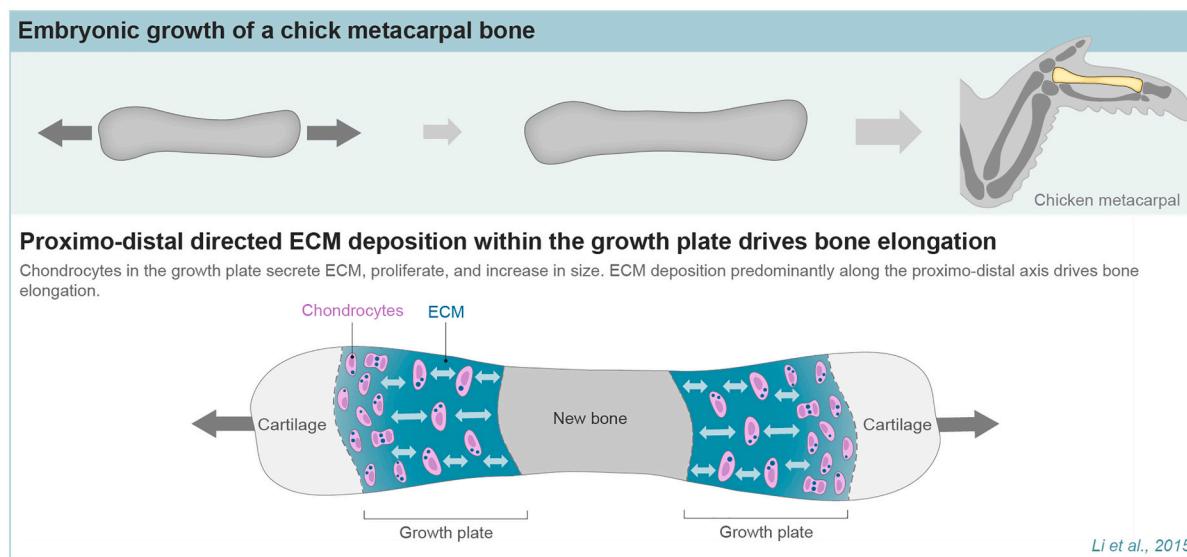


Fig. 6. Tissue elongation through anisotropic expansion of interstitial matrix. Growth of the embryonic chick metacarpal bone involves asymmetric expansion of the growth plate. Polarized deposition of ECM by chondrocytes along a proximo-distal axis leads to an asymmetric pressure that drives anisotropic expansion of the tissue.

distal axis with little evidence for convergent-extension driven cellular rearrangements, which was previously proposed as a mechanism of anisotropic growth plate expansion (Ahrens et al., 2009). Additionally, live imaging revealed that the rate and orientation of chondrocyte cell divisions could also not explain the homogeneous cellular displacements along a proximo-distal axis. Quantification of the increase in cell volume showed that the chondrocytes expand anisotropically in line with the direction of growth plate elongation, suggesting that asymmetric cellular growth was involved – but not the entire story. The increase in volume of the surrounding ECM was more than double that of the cells suggesting that the expansion of the growth plate is predominantly driven by asymmetric production and deposition of matrix. However, it is unclear how the cartilaginous ECM can expand anisotropically. The chondrocytes are entirely embedded in ECM and for anisotropic

expansion to occur, the cells need to somehow assemble the matrix network predominantly along a proximo-distal axis. There is precedent for polarized secretory mechanisms controlling ECM deposition. For example, the follicular epithelial cells of the *Drosophila* egg chamber specifically deposit BM components basally through Rab-mediated trafficking of components, which is critical for proper morphogenesis of the tissue (Isabella and Horne-Badovinac, 2016). Interestingly, planar polarity signals are involved in chondrocyte organization (Gao et al., 2011) and it is intriguing to speculate that this may lead to a similar directional bias in ECM assembly within the growth plate.

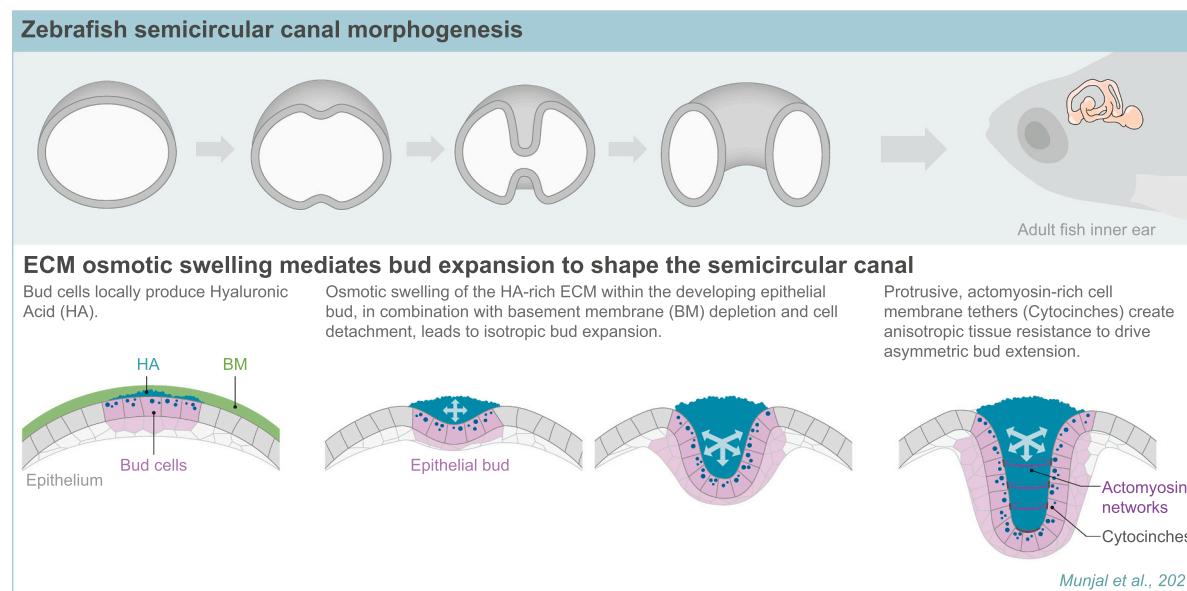


Fig. 7. Tissue expansion through an ECM-dependent increase in hydrostatic pressure. The developing zebrafish semicircular canal is shaped by epithelial buds, which expand asymmetrically into a lumen. Bud expansion is driven by isotropic swelling of an ECM, and a cellular acto-myosin network within the epithelium sculpts this ECM-driven stress to polarize growth of the canal.

4.2. Tissue expansion through an ECM-dependent increase in hydrostatic pressure

The semicircular canals of the inner ear form from invaginations of the otic epithelium, which eventually fuse to configure the complex canal architecture. Recent work in zebrafish revealed that initial bud formation cannot be explained by an increase in localized epithelial proliferation, epithelial buckling, or cellular rearrangements, which led to an intriguing role for the ECM (Munjal et al., 2021) (Fig. 7). Analysis of hyaluronan synthesis enzymes during bud formation showed that they are locally produced by the bud cells, which leads to a dense HA-rich ECM network within the bud. This HA-rich matrix results in osmotic swelling of the ECM thus expanding the developing bud. An ECM-dependent increase in hydrostatic pressure has previously been proposed to drive lumen expansion in the sea urchin gastrula (Lane et al., 1993) and the *Drosophila* hindgut (Syed et al., 2012). However, in contrast to these examples, the zebrafish otic bud expands such that its aspect ratio is anisotropic, which cannot be explained by a simple increase in osmotic pressure as this would result in isotropic bud expansion. The anisotropy of the bud is controlled by cells circumferentially extending protrusive membrane tethers ('cytocinchies') around the bud, constraining the isotropic osmotic pressure leading to oriented bud extension. This is a cell/ECM role reversal compared with previously discussed morphogenetic processes, such as anisotropic growth of the fly egg chamber. The force behind egg expansion is from isotropic pressure due to cellular growth within the egg chamber, which is subsequently 'sculpted' by an anisotropic ECM surrounding the tissue (Crest et al., 2017; Jayadev and Sherwood, 2016). In contrast, the force behind the asymmetric expansion of the otic bud is from isotropic osmotic swelling of the ECM, which is subsequently 'sculpted' by protrusive structures from the epithelial cells. Regardless, neither morphogenetic process could be explained without understanding the dynamics of the ECM. A recent preprint also suggests that HA-driven swelling of the ECM may be more widespread during morphogenesis; HA production in the developing chick presomitic mesoderm coupled with tissue confinement is hypothesized to create pushing forces that drive posterior elongation of the embryo (Michaut et al., 2022).

4.3. Alteration in tissue morphology by intrinsic stresses in an assembling BM network

The *Drosophila* ventral nerve cord (VNC) is a long tubular structure attached to the brain containing a central bundle of axons that is surrounded by glia and a BM, which ensheathes the entire tissue. Midway through embryonic development the VNC suddenly shrinks in length in a posterior to anterior direction. While this is known to require ECM producing cells (hemocytes) along with BM components, such as Collagen-IV and Laminin (Borchiellini et al., 1996; Matsubayashi et al., 2017; Olofsson and Page, 2005; Urbano et al., 2009) (Fig. 8), the precise role of the ECM during this process has been unclear. Recent work has revealed that activity of the cells within the VNC (i.e., glia and neurons) is not essential for initiation of VNC morphogenesis; driving dominant negative GTPases, inhibiting Myosin, and even killing the cells surrounding the tissue have little effect on the initial stages of the process (Karkali et al., 2022; Serna-Morales et al., 2023). In contrast, removing ECM components, such as Collagen-IV, or inhibiting component distribution within the embryo leads to very severe VNC morphogenetic defects from the start (Fagotto, 2023; Serna-Morales et al., 2023). Live imaging revealed that prior to VNC morphogenesis there is little Collagen-IV within the embryo, and coincident with initiation of VNC condensation, Collagen-IV begins to be exponentially expressed and assembled around the tissue. Finite element modelling suggested that the morphodynamics of the process could be explained by a sudden anisotropic increase in surface tension, which was experimentally confirmed to occur during Collagen-IV induction by atomic force microscopy. Timelapse imaging of glia and Collagen-IV dynamics during the initial assembly of the BM showed a viscous flow of ECM on the tissue surface, which is convective – similar to the coherent ECM motion discussed earlier – and independent of glial remodeling. This convective ECM motion was hypothesized to be driven by anisotropic stresses in the developing network as a result of a transient gradient of Collagen-IV deposition on the tissue surface. Furthermore, subtle perturbation of Collagen-IV polymerization with dominant negative mutant Collagen-IV transgenes was sufficient to alter the overall rate of VNC morphogenesis. As mentioned previously, all polymer networks can generate stresses during their initial assembly and this work suggests that de novo

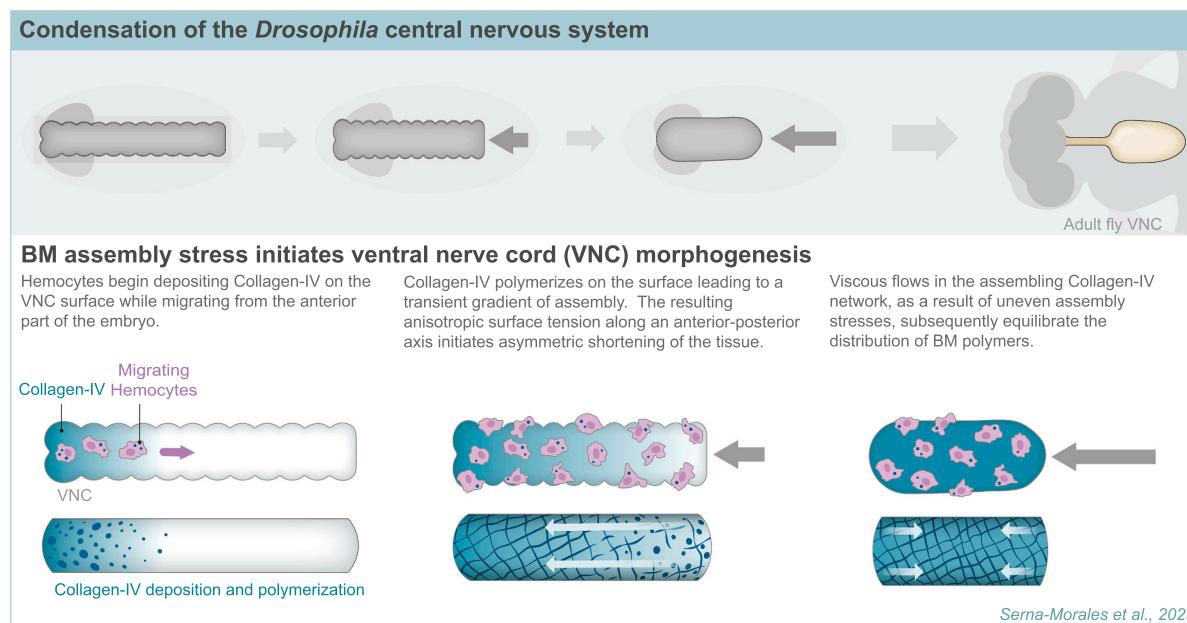


Fig. 8. Alteration in tissue morphology by intrinsic stresses in an assembling BM network. The *Drosophila* ventral nerve cord (VNC) undergoes a condensation process in which the tissue reduces in length asymmetrically from a tail to head direction. Assembly of Collagen-IV on the tissue surface by migrating hemocytes leads to an anisotropic increase in surface tension, which drives shortening of the VNC.

production of an ECM network can indeed create forces and convective movements that shape developing tissues.

5. How to highlight active ECM mechanisms during animal development

Highlighting active mechanisms for an ECM network during developmental processes is not trivial. Our toolkit of approaches that allows for examination of cellular behaviors in developing model organisms is far more extensive than techniques to interrogate the ECM. It is therefore unsurprising that tissue morphogenesis is historically assumed to be a cellular-driven phenomenon. Yet it is increasingly possible to live image and perturb ECM dynamics and organization in several model organisms such that a perceived difficulty to interrogate precise morphogenetic roles of the ECM may no longer be an excuse. Here we will list a few general approaches that have been consistently utilized to highlight active mechanisms for the ECM during tissue morphogenesis.

5.1. Live imaging ECM dynamics

The most obvious approach to highlight active ECM roles during embryonic development involves an ability to live image the network. For example, it is now possible to endogenously tag several different BM components in *Drosophila* and *C. elegans*, which revealed that the ECM is far more dynamic during development than many may assume (Haigo and Bilder, 2011; Horne-Badovinac, 2020; Ihara et al., 2011; Keeley et al., 2020; Matsubayashi et al., 2017; Matsubayashi et al., 2020; Serna-Morales et al., 2023). More recently, fluorescent tagging of BM was also developed for zebrafish (Soans et al., 2022; Yamaguchi et al., 2022) and mouse (Futaki et al., 2023; Morgner et al., 2023). However, genetically encoded fluorescent tagging is not the only way to image BM dynamics. Indirect labelling approaches to highlight BM dynamics by injecting fluorescently tagged antibodies have also been developed for several model systems (Harunaga et al., 2014; Zamir et al., 2008). Unsurprisingly, due to the ability to live image the developing BM, most of our advances in highlighting an active role for the ECM during development have focused on this type of ECM. However, interstitial matrices found within the developing mesenchyme are also amenable to labelling approaches. Fibrillar collagen structure and dynamics can be analyzed through second harmonic generation microscopy (Chen et al., 2012; Weigert et al., 2013). Additionally, a GFP-tagged Collagen Type I line has been developed for zebrafish (Morris et al., 2018) and indirect, antibody labelling strategies are also possible for fibrillar matrices (Czirok et al., 2004). In order to unveil cellular vs. ECM movement it is essential to simultaneously image both components to understand how much of the ECM motion may be from cellular remodeling (and vice versa: how much of the cellular drift may be from unappreciated ECM flows). Cell migration involves relatively rapid and non-persistent motion relative to the local ECM network, while convective ECM movements will be slower yet persistent in time and space, and there are computational frameworks in place to decompose these movements if both cells and ECM can be imaged simultaneously (Loganathan et al., 2016).

There are also unappreciated ECM dynamics that cannot be visualized by simple labelling approaches. For example, different BM components have been shown to undergo relatively high rates of turnover (i.e., constant loss and replacement) in developing *Drosophila* (Matsubayashi et al., 2020) and *C. elegans* (Keeley et al., 2020), and analyzing ECM half-life requires more complex imaging approaches, such as pulse-chase analysis and fluorescence recovery after photobleaching. Understanding differential rates of homeostatic turnover during morphogenesis and growth will be informative in explaining some alterations in tissue shape. Indeed, the bending of the fly wing disc because of localized growth of the underlying BM and tissue-specific growth observed in *C. elegans* may both be explained by localized changes to component turnover (Harmansa et al., 2023; Keeley et al., 2020). Importantly,

tagging of BM components with photoactivatable constructs has recently been performed in both zebrafish (Soans et al., 2022) and mice (Morgner et al., 2023), which will open up the possibility of examining alterations in turnover during vertebrate development.

5.2. Mathematical modelling and computational simulation

A consistent feature observed in the publications discussed in this review highlighting an active role for ECM during morphogenesis is that many involve some amount of computational modelling. To understand bending of the wing disc pouch a finite element model helped to rule out differences in epithelial expansion and explain how differential growth anisotropy of the BM drives the morphological changes of the tissue (Harmansa et al., 2023). A 3D vertex model showed that reductions in basal tension within the hinge of the wing disc can drive basal-directed folding (Sui et al., 2018). Finite element modelling revealed that local accumulation of interstitial ECM and associated stiffness asymmetries control branching angles in the developing mammary gland (Nerger et al., 2021). Computational simulation of cellular trajectories during growth plate expansion allowed the authors to reveal that increases in cell and ECM volume were sufficient on their own to explain the anisotropic growth of the tissue (Li et al., 2015). A 2D vertex model showed how cytocinches could synergistically interact with an increase in ECM-driven hydrostatic pressure to sculpt semicircular canal budding (Munjal et al., 2021). Finally, a finite element model revealed how an increase in BM-driven surface tension could explain the isovolumetric alteration in VNC shape during condensation of the tissue (Serna-Morales et al., 2023). Modelling allows one to test biased, preconceived notions to determine whether simple cellular behaviors are sufficient to explain changes in tissue shape. Simulations further enable a greater understanding of complex dynamics and synergistic interactions between cells and their environment, which often lead to unintuitive effects. Finally, these computational approaches help generate novel hypotheses that can be experimentally tested to determine whether the ECM is indeed playing a more active morphogenetic function than appreciated.

5.3. Chemical and genetic perturbation of ECM

One of the most challenging experimental hurdles to understanding active roles for the ECM during development is related to the difficulty of specifically perturbing ECM mechanics or dynamics. Compared to our comprehension of how cells regulate their dynamics and mechanical properties, we know virtually nothing about mechanisms regulating ECM during development. Understanding how the ECM forms and is dynamically altered during embryogenesis will be critical to elucidate how mechanical or geometrical anisotropies develop in an ECM network to regulate morphogenetic processes, which is an outstanding question in most of the examples highlighted in this review. Experimentally, there are brute force approaches to interrogate developmental ECM functions, such as enzymatic degradation, but this may be too detrimental to interpret due to gross changes to tissue structure. If one is lucky, it is possible that obvious regulators of ECM dynamics are involved in a process of interest, such as MMPs, which can be inhibited by specific chemical perturbation or genetic manipulation [used successfully to understand fly wing disc folding (Harmansa et al., 2023) and growth of pre-gastrulation stage mouse embryos (Kyrianiou et al., 2020)]. However, ECM regulation will involve far more than just MMPs: ECM components are covalently crosslinked by several enzymes; ECM networks involve myriad components controlled in a tissue-specific fashion; components are known to involve numerous post-translational modifications that are thought to alter stability; non-covalent interactions are critical for initial ECM assembly and are likely sufficient to explain a lot about overall stabilization of the network. Furthermore, ECM stiffness is not the only instructive mechanical parameter that we need a capacity to modulate in order to understand tissue morphogenesis; cells and

organoids exhibit unique responses to changes in ECM viscoelasticity (Elosegui-Artola, 2021; Elosegui-Artola et al., 2023) and it is plausible that developing embryos modulate viscous properties of the ECM to accommodate rapid tissue growth and alterations in shape. Until we understand how ECM mechanical and molecular alterations are controlled from a developmental context, it will be difficult to generate subtle perturbations that inform on a precise role for the ECM during tissue morphogenesis. This is made more complex given the composite nature of the ECM, which consists of several different proteins contributing distinct mechanical properties to the network (e.g., elasticity vs viscosity) and it will be critical to understand how these components individually alter ECM characteristics.

6. Conclusion

While it is clearly a challenge to interrogate the role of ECM dynamics (i.e., movement, remodeling, and homeostatic turnover) during morphogenesis, tools and approaches are rapidly maturing, which will reveal new functions in other developmental processes. However, the biggest barrier to unveiling active ECM roles during development is conceptual. It requires an understanding that tissue morphogenesis involves more than just a limited repertoire of cellular processes (Barresi and Gilbert, 2020) if cellular behaviors synergistically interact with a dynamic ECM. Additionally, it must be appreciated that the change in embryonic form is not just the result of cellular forces (Wolpert, 2019), but in the right conditions may also come directly from the ECM itself. Altering developmental biology textbooks may be the best place to start.

CRediT authorship contribution statement

Both authors contributed equally to writing the manuscript.

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References

- Abercrombie, M., Flint, M., James, D., 1956. Wound contraction in relation to collagen formation in scorbutic guinea-pigs. *Development* 4, 167–175.
- Ahrens, M.J., Li, Y., Jiang, H., Dudley, A.T., 2009. Convergent extension movements in growth plate chondrocytes require gpi-anchored cell surface proteins. *Development* 136, 3463–3474.
- Aigouy, B., Farhadifar, R., Staple, D.B., Sagner, A., Roper, J.C., Julicher, F., Eaton, S., 2010. Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. *Cell* 142, 773–786.
- Aleksandrova, A., Czirok, A., Szabo, A., Filla, M.B., Hossain, M.J., Whelan, P.F., Lansford, R., Rongish, B.J., 2012. Convective tissue movements play a major role in avian endocardial morphogenesis. *Dev. Biol.* 363, 348–361.
- Barresi, M.J.F., Gilbert, S.F., 2020. *Developmental Biology*. Sinauer Associates, New York.
- Bonnans, C., Chou, J., Werb, Z., 2014. Remodelling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell Biol.* 15, 786–801.
- Borchiellini, C., Coulon, J., Le Parco, Y., 1996. The function of type IV collagen during *Drosophila* muscle development. *Mech. Dev.* 58, 179–191.
- Bronner-Fraser, M., 1982. Distribution of latex beads and retinal pigment epithelial cells along the ventral neural crest pathway. *Dev. Biol.* 91, 50–63.
- Brown, N.H., 2011. Extracellular matrix in development: insights from mechanisms conserved between invertebrates and vertebrates. *Cold Spring Harb. Perspect. Biol.* 3.
- Brownfield, D.G., Venugopalan, G., Lo, A., Mori, H., Tanner, K., Fletcher, D.A., Bissell, M. J., 2013. Patterned collagen fibers orient branching mammary epithelium through distinct signaling modules. *Curr. Biol.* 23, 703–709.
- Carrel, A., 1910. The treatment of wounds. A first article. *JAMA* 55, 2148–2150.
- Carrel, A., Hartmann, A., 1916. Cicatrization of wounds : I. The relation between the size of a wound and the rate of its cicatrization. *J. Exp. Med.* 24, 429–450.
- Chen, X., Nadiarynk, O., Plotnikov, S., Campagnola, P.J., 2012. Second harmonic generation microscopy for quantitative analysis of collagen fibrillar structure. *Nat. Protoc.* 7, 654–669.
- Christiansen, D.L., Huang, E.K., Silver, F.H., 2000. Assembly of type I collagen: fusion of fibril subunits and the influence of fibril diameter on mechanical properties. *Matrix Biol.* 19, 409–420.
- Chu, W.C., Hayashi, S., 2021. Mechano-chemical enforcement of tendon apical ECM into nano-filaments during *Drosophila* flight muscle development. *Curr. Biol.* 31 (1366–1378), e1367.
- Crest, J., Diz-Munoz, A., Chen, D.Y., Fletcher, D.A., Bilder, D., 2017. Organ sculpting by patterned extracellular matrix stiffness. *Elife* 6.
- Czirok, A., Rongish, B.J., Little, C.D., 2004. Extracellular matrix dynamics during vertebrate axis formation. *Dev. Biol.* 268, 111–122.
- Czirok, A., Zamir, E.A., Filla, M.B., Little, C.D., Rongish, B.J., 2006. Extracellular matrix macroassembly dynamics in early vertebrate embryos. *Curr. Top. Dev. Biol.* 73, 237–258.
- Daley, W.P., Yamada, K.M., 2013. ECM-modulated cellular dynamics as a driving force for tissue morphogenesis. *Curr. Opin. Genet. Dev.* 23, 408–414.
- Danielsen, C.C., 1981. Mechanical properties of reconstituted collagen fibrils. A study on reconstitution methodology and influence of in vitro maturation. *Connect. Tissue Res.* 9, 51–57.
- Derrick, C.J., Sanchez-Posada, J., Hussein, F., Tessadori, F., Pollitt, E.J.G., Savage, A.M., Wilkinson, R.N., Chico, T.J., van Eeden, F.J., Bakkers, J., Noel, E.S., 2022. Asymmetric Hapln1a drives regionalized cardiac ECM expansion and promotes heart morphogenesis in zebrafish development. *Cardiovasc. Res.* 118, 226–240.
- Desmouliere, A., Hinz, B., 2021. The myofibroblast and Giulio Gabbianni: an inseparable couple celebrates their 50 years golden wedding anniversary. *Wound Repair Regen.* 29, 511–514.
- Díaz-de-la-Loza, M.D., Ray, R.P., Ganguly, P.S., Alt, S., Davis, J.R., Hoppe, A., Tapon, N., Salbreux, G., Thompson, B.J., 2018. Apical and basal matrix remodeling control epithelial morphogenesis. *Dev. Cell* 46 (23–39), e25.
- Díaz-de-la-Loza, M.D., Loker, R., Mann, R.S., Thompson, B.J., 2020. Control of tissue morphogenesis by the HOX gene Ultrabithorax. *Development* 147.
- Elosegui-Artola, A., 2021. The extracellular matrix viscoelasticity as a regulator of cell and tissue dynamics. *Curr. Opin. Cell Biol.* 72, 10–18.
- Elosegui-Artola, A., Gupta, A., Najibi, A.J., Seo, B.R., Garry, R., Tringides, C.M., de Lazar, I., Darnell, M., Gu, W., Zhou, Q., Weitz, D.A., Mahadevan, L., Mooney, D.J., 2023. Matrix viscoelasticity controls spatiotemporal tissue organization. *Nat. Mater.* 22, 117–127.
- Etournay, R., Popovic, M., Merkel, M., Nandi, A., Blasse, C., Aigouy, B., Brandl, H., Myers, G., Salbreux, G., Julicher, F., Eaton, S., 2015. Interplay of cell dynamics and epithelial tension during morphogenesis of the *Drosophila* pupal wing. *Elife* 4, e07090.
- Fagotto, F., 2023. Getting reshaped by the building of your substrate: extracellular matrix assembly joins the morphogenesis toolkit. *Dev. Cell* 58, 823–824.
- Forgacs, G., Jaikaria, N.S., Frisch, H.L., Newman, S.A., 1989. Wetting, percolation and morphogenesis in a model tissue system. *J. Theor. Biol.* 140, 417–430.
- Freund, L.B., Suresh, S., 2009. *Thin Film Materials: Stress, Defect Formation, and Surface Evolution*, xviii. Cambridge University Press, Cambridge, England ; New York (750 p. pp).
- Futaki, S., Horimoto, A., Shimono, C., Norioka, N., Taniguchi, Y., Hamaoka, H., Kaneko, M., Shigeta, M., Abe, T., Sekiguchi, K., Kondo, Y., 2023. Visualization of basement membranes by a nitrogen-based fluorescent reporter in mice. *Matrix Biol. Plus*. 18, 100133.
- Gao, B., Song, H., Bishop, K., Elliot, G., Garrett, L., English, M.A., Andre, P., Robinson, J., Sood, R., Minami, Y., Economides, A.N., Yang, Y., 2011. Wnt signaling gradients establish planar cell polarity by inducing *Vangl2* phosphorylation through *Ror2*. *Dev. Cell* 20, 163–176.
- Green, J.B.A., 2022. Resolving morphogenesis into quantifiable cell behaviours. *Development* 149.
- Haigo, S.L., Bilder, D., 2011. Global tissue revolutions in a morphogenetic movement controlling elongation. *Science* 331, 1071–1074.
- Harmansa, S., Erlich, A., Eloy, C., Zurlo, G., Lecuit, T., 2023. Growth anisotropy of the extracellular matrix shapes a developing organ. *Nat. Commun.* 14, 1220.
- Harunaga, J.S., Doyle, A.D., Yamada, K.M., 2014. Local and global dynamics of the basement membrane during branching morphogenesis require protease activity and actomyosin contractility. *Dev. Biol.* 394, 197–205.
- Hohenester, E., Yurchenco, P.D., 2013. Laminins in basement membrane assembly. *Cell Adhes. Migr.* 7, 56–63.
- Horne-Badovinac, S., 2020. Mobilizing the matrix for organ morphogenesis. *Dev. Cell* 54, 1–2.
- Hughes, A.J., Miyazaki, H., Coyle, M.C., Zhang, J., Laurie, M.T., Chu, D., Vavrusova, Z., Schneider, R.A., Klein, O.D., Gartner, Z.J., 2018. Engineered tissue folding by mechanical compaction of the mesenchyme. *Dev. Cell* 44 (165–178), e166.
- Ihara, S., Hagedorn, E.J., Morrissey, M.A., Chi, Q., Motegi, F., Kramer, J.M., Sherwood, D.R., 2011. Basement membrane sliding and targeted adhesion remodels tissue boundaries during uterine-vulval attachment in *Caenorhabditis elegans*. *Nat. Cell Biol.* 13, 641–651.
- Isabella, A.J., Horne-Badovinac, S., 2016. Rab10-mediated secretion synergizes with tissue movement to build a polarized basement membrane architecture for organ morphogenesis. *Dev. Cell* 38, 47–60.
- Iyer, K.V., Piscitello-Gomez, R., Pajimans, J., Julicher, F., Eaton, S., 2019. Epithelial viscoelasticity is regulated by mechanosensitive E-cadherin turnover. *Curr. Biol.* 29 (578–591), e575.
- Jayadev, R., Sherwood, D.R., 2016. Tissue sculpting by fibrils. *Dev. Cell* 38, 1–3.

- Karkali, K., Tiwari, P., Singh, A., Tlili, S., Jorba, I., Navajas, D., Munoz, J.J., Saunders, T.E., Martin-Blanco, E., 2022. Condensation of the *Drosophila* nerve cord is oscillatory and depends on coordinated mechanical interactions. *Dev. Cell* 57 (867–882), e865.
- Keeley, D.P., Hasteie, E., Jayadev, R., Kelley, L.C., Chi, Q., Payne, S.G., Jeger, J.L., Hoffman, B.D., Sherwood, D.R., 2020. Comprehensive endogenous tagging of basement membrane components reveals dynamic movement within the matrix scaffolding. *Dev. Cell* 54 (60–74), e67.
- Ku, H.Y., Harris, L.K., Bilder, D., 2023. Specialized cells that sense tissue mechanics to regulate *Drosophila* morphogenesis. *Dev. Cell* 58 (211–223), e215.
- Kubow, K.E., Vukmirovic, R., Zhe, L., Klotzsch, E., Smith, M.L., Gourdon, D., Luna, S., Vogel, V., 2015. Mechanical forces regulate the interactions of fibronectin and collagen I in extracellular matrix. *Nat. Commun.* 6, 8026.
- Kurpios, N.A., Ibanes, M., Davis, N.M., Lui, W., Katz, T., Martin, J.F., Izpisua Belmonte, J.C., Tabin, C.J., 2008. The direction of gut looping is established by changes in the extracellular matrix and in cell-cell adhesion. *Proc. Natl. Acad. Sci. U. S. A.* 105, 8499–8506.
- Kyprianou, C., Christodoulou, N., Hamilton, R.S., Nahaboo, W., Boomgaard, D.S., Amadei, G., Migeotte, I., Zernicka-Goetz, M., 2020. Basement membrane remodelling regulates mouse embryogenesis. *Nature* 582, 253–258.
- Lane, M.C., Koehl, M.A., Wilt, F., Keller, R., 1993. A role for regulated secretion of apical extracellular matrix during epithelial invagination in the sea urchin. *Development* 117, 1049–1060.
- Li, Y., Trivedi, V., Truong, T.V., Koos, D.S., Lansford, R., Chuong, C.M., Warburton, D., Moats, R.A., Fraser, S.E., 2015. Dynamic imaging of the growth plate cartilage reveals multiple contributors to skeletal morphogenesis. *Nat. Commun.* 6, 6798.
- Loeb, L., 1920. A comparative study of the mechanism of wound healing. *J. Med. Res.* 41, 247–281.
- Loganathan, R., Rongish, B.J., Smith, C.M., Fillia, M.B., Czirok, A., Benazeraf, B., Little, C.D., 2016. Extracellular matrix motion and early morphogenesis. *Development* 143, 2056–2065.
- Malhotra, N., Kundabala, M., Shashirashmi, A., 2010. Strategies to overcome polymerization shrinkage—materials and techniques. A review. *Dent. Update* 37, 115–118 (120–112, 124–115).
- Masic, A., Bertinetti, L., Schuetz, R., Chang, S.W., Metzger, T.H., Buehler, M.J., Fratzl, P., 2015. Osmotic pressure induced tensile forces in tendon collagen. *Nat. Commun.* 6, 5942.
- Matsubayashi, Y., Louani, A., Dragu, A., Sanchez-Sanchez, B.J., Serna-Morales, E., Yolland, L., Gyoergy, A., Vizcay, G., Fleck, R.A., Heddleston, J.M., Chew, T.L., Siekhaus, D.E., Stramer, B.M., 2017. A moving source of matrix components is essential for de novo basement membrane formation. *Curr. Biol.* 27 (3526–3534), e3524.
- Matsubayashi, Y., Sanchez-Sanchez, B.J., Marcotti, S., Serna-Morales, E., Dragu, A., Diaz-de-la-Loza, M.D., Vizcay-Barrena, G., Fleck, R.A., Stramer, B.M., 2020. Rapid homeostatic turnover of embryonic ECM during tissue morphogenesis. *Dev. Cell* 54 (33–42), e39.
- Medawar, P., 1980. Michael Abercrombie, 14 August 1912–28 May 1979. In: *Biographical Memoirs of Fellows of the Royal Society*, 26, pp. 1–15.
- Michaut, A., Mongera, A., Gupta, A., Serra, M., Rigoni, P., Lee, J., Duarte, F., Hall, A., Mahadevan, A., Guevorkian, K., Pourquie, O., 2022. Activity-driven Extracellular Volume Expansion Drives Vertebrate Axis Elongation. *bioRxiv*.
- Morgner, J., Bornes, L., Hahn, K., Lopez-Iglesias, C., Kroese, L., Pritchard, C.E.J., Vennin, C., Peters, P.J., Huijbers, I., van Rheenen, J., 2023. A Lamb1Dendra2 mouse model identifies basement-membrane-producing origins and dynamics in PyMT breast tumors. *Dev. Cell* 58 (535–549), e535.
- Morris, J.L., Cross, S.J., Lu, Y., Kadler, K.E., Lu, Y., Dallas, S.L., Martin, P., 2018. Live imaging of collagen deposition during skin development and repair in a collagen I-GFP fusion transgenic zebrafish line. *Dev. Biol.* 441, 4–11.
- Munjal, A., Hannezo, E., Tsai, T.Y., Mitchison, T.J., Megason, S.G., 2021. Extracellular hyaluronate pressure shaped by cellular tethers drives tissue morphogenesis. *Cell* 184 (6313–6325), e6318.
- Nelson, C.M., 2016. On Buckling Morphogenesis. *J. Biomech. Eng.* 138, 021005.
- Nerger, B.A., Jaslove, J.M., Elashai, H.E., Mao, S., Kosmrlj, A., Link, A.J., Nelson, C.M., 2021. Local accumulation of extracellular matrix regulates global morphogenetic patterning in the developing mammary gland. *Curr. Biol.* 31 (1903–1917), e1906.
- Newman, S., Cloitre, M., Allain, C., Forgacs, G., Beyens, D., 1997. Viscosity and elasticity during collagen assembly in vitro: relevance to matrix-driven translocation. *Biopolymers* 41, 337–347.
- Newman, S.A., Frenz, D.A., Tomasek, J.J., Rabuzzi, D.D., 1985. Matrix-driven translocation of cells and nonliving particles. *Science* 228, 885–889.
- Newman, S.A., Frenz, D.A., Hasegawa, E., Akiyama, S.K., 1987. Matrix-driven translocation: dependence on interaction of amino-terminal domain of fibronectin with heparin-like surface components of cells or particles. *Proc. Natl. Acad. Sci. U. S. A.* 84, 4791–4795.
- Olofsson, B., Page, D.T., 2005. Condensation of the central nervous system in embryonic *Drosophila* is inhibited by blocking hemocyte migration or neural activity. *Dev. Biol.* 279, 233–243.
- Pastor-Pareja, J.C., Xu, T., 2011. Shaping cells and organs in *Drosophila* by opposing roles of fat body-secreted collagen IV and perlecan. *Dev. Cell* 21, 245–256.
- Qin, X., Park, B.O., Liu, J., Chen, B., Choesmel-Cadamuro, V., Belguise, K., Heo, W.D., Wang, X., 2017. Cell-matrix adhesion and cell-cell adhesion differentially control basal myosin oscillation and *Drosophila* egg chamber elongation. *Nat. Commun.* 8, 14708.
- Ray, R.P., Matamoro-Vidal, A., Ribeiro, P.S., Tapon, N., Houle, D., Salazar-Ciudad, I., Thompson, B.J., 2015. Patterned Anchorage to the apical extracellular matrix defines tissue shape in the developing appendages of *Drosophila*. *Dev. Cell* 34, 310–322.
- Revell, C.K., Jensen, O.E., Shearer, T., Lu, Y., Holmes, D.F., Kadler, K.E., 2021. Collagen fibril assembly: new approaches to unanswered questions. *Matrix Biol. Plus* 12, 100079.
- Revell, C.K., Herrera, J.A., Lawless, C., Lu, Y., Kadler, K.E., Chang, J., Jensen, O.E., 2023. Modeling collagen fibril self-assembly from extracellular medium in embryonic tendon. *Biophys. J.* 122, 3219–3237.
- Rozario, T., DeSimone, D.W., 2010. The extracellular matrix in development and morphogenesis: a dynamic view. *Dev. Biol.* 341, 126–140.
- Rupp, P.A., Czirok, A., Little, C.D., 2004. alphavbeta3 integrin-dependent endothelial cell dynamics in vivo. *Development* 131, 2887–2897.
- Sakar, M.S., Baker, B.M., 2018. Engineering control over 3D morphogenesis by tissue origami. *Dev. Cell* 44, 131–132.
- Santillan, K., Dahmann, C., Fischer-Friedrich, E., 2022. Hydrostatic Presser and Lateral Actomyosin Tension Control Stretch and Tension of the Basement Membrane in Epithelia. *bioRxiv*.
- Serna-Morales, E., Sanchez-Sanchez, B.J., Marcotti, S., Nichols, A., Bhargava, A., Dragu, A., Hirvonen, L.M., Diaz-de-la-Loza, M.D., Mink, M., Cox, S., Rayfield, E., Lee, R.M., Hobson, C.M., Chew, T.L., Stramer, B.M., 2023. Extracellular matrix assembly stress initiates *Drosophila* central nervous system morphogenesis. *Dev. Cell* 58 (825–835), e826.
- Sivakumar, A., Mahadevan, A., Lauer, M.E., Narvaez, R.J., Ramesh, S., Demler, C.M., Souchet, N.R., Hascall, V.C., Midura, R.J., Garantziotis, S., Frank, D.B., Kimata, K., Kurpios, N.A., 2018. Midgut laterality is driven by hyaluronan on the right. *Dev. Cell* 46 (533–551), e535.
- Soans, K.G., Ramos, A.P., Sidhaye, J., Krishna, A., Solomatina, A., Hoffmann, K.B., Schlussler, R., Guck, J., Sbalzarini, I.F., Modes, C.D., Norden, C., 2022. Collective cell migration during optic cup formation features changing cell-matrix interactions linked to matrix topology. *Curr. Biol.* 32 (4817–4831), e4819.
- Spurlin, J.W., Siedlik, M.J., Nerger, B.A., Pang, M.F., Jayaraman, S., Zhang, R., Nelson, C.M., 2019. Mesenchymal proteases and tissue fluidity remodel the extracellular matrix during airway epithelial branching in the embryonic avian lung. *Development* 146.
- Sui, L., Alt, S., Weigert, M., Dye, N., Eaton, S., Jug, F., Myers, E.W., Julicher, F., Salbreux, G., Dahmann, C., 2018. Differential lateral and basal tension drive folding of *Drosophila* wing discs through two distinct mechanisms. *Nat. Commun.* 9, 4620.
- Sussman, M.V., Katchalsky, A., 1970. Mechanocalchemical turbine: a new power cycle. *Science* 167, 45–47.
- Syed, Z.A., Bouge, A.L., Byri, S., Chavoshi, T.M., Tang, E., Bouhin, H., van Dijk-Hard, I.F., Uv, A., 2012. A luminal glycoprotein drives dose-dependent diameter expansion of the *Drosophila melanogaster* hindgut tube. *PLoS Genet.* 8, e1002850.
- Tajiri, R., Ogawa, N., Fujiwara, H., Kojima, T., 2017. Mechanical control of whole body shape by a single cuticular protein obstructor-E in *Drosophila melanogaster*. *PLoS Genet.* 13, e1006548.
- Tajiri, R., Fujiwara, H., Kojima, T., 2021. A corset function of exoskeletal ECM promotes body elongation in *Drosophila*. *Commun. Biol.* 4, 88.
- Topfer, U., Guerra Santillan, K.Y., Fischer-Friedrich, E., Dahmann, C., 2022. Distinct contributions of ECM proteins to basement membrane mechanical properties in *Drosophila*. *Development* 149.
- Tsoboi, A., Fujimoto, K., Kondo, T., 2023. Spatiotemporal remodeling of extracellular matrix orients epithelial sheet folding. *Sci. Adv.* 9, eadh2154.
- Urbano, J.M., Torgler, C.N., Molnar, C., Tepass, U., Lopez-Varea, A., Brown, N.H., de Celis, J.F., Martin-Bermudo, M.D., 2009. *Drosophila* laminins act as key regulators of basement membrane assembly and morphogenesis. *Development* 136, 4165–4176.
- Van de Bor, V., Zimniak, G., Papone, L., Cerezo, D., Malbouyres, M., Juan, T., Ruggiero, F., Noselli, S., 2015. Companion blood cells control ovarian stem cell niche microenvironment and homeostasis. *Cell Rep.* 13, 546–560.
- Walma, D.A.C., Yamada, K.M., 2020. The extracellular matrix in development. *Development* 147.
- Watts, G.T., Grillo, H.C., Gross, J., 1958. Studies in wound healing: II. The role of granulation tissue in contraction. *Ann. Surg.* 148, 153–160.
- Weigert, R., Porat-Shliom, N., Amornphimoltham, P., 2013. Imaging cell biology in live animals: ready for prime time. *J. Cell Biol.* 201, 969–979.
- Wolpert, L., 2019. *Principles of Development*. Oxford University Press, Oxford, United Kingdom, New York, NY.
- Yamaguchi, N., Zhang, Z., Schneider, T., Wang, B., Panizzo, D., Knaut, H., 2022. Rear traction forces drive adherent tissue migration in vivo. *Nat. Cell Biol.* 24, 194–204.
- Yurchenco, P.D., Furthmayr, H., 1984. Self-assembly of basement membrane collagen. *Biochemistry* 23, 1839–1850.
- Yurchenco, P.D., Tsilibary, E.C., Charonis, A.S., Furthmayr, H., 1986. Models for the self-assembly of basement membrane. *J. Histochem. Cytochem.* 34, 93–102.
- Zamir, E.A., Czirok, A., Cui, C., Little, C.D., Rongish, B.J., 2006. Mesodermal cell displacements during avian gastrulation are due to both individual cell-autonomous and convective tissue movements. *Proc. Natl. Acad. Sci. U. S. A.* 103, 19806–19811.
- Zamir, E.A., Rongish, B.J., Little, C.D., 2008. The ECM moves during primitive streak formation—computation of ECM versus cellular motion. *PLoS Biol.* 6, e247.