Review

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On folding morphogenesis, a mechanical problem

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Tissue folding is a fundamental process that sculpts a simple flat epithelium into a complex three-dimensional organ structure. Whether it is the folding of the brain, or the looping of the gut, it has become clear that to generate an invagination or a fold of any form, mechanical asymmetries must exist in the epithelium. These mechanical asymmetries can be generated locally, involving just the invaginating cells and their immediate neighbours, or on a more global tissue-wide scale. Here, we review the different mechanical mechanisms that epithelia have adopted to generate folds, and how the use of precisely defined mathematical models has helped decipher which mechanisms are the key driving forces in different epithelia.

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1. Tissue folding: a mechanical process

Like an origami crane emerging from a flat sheet of paper, epithelia must fold during development to generate complex three-dimensional organ structures. Multiple studies have investigated the biochemical signalling pathways that are required to generate an invagination or a fold [1,2]. However, folding is inherently a physical process, requiring mechanical force asymmetries in an epithelium, especially between the apical and basal surfaces, to generate an invagination or a fold. In this review, we deliberately refrain from a detailed discussion of the different biochemical pathways that are upstream of fold formation, and focus on the mechanical aspects of the fold driving process. This is to put biological tissues in the context of the physical world they inhabit, moving away from the biochemical pathway abstraction.

An emerging picture is that the mechanical forces driving fold formation can occur at a local scale, involving just the cells in and around the fold, or on a more global tissue-wide scale. As we move towards a more holistic understanding of how tissues are sculpted, and the complex interplay between local and global forces, there is an increasing need to use mathematical models to capture the global mechanical properties of the tissue, as well as the local cellular behaviours. In this review, we discuss how different local and global force asymmetries are generated in different tissues, to form precise folds, and how mathematical modelling approaches, in particular computational models, have been used to reveal the key force generating cellular behaviours.

2. Local active mechanisms: forces from the folding cells

The most direct way of initiating a tissue fold is via active forces generated by the cells forming the indentation. These local mechanisms range through constriction or relaxation of cell surfaces, cell shape changes and extracellular matrix (ECM) modifications, occurring in different combinations. Relative constriction of the invaginating concave surface is embedded in the geometric definition of a fold. Here we make the distinction that the cells of the folding region can directly apply forces to bring about the shape change, thereby referring to these as local active mechanisms.

Figure 1. Local active mechanisms driving fold formation. (a) A combination of apical constriction, lateral shortening and basal relaxation, together with cell volume conservation, drives folds, in tissues such as the *Drosophila* embryo, during ventral furrow formation. Similar mechanisms also occur in generating some of the folds of the wing disc. (b) Apical constriction and apoptosis drive fold formation in the *Drosophila* leg disc. (c) Apical junction shifting followed by cell shortening drives folding in the dorsal folds of *Drosophila* embryos.

Since the early observations of bottle cells in avian gastrulation [3], apical constriction via accumulation of non-muscle myosin II (myosin) has been central in tissue folding research, and is arguably the most extensively studied [3–6]. A classic example demonstrating apical constriction is the formation of the ventral furrow during *Drosophila melanogaster* gastrulation, which has been extensively reviewed [7,8]. In ventral furrow formation, the thin strip of prospective furrow cells accumulates myosin on their apical surface, with a gradient that is highest at the centre of the strip. Myosin leads to apical constriction with pulsatile contractions [9–12]. This is followed by an initial cell elongation, then cell shortening and basal expansion to fully form the ventral furrow ([10], figure 1a).

Mathematical models can be used to reveal mechanisms that are hard to access experimentally. Understanding of the mechanisms driving tissue folding has benefited immensely from different computational models. Mechanical models have been used to reverse-engineer the cellular mechanical states required to generate the cell shape changes to form a fold. Two-dimensional vertex models of the cross-section demonstrated active apical constriction, coupled with volume conservation of cells and higher tensile forces on the lateral sides relative to basal side are sufficient to capture ventral furrow geometries for healthy and mutant tissues [6,13]. These models allow the mechanical sequence of events: once apical constriction starts reducing the apical area of invaginating cells, an initial elongation of the cells compensates for the accompanying area reduction when the lateral and basal tensile forces are comparable. As the basal stiffness is gradually reduced or the lateral tension increased, it becomes more energetically favourable to expand the basal surface and shorten the cells into the characteristic wedge shape, forming the correct furrow geometry. The emerging shape is fine-tuned with the gradient of myosin accumulation, which is responsible for the sharp curvature of the emerging furrow [9]. Three-dimensional computational modelling has further identified flows in the yolk accompanying the furrow formation, which can link local mechanics of the furrow-forming cells to embryo-scale mechanical signals [14]. The importance of

cytoplasmic flows in promoting global cell shape changes was later experimentally verified [15], showing the power of computational modelling predictions. These studies have not only thoroughly deciphered the mechanics of individual cellular sub-domains, they have also demonstrated that generation of a single epithelial fold requires orchestration of complex dynamics on each of the apical, basal and lateral surfaces. Indeed, this coupling of apical and basolateral mechanisms is a recurring theme that we see in all forms of fold formation.

During the larval development of *Drosophila*, the leg imaginal disc forms multiple folds, which mark the future segment joints of the adult fly leg [16]. The fold formation depends on mechanical forces generated by both apical constriction and apoptosis in an interweaved machinery [16,17]. The apoptotic cells generate myosin cables spanning the apical–basal height of the cell, which transiently pull the apical surface inwards (figure 1b). Although this transient indentation is released upon fragmentation of the apoptotic cell, the apoptotic event induces stabilized actomyosin accumulation in its neighbours. With each apoptotic cell, the apically constricted cells spread, to form a ring spanning the entire tissue circumference, inducing the leg fold. When tested in a vertex model, this sequence of events reveals that both the transient pulling forces of the apoptotic cells, and their apical constriction stabilization on their neighbours, are necessary for successful fold formation, neither mechanism succeeding individually. Modelling also suggests a threshold density of apoptotic cells, below which folds cannot be induced. In a well rounded study, this threshold effect has also been experimentally validated by induction of ectopic folding in the *Drosophila* wing imaginal disc [17]. The apoptotic machinery of the leg disc, while inducing the pulling forces, also transiently increases the density of its apical junctions, potentially to resist tissue rupture against the increased forces, bringing our attention to the cell–cell junctions.

While the active force generation by myosin motors can constrict the tissue, one fundamental pre-requisite of this mechanism is that the tissue maintains its integrity under the applied forces. The contractile forces generated by individual cells are extended to a tissue-scale morphogenetic event via the

connectivity of the cells, through cell–cell junctions [18,19]. Beyond this fundamental yet passive role, cell–cell junctions can also take on more active roles in fold formation. During *Drosophila* gastrulation, dorsal folds of the embryo are formed with junctional shifting followed by cell shortening [20]. In a mechanism independent of asymmetric myosin accumulation, apical junctions of the thin strip of cells of the prospective dorsal fold start lowering of the cell surface (figure 1c). Since the cells of the epithelia are connected through their adherens junctions (AJ), that immediately causes a sheer deformation of the neighbouring cells with apically positioned AJ. The uncharacteristically large apical domain of the initiating cells then collapses down by weakening of the microtubule scaffold holding the cell apical dome [20,21]. This leads to cell shortening and creates an apical invagination. The deformation of the neighbouring cells is then relieved in bending of the epithelium, further establishing the apical fold.

Multiple similar basolateral regulatory mechanisms indeed can act independently of apical constriction, coupled with higher-scale mechanisms. During the larval stage of *Drosophila* development, wing imaginal disc forms three folds spanning the anterior–posterior width of the tissue. There are multiple tissue-scale mechanical regulators of these folds, which we will cover in following sections [22]. At the local scale, lateral constriction, the consequent cell shortening and basement membrane (BM) modulation facilitate these folds [23–25]. The lateral constriction observed in the central hinge–hinge fold is driven by an active tension increase as measured in laser ablations and is concurrent with increased filamentous actin. BM degradation with release of metalloproteases facilitates a relaxation of the basal side for the pouch–hinge fold. In a close analogy to apical constriction, basal relaxation of tension coupled to volume conservation then allows for folding from the apical surface [23,24].

The relationship between the cells and the BM is evident in other active mechanisms driven by the basal surface of the tissue. Fold formation in development appears to be overwhelmingly dominated by apical indentations. Yet, there are cases where active basal constrictions have been identified for tissue folding through basal indentations, such as in optic cup morphogenesis [26,27] and midbrain–hindbrain boundary formation in zebrafish [28]. Actomyosin-driven basal constriction is a main driver in optic cup folding [27,29]. The mid–hind brain boundary formation combines multiple driving mechanisms to indent the basal surface. First, cells shorten with myosin IIA activity. This is followed by microtubule and myosin IIB-dependent basal constriction, and finally a passive apical expansion phase which is thought to be a mechanical consequence of basal constriction [28,30–32]. A key component of the BM, laminin, as well as the focal adhesion complex and integrins, are required for basal constriction [26,28,30,33]. In a conceptual analogy to the AJ of the apical surface, both the integrity of the BM and the adhesion machinery between the cells and the BM are essential for functional basal constriction.

In an approach analogous to basal constriction, it has been shown that epithelia can be folded by patterned compaction of the ECM by mesenchymal cells [34]. In the current set-up, complex three-dimensional structures, from cubes to helices, can be constructed. Albeit still being on the abstract geometrical end, these three-dimensional structures strongly point to the future possibilities in constructing complex tissue architectures *in vivo*.

This approach also introduces us to a slight conceptual shift: while the acting forces are still local to the invaginating region, the cell population that is the source of the active mechanism is distinct from the displaced cells. In the above *in vitro* set-up, the active mesenchymal cells bend the passive ECM and the connected epithelia. Similar active mechanisms *in vivo* have recently been reviewed [35], where cells use protrusions to displace their immediate neighbours into forming invaginations in vertical telescoping, or stratified epithelia buckling with suprabasal intercalations. This detail on the source of the active mechanisms brings us closer to our next scale of interest, where the local surroundings of the folding region can provide the active forces.

3. Neighbouring active mechanisms: forces from surrounding cells

Zooming slightly out of the immediate fold-forming cells, the mechanics of the surrounding tissue can act as a driver for fold formation. Here, the surrounding tissue is still defined as being relatively local to the fold neighbourhood, rather than at the whole-tissue scale, which will be discussed in the next section. In all the scenarios we investigate, the invaginating cells and the surrounding tissue have spatially segregated but temporally aligned active mechanisms, most likely regulated via upstream biochemical signalling. Yet the coupling of the two, the signal transmitted from the surrounding tissue to the folding cells, is a mechanical one.

In the *Drosophila* embryo, salivary gland formation starts by invagination of an oval patch of cells, the salivary gland placode, from the surface of the embryo [36]. Similar to ventral furrow formation, the invaginating cells are apically constricted, by accumulation of myosin and pulsatile contractions [36,37]. Yet unlike ventral furrow formation, the apical constriction has been shown to be expendable for salivary gland formation [38]. In the absence of apical constriction, the gland can successfully be internalized, albeit with some deformation in the shape of the invaginating patch. By contrast, a supra-cellular actomyosin cable that surrounds the placode (figure 2a) exerts compressive forces essential for invagination [38,39]. Here, the existence of apical constriction, and the ability to invaginate from the correct patch upon its elimination, highlights multiple local active mechanisms that select the placode cells for invagination. Still, the compressive forces driven by the activity of surrounding tissue are an essential driver of folding. The compressive forces of such supra-cellular myosin cables are indeed recurrent in folding morphogenesis of many organs [40].

In a very similar topology, tracheal placode of *Drosophila* also invaginates from the embryo surface, in two distinct phases: the slow and fast phase [41]. The slow phase demonstrates apical constriction of central cells [42] and cell intercalations at the boundaries of the forming pit [43]. The boundary cells also form a compressive ring with increased myosin, which is likely to be driving the central apical constriction [44]. In a mechanical and philosophical opposite of the apoptotic forces seen in leg disc folding, the following fast phase of tracheal placode invagination coincides with mitotic entry in the central cells. This mitosis is distinct from the apical cell rounding observed in epithelia [45] such that cells round internally. It has been identified that it is the rounding, and not the mitosis, that drives this fast invagination. The BM

Figure 2. Neighbouring active mechanisms driving fold formation. (a) In *Drosophila* salivary gland formation, the folding force primarily comes from the surrounding actomyosin cable that compresses the invaginating cells. Apical constriction of the invaginating cells is not essential. (b) During lung branching morphogenesis, smooth muscle wraps around the growing buds to constrict and fold the epithelia into shape. Without smooth muscle, the lung epithelium folds randomly as it grows against the mesenchyme.

is also expendable in this scenario. As the cells start rounding from their columnar structure, they acquire a larger surface area while shortening the tissue. It is thought that, coupled with compression of the invaginating pit via the contractile forces of the myosin ring at the boundary, it becomes energetically more favourable to relax the surface requirement by buckling the tissue [44]. Mutants suggest both mechanisms can eventually drive the invagination individually, albeit slowly. Therefore while there is redundancy in the system, it demonstrates a coupling of the active shape changes of the invaginating cells with the compressive forces emerging from the surrounding tissue. It is important to note that further physical property alterations of the cells during mitotic rounding could also prime these cells for faster invagination [35].

The external constriction of epithelia to regulate folding emerges as a mechanical solution not only to induce folds, but also to regulate the emergent fold topology. During lung development, the complex branched structure is achieved by repetitive formation of new sequential buds on the epithelial tubes; their bifurcation then grows into branches, which are used to generate new buds [46]. Each of these newly formed buds and its bifurcations is essentially an epithelial folding event. It has been shown that the bifurcation is regulated by wrapping of smooth muscle around the growing buds, constricting the growing epithelia into shape (figure 2b). In the absence of smooth muscle, the lung epithelia still induce folds, in the form of random wrinkles. These are due to the restricted growth of the epithelia against the embedding mesenchyme, as demonstrated experimentally and explained with a computational continuum model [47,48], which was instrumental in revealing the mechanism of folding. Indeed, as forces move away from the immediate local environment of the fold, computational modelling becomes more and more important in deciphering the forces that drive folding. Beyond demonstrating a clear mechanical regulation by constriction in the formation of branched structures, lung development also exemplifies the role of differential growth and physical rules of wrinkling in folding morphogenesis. Indeed, differential growth, planar or along the apical–basal axis, is a tissue-scale mechanism to regulate folding, which is the focus of the next section.

4. Global mechanisms

In the mechanical regulation of folding morphogenesis via differential growth, residual strains emerge as the tissue accommodates the size differences accumulating between neighbouring fast- and slow-growing regions, and drive three-dimensional shape changes [49]. Through the following case studies of differential growth, macro-level tissue properties will emerge as influencers, such as the overall size and shape of the tissue, or the influence of encapsulating bodies such as the skull in brain folding. Unlike local properties or specific gene expressions, these properties are extremely unyielding to experimental manipulation. Therefore, tissue-scale computational modelling becomes the main guide in our understanding of folding morphogenesis with differential growth at the global scale.

(a) Differential growth in different layers of the tissue

In animal tissues, the differential growth of composite tissue layers is a well established folding mechanism, demonstrated in detail for the gut. At a macro-level, the looping of the whole organ is explained by the fast growth of gut tissue on top of the slow-growing mesentery (figure 3c), as demonstrated by experimentation, and explained by physical and computational modelling [50]. Its inner surface morphology, formation of villi, is governed by a series of oriented differential growth patterns [51]. During the development of the chick gut, three distinct smooth muscle layers form around the tubular epithelial layer, inducing its different folding stages. The first smooth muscle layer to form has circumferential orientation, and as expected, induces longitudinal ridges in the epithelial layer (figure 3d). Next, two layers of longitudinal smooth muscles form first outside then inside the circumferential layer. These two layers, respectively, transform the epithelial ridges into zigzags, and the zigzags into villi, both steps agreeing with increasing compression in the longitudinal direction. In line with the observations in plant leaves (see §4b [52]), the relief of the constraint of the muscle layers, by inhibiting their development at each stage, relaxes corresponding morphological change in the epithelia, therefore exemplifying residual

Figure 3. Differential growth as a global mechanism that drives tissue folding. (a) In simple one dimension, differential expansion of two connected layers will cause bending. (b) In two dimensions, differential growth between different regions or layers of connected material will cause wrinkling or doming. (c–e) Real examples of differential growth in different layers of the tissue causing folding include the looping of the gut (c), the formation of villi in the gut (d) and the folds in the brain (e).

stresses driving tissue shape formation. Importantly, the formation of the epithelial folding resulting in the formation of the villi is not dependent on the active contractile forces generated by the muscles, but due to the slower growth of muscles, which compresses the epithelia. Indeed, surgical separation of the muscle layer from the epithelia leads to relaxation of both layers. Computational modelling of the folding of the gut, with inclusion of directional differential growth in the measured scales, confirms this compression is sufficient for the emergence of observed folding morphogenesis [51]. The chick gut represents the distinct phases of villi formation at consecutive steps, but the animal kingdom offers an exquisite variability in the dynamics and form of its gut surface. In the mouse, the intermediate steps are skipped, as multiple muscle layers develop rapidly in a short time window. *Xenopus* comes short of the third muscle layer, and has zigzag villi [51]. In all cases, the physics of differential growth are fundamental to the emergence of the variable forms. Indeed, similar differential growth profiles explain the folded surface of other tubular epithelia, such as the trachea and oesophagus [51].

In the gut, the folding is a result of connected but differential growth of explicitly distinct tissue layers, yet this does not need to be the case for all folding organs. Another extensively studied folded organ, the brain, is thought to fold its cortex with differential faster growth of the cortical layer with respect to the underlying white matter (figure 3e). Simulations of

brain as soft matter with a relatively expanding cortex can successfully generate a range of brain morphologies, from the small smooth brains of rats to highly convoluted humans.

There are empirical observations relating on the volumes of grey matter and white matter and the thickness of the cortical layer that hold true inter-species and through spatial variation within the large human brain [53,54]. These conserved relationships combined with cortical thickness measurements leave only one free variable in the system, the ratio of the cortex thickness to the size of the brain. With these experimental bounds, simulations predict there is a brain size limit below which folds do not form, and it is at the scale of the smooth rat brains. As the brains get progressively larger, the folding increases, such as in the human brain [54]. In pathology, models demonstrate the experimentally observed thinning of the cortical layer in polymicrogyria is sufficient to induce the increased folding phenotype, and implementing the known reduction in cortical growth explains the reduction in folding in lissencephalia [55]. In details of topology, as opposed to being a once hypothesized driver of cortical folding, the spatial constraints of the skull alter the morphology of the emerging folds, flattening the tops of gyri, without influencing their emergence. As the brain shape deviates from a sphere, the folds start following the long axis, which is exemplified in ferret brain [56]. In a key experimental test of the cortical expansion model, swelling gels in the shape of developing brains

successfully reproduce position and orientation of multiple major sulci [57]. Still, the precise positioning of the finer folds have not been accessible, and correct ratios of different cell types are shown to be essential for cortical folding [58]. There possibly are further finer heterogeneities that currently elude us and fine-tune the highly reproducible folding pattern of the brain. A uniform growth of the cortical layer has been adapted in all discussed modelling approaches. By contrast, in modelling of the cerebellum, experimentally observed oriented growth has been shown to be sufficient to induce its distinct parallel folding structure [59]. It is therefore becoming clear that differential growth, and even minute heterogeneities in growth rates and orientations, can fine-tune emergent fold structures.

(b) Differential growth in the plane of the tissue

Visualizing the influence of differential growth on emergent shape in lower dimensions is intuitive. In a classical one-dimensional example from physics, a composite thin rod with two layers of materials with differing coefficients of thermal expansion will bend once heated, with the faster-expanding side forming the convex edge (figure 3*a*). Equally, the differential growth of a plant stem will enable its rotation towards the sun [60]. The behaviour of two-dimensional planes in nature can be exemplified in the plant domain with buckling of leaves. A growth gradient from a slow-growing leaf core to fast-growing leaf edges is sufficient to form both the saddle shape of a leaf surface, and the rippled edges [52]. The existence of the residual strains can simply be demonstrated by segmenting the leaf into strips, whereby each thin strip flattens and expands in size as it relaxes, the scale of the expansion varying with the distance to the core [52]. On top of the differential growth scale, its orientation will also determine the emergent shape. Depending on the orientation of the growth, the material to be accommodated will lie on different axes. Mathematical modelling demonstrates that in the case of a simple disc, a faster circumferential growth relative to the radial direction leads to saddle shapes, whereas a faster radial growth induces a cone shape (figure 3*b*). The former is equivalent to plant leaf behaviour, and the latter can be observed in mushroom cap formation [61]. Beyond the two dimensions, differential growth in plants has been shown to shape many complex three-dimensional structures, such as carnivorous cup leaves [62] and flowers, where differential growth patterns induce the initial folding of the bud, thereby specifying the future shape of the bloom [49]. The observations from plants show us that differential growth can directly fold and shape the final tissue, such as in leaves, or induce folds in precise positions, delivering the spatial mapping of further development by mechanical means. This positioning idea is something we will re-visit in detail in developing animal tissues.

Tissue folding is observed not only in healthy development but also in pathology, specifically with overgrowth in cancer. In the simplest example, excess local growth constraint by non-growing surrounding tissue can lead to buckling in the third dimension, inducing fold formation. A well-studied example is the ectopic folding induced in pancreatic cancer [63]. Recent work has demonstrated that in carcinogenic transformation of pancreatic cells, the apical–basal tension polarity is perturbed [64]. The wild-type tissue retains an increased apical tension, whereas the transformed cells lose this polarity, with both a reduction in apical and an increase in basal

tensions. In experimentation and a coupled computational three-dimensional vertex model, this mechanical polarity perturbation in hyper-proliferative cells is shown to be sufficient for folded lesion formation. With both analytical and vertex modelling approaches, Messal *et al.* have shown that a tissue-scale property, the diameter of the tube hosting the carcinogenic tissue patch, will determine the direction of the folding, with consequences relating directly to the prognosis of the disease. On larger ducts, ectopic folds will tend to invaginate, whereas on smaller ducts, they will tend to evaginate. The threshold of transition depends on the mechanical properties, specifically bending resistance, of the tissue. The mechanics of ductal systems such as lung and pancreas place the directionality transition diameter at approximately 20 μm , and the predicted behaviour switch, have been confirmed in experiments. The clinical significance of the observation is that the evaginating folds are better positioned for metastasis, recruiting cancer-associated fibroblasts and going through epithelial to mesenchymal transition more effectively [64]. Ability to evaginate folds in the form of new buds is the basis for the development of any branched structure, pancreas being one. Computational modelling of folding morphogenesis on the scale of the entire ductal tube made it evident cancer hijacks not only the biochemical, but also the physical and topological pathways of healthy development.

More complex patterns of differential growth on the plane of the tissue can orchestrate complex multi-folded patterns, as exemplified in the wing imaginal disc of *D. melanogaster*. The wing imaginal disc is the larval tissue that develops into the wing, the notum (back of the fly), and the hinge connecting the wing to the body. During the development of the larva, three folds emerge on the hinge region of the wing disc, compartmentalizing the tissue. Although their exact purposes are not clear, these folds form in highly reproducible and precise positions, making the wing disc an ideal system to study the emergence of complex tissue folds. It has previously been shown that there is differential growth within the pouch region of the wing disc, and this can induce morphological changes in cell shapes, stretching the slow-growing edges while compressing the fast-growing core [65]. Extending the measurement beyond the pouch, it has been shown that the wing disc demonstrates high spatial and temporal variability in its local growth rates and orientations across the entire tissue [22]. In a finite-element model of wing disc development, when combined with constraining effects of the BM and a relatively stiffer apical actomyosin layer, this differential growth is shown to determine the precise positioning of the three folds of the wing disc epithelium (figure 4). Differential growth without the mechanical heterogeneity of external constraints fails to build up sufficient strains to initiate folds, and the correct mechanical properties in the absence of growth heterogeneity drive uniform wrinkling of the tissue. The mechanical heterogeneity and external resistance generate a form of layered differential growth in addition to the planar differential growth, where the stiffer layers can generate sufficient forces to expand more than the softer layers.

The role of differential growth in the positioning of the folds is clearly demonstrated in simulations where growth rates are perturbed, with all other tissue properties being constant. The predicted perturbed morphologies of over- and undergrowth mutants are then validated in experiments, solidifying differential growth as the main driver of precise fold positioning. Modelling predicts overgrowth will induce

Figure 4. Differential growth in the plane of the tissue drives the position of folds in the *Drosophila* wing disc. A computational model (a), has shown that uniform growth gives uniform ripples in the wing (b), whereas heterogeneous growth, as measured in the real wing disc, gives the non-uniform fold topology that matches experiments (c).

ectopic folding at the boundaries of the overgrown patch, as validated by experimental overgrowth clones. By contrast, patterned undergrowth will alter the emergent fold topology, reducing the fold number from three to two, as validated via *wingless* mutants. One key observation is that the differential growth experienced by the tissue prior to formation of any observable folding is sufficient to position the folds correctly, even upon severe perturbations to the growth rates and orientations. The mechanics grant a memory to the tissue, where the stored forces can be used in precise morphogenesis further down the timeline. Together with biochemical pathways and morphogen signalling, emergent mechanics will be an inseparable part of morphogenesis, and we are now starting to understand the utilization of this aspect. Currently observed for the wing disc of the fruit fly, it is highly likely this will emerge as a fundamental mechanism in other tissues.

5. Conclusion

In this review, we have discussed the different length scales in which physical forces act to generate folds in tissues. Experimentally, we can look into the individual behaviours of cells, we can investigate the effects of immediate surroundings, and we can start to observe tissue-scale orchestration with differential growth and macro-properties of the tissue. As the length scale grows, the need for combining experiments with mathematical modelling also grows. In fact, for studying effects such as differential growth on a global scale, it is hard to find examples of purely experimental approaches. At this scale, the effects are tied in with such fundamental properties of

the tissue, for example its shape, that the explanations require holistic studies.

With the prominent appearance of tissue folds in development and disease, an understanding of the active mechanisms will strengthen our approaches in tissue engineering and diagnostics. The ability to artificially sculpt tissues would have immense significance in tissue engineering. The most direct approach to artificially govern tissue architecture would be to use local active mechanisms to bend the tissue into the desired shape. We are beginning to mimic folding behaviour in organ-on-a-chip models, and control them in organoids, through the precise design of extracellular structures [66]. By combining different forces, we can improve the accuracy of folding *in vitro* tissues, and come closer to real organ models. We should continue pushing the holistic approach, taking into account the three-dimensional nature of the folding phenomena and the long-range influences of the whole tissue. As we further increase in length scale, and begin to study how different tissues and organs interact on a more systemic level, the use of mathematical modelling to guide experimentation will become even more necessary.

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