REVIEW

doi:10.1038/nature21348

From morphogen to morphogenesis and back

Darren Gilmour[1], Martina Rembold[2]† & Maria Leptin[1,2]

A long-term aim of the life sciences is to understand how organismal shape is encoded by the genome. An important challenge is to identify mechanistic links between the genes that control cell-fate decisions and the cellular machines that generate shape, therefore closing the gap between genotype and phenotype. The logic and mechanisms that integrate these different levels of shape control are beginning to be described, and recently discovered mechanisms of cross-talk and feedback are beginning to explain the remarkable robustness of organ assembly. The 'full-circle' understanding of morphogenesis that is emerging, besides solving a key puzzle in biology, provides a mechanistic framework for future approaches to tissue engineering.

The complex 3D form of tissues, organs and organisms emerges from the coordinated behaviour of cell groups through mechanisms that are collectively termed morphogenesis (the generation of shape). With literature dating back to the Greek philosopher and scientist Aristotle[1], the study of morphogenesis pre-dates many central principles of modern biology, including cell theory and the laws of genetic inheritance. Most early work in the field did not therefore consider the role of genes or cells, but instead approached morphogenesis purely from the perspective of tissue mechanics. This school of thought, named *Entwicklungsmechanik* (developmental mechanics) by eighteenth century German embryologists, identified important roles for mechanical forces in coordinating tissue-scale behaviours, many of which are now experiencing renewed interest[2,3]. However, the mechanics-centred approach was unable to provide explanations for other fundamental processes such as tissue patterning and cell-type differentiation. Solutions for these problems were not found until the late 1970s, when Christiane Nüsslein-Volhard and Eric Wieschaus performed large-scale screens for genes that are required for early development in *Drosophila*[4]. The initial landmark publication from this herculean project revealed that the first step in morphogenesis is the subdivision of the embryo into discrete regions by a cascade of 'patterning' genes[4]. Only then is each domain converted to the corresponding region of the body through a bespoke morphogenetic program, therefore establishing that the timing, positioning and inheritance of tissue-shaping events is controlled genetically. Subsequent molecular characterization in *Drosophila* and other systems revealed that patterning genes mainly encode signalling pathways that mediate long-range tissue patterning[5,6] and gene-regulatory networks that control fate decisions; however, such genes do not control cell and tissue shape directly. Rather, the task of physically shaping cells and tissues is performed using a toolbox of essential cellular machines discovered by cell biologists, which are present in all cells in the embryo. Collectively, these studies reveal a picture in which the shape of tissues is determined by the combined actions of genetic, cellular and mechanical inputs (Box 1). Although a number of the main players are now known, and their functions understood, we still know surprisingly little about how the various levels of shape control are integrated during morphogenesis.

The focus of this Review is the logic and mechanisms that connect gene regulation, cellular effectors and tissue-scale mechanics — the troika of tissue shaping. We describe how shape, at the local level, emerges from the interaction of tissue-specific genetic inputs and the self-organizing behaviour of core intracellular machines. We then discuss how this mechanistic logic is used in several modified forms to produce a variety of shaping modes. It is becoming clear that the chain of command from gene to shape is not unidirectional, owing to the discovery of mechanisms that enable changes in tissue architecture and mechanics to feed back to 'upstream' patterning networks. The emerging integrated view of tissue shaping therefore goes full circle, from morphogen to morphogenesis and back.

Epithelial sheets as the substrate of morphogenesis

Much of our knowledge about the cellular mechanisms of morphogenesis comes from studies on epithelia, the main building block of multicellular systems. Epithelia consist of cohesive sheets of cells that are connected through cell–cell contacts such as adherens junctions. This direct coupling enables the behaviour of intracellular machines to be coordinated across many cells and facilitates the emergence of tissue-scale organization. Another characteristic that defines epithelia is a clear apicobasal polarity, established and maintained by polarity complex proteins, which enables opposing apical and basolateral plasma membrane domains to adopt distinct features. Cell polarity plays a crucial part both in epithelial organ function, in which it is required for physiological features such as polarized absorption and secretion, and in morphogenesis, in which the polarized recruitment and regulation of core adhesive and contractile machineries inside cells is the main driver of tissue shaping[7,8].

The central features of epithelial morphogenesis have been highly conserved during metazoan evolution, making it possible to integrate findings from a number of model systems. As main examples, we use the early blastoderm stage of the *Drosophila* embryo as well as a selection of vertebrate tissues. The blastoderm is a homogeneous epithelial monolayer of around 6,000 columnar cells that are formed through the subdivision of a syncytium. Because all of the cells have a similar biochemical composition and low hysteresis (the influence of previous developmental decisions on them is minimal), the blastoderm provides a uniform and naive 'canvas' on which the effects of gene expression on tissue shape can be studied efficiently[9–11].

[1]European Molecular Biology Laboratory, 69117 Heidelberg, Germany. [2]Institute for Genetics, University of Cologne, 50674 Cologne, Germany. †Present address: European Molecular Biology Organization, 69117 Heidelberg, Germany.

BOX 1

From gene to tissue shape

The molecular regulators of morphogenesis can be organized into a regulatory cascade with four distinct levels of control.

- **Global strategists** Large-scale patterning in embryos is provided by morphogens, which are molecules that can specify cell fates in a concentration-dependent manner. The concept of morphogens originates from the work of Theodor Boveri and Thomas Hunt Morgan[5], and the term morphogen was coined by Alan Turing[145]. However, the first bona fide morphogens were not identified until large-scale genetic screens were performed in *Drosophila*. In most cases, morphogens are secreted signalling proteins, such as Wingless, Decapentaplegic and fibroblast growth factor, that either form long-range concentration gradients in the extracellular environment or act as short-range signals. The mechanisms and regulation of morphogens is a topic of intense and perpetual debate in developmental biology research[6]. Morphogens do not shape cells directly; instead, they act at the top of a regulatory cascade by controlling gene-regulatory networks in discrete patterns across the embryo or developing organs.
- **Local decision makers** The role of the morphogen-activated gene-regulatory networks is to demarcate defined domains (germ layers, organ primordia or subdomains of developing organs) and to determine the fate of the cells within them. In most cases, this involves the activation of transcription factors such as Twist, Snail or Pitx3, which control cell differentiation through the establishment of tissue-specific gene-expression profiles. These 'master' regulators of tissue fate are expressed with exquisite spatiotemporal specificity and precision. The gene-regulatory networks therefore control exactly when and where a specific tissue-shaping process will take place. However, similar to morphogens, many features of the transcriptional regulators in such networks, including their localization to the nucleus, exclude them from having a direct role in tissue shaping.
- **Go-betweens** How do tissue-specific gene-regulatory networks influence cell and tissue shape? Rather than regulating the expression of the cellular machinery that shapes cells directly, such networks activate or repress the tissue-specific expression of proteins that modulate their activity in space and time. It is the precise activation of these 'go-between' proteins that enables the morphogenetic cellular machines, and therefore tissue shaping, to be placed under spatial and temporal control.
- **Movers and shakers** The diversity of tissue-shaping events involves surprisingly similar, or even identical, sets of effectors that act mostly at the cell cortex. This core toolbox includes the contractile actomyosin system, adherens junctions that facilitate cell–cell contact, and polarity complexes that provide positional information in the cell. Many of these cellular multitasking proteins are expressed by all cells at all times, which means that they are regulated mainly through non-transcriptional mechanisms. Changes in the cortical recruitment or activation of these complexes by subcellular regulators therefore determine the outcome of many morphogenetic events.

Genetic control of tissue shaping

Epithelial morphogenesis is driven by three main cellular processes: cell migration, changes in cell shape and cell rearrangements. The transition of epithelial cells into a migratory state requires a reduction in epithelial integrity and the adoption of mesenchymal features such as increased motility and reduced cell–cell adhesion. This process is termed an epithelial mesenchymal transition when the tissues are completely converted to mesenchymal cells, or collective migration when only defined 'leader' cells adopt mesenchymal features while maintaining stable cell–cell contacts with 'follower' epithelial cells[12,13]. As both epithelial mesenchymal transition and collective migration have been reviewed extensively elsewhere[14–16], here we focus on changes in cell shape and cell rearrangements, the two main mechanisms that shape sheets of cells without the considerable loss of epithelial character.

Bending tissues

Tissue bending transforms initially flat epithelial sheets into 3D folds, tubes or spheres, depending on the context. Embryological studies performed in a variety of model organisms, most notably the Amphibia, revealed that tissue bending occurs when groups of cells adopt a bottle-shaped morphology through the coordinated constriction of their apical surfaces[17,18]. A powerful model for addressing the genetic regulation of apical constriction is the formation of the *Drosophila* ventral furrow (Fig. 1). This ten-cell-wide band of cells runs along the ventral side of the embryo and invaginates to initiate the movement of the future mesoderm into the embryo's interior[19,20].

The force for apical constriction is generated by a dynamic, contractile meshwork of actomyosin that becomes enriched at the apical cortex of cells that will undergo invagination[8,21]. Cycles of mediolateral flow of actomyosin and its regulators drive apical constriction in incremental, ratchet-like steps[21–23], producing a dynamic pulsing behaviour of epithelia that was also described during the related morphogenetic process of dorsal closure in *Drosophila*[24]. Before the meshwork forms, the adherens junctions and many of their associated proteins are repositioned from a subapical position to the most apical position in the lateral membranes[25,26], where they act as anchors for the contracting apical actomyosin network[8,22,27].

The positioning and timing of ventral furrow formation is determined genetically by the local expression of two transcription factors under the control of the maternal morphogen Dorsal — the basic helix–loop–helix protein Twist, which is directly activated by Dorsal, and the zinc-finger protein Snail, which is activated by Dorsal and Twist in combination (Box 1). Twist and Snail later regulate the differentiation of the mesoderm through the activation of a large number of genes. However, their immediate effect is to control tissue folding though the expression of specific proteins that are involved in the regulation and positioning of the adherens junctions and actomyosin. These effectors are regulated by two transcriptional mechanisms (Box 1). Snail is best known as a transcriptional repressor, a function in which it downregulates ectodermal genes in the prospective mesodermal region, including those that encode DE-cadherin and the polarity complex protein Crumbs[28,29]. The early morphogenetic function of Snail is to enable the disassembly of adherens junctions — a function conserved in all animals and often associated with epithelial-mesenchymal transitions[25,30]. Curiously, the transcriptional repression of DE-cadherin is not the process that leads to the remodelling of the adherens junctions at this stage. Instead, Snail acts indirectly on the ubiquitous pool of DE-cadherin by preventing the expression of proteins from the Bearded family, which are transcriptional regulators that repress Neuralized, an ubiquitin ligase needed to disassemble subapical junctions[31]. Junctional repositioning and the assembly of the actomyosin meshwork is subsequently achieved through the Twist- and Snail-dependent transcriptional activation of four regulators, three of which function to recruit Ras-like GTP-binding protein (Rho) guanine nucleotide exchange factor (RhoGEF)2 to the apical domain[25,29,32–34]. A G-protein-coupled receptor Mist (Mesoderm-invagination signal transducer, also known as Methuselah-like 1)[34], its ligand Folded gastrulation (Fog)[8,33], which is an apically secreted peptide, and the transmembrane protein T48 (ref. 25), which directly binds the PDZ domain of RhoGEF, cooperate to accumulate RhoGEF at the apex, where it can activate Rho1 and concentrate active myosin. The fourth target of Twist, TNF-receptor-associated factor 4 (*Traf4*), is needed for the efficient apical accumulation of adherens junctions

through a poorly understood mechanism that involves the direct binding of Traf4 to β-catenin[32] (Fig. 2a and Table 1).

The invagination of the posterior midgut (Fig. 1) is driven by a similar localized apical constriction to that of the ventral furrow and is mediated by an almost identical pathway, which comprises the Fog–Concertina cassette and RhoGEF2, but not T48. Instead of Twist and Snail providing the genetic control, in this case, the proteins Huckebein and Tailless enable the same mechanism to be activated in this region of the embryo[33]. The same mediators and cellular effectors can therefore be used by different tissue-specific patterning systems to control morphogenetic processes in space and time.

Tissue folding

Apical constriction has an equally important role during vertebrate development[18], in which it drives local tissue bending during processes such as closure of the neural tube, branching morphogenesis in the lung[35] and the formation of the optic cup[36–38] that later become the eye. To achieve this, the same toolbox of cortical machines are engaged as in invertebrates, including myosin regulatory light chain (a component of Myosin II), Rho-associated coiled-coil-containing protein kinase (ROCK) and regulators of actin such as Mena and VASP (mouse orthologues of *Drosophila* Enabled) (Fig. 2b, Table 1 and Box 1). However, it seems the tissue-specific factors that are responsible for the spatial regulation of apical constriction during ventral furrow formation, such as Fog or T48, are not conserved in vertebrates. Apical constriction must therefore be under the control of a different set of genetically regulated mediators of cortical actomyosin. In vertebrates, Shroom family proteins can play the part of the mediator that connects the regulators of patterning to the cortical effectors of morphogenesis[39–41]. Their involvement in apical constriction was first demonstrated when Shroom3 was shown to be required for neural-tube closure in mice[42]. The tissue-specific expression of Shroom3 has since been shown to control apical constriction in several morphogenetic processes[43], including formation of the lens[44] and the gut[45], as well as in the lateral line primordium of zebrafish[46]. The presence of Shroom3 seems to be sufficient to trigger apical constriction, as its misexpression leads to the apicobasal lengthening of epithelial cells and the promotion of apical constriction[45]. Similar to T48 and Fog signalling in the *Drosophila* embryo, Shroom3 regulates cell shape through the polarized recruitment of Rho–ROCK, which are core regulators of actomyosin[40,41,47]. The patterning systems responsible for the spatiotemporal control of Shroom3 expression and, consequently, tissue bending have been identified in several cases. In the placode of the *Xenopus* gut, Shroom3 is directly controlled by the transcription factor Pitx[45] and in the mouse its expression in the lens placode depends on Pax6 (ref. 36); however, in the zebrafish lateral line primordium, Shroom3a is controlled by fibroblast growth factor (FGF)-signalling[46]. The connection of patterning systems and universal cellular effectors through the expression of specific mediator proteins seems to be a widely used strategy for the genetic regulation of tissue-folding events.

Tissue elongation

Tissue folding through apical constriction is only one of several conserved mechanisms for shaping tissues. Another important driver of embryonic morphogenesis is tissue elongation through convergent extension, first described in Amphibia, in which cells converge in one direction while extending the tissue in the orthogonal axis. Although many convergent extension processes are driven by the rearrangement of cells, rather than by changes in cell shape, the precise underlying

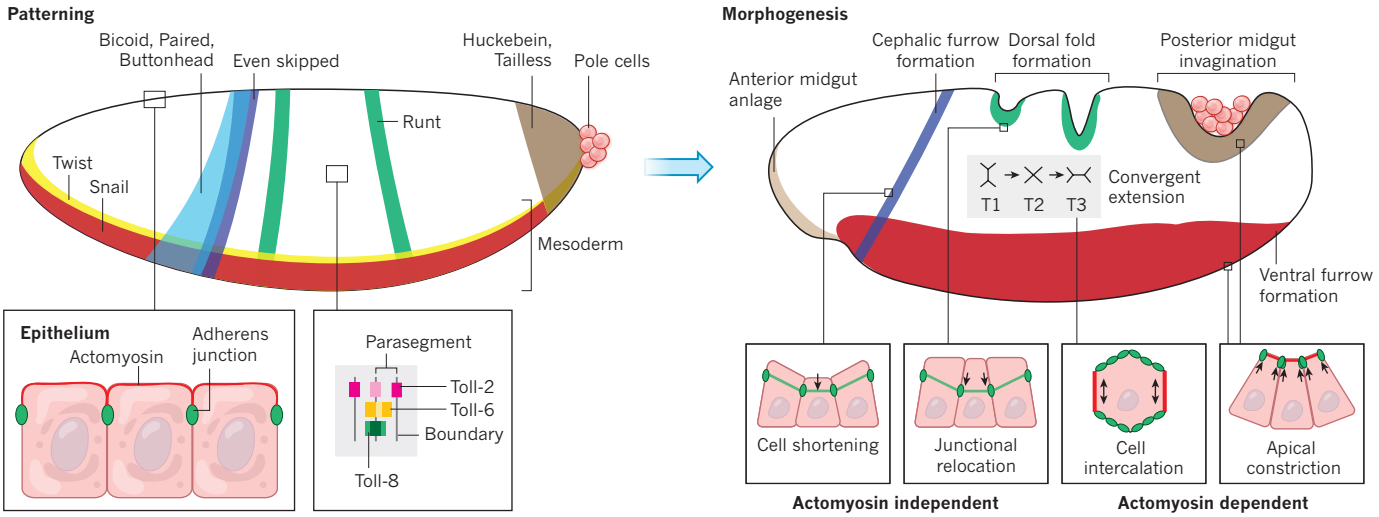

Figure 1 | From patterning to morphogenesis in the *Drosophila* blastoderm. Morphogens set up the expression domains of genes at the blastoderm stage of *Drosophila* development (left). Broad patterns of morphogen expression are gradually refined by hierarchical zygotic gene-regulatory networks, and eventually each cell in the embryo contains defined positional information in the form of the set of transcription factors that it expresses. These gene-regulatory networks determine the morphogenetic activities of the gene-expression domains (right). Along the head-to-tail (anterior–posterior) axis, the anterior morphogen Bicoid, through the expression of gap genes *paired* and *buttonhead* (light blue), sets up the first of seven stripes of expression of the pair-rule gene *even skipped* (dark blue). Even skipped then specifies cell behaviour in the cephalic furrow, where cells shorten along their apical–basal axis, independently of myosin activity. During convergent extension, the pair-rule transcription factors Even skipped and Runt control the spatial expression of three Toll-family receptors (Toll-2, Toll-6 and Toll-8) in overlapping patterns (with darker shading representing a higher level of expression), which creates an anterior–posterior asymmetry that is necessary for the polarized distribution of myosin. This results in polarized shrinkage of cell–cell boundaries along the dorsal–ventral axis and the exchange of neighbouring cells (known as a T1–T2–T3 transition). The second and fifth stripes of Runt expression (green) coincide with the domain in which dorsal-fold formation takes place when the basal shift of adherens junctions in initiator cells (and the resulting junctional asymmetry with neighbouring cells) induces local bending. The dorsal–ventral axis is established by a gradient of nuclear Dorsal (not shown), and the highest levels of Dorsal activate the expression of genes *twist* and *snail* in the prospective mesoderm. Together, the transcriptional activator Twist (yellow) and the dual-function transcription factor Snail (red) control the expression of mesoderm-specific regulators of cell behaviour that control actomyosin-dependent apical constriction and mesoderm invagination. At the posterior of the embryo, the gap genes *huckebein* and *tailless* are required for the expression of Fog in the posterior midgut and for posterior-midgut invagination by a similar apical constriction mechanism that drives ventral-furrow formation. For simplicity, only relevant part of the expression domains of Even skipped, Runt and Huckebein are shown.

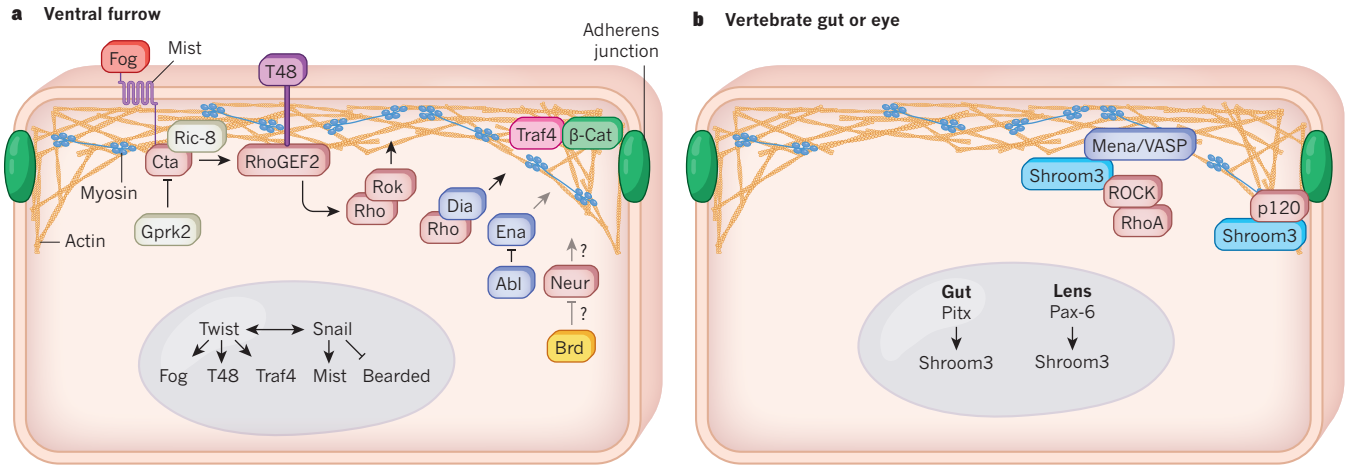

Figure 2 | Cellular regulation of apical constriction and cell intercalation. **a**, Apical constriction in the *Drosophila* mesoderm. Twist activates the expression of the genes *fog*, *T48* and *traf4* and Snail activates *Mist* and represses genes from the Bearded family. The effects of the protein products of these genes on globally expressed components (grey and brown) are shown at their sites of action at the apical cell cortex (a layer of actin and myosin at the inner cell periphery) or outside the cell. The secreted ligand Fog activates RhoGEF–Rho signalling through the G-protein-coupled receptor Mist. The Rho–Rok complex activates myosin and regulates the junctional actin cytoskeleton through Diaphanous (Dia). The Twist-target Traf4 associates physically with β-catenin (β-cat) and contributes to the efficient relocalization of the adherens junctions to the apical region of the cell. Ric-8 (also known as Synembryn) supports the apical localization of Concertina (Cta). Tyrosine-protein kinase Abl inhibits Enabled (Ena) in the mesoderm to provide a functional cortical actin cytoskeleton. For the E3 ubiquitin ligase Neuralized (Neur) to be active, the expression of Bearded (Brd) has to be inhibited in the mesoderm. This enables the apical relocalization of adherens junctions through an unknown mechanism. **b**, Apical constriction in vertebrates. In *Xenopus*, the transcription factor Pitx directly activates Shroom3 expression in the gut, whereas the transcription factor Pax-6 is required for Shroom3 expression in lens cells. Shroom3 is recruited to the apical region of the cell through the protein's actin-binding domain, and the Shroom Domain 2 of Shroom3 recruits ROCK. In lens cells, p120–N-cadherin can recruit Shroom3 to adherens junctions.

mechanism often depends on the context of the organism and tissue. During germband extension in *Drosophila*, elongation of the body axis is driven by local cell intercalation[48] (Fig. 1). Cell interfaces that run orthogonally to the axis of extension shrink, whereas those that are aligned expand. These cell-interface manoeuvres (also termed T1 transitions) are achieved mainly through the remodelling of apical cell–cell junctions and lead to cell intercalation and the formation of multicellular rosettes[49,50]. A similar mechanism of junctional remodelling and rosette rearrangement underlies a number of convergent extension processes in vertebrate embryos, including morphogenesis of the kidney[51] and the neural plate[41,52] and formation of the chick primitive streak[53]. Frog gastrulation, arguably the archetypal example of convergent extension, was initially proposed to be driven by an alternative mechanism in which dynamic mediolateral cell protrusions generate tractions that enable cells to interdigitate through cell crawling[54,55]. Such a mechanism of cell motility is consistent with the proposal that convergent-extension movements are driven by collective migration during zebrafish gastrulation[56]. However, other studies support a model in which mesodermal convergent extension in *Xenopus* is also influenced by the planar, polarized contraction of mediolateral cell interfaces[57], a mechanism that is remarkably similar to germband extension in *Drosophila*. It remains to be established whether dynamic basal protrusions, which are known to drive collective cell migration, play any part in convergent extension in these species.

Convergent extension processes share a dependence on the contractile actomyosin machinery, which is recruited to defined cell–cell interfaces to drive coordinated cell rearrangement across the plane of the epithelium[49,58–60]. Therefore, an important question is how this planar, polarized recruitment of actomyosin is achieved and coordinated across tissues. Notably, the core planar cell polarity pathways are not required for germband extension[58]. Instead, cell intercalation is placed under the genetic control of the pair-rule transcription factors Eve and Runt[48,58], which direct the spatial expression of a set of Toll-family receptors that were previously not known to have roles in planar cell polarity[61]. Three Toll receptors (Toll-2, Toll-6 and Toll-8) are expressed in overlapping patterns along the anterior–posterior axis, which results in cells expressing a code comprised of various combinations of Toll receptors[61]. Heterophilic interactions between neighbouring cells with differing compositions of Toll receptors break cell symmetry to promote the planar, polarized localization of actomyosin and cell intercalation. Therefore, the genetic regulation of tissue elongation is achieved again by the patterned expression of key interactors that drive changes in the subcellular localization of the ubiquitous contractile actomyosin apparatus.

Emerging principles of tissue shaping

A general mechanistic logic for the genetic regulation of tissue shaping is beginning to emerge, owing to work on a number of models. Although the processes that shape tissues are controlled in space and time by specific programs of gene regulation, the generation of shape itself is achieved through a more standard set of cellular machinery. This toolbox includes the contractile actomyosin system, adherens junctions and the polarity complexes that provide positional information in cells. Many of these players, including actomyosin, are required for a variety of basic cellular processes, which means that they are not generally the targets of tissue-specific gene regulation. Rather, it is precise changes in the subcellular localization and activity of these ubiquitously expressed machines that determine whether a tissue folds through apical constriction or extends through convergent extension. Direct links between these two levels of shape control — gene expression and the universal cell-shaping apparatus — are provided by interacting proteins that 'plug in' to generate tissue-specific patterns of actomyosin localization and resultant shape changes. For example, actomyosin-mediated activity is recruited specifically to the apical domains of cells through the local expression of intracellular regulators, including Fog and T48 in invertebrates and Shroom3 in vertebrates, to drive apical constriction and tissue-scale folding. In other contexts, actomyosin contractility is increased at specific cell–cell interfaces in the plane of the epithelium through the expression of regulators of planar polarity signalling such as Celsr1 (ref. 62) and Ptk7 (ref. 52) in vertebrates, or Toll receptors in *Drosophila*[61], to drive cell intercalation and tissue elongation.

In many of the examples elucidated so far, the mechanism that links

genes to shape is linear, with the same transcription factors that control the specification and differentiation of tissue domains, such as Twist and Snail, directly regulating the factors that affect subcellular actomyosin dynamics. However, a similar morphogenetic logic can be found in several context-specific variants. In numerous situations, the subcellular regulation of actomyosin activity may depend less on hardwired gene-regulatory inputs and more on dynamic cell–cell signalling systems such as the planar cell polarity pathways. For example, the two main processes that shape the *Drosophila* blastoderm — apical constriction (for ventral furrow formation) and convergent extension (for germband extension) — both require a single G-protein-coupled receptor called Smog[63]. To elicit such different effects, Smog must regulate actomyosin in distinct subcellular domains. However, it is expressed ubiquitously, which means that these local differences in function are probably the result of the position-specific modulation of Smog's signalling activity.

A further source of variation in morphogenetic programs is found at the level of the cell-shaping machinery. We have already described how actomyosin-driven apical constriction controls tissue folding in a variety of species. However, even in the simple blastoderm monolayer, there is more than one to way to make a fold. For example, the formation of two transverse dorsal folds (Fig. 1) is achieved through the basal repositioning of adherens junctions in two stripes of initiator cells — a relocation of the junctions in the direction opposite to that observed in the ventral furrow[64]. The resulting junctional asymmetry forces neighbouring cells to bend towards initiator cells. Another fold, the cephalic furrow, also forms when initiator cells force neighbouring cells to bend towards them[65,66], but there is no detectable relocation of adherens junctions (Fig. 1). Instead, formation of this fold seems to be driven by a shortening as well as a basal widening of the initiator cells[67]. Interestingly, neither of these tissue-folding mechanisms involves actomyosin contractility[64,67,68], which indicates that other cell-shaping machineries are at work. Although tissue shaping can potentially be controlled through a number of other mechanisms, including regulated changes in the adhesion of cells to the extracellular matrix, or hydrostatic pressure, it is probable that the spatiotemporal control of these and other drivers of shape changes will be achieved through the tissue-specific expression of key regulators.

Coordination of tissue shaping by mechanical cross-talk

Most progress in bridging the gap between genotype and morphological phenotype has come from studies that focus on individual tissues such as the ventral furrow, which are defined through the expression of patterning genes. This reductionist approach is a logical consequence of the fact that the subdivision of embryos into distinct domains is a hallmark of all developmental programs. However, embryos are not simple patchworks of autonomously developing tissues; rather, they are integrated systems in which the final shape emerges from physical interactions between domains. For example, mechanical interactions between tissue domains can play an important part in shaping individual organs such as the wing[69]. The development of methods for whole-organism live imaging, such as light-sheet microscopy[70], as well as high-precision perturbations[71–73], means that it is now possible to address cross-talk between tissues and domains across entire embryos. Tissue-scale 'pulling' forces that are generated during posterior midgut invagination participate actively in germband extension by orientating the growth of cell junctions and promoting cell intercalation in neighbouring cells, linking two morphogenetic processes that were previously studied separately[71,74,75]. Similarly, ventral furrow formation in the *Drosophila* embryo requires a coordination that extends beyond the tissue, with the mechanical properties of cells throughout the embryo contributing to orderly and reproducible invagination[72,76,77]. Understanding the full extent of such interactions, as well as their physico-mechanical bases, will be essential for the production of integrated models of tissue behaviour, irrespective of the species or level of complexity. For example, an epithelial invagination in the early ascidian embryo, a simple model with a small number of defined cell types that is also driven by apical constriction, relies on cooperation of the central endoderm with two adjacent populations of cells that have different cortical tensions[78].

Morphogenetic feedback enables tissue self-assembly

The patterns of gene expression that control cell-fate decisions are often refined as tissues physically take shape, not just before. Furthermore, the remarkable ability of cultured pluripotent cells to self-assemble into differentiated organoids such as optic cups and 'mini' guts and brains confirms that patterns of gene expression and cell fate can emerge *de novo* as tissues assemble. Rather than being organized in a strict hierarchical relationship, genetic patterning and physical tissue morphogenesis must therefore be interlinked through a form of dynamic feedback. Biological feedback is classically defined as the regulation of processes by their products, and is best demonstrated by the allosteric regulation of enzyme kinetics. As the main 'product' of all morphogenetic programs is a change in multicellular organization, a crucial question is how such tissue-scale changes in shape are detected to control gene expression and fate at the level of the individual cell. These emerging mechanisms of morphogenetic feedback are bringing us closer to achieving a full-circle understanding of tissue self-assembly.

Table 1 | Tissue-specific control of cellular behaviour

Function	Ventral furrow	Posterior-midgut invagination	Cephalic furrow	Dorsal fold	Vertebrate tissue folding	Convergent extension
Transcriptional control	Twist, Snail	Huckebein, Tailless	Buttonhead, Paired, Even skipped	Anterior–posterior patterning system	Pitx or Pax-6?	Even skipped, Runt
Tissue-specific mediation	Fog[8,33], Mist[34], T48 (ref. 25), Traf4 (ref. 32)	Fog[33]	Unknown	RapGAP[137]	Shroom3 (refs 36–41)	Toll-2, Toll-6, Toll-8 (ref. 61)
Signalling or polarity regulation	Gαβγ[128,129], Rho/Rok[68], Rho/Dia/Abl/Ena[22,130,131], Ric-8 (ref. 132), Gprk2 (ref. 133), Dizzy/Rap1 (ref. 134), Canoe[135], Xit[136]	Gαβγ[128], Rho/Rok[68]	Unknown	Rap1 (ref. 137), Bazooka/Par-1 (ref. 64)	RhoA/ROCK[41,47], Mena/VASP[138], Lulu1/Lulu2 (ref. 139)	Rho/Rok[140,141], Abl/Dia[142,143], Shroom[144], Bazooka[135], Canoe[58,140], Xit[136]
Cellular effectors	Adherens junctions, myosin, actin	Adherens junctions, myosin, actin	Adherens junctions? Actin?	Adherens junctions	Adherens junctions, myosin, actin	Adherens junctions, myosin, actin
Cell-behaviour modification	Apical constriction	Apical constriction	Cell shortening	Junctional relocation	Apical constriction	Cell rearrangement

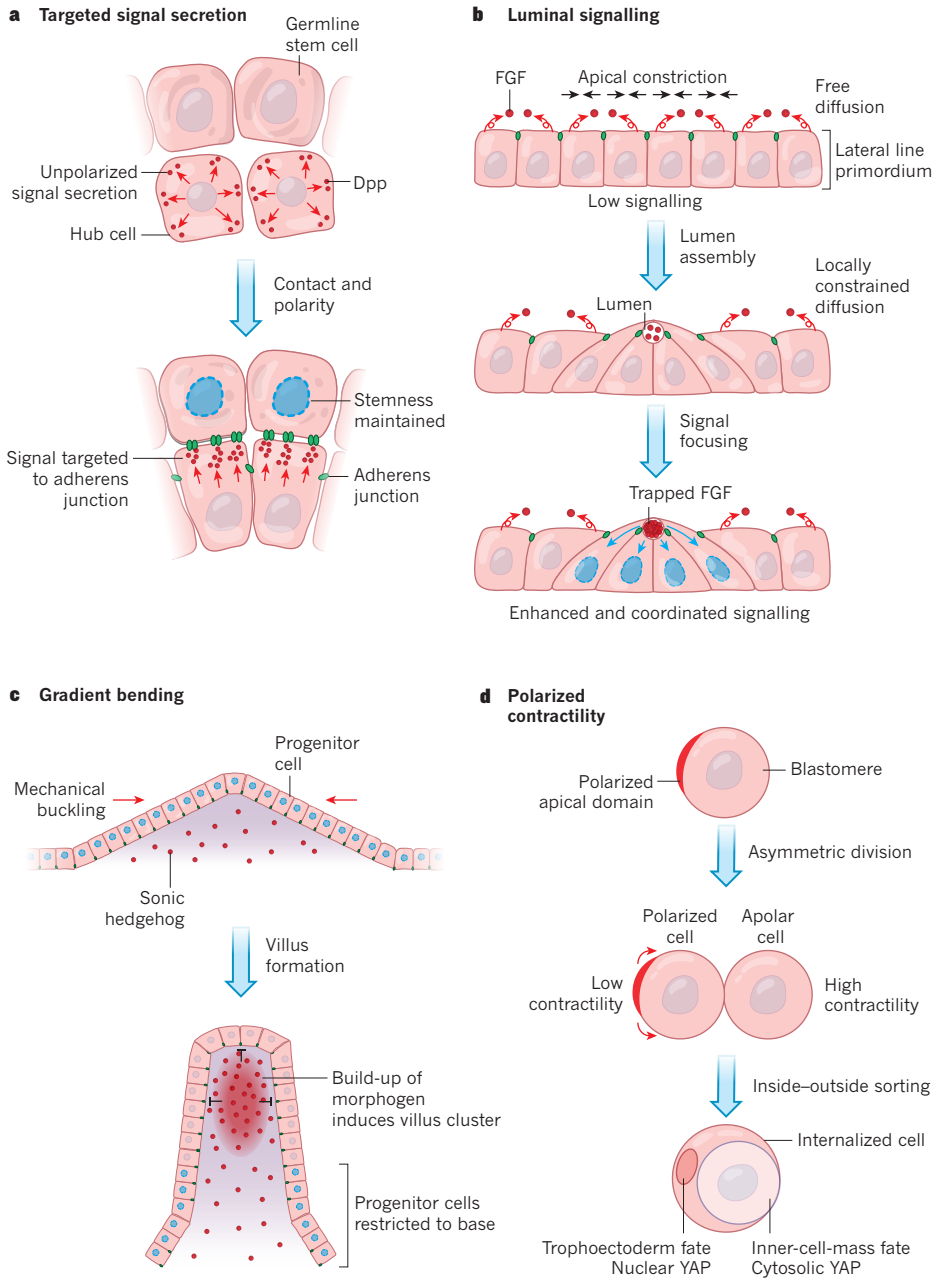

Figure 3 | Morphogenetic feedback mechanisms. **a**, In targeted signal secretion, source-cell polarization is required for efficient morphogen signalling in germline stem-cell niches. Decapentaplegic (Dpp) is secreted from hub cells in the absence of polarity, but the levels of Dpp signalling are insufficient to maintain the stemness of the niche (top). Hub cells and the germline stem-cell niche are coupled through adherens junctions (bottom). Dpp secretion is targeted to adherens junctions, which leads to a higher level of signal reception and maintains the stemness of the niche. **b**, In luminal signalling, morphogen signalling is focused in the migrating lateral line primordium. FGF secretion occurs before assembly of the lumen, and free diffusion of FGF results in low levels of signalling. The lumen is assembled through concerted apical constriction (middle). The local trapping of FGF results in high levels of signalling in participating cells. Adapted from ref. 98. **c**, The formation of villi in the gut bends Sonic hedgehog gradients, which restricts progenitor cells to the base of these structures. Sonic hedgehog secretion occurs before villus formation (top). Because the levels of ligand are insufficient to induce 'villus cluster' genes that repress progenitor fate, progenitor cells are found throughout the epithelium. Villi are formed through a buckling process that results from their continued growth under mechanical constraint (bottom). The geometry of the villus leads to the accumulation of higher levels of Sonic hedgehog at the tip and the induction of the villus cluster, restricting progenitor cell fate to cells at the base. **d**, Differences in cell contractility control the first cell-fate decision of mouse development. The mini-blastocyst approach enables fate decisions to be studied in single polarized cells (top). Asymmetric inheritance of the apical domain (middle) results in a polarized daughter cell that has lower levels of cortical contractility than its apolar sister cell. The difference in contractility leads to the internalization of the apolar cell, which adopts the inner-cell-mass fate (cytosolic YAP localization), and the trophoectoderm fate (nuclear YAP localization) is adopted by the outer polar cell.

Architectural control of cell fate by signalling

Intercellular signalling pathways that control cell-fate decisions and tissue patterning are emerging as central mediators of morphogenetic feedback control. Precisely how the activity of these signals is coupled to changes in tissue architecture depends on their specific mode of action. The cell-signalling pathways that control development can be subdivided into those that mediate short-range inductive interactions, such as Delta–Notch juxtacrine signalling, and those that confer positional identity at the tissue scale, most typically in the form of extracellular morphogen gradients. However, even the range over which morphogen signals act remains a topic of intense research, decades after their original identification. For example, two studies have shown that the morphogen Wingless, which was classically thought to form long-range diffusive gradients, acts through short-range signalling in both *Drosphila* and mice. The requirement for Wingless diffusion in *Drosophila* development was tested directly by replacing the endogenous protein with a membrane-tethered version[79]. Although this prevented the diffusion of Wingless from source cells and considerably reduced the range of signalling, remarkably, it did not compromise the function of Wingless as a morphogen[79]. Similarly, in the intestinal stem cell niches of mice, Wnt-3, a vertebrate homologue of Wingless, remains bound to the surface of source cells and acts over short ranges[80]. In the wing disc of *Drosophila*, as well as other contexts, morphogens can be delivered to or sensed by target cells through long filopodial protrusions termed cytonemes[81]. A corollary to the discovery that many proteins of importance to developmental signalling act through direct delivery is that these patterning signals 'flow' with the repositioning or growth of source cells — a phenomenon termed morphogen advection[82]. Any morphogenetic events that alter the number or arrangement of cells, such as proliferation or convergent extension, therefore have the potential to feed back on genetic patterning by changing the relative positioning of cells that produce or receive signals.

Many archetypal features of epithelial cells, such as apicobasal polarity and adhesion, are not apparent in single cells. Instead, they depend on the population context of the tissue[83], which constitutes tissue-level parameters such as the local cell density[84] or distance from the tissue's edge[85]. The population context also feeds back on cell-fate decisions to ensure that cells differentiate in a way that is appropriate for their local

tissue environment. Many of the systems that enable such contextual control of differentiation exploit polarity sensitive aspects of the cell–cell signalling pathways that control tissue patterning and cell-fate acquisition. For example, a common mechanism involves the sequestration of signalling receptors into organized plasma membrane domains, either to restrict receptor mobility or to prevent receptor–ligand interactions in a polarity-dependent manner, in epithelial cells in culture[86,87] and *in vivo*[88]. For example, the establishment of contact inhibition of proliferation in confluent epithelial cultures has been shown to require the basolateral recruitment of receptors for transforming growth factor β, to prevent their interaction with any ligand presented to the apical surface and to suppress signalling and proliferation in regions of high cell density[89,90]. Changes in cell adhesion and polarity can modulate signalling by influencing the trafficking and presentation of the ligands themselves. An *in vivo* example is provided by the stem-cell niche of the *Drosophila* testis, in which the morphogen Decapentaplegic is secreted by cells of the surrounding hub and acts as a short-range niche signal to maintain 'stemness' in germline stem cells[91] (Fig. 3a). Here, efficient signalling depends on the polarized trafficking and presentation of the Decapentaplegic signal at adherens junctions that physically couple germline stem cells to hub cells[92]. A similar dependence on polarized localization and cell–cell contact has been described for other important developmental signalling pathways, such as the Delta–Notch pathway. Cell-fate acquisition can therefore be coupled to tissue-scale changes in cell polarity and adhesion through negative and positive feedback. The choice of trafficking pathway used to release morphogens from secreting cells can also influence their range of action. For example, the basolateral secretion of Hedgehog in the *Drosophila* wing disc activates short-range, high-threshold target genes, whereas its secretion into the apical lumen of the wing disc activates long-range, low-threshold target genes[93].

Although there is growing support for the idea that many key signals can act over a short range, in other contexts, long-range extracellular diffusion is essential for correct tissue-scale patterning. For example, one study that specifically altered the extracellular distribution of the morphogen Decapentaplegic by expressing a 'morphotrap' — a blocking antibody that sequesters target proteins — demonstrated strong effects on the patterning of the wing[94]. How can physical changes in multicellular architecture feed back on the activity of molecules that encode such long-range positional information? Answers to this crucial question came from two studies that showed that 3D tissue shape can overwrite patterns of gene expression by regulating the extracellular distribution of morphogen signals. Ligands of the FGF family control the assembly of rosette-like mechanosensory organs in the migrating lateral line primordium of the zebrafish[95,96] through a Shroom3–Rho-dependent apical constriction process[46,97]. This leads to the assembly of a central shared microlumen that traps the secreted FGF, resulting in increased local concentrations of ligand and activation of target genes[98]. Targeted perturbation experiments have revealed that such 'focusing' of the morphogen in these multicellular cavities is required to restrict, coordinate and enhance FGF-mediated signalling in the migrating tissue. When the content of the microlumen is released by two-photon laser micropuncture, the cells that surround it show a specific reduction in signalling and do not undergo the normal FGF-driven behavioural transition. Such luminal signalling therefore represents a bona fide mechanism of morphogenetic feedback because the activity of an important cell–cell signalling pathway is further enhanced by the change in tissue shape that it promotes[98] (Fig. 3b). Studies on the positioning of the stem-cell niche in the vertebrate gut have uncovered another mechanism, termed gradient bending, that enables tissue shape to influence morphogen signalling[99] (Fig. 3c). Initially, the precursors of intestinal stem cells are distributed throughout the epithelium of the gut endoderm, but eventually they become excluded from the tips of intestinal villi. The exclusion is mediated by mesenchymal cells of the villus cluster, a group of cells at the tip of the villus that secrete a number of signalling factors that inhibit stem-cell fate in the overlying epithelium. The induction of the villus cluster requires Sonic hedgehog signalling[100], but the gene sonic hedgehog is transcribed uniformly throughout the gut endoderm at this stage, which indicates that another feature must focus Sonic-hedgehog activity at the villus tip. A model[99] has been proposed in which the curved shape of the villus tip 'bends' the morphogen distribution, owing to the target cells being surrounded by epithelial cells that secrete Sonic hedgehog, a mechanism that is supported by computational models. Opening up the tightly folded epithelium, through gut inversion, resulted in the reduced expression of Sonic hedgehog target genes. Conversely, when villus formation is induced artificially, by culturing slabs of intestine under a grid that mimics mechanical buckling, Sonic hedgehog target-gene expression was increased. A shared feature of both luminal-signalling and gradient-bending mechanisms is that they directly link the activity of key patterning signals to changes in 3D tissue architecture, and therefore could be widespread. Moreover, morphogenetic feedback mechanisms represent an important step towards understanding the remarkable self-assembly properties of developing tissues, both *in vivo* and *in vitro*.

Tissue mechanics as a feedback regulator of cell fate

The mechanical forces that drive tissue-shaping processes provide another important channel for feedback between cell form and fate[101–103]. The influence of mechanics on developmental decisions is strikingly demonstrated by biophysical studies showing that the stiffness of the substrate on which mesenchymal stem cells are plated determines their path of differentiation[104]. Mesenchymal stem cells that were plated on soft substrates differentiated into neurons, which are cells that would normally form in soft environments *in vivo*, whereas cells that were plated on stiff substrates differentiated into osteoclasts, a type of bone cell. Sensing the stiffness of culture substrates is known to require the activity of the actinomyosin system[104,105] and can influence gene expression through nuclear mechanics[106]. The forces that shape tissues during embryogenesis can be detected by a plethora of mechanosensitive systems, including machinery that was directly involved in the initial generation of these forces. An example of such a system is the catch bond that links the cadherin–catenin complexes of adherens junctions to actin, in which increased force shifts the bond from a weakly bound state to a strongly bound state, potentially enhancing cell–cell adhesion in a force-dependent manner[107]. Similarly, the aligned assembly of contractile actomyosin cables at the interface between cells is regulated positively by increased cortical tension[60]. Mechanisms of this kind play an important part in providing feedback to enable a rapid response to changes in adhesion and contractility. To control cell differentiation, however, changes in forces must be sensed and signalled to the nucleus to regulate the expression of fate-determining genes. An important breakthrough in understanding was the discovery of transcription factors that shuttle from the cytosol to the nucleus in response to changes in the actin cytoskeleton[108], cell shape[109] or mechanical forces[110–112]. Most notable among these are the transcription factors YAP and TAZ of the Hippo signalling pathway, which have been shown to act as both sensors and mediators of mechanical inputs in several *in vitro* and *in vivo* contexts[111,113].

Examples of how tissue mechanics can feed back into cell fate *in vivo* can be found in mouse, zebrafish and *Drosophila* models. A key role has been identified for tissue mechanics in controlling the first cell-fate decision of early mouse development, during which cells that will become the inner cell mass of the embryo move inwards, leaving those that are fated to become extra-embryonic cells as a polarized outer layer known as the trophectoderm[114]. This decision is influenced by a number of parameters, including whether cells are at the external surface or the interior of the embryo ('inside–outside' positioning), the orientation of cell division, apicobasal polarity and the expression of key genes. However, how these different types of input are integrated to control cell fate was unclear. This important question has now been resolved by the discovery that the asymmetric division of polarized apical domains generates differences in cortical contractility that subsequently direct the positioning of daughter blastomeres. Using a combination of targeted perturbations and computational modelling, blastomeres that

inherit the polarized apical domain were shown to have reduced contractility and spreading in comparison to their more contractile, apolar sister cells, and could therefore internalize them (Fig. 3d). Interestingly, loss of contractility also led to a loss of YAP in the nuclei of externally positioned cells. Because exclusion of the mechanosensitive YAP had previously been shown to be important for the acquisition of the trophectoderm fate, this reveals a possible role for mechanosensation in this key cell-fate decision. An alternative mechanosensitive pathway, involving the Wnt–cadherin effector β-catenin, has been shown to regulate mesodermal identity *in vivo*[115]. Mechanical compression during epiboly in zebrafish embryos was sufficient to drive the membrane-to-nucleus translocation of β-catenin and the transcriptional activation of the mesoderm-specific target gene *no tail*, which encodes the zebrafish brachyury orthologue. Blocking the endogenous forces of epiboly with blebbistatin suppressed the nuclear translocation of β-catenin and the induction of *no tail*. However, both of these mesodermal characteristics were rescued when epiboly was mimicked in these embryos by applying magnetically induced external forces. The same study found that the mechanosensitive translocation of β-catenin might also be involved in mesoderm induction in *Drosophila*, which raises the possibility that this represents an evolutionary conserved mechanosensitive pathway for regulating a crucial developmental decision *in vivo*. Further insights into how these pathways sense forces was provided by cell-culture experiments that showed that the nuclear translocation of both YAP and β-catenin requires the extracellular engagement of E-cadherin, and therefore identified a potential general role for cadherin in mediating responses to mechanical stress[116].

From embryo morphogenesis to tissue engineering

As the genetics and cell-biology principles of morphogenetic cascades are becoming clearer, physicists are again joining forces with biologists to address the physical mechanisms that drive embryonic development[2,3]. Quantitative models are being produced that are enabling the first multiscale views of the dynamic processes that drive morphogenesis, including subcellular interactions between the actomyosin meshwork and the plasma membrane[117] and cell–cell and tissue–tissue interactions across organs and organisms[118,119]. One goal of computational modelling is to generate theoretical frameworks that will facilitate the integration of knowledge at the mechanical, cellular and gene-regulatory levels[120].

A stunning demonstration of the full-circle nature of morphogenesis, in which genes regulate tissue shaping and vice versa, comes from the study of organoids. Here, cultured pluripotent cells self-assemble into organ-like structures that are remarkably similar to those formed in the embryo[121–123]. Organoids can even be generated from patient-derived induced pluripotent stem cells, which means that this technology has the potential to herald a new era in tissue engineering for the modelling of disease and the development of therapies that is based on the principles of developmental biology[124]. Indeed, synthetic approaches are already progressing towards the manipulation of developmental mechanisms to control tissue differentiation, both *in vitro* and *in vivo*. For example, synthetic Notch-based signalling receptors have been developed that enable the user-defined control of cell behaviour through numerous orthogonal signalling circuits[125]. Conversely, breakthroughs from the realm of synthetic biology are providing fresh insights into the central mechanisms of development. Organoid formation itself demonstrates that cells can become organized in the absence of predetermined long-range external patterning influences such as morphogen gradients or mechanical forces, which are a cornerstone of classic developmental biology[121]. This unexpected lack of requirement for long-range pre-patterning has led to organoid formation being described as an example of 'self-organization', which is defined classically as the spontaneous emergence of order through the interaction of initially homogeneous components. Although some aspects of organoid formation may show self-organizing properties, it is already clear that cell heterogeneity and patterned gene expression play a crucial part throughout[121,126]. A more accurate definition by Alfonso Martinez-Arias and his colleagues describes organoid formation as a process of genetically encoded self-assembly, in which the local activation of genetic patterning systems generates non-equivalent groups of cells that interact to drive each step of tissue assembly[127]. It is not yet known how organoids are able to trigger and refine these self-assembly events, which means that this exciting field will probably yield further surprises. As organoids can exploit only pre-existing mechanisms, these studies highlight that tissue self-assembly has a potentially greater role in organogenesis *in vivo* than was previously appreciated. Future mechanistic investigations into how tissue shaping is encoded genetically and how, conversely, tissue shaping feeds back into gene expression to control cell fate will therefore both increase our understanding of embryonic development and help to advance the development of therapies that are based on controlling morphogenesis in the culture dish. ■

Received 29 March; accepted 18 November 2016.

1. Thomasen, A. L. "*Historia animalium*" compared to "*Gynaecia*" in the literature of the Middle Ages. *Clio Med.* **15,** 5–24 (1980).
2. Keller, R. Physical biology returns to morphogenesis. *Science* **338,** 201–203 (2012).
3. Heisenberg, C.-P. & Bellaïche, Y. Forces in tissue morphogenesis and patterning. *Cell* **153,** 948–962 (2013).
4. Nüsslein-Volhard, C. & Wieschaus, E. Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287,** 795–801 (1980).
   The first systematic study on the genetic regulation of embryonic development; it remains unparalleled in impact and importance.
5. Lawrence, P. A. Morphogens: how big is the big picture? *Nature Cell Biol.* **3,** E151–E154 (2001).
6. Green, J. B. A. & Sharpe, J. Positional information and reaction-diffusion: two big ideas in developmental biology combine. *Development* **142,** 1203–1211 (2015).
7. Munro, E. M. & Odell, G. M. Polarized basolateral cell motility underlies invagination and convergent extension of the ascidian notochord. *Development* **129,** 13–24 (2002).
8. Dawes-Hoang, R. E. *et al.* Folded gastrulation, cell shape change and the control of myosin localization. *Development* **132,** 4165–4178 (2005).
9. Lecuit, T. Adhesion remodeling underlying tissue morphogenesis. *Trends Cell Biol.* **15,** 34–42 (2005).
10. Settleman, J. & Baum, B. Cell shape and tissue morphogenesis. *Semin. Cell Dev. Biol.* **19,** 213–214 (2008).
11. Zallen, J. A. & Blankenship, J. T. Multicellular dynamics during epithelial elongation. *Semin. Cell Dev. Biol.* **19,** 263–270 (2008).
12. Barrallo-Gimeno, A. & Nieto, M. A. The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development* **132,** 3151–3161 (2005).
13. Revenu, C. & Gilmour, D. EMT 2.0: shaping epithelia through collective migration. *Curr. Opin. Genet. Dev.* **19,** 338–342 (2009).
14. Thiery, J.-P., Acloque, H., Huang, R. Y. J. & Nieto, M. A. Epithelial–mesenchymal transitions in development and disease. *Cell* **139,** 871–890 (2009).
15. Friedl, P., Locker, J., Sahai, E. & Segall, J. E. Classifying collective cancer cell invasion. *Nature Cell Biol.* **14,** 777–783 (2012).
16. Friedl, P. & Gilmour, D. Collective cell migration in morphogenesis, regeneration and cancer. *Nature Rev. Mol. Cell Biol.* **10,** 445–457 (2009).
17. Keller, R. E. An experimental analysis of the role of bottle cells and the deep marginal zone in gastrulation of *Xenopus laevis*. *J. Exp. Zool.* **216,** 81–101 (1981).
18. Martin, A. C. & Goldstein, B. Apical constriction: themes and variations on a cellular mechanism driving morphogenesis. *Development* **141,** 1987–1998 (2014).
19. Leptin, M. & Grunewald, B. Cell shape changes during gastrulation in *Drosophila*. *Development* **110,** 73–84 (1990).
20. Sweeton, D., Parks, S., Costa, M. & Wieschaus, E. Gastrulation in *Drosophila*: the formation of the ventral furrow and posterior midgut invaginations. *Development* **112,** 775–789 (1991).
21. Martin, A. C., Kaschube, M. & Wieschaus, E. F. Pulsed contractions of an actin–myosin network drive apical constriction. *Nature* **457,** 495–499 (2009).
    This paper shows that apical constriction in the *Drosophila* mesoderm is driven by a medially localized pulsatile actin–myosin network in incremental, contractile steps.
22. Mason, F. M., Tworoger, M. & Martin, A. C. Apical domain polarization localizes actin–myosin activity to drive ratchet-like apical constriction. *Nature Cell Biol.* **15,** 926–936 (2013).
23. Vasquez, C. G., Tworoger, M. & Martin, A. C. Dynamic myosin phosphorylation regulates contractile pulses and tissue integrity during epithelial morphogenesis. *J. Cell Biol.* **206,** 435–450 (2014).
24. Solon, J., Kaya-Çopur, A., Colombelli, J. & Brunner, D. Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell* **137,** 1331–1342 (2009).
25. Kölsch, V., Seher, T., Fernandez-Ballester, G. J., Serrano, L. & Leptin, M. Control of *Drosophila* gastrulation by apical localization of adherens junctions and

RhoGEF2. *Science* **315,** 384–386 (2007).
This study identifies a target of Twist called T48 that recruits RhoGEF2 specifically to the apical membrane in ventral-furrow cells and elucidated the interplay between Rho1 signalling and Snail in the apical relocalization of adherens junctions.
26. Weng, M. & Wieschaus, E. Myosin-dependent remodeling of adherens junctions protects junctions from Snail-dependent disassembly. *J. Cell Biol.* **212**, 219–229 (2016).
27. Sawyer, J. K., Harris, N. J., Slep, K. C., Gaul, U. & Peifer, M. The *Drosophila* afadin homologue Canoe regulates linkage of the actin cytoskeleton to adherens junctions during apical constriction. *J. Cell Biol.* **186,** 57–73 (2009).
28. Oda, H., Tsukita, S. & Takeichi, M. Dynamic behavior of the cadherin-based cell–cell adhesion system during *Drosophila* gastrulation. *Dev. Biol.* **203,** 435–450 (1998).
29. Leptin, M. twist and snail as positive and negative regulators during *Drosophila* mesoderm development. *Genes Dev.* **5,** 1568–1576 (1991).
30. Nieto, M. A. Epithelial plasticity: a common theme in embryonic and cancer cells. *Science* **342,** 1234850 (2013).
31. Chanet, S. & Schweisguth, F. Regulation of epithelial polarity by the E3 ubiquitin ligase Neuralized and the Bearded inhibitors in *Drosophila*. *Nature Cell Biol.* **14,** 467–476 (2012).
32. Mathew, S. J., Rembold, M. & Leptin, M. Role for Traf4 in polarizing adherens junctions as a prerequisite for efficient cell shape changes. *Mol. Cell. Biol.* **31,** 4978–4993 (2011).
33. Costa, M., Wilson, E. T. & Wieschaus, E. A putative cell signal encoded by the folded gastrulation gene coordinates cell shape changes during *Drosophila* gastrulation. *Cell* **76,** 1075–1089 (1994).
34. Manning, A. J., Peters, K. A., Peifer, M. & Rogers, S. L. Regulation of epithelial morphogenesis by the G protein-coupled receptor mist and its ligand fog. *Sci. Signal.* **6,** ra98 (2013).
Refs 34 and 63 report the identification of the long sought-after receptors for Fog, which function additively in the mesoderm.
35. Kim, H. Y., Varner, V. D. & Nelson, C. M. Apical constriction initiates new bud formation during monopodial branching of the embryonic chicken lung. *Development* **140,** 3146–3155 (2013).
36. Plageman, T. F. *et al.* Pax6-dependent *Shroom3* expression regulates apical constriction during lens placode invagination. *Development* **137,** 405–415 (2010).
37. Plageman, T. F. *et al.* A Trio–RhoA–Shroom3 pathway is required for apical constriction and epithelial invagination. *Development* **138,** 5177–5188 (2011).
38. Eiraku, M., Adachi, T. & Sasai, Y. Relaxation–expansion model for self-driven retinal morphogenesis. *Bioessays* **34,** 17–25 (2012).
39. Haigo, S. L., Hildebrand, J. D., Harland, R. M. & Wallingford, J. B. Shroom induces apical constriction and is required for hingepoint formation during neural tube closure. *Curr. Biol.* **13,** 2125–2137 (2003).
40. Hildebrand, J. D. Shroom regulates epithelial cell shape via the apical positioning of an actomyosin network. *J. Cell Sci.* **118,** 5191–5203 (2005).
41. Nishimura, T. & Takeichi, M. Shroom3-mediated recruitment of Rho kinases to the apical cell junctions regulates epithelial and neuroepithelial planar remodeling. *Development* **135,** 1493–1502 (2008).
42. Hildebrand, J. D. & Soriano, P. Shroom, a PDZ domain-containing actin-binding protein, is required for neural tube morphogenesis in mice. *Cell* **99,** 485–497 (1999).
43. Lee, C., Le, M. P. & Wallingford, J. B. The shroom family proteins play broad roles in the morphogenesis of thickened epithelial sheets. *Dev. Dyn.* **238,** 1480–1491 (2009).
44. Lang, R. A., Herman, K., Reynolds, A. B., Hildebrand, J. D. & Plageman, T. F. p120-catenin-dependent junctional recruitment of Shroom3 is required for apical constriction during lens pit morphogenesis. *Development* **141,** 3177–3187 (2014).
45. Chung, M.-I., Nascone-Yoder, N. M., Grover, S. A., Drysdale, T. A. & Wallingford, J. B. Direct activation of *Shroom3* transcription by Pitx proteins drives epithelial morphogenesis in the developing gut. *Development* **137,** 1339–1349 (2010).
46. Ernst, S. *et al.* Shroom3 is required downstream of FGF signalling to mediate proneuromast assembly in zebrafish. *Development* **139,** 4571–4581 (2012).
47. Das, D. *et al.* The interaction between Shroom3 and Rho-kinase is required for neural tube morphogenesis in mice. *Biol. Open* **3,** 850–860 (2014).
48. Irvine, K. D. & Wieschaus, E. Cell intercalation during *Drosophila* germband extension and its regulation by pair-rule segmentation genes. *Development* **120,** 827–841 (1994).
49. Bertet, C., Sulak, L. & Lecuit, T. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* **429,** 667–671 (2004).
Using the *Drosophila* embryo, this paper reveals a mechanism of cell intercalation that was subsequently shown to drive tissue elongation in a variety of organs and organisms.
50. Blankenship, J. T., Backovic, S. T., Sanny, J. S. P., Weitz, O. & Zallen, J. A. Multicellular rosette formation links planar cell polarity to tissue morphogenesis. *Dev. Cell* **11,** 459–470 (2006).
51. Lienkamp, S. S. *et al.* Vertebrate kidney tubules elongate using a planar cell polarity–dependent, rosette-based mechanism of convergent extension. *Nature Genet.* **44,** 1382–1387 (2012).
52. Williams, M., Yen, W., Lu, X. & Sutherland, A. Distinct apical and basolateral mechanisms drive planar cell polarity-dependent convergent extension of the mouse neural plate. *Dev. Cell* **29,** 34–46 (2014).
53. Rozbicki, E. *et al.* Myosin-II-mediated cell shape changes and cell intercalation contribute to primitive streak formation. *Nature Cell Biol.* **17,** 397–408 (2015).
54. Shih, J. & Keller, R. Cell motility driving mediolateral intercalation in explants of *Xenopus laevis*. *Development* **116,** 901–914 (1992).
55. Keller, R. *et al.* Mechanisms of convergence and extension by cell intercalation. *Phil. Trans. R. Soc. Lond. B* **355,** 897–922 (2000).
56. Tada, M. & Heisenberg, C.-P. Convergent extension: using collective cell migration and cell intercalation to shape embryos. *Development* **139,** 3897–3904 (2012).
57. Shindo, A. & Wallingford, J. B. PCP and septins compartmentalize cortical actomyosin to direct collective cell movement. *Science* **343,** 649–652 (2014).
58. Zallen, J. A. & Wieschaus, E. Patterned gene expression directs bipolar planar polarity in *Drosophila*. *Dev. Cell* **6,** 343–355 (2004).
59. Rauzi, M., Verant, P., Lecuit, T. & Lenne, P.-F. Nature and anisotropy of cortical forces orienting *Drosophila* tissue morphogenesis. *Nature Cell Biol.* **10,** 1401–1410 (2008).
60. Fernandez-Gonzalez, R. *et al.* Dynamics are regulated by tension in intercalating cells. *Dev. Cell* **17,** 736–743 (2009).
61. Paré, A. C. *et al.* A positional Toll receptor code directs convergent extension in *Drosophila*. *Nature* **515,** 523–527 (2014).
The patterned expression of three Toll receptor family members is shown to link anterior–posterior tissue patterning to cellular behaviour that drives cell intercalation and germband elongation.
62. Nishimura, T., Honda, H. & Takeichi, M. Planar cell polarity links axes of spatial dynamics in neural-tube closure. *Cell* **149,** 1084–1097 (2012).
63. Kerridge, S. *et al.* Modular activation of Rho1 by GPCR signalling imparts polarized myosin II activation during morphogenesis. *Nature Cell Biol.* **18,** 261–270 (2016).
64. Wang, Y.-C., Khan, Z., Kaschube, M. & Wieschaus, E. F. Differential positioning of adherens junctions is associated with initiation of epithelial folding. *Nature* **484,** 390–393 (2012).
This paper shows that epithelial folding in the *Drosophila* embryo can be initiated by the basal movement of adherens junctions, independently of myosin activity.
65. Turner, F. R. & Mahowald, A. P. Scanning electron microscopy of *Drosophila melanogaster* embryogenesis: II. gastrulation and segmentation. *Dev. Biol.* **57,** 403–416 (1977).
66. Vincent, A., Blankenship, J. T. & Wieschaus, E. Integration of the head and trunk segmentation systems controls cephalic furrow formation in *Drosophila*. *Development* **124,** 3747–3754 (1997).
67. Spencer, A. K., Siddiqui, B. A. & Thomas, J. H. Cell shape change and invagination of the cephalic furrow involves reorganization of F-actin. *Dev. Biol.* **402,** 192–207 (2015).
68. Barrett, K., Leptin, M. & Settleman, J. The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell* **91,** 905–915 (1997).
69. Aigouy, B. *et al.* Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. *Cell* **142,** 773–786 (2010).
70. Huisken, J., Swoger, J., Del Bene, F., Wittbrodt, J. & Stelzer, E. H. K. Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science* **305,** 1007–1009 (2004).
71. Collinet, C., Rauzi, M., Lenne, P.-F. & Lecuit, T. Local and tissue-scale forces drive oriented junction growth during tissue extension. *Nature Cell Biol.* **17,** 1247–1258 (2015).
72. Rauzi, M. *et al.* Embryo-scale tissue mechanics during *Drosophila* gastrulation movements. *Nature Commun.* **6,** 8677 (2015).
73. Desprat, N., Supatto, W., Pouille, P., Beaurepaire, E. & Farge, E. Tissue deformation modulates twist expression to determine anterior midgut differentiation in *Drosophila* embryos. *Dev. Cell* **15,** 470–477 (2008).
74. Butler, L. C. *et al.* Cell shape changes indicate a role for extrinsic tensile forces in *Drosophila* germ-band extension. *Nature Cell Biol.* **11,** 859–864 (2009).
75. Lye, C. M. *et al.* Mechanical coupling between endoderm invagination and axis extension in *Drosophila*. *PLoS Biol.* **13,** e1002292 (2015).
76. Martin, A. C., Gelbart, M., Fernandez-Gonzalez, R., Kaschube, M. & Wieschaus, E. F. Integration of contractile forces during tissue invagination. *J. Cell Biol.* **188,** 735–749 (2010).
77. Spahn, P. & Reuter, R. A vertex model of *Drosophila* ventral furrow formation. *PLoS ONE* **8,** e75051 (2013).
78. Hashimoto, H., Robin, F. B., Sherrard, K. M. & Munro, E. M. Sequential contraction and exchange of apical junctions drives zippering and neural tube closure in a simple chordate. *Dev. Cell* **32,** 241–255 (2015).
79. Alexandre, C., Baena-Lopez, A. & Vincent, J.-P. Patterning and growth control by membrane-tethered Wingless. *Nature* **505,** 180–185 (2014).
80. Farin, H. F. *et al.* Visualization of a short-range Wnt gradient in the intestinal stem-cell niche. *Nature* **530,** 340–343 (2016).
81. Kornberg, T. B. Cytonemes and the dispersion of morphogens. *Wiley Interdiscip. Rev. Dev. Biol.* **3,** 445–463 (2014).
82. Averbukh, I., Ben-Zvi, D., Mishra, S. & Barkai, N. Scaling morphogen gradients during tissue growth by a cell division rule. *Development* **141,** 2150–2156 (2014).
83. Snijder, B. & Pelkmans, L. Origins of regulated cell-to-cell variability. *Nature Rev. Mol. Cell Biol.* **12,** 119–125 (2011).
84. Curto, M., Cole, B. K., Lallemand, D., Liu, C.-H. & McClatchey, A. I. Contact-dependent inhibition of EGFR signaling by Nf2/Merlin. *J. Cell Biol.* **177,** 893–903 (2007).
Refs 84–90 provide mechanistic insights into how changes in cell contact and density can feed back into the activity of key signalling pathways.
85. Frechin, M. *et al.* Cell-intrinsic adaptation of lipid composition to local crowding

drives social behaviour. *Nature* **523,** 88–91 (2015).
86. Etoc, F. *et al.* A balance between secreted inhibitors and edge sensing controls gastruloid self-organization. *Dev Cell* **39,** 302–315 (2016).
87. Klingner, C. *et al.* Isotropic actomyosin dynamics promote organization of the apical cell cortex in epithelial cells. *J. Cell Biol.* **207,** 107–121 (2014).
88. Haag, A. *et al.* An *in vivo* EGF receptor localization screen in *C. elegans* identifies the Ezrin homolog ERM-1 as a temporal regulator of signaling. *PLoS Genet.* **10,** e1004341 (2014).
89. Nallet-Staub, F. *et al.* Cell density sensing alters TGF-β signaling in a cell-type-specific manner, independent from Hippo pathway activation. *Dev. Cell* **32,** 640–651 (2015).
90. Narimatsu, M., Samavarchi-Tehrani, P., Varelas, X. & Wrana, J. L. Distinct polarity cues direct Taz/Yap and TGFβ receptor localization to differentially control TGFβ-induced Smad signaling. *Dev. Cell* **32,** 652–656 (2015).
91. Wang, H. *et al.* Rap–GEF signaling controls stem cell anchoring to their niche through regulating DE-Cadherin-mediated cell adhesion in the *Drosophila* testis. *Dev. Cell* **10,** 117–126 (2006).
92. Michel, M., Raabe, I., Kupinski, A. P., Pérez-Palencia, R. & Bökel, C. Local BMP receptor activation at adherens junctions in the *Drosophila* germline stem cell niche. *Nature Commun.* **2,** 415 (2011).
93. Matusek, T. *et al.* The ESCRT machinery regulates the secretion and long-range activity of Hedgehog. *Nature* **516,** 99–103 (2014).
94. Harmansa, S., Hamaratoglu, F., Affolter, M. & Caussinus, E. Dpp spreading is required for medial but not for lateral wing disc growth. *Nature* **527,** 317–322 (2015).
95. Nechiporuk, A. & Raible, D. W. FGF-dependent mechanosensory organ patterning in zebrafish. *Science* **320,** 1774–1777 (2008).
96. Lecaudey, V., Cakan-Akdogan, G., Norton, W. H. J. & Gilmour, D. Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development* **135,** 2695–2705 (2008).
97. Harding, M. J. & Nechiporuk, A. V. Fgfr–Ras–MAPK signaling is required for apical constriction via apical positioning of Rho-associated kinase during mechanosensory organ formation. *Development* **139,** 3130–3135 (2012).
98. Durdu, S. *et al.* Luminal signalling links cell communication to tissue architecture during organogenesis. *Nature* **515,** 120–124 (2014).
Refs 98 and 99 reveal crucial feedback roles for 3D tissue architecture in regulating cell fate and behaviour during organogenesis *in vivo*.
99. Shyer, A. E., Huycke, T. R., Lee, C., Mahadevan, L. & Tabin, C. J. Bending gradients: how the intestinal stem cell gets its home. *Cell* **161,** 569–580 (2015).
100. Karlsson, L., Lindahl, P., Heath, J. K. & Betsholtz, C. Abnormal gastrointestinal development in PDGF-A and PDGFR-α deficient mice implicates a novel mesenchymal structure with putative instructive properties in villus morphogenesis. *Development* **127,** 3457–3466 (2000).
101. Discher, D. E., Janmey, P. & Wang, Y.-L. Tissue cells feel and respond to the stiffness of their substrate. *Science* **310,** 1139–1143 (2005).
102. Bellas, E. & Chen, C. S. Forms, forces, and stem cell fate. *Curr. Opin. Cell Biol.* **31,** 92–97 (2014).
103. Chanet, S. & Martin, A. C. Mechanical force sensing in tissues. *Prog. Mol. Biol. Transl. Sci.* **126,** 317–352 (2014).
104. Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126,** 677–689 (2006).
Refs 104 and 105 elegantly demonstrate that changes in substrate mechanics can influence lineage decisions in cultured stem cells.
105. McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K. & Chen, C. S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **6,** 483–495 (2004).
106. Swift, J. *et al.* Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* **341,** 1240104 (2013).
107. Buckley, C. D. *et al.* The minimal cadherin–catenin complex binds to actin filaments under force. *Science* **346,** 1254211 (2014).
108. Mouilleron, S., Langer, C. A., Guettler, S., McDonald, N. Q. & Treisman, R. Structure of a pentavalent G-actin•MRTF-A complex reveals how G-actin controls nucleocytoplasmic shuttling of a transcriptional coactivator. *Sci. Signal.* **4,** ra40 (2011).
109. Sero, J. E. *et al.* Cell shape and the microenvironment regulate nuclear translocation of NF-κB in breast epithelial and tumor cells. *Mol. Syst. Biol.* **11,** 790 (2015).
110. Farge, E. Mechanical induction of Twist in the *Drosophila* foregut/stomodeal primordium. *Curr. Biol.* **13,** 1365–1377 (2003).
111. Dupont, S. *et al.* Role of YAP/TAZ in mechanotransduction. *Nature* **474,** 179–183 (2011).
The transcriptional regulators YAP and TAZ are shown to act as sensors and mediators of mechanical inputs, therefore providing a mechanism by which force can control gene expression and fate.
112. Wei, S. C. *et al.* Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1–G3BP2 mechanotransduction pathway. *Nature Cell Biol.* **17,** 678–688 (2015).
113. Piccolo, S., Dupont, S. & Cordenonsi, M. The biology of YAP/TAZ: Hippo signaling and beyond. *Physiol. Rev.* **94,** 1287–1312 (2014).
114. Maître, J.-L. *et al.* Asymmetric division of contractile domains couples cell positioning and fate specification. *Nature* **536,** 344–348 (2016).
This study uncovers a role for mechanics in regulating the first cell-fate decision in the early mouse embryo.
115. Brunet, T. *et al.* Evolutionary conservation of early mesoderm specification by mechanotransduction in Bilateria. *Nature Commun.* **4,** 2821 (2013).
116. Benham-Pyle, B. W., Pruitt, B. L. & Nelson, W. J. Mechanical strain induces E-cadherin-dependent Yap1 and β-catenin activation to drive cell cycle entry. *Science* **348,** 1024–1027 (2015).
117. Saha, A. *et al.* Determining physical properties of the cell cortex. *Biophys. J.* **110,** 1421–1429 (2016).
118. Etournay, R. *et al.* TissueMiner: a multiscale analysis toolkit to quantify how cellular processes create tissue dynamics. *eLife* **5,** e14334 (2016).
119. Bielmeier, C. *et al.* Interface contractility between differently fated cells drives cell elimination and cyst formation. *Curr. Biol.* **26,** 563–574 (2016).
120. Morelli, L. G., Uriu, K., Ares, S. & Oates, A. C. Computational approaches to developmental patterning. *Science* **336,** 187–191 (2012).
121. Eiraku, M. *et al.* Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472,** 51–56 (2011).
A groundbreaking study that describes the self-assembly of embryonic stem cells into optic-cup organoids in the absence of pre-patterned chemical or mechanical gradients.
122. Lancaster, M. A. & Knoblich, J. A. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* **345,** 1247125 (2014).
123. Clevers, H. Modeling development and disease with organoids. *Cell* **165,** 1586–1597 (2016).
124. Davies, J. A. & Cachat, E. Synthetic biology meets tissue engineering. *Biochem. Soc. Trans.* **44,** 696–701 (2016).
125. Morsut, L. *et al.* Engineering customized cell sensing and response behaviors using synthetic Notch receptors. *Cell* **164,** 780–791 (2016).
126. Eiraku, M., Adachi, T. & Sasai, Y. Relaxation–expansion model for self-driven retinal morphogenesis. *Bioessays* **34,** 17–25 (2012).
127. Turner, D. A., Baillie-Johnson, P. & Martinez Arias, A. Organoids and the genetically encoded self-assembly of embryonic stem cells. *Bioessays* **38,** 181–191 (2016).
128. Parks, S. & Wieschaus, E. The *Drosophila* gastrulation gene *concertina* encodes a Gα-like protein. *Cell* **64,** 447–458 (1991).
129. Kanesaki, T., Hirose, S., Grosshans, J. & Fuse, N. Heterotrimeric G protein signaling governs the cortical stability during apical constriction in *Drosophila* gastrulation. *Mech. Dev.* **130,** 132–142 (2013).
130. Homem, C. C. F. & Peifer, M. Diaphanous regulates myosin and adherens junctions to control cell contractility and protrusive behavior during morphogenesis. *Development* **135,** 1005–1018 (2008).
131. Fox, D. T. & Peifer, M. Abelson kinase (Abl) and RhoGEF2 regulate actin organization during cell constriction in *Drosophila*. *Development* **134,** 567–578 (2006).
132. Hampoelz, B., Hoeller, O., Bowman, S. K., Dunican, D. & Knoblich, J. A. *Drosophila* Ric-8 is essential for plasma-membrane localization of heterotrimeric G proteins. *Nature Cell Biol.* **7,** 1099–1105 (2005).
133. Fuse, N., Yu, F. & Hirose, S. Gprk2 adjusts Fog signaling to organize cell movements in *Drosophila* gastrulation. *Development* **140,** 4246–4255 (2013).
134. Spahn, P., Ott, A. & Reuter, R. The PDZ-GEF protein Dizzy regulates the establishment of adherens junctions required for ventral furrow formation in *Drosophila*. *J. Cell Sci.* **125,** 3801–3812 (2012).
135. Sawyer, J. K. *et al.* A contractile actomyosin network linked to adherens junctions by Canoe/afadin helps drive convergent extension. *Mol. Biol. Cell* **22,** 2491–2508 (2011).
136. Zhang, Y. *et al.* The glucosyltransferase Xiantuan of the endoplasmic reticulum specifically affects E-Cadherin expression and is required for gastrulation movements in *Drosophila*. *Dev. Biol.* **390,** 208–220 (2014).
137. Wang, Y.-C., Khan, Z. & Wieschaus, E. F. Distinct Rap1 activity states control the extent of epithelial invagination via β-catenin. *Dev. Cell* **25,** 299–309 (2013).
138. Menzies, A. S. Mena and vasodilator-stimulated phosphoprotein are required for multiple actin-dependent processes that shape the vertebrate nervous system. *J. Neurosci.* **24,** 8029–8038 (2004).
139. Nakajima, H. & Tanoue, T. Epithelial cell shape is regulated by Lulu proteins via myosin-II. *J. Cell Sci.* **123,** 555 (2010).
140. Simões, S. de M. *et al.* Rho-kinase directs Bazooka/Par-3 planar polarity during *Drosophila* axis elongation. *Dev. Cell* **19,** 377–388 (2010).
141. Munjal, A., Philippe, J.-M., Munro, E. & Lecuit, T. A self-organized biomechanical network drives shape changes during tissue morphogenesis. *Nature* **524,** 351–355 (2015).
142. Tamada, M., Farrell, D. L. & Zallen, J. A. Abl regulates planar polarized junctional dynamics through β-catenin tyrosine phosphorylation. *Dev. Cell* **22,** 309–319 (2012).
143. Levayer, R., Pelissier-Monier, A. & Lecuit, T. Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis. *Nature Cell Biol.* **13,** 529–540 (2011).
144. Simões, S. D. M., Mainieri, A. & Zallen, J. A. Rho GTPase and Shroom direct planar polarized actomyosin contractility during convergent extension. *J. Cell Biol.* **204,** 575–589 (2014).
145. Turing, A. M. The chemical basis of morphogenesis. *Phil. Trans. R. Soc. Lond. B* **237,** 37–72 (1952).

Acknowledgements We are grateful to S. De Renzis, F. Peri and the Gilmour and Leptin groups for discussions. We thank C. Böckel, S. Durdu, A. Shyer and T. Hiiragi for help with Fig. 3.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this paper at go.nature.com/2hxuecb. Correspondence should be addressed to D.G. (gilmour@embl.de) or M.L. (mleptin@uni-koeln.de).

Reviewer Information *Nature* thanks C.-P. Heisenberg and the other anonymous reviewer(s) for their contribution to the peer review of this work.