Mechanotransduction in development: a growing role for contractility

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Abstract | Mechanotransduction research has focused historically on how externally applied forces can affect cell signalling and function. A growing body of evidence suggests that contractile forces that are generated internally by the actomyosin cytoskeleton are also important in regulating cell behaviour, and suggest a broader role for mechanotransduction in biology. Although the molecular basis for these cellular forces in mechanotransduction is being pursued in cell culture, researchers are also beginning to appreciate their contribution to *in vivo* developmental processes. Here, we examine the role for mechanical forces and contractility in regulating cell and tissue structure and function during development.

Morphogen
A diffusible signalling molecule that is usually found in a concentration gradient. Morphogens regulate tissue patterning during development.

Stiffness
The degree to which the surrounding adhesive scaffold resists deformation. Stiffness is also defined as the elastic modulus of a material.

Contractility
The ability of a cell to shorten or shrink in response to a stimulus. It is generated by the motor myosin II, which uses ATP hydrolysis to walk along actin filaments.

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Although early research of embryogenesis focused on both the structural rearrangements that give rise to complex morphological body plans and the mechanical origins of such rearrangements[1,2], much of our modern descriptions of the process are presented in terms of spatiotemporally coordinated changes in gene expression patterning. Only recently have investigators begun to integrate these two approaches to provide early hints of a more global model that incorporates the contribution of mechanics to our modern molecular model of development.

The early developmental stages from an egg to a detailed body plan differ between species, but in general they are often characterized by common structural rearrangements (BOX 1). At the cellular level, many of these stereotypic events arise from the coordinated and iterative regulation of many basic cellular processes, including proliferation, differentiation and spatial rearrangements (BOX 1). In addition to the indispensable functions of different genetic programmes and soluble morphogens, these cellular processes are also regulated by mechanical forces. Much work has uncovered how mechanical forces are transduced into biochemical signals (mechanotransduction) and how mechanotransduction in turn affects numerous cell functions[3]. In parallel, recent studies *in vivo* have begun to characterize the forces that cells might experience during development.

Here, we explore our nascent understanding of mechanical forces during embryogenesis and examine how these forces might specifically regulate basic cellular processes (such as proliferation, differentiation and organizational changes) in the broader context of embryogenesis. For this reason, this Review is not tailored to one specific species, but rather is written to be a general perspective. Drawing from both *in vitro* and *in vivo* studies from several model systems, we explore how actomyosin-mediated contractile forces regulate these cellular processes and discuss how they might be mechanistically controlled during development. By focusing specifically on how forces in embryogenesis might drive changes in cell proliferation, differentiation and organizational changes that are associated with development, we hope to integrate recent data within a broader picture of the biology of mechanotransduction.

Biomechanics during embryogenesis

Two main factors contribute to the mechanical stresses that are experienced by cells and influence cell behaviour in early development — the mechanical stiffness of the local tissue environment and the contractile activity of the cells that are pulling on that environment. Stiffness and contractility both contribute to the cellular mechanical stresses that are essential for mechanotransduction. Cells routinely contract to pull on the scaffolds to which they are attached (the extracellular matrix (ECM) or other cells), thereby generating tension in the cell (internal mechanical stress). The magnitude of such stress is affected both by the strength of contractile activity in the cell and the substrate stiffness. In development, understanding the interplay between cellular contractile activity, stiffness of surrounding tissues and the resulting mechanical deformations and stresses is crucial for refining our model of embryogenesis.

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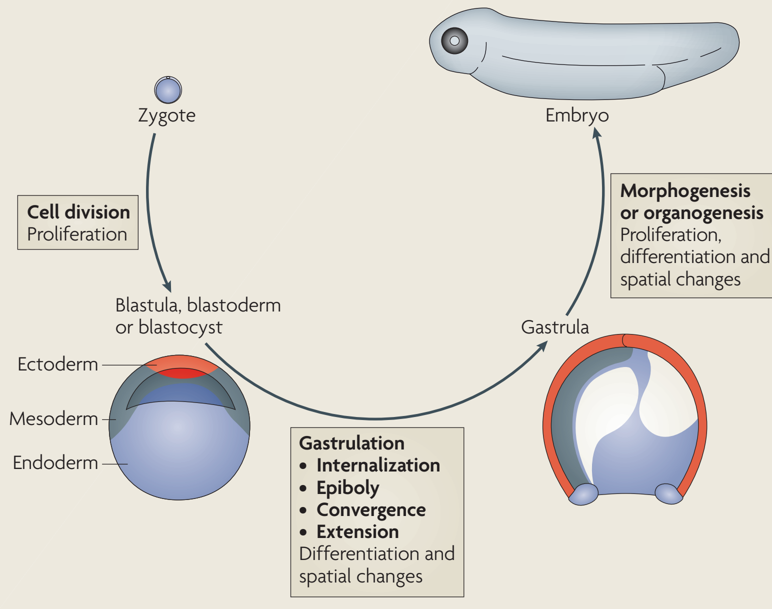
Box 1 | **Key developmental steps of embryogenesis**

Throughout development, and particularly during embryogenesis, there is tight coupling between changes in gene expression, cell shape and multicellular organization. In the figure, the specific sketches of various stages are modelled on *Xenopus laevis* embryogenesis, and the key cellular processes of embryogenesis are shown. Zygotic cell proliferation gives rise to a blastula, which then forms an inner cell mass to become a blastocyst. Gastrulation is the process by which the blastocyst is transformed into a gastrula, which has different germ layers (in most organisms the gastrula has three germ layers — the mesoderm, the ectoderm and the endoderm). Gastrulation consists of several different steps. First, after progenitor cells are sorted, apical constriction and internalization movements position the nascent mesoderm and endoderm beneath the prospective ectoderm. Epiboly events (including intercalation) then expand and thin these nascent germ layers. Finally, convergence and extension mediolaterally narrow and anterioposteriorly lengthen the embryo, respectively, to form the gastrula. After gastrulation, the gastrula undergoes several morphogenetic movements that give rise to specialized tissues and organs of the embryo.



Convergence
Embryonic movements that mediolaterally narrow the tissue.

Mesoderm
One of the three germ layers that are produced by gastrulation. It gives rise to bone, muscle and connective tissue.

Notochord
A flexible rod of mesodermal cells that defines the axis of the embryo to provide support.

Involuting marginal zone
The vegetal portion of the marginal zone (a region between the animal and vegetal hemispheres) of the *X. laevis* embryo that turns inside the embryo during involution.

Stiffness of embryos. There is *in vivo* evidence that stiffness is important during embryogenesis. For example, during *Xenopus laevis* gastrulation, convergence and extension movements can only occur if the mesoderm and notochord remain stiff enough to resist buckling[4,5]. During this same process, the involuting marginal zone actively stiffens so that this tissue does not collapse or deform during gastrulation[6]. Whether these changes in tissue stiffness at various stages are strictly to provide mechanical strength for morphogenetic events or also present a mechanotransduction stimulus to orchestrate other cellular processes (such as proliferation or differentiation) remains unclear. Nonetheless, these data suggest that mechanisms exist to modulate tissue stiffness and, furthermore, that these changes in stiffness are required for development to proceed.

Because of the technical challenges of accurately measuring mechanical parameters *in vivo* (BOX 2), only a few studies have directly measured embryo stiffness. Stiffness is determined empirically by applying a defined force across a specific area (stress), and then measuring the resulting deformation (strain). The slope of the stress–strain plot is the stiffness, reported in units of Pascals (Pa). Stiffness values of different tissues during *X. laevis* gastrulation range from 3 to 14.2 Pa[6]. Likewise, the estimated stiffness of *Ambystoma mexicanum* early stage embryos is approximately 20 Pa[7]. Stiffness of embryonic tissues is low in comparison to adult tissues[6–8], which range from 17 Pa (human fat) to 310 MPa (the rat Achilles tendon)[9]. Given the apparent importance of stiffness to cell function, it will be especially meaningful to develop approaches to track how stiffness values change in different tissues during the many movements and tissue rearrangements that occur in embryogenesis.

Tissue stiffness might arise from several different factors, including the stiffness of the cells (which is usually regulated by the cytoskeleton[10]), the strength of cell–ECM or cell–cell contacts, the biochemical identity of ECM proteins, and ECM organization and maturation. It is proposed that during convergence and extension movements in *X. laevis*, stiffness arises primarily from changes in the cytoskeleton and the ECM[6]. Given the dramatic changes in cell–cell and cell–matrix adhesion that also occur during this complex rearrangement of cells, it is likely that changes in adhesion also contribute to tissue stiffness. However, because manipulations that target any one of these systems (the cytoskeleton, cell adhesions or ECM) often feed back to affect all three, it has been difficult to develop an appropriate *in vivo* model to study how these different factors independently contribute to the stiffness of a tissue.

The stiffness of the ECM *in vitro* has emerged as an important regulator of cell function. Decreasing substrate stiffness seems to alter cell structure in many cell types: cell spreading against the substrate and the formation of focal adhesions and stress fibres are reduced[11]. Changes in stiffness also have potent effects on behaviour. In direct contrast to traditional culture on plastic, many cell types maintain a more differentiated phenotype when cultured on less stiff substrates, as these are more reflective of the stiffness of their *in vivo* tissue environment[9,12–14]. Mesenchymal stem cell (MSC) specification to different lineages is also strongly influenced by substrate stiffness[15]. When MSCs are cultured on soft substrates, which resemble the stiffness of brain tissue, genetic profiling suggests that these cells undergo neuronal differentiation. However, on substrates of intermediate stiffness similar to striated muscle, MSCs differentiate into myoblasts. On stiff substrates that mimic bone stiffness, MSCs undergo osteogenesis[15]. These *in vitro* studies highlight the important influence that stiffness can have on cell function and suggest that changes in stiffness might also regulate cell function during embryogenesis.

Cell-generated forces during embryogenesis. In addition to changes in stiffness, various internal and external forces also contribute to mechanical stresses during embryogenesis. For the purposes of this Review, we define these forces at the cellular level: 'internal forces' refer to contractile forces that are generated internally by the actomyosin cytoskeleton, whereas 'external forces' refer to forces that are generated outside of the cell that

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Mesenchymal stem cell
A multipotent stem cell that retains the ability to differentiate into multiple cell types.

Dorsal closure
The process during *D. melanogaster* embryogenesis whereby the two sides of epidermal tissue grow to close and cover the dorsal opening. During this time, the underlying amnioserosa is also stretched to separate the yolk sac from the vitelline envelope.

Egg chamber
A chamber in *D. melanogaster* that consists of a germline cyst that is covered by a somatic epithelium. During morphogenesis, the cyst grows in a proliferation-independent manner, whereas the epithelium grows by proliferation.

is responding to the force. Therefore, by these definitions, cell-generated forces in one part of a mechanically active tissue (in which the force is internal) might cause passive deformation of a neighbouring tissue, such that the tissue responds to an external force. However, at the tissue level, the definition of external forces refers to forces that are developed outside of the system (for example, intracardiac fluid forces that are required for embryonic cardiogenesis[16]). Although these types of external tissue forces are also crucial during embryonic development[16], their analysis is beyond the scope of this Review.

An example of cellular internal forces (cell-generated contractile forces) that regulate embryogenesis is shown in the *X. laevis* dorsal involuting and non-involuting marginal zones. Cultured explants of these tissues still converge and extend, which suggests that the tissue itself — and not the external forces that are generated in a different place in the embryo — actively regulates these movements in gastrulation[17]. Although there are several methods used to observe and measure cell-generated forces at the *in vitro* single-cell level (BOX 3), these methods are difficult to translate to embryos (BOX 2).

One method that has been used to give insight into the forces that are required for embryogenesis is laser ablation[18–24] (BOX 2). Laser-ablation studies in *Caenorhabditis elegans* and *Drosophila melanogaster* have reported changes in global movements that are too fast to be explained by signal transduction cascades, which suggests that such movements are mechanical in nature[20,23,25]. In conjunction with laser-ablation studies, quantitative mechanical modelling has been used to form hypotheses regarding how these forces regulate movements in the embryo[21,23]. Based on laser-ablation studies to examine how different tissues mechanically contribute to *D. melanogaster* dorsal closure[21,22], four forces have been proposed to contribute to dorsal closure: first, the leading edge of the lateral epidermis is under tension and behaves like a 'supracellular purse string'; second, the amnioserosa is under tension; third, the amnioserosa contracts; and fourth, tension in the ventral lateral epidermis opposes the dorsally located contraction[22,23] (FIG. 1). These forces probably arise from non-muscle myosin II-dependent contraction[21,22], because restoration of myosin motor activity in any one of the three areas that generate tension can restore dorsal closure in myosin-mutant embryos[26].

Recent work that used laser microdissection in *D. melanogaster* embryos revealed apoptosis as another unexpected source of force generation. Although apoptosis was known to occur during dorsal closure to remove supernumerary cells, it also contributes between one-half and one-third of the forces needed during dorsal closure[27]. *In vitro* studies in epithelial monolayers have shown that neighbouring cells increase contractility to actively extrude the apoptotic cell[28] from the monolayer. Therefore, these apoptotic events in *D. melanogaster* embryos might act as triggers of local contraction that can spread through the amnioserosa to propagate the force generation that is required for dorsal closure to proceed[27,29].

Box 2 | **Techniques that are used to study mechanics in embryos**

Characterizing mechanics is difficult at the single-cell level because cells are dynamic — they generate and respond to force. At the embryonic level, this task becomes even more complicated. Embryos offer more challenges than single cells, including increased tissue fragility, difficulty in defining the regions of interest in a small embryo and the continuous dynamic movements that cause gross tissue deformations[7,24]. However, several methods have been used to understand tissue mechanics during embryogenesis. Most of these methods rely on applying forces to explanted embryonic tissues and observing their behaviour to define the mechanical parameters. For example, in the stress-relaxation test, tissue explants of starting length L_o are compressed to length L, and the force required to maintain L is determined[6]. Similar tests can be done with parallel plates that compress the explanted tissue and measure its viscoelastic responses[101,102]. By contrast, cantilever tissue testers separate the embryonic epithelia from the embryo; cantilever wires are then used to elongate the tissue at a constant true strain rate. Force is then determined by measuring the bending of the wires[7]. A fibre-optic system has also been described that uses a flexible cantilevered optical fibre probe to apply force or deformation to an explanted tissue. The probe tip position and deflection measure tissue deformation and force, respectively[103]. Another method that is commonly used to assess the mechanical contributions of different tissues is laser ablation. By locally cutting a tissue, one can determine indirectly whether that tissue was under tension (by observing the tissue pulling away from the cutting site, as with a spring)[18,19,21,22,26].

Although all of these methods require external interventions, there are more recently described methods that do not require contact between a probe and the tissue. Particle tracking microrheology has been modified for use in *Caenorhabditis elegans* embryos. In this procedure, nanoparticles are microinjected into zygotes and particle movement is monitored to determine the local viscoelastic properties (including the diffusion coefficient and shear viscocity)[104]. In addition, a micromanipulation assay has recently been described in *Drosophila melanogaster* embryos. Ferrofluid can be injected into specific locations in the embryo and then magnetic tweezers can be used to manipulate the magnetized cells to apply tissue deformations[60]. Further refinement of these methods and other methods amenable to single cells will undoubtedly shed light on tissue mechanics during embryogenesis.

Proliferation and mechanical stress

Proliferation is an absolute requirement for development because it provides the necessary cellular mass for developing tissues. Proliferation must be tightly regulated during embryogenesis so that cells do not grow uncontrollably, as this would, among other consequences, disrupt the shape of the embryo and its developing tissues.

Mechanical feedback regulates proliferation. Wang and Riechmann have recently described a mechanism to explain how localized proliferation is controlled by mechanical stresses during *D. melanogaster* egg chamber morphogenesis[30]. As the epithelial cyst grows, epithelial cells proliferate to increase the surface area of the epithelium[30]. Localized myosin activity at the apical face of the epithelia leads to increased tension in the growing epithelial cyst, which results in localized proliferation to regulate tissue growth during oogenesis[30]. Furthermore, the cyst deforms when myosin activity is reduced or absent but blocking cyst growth suppresses these deformations, which indicates that there is a link between cyst tissue growth and cell proliferation[30]. These data suggest that tensional stresses increase proliferation, whereas compression slows growth[30,31]. Recent mathematical models corroborate that this type of mechanical feedback could stabilize growth to maintain *D. melanogaster* tissue shape and form[32,33].

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Box 3 | **Techniques used to study mechanics in single cells**

Two approaches are commonly used to study cell mechanics. Cell-generated forces can be measured or external forces can be applied to cells and their responses recorded to obtain information regarding the cellular mechanical parameters. Both approaches have been used successfully in single cells and in embryos (BOX 2).

To observe cell-generated forces in single cells, Harris *et al.*[105] first introduced wrinkling substrates more than 25 years ago. In this method, cells are cultured on thin films of silicone that wrinkle when cells pull on them[105]. Over the years, this procedure has been modified to be more quantitative. By plating cells on thin micropatterned elastomer substrates, cells will distort the substrates (and thus the patterns) so their displacements can be mapped and cell-generated forces can be calculated[106]. In addition, traction force microscopy also allows quantitative measures of force. In this technique, fluorescent beads are embedded in a flexible non-wrinkling material. As the cells pull the underlying material, the bead displacements are tracked, which can then be used to calculate cell-generated forces[107].

Laser tweezers and microneedles are capable of both measuring cell-generated forces and applying forces to cells. The laser tweezer technique uses a focused laser beam to physically hold an extracellular matrix-coated bead on cells. The amount of cell-generated force that is required to move the bead out of the laser trap can be calculated[108,109]. In addition, the strength of the laser trap can be increased to apply increasing force to the cell[108]. Microneedles are arrays of elastic posts that act as microcantilevers. When cells plated on these post arrays apply forces, the posts bend. Force can then be calculated by measuring the bending (post deflection)[58]. Forces can also be applied to cells using a magnetic modification of this system, whereby nanowires are interspersed in the posts so that a magnetic field will induce torque in the nanowires, causing post deflection to apply external force to the attached cell[110]. To understand how forces dynamically regulate cell behaviour, these methods have been used in conjunction with studies on focal adhesion formation and migration.

This mechanical feedback model was actually first proposed over 25 years ago by Ingber and colleagues, who suggested that the tensional and compressional forces that are transmitted through a tissue might continually feed back to regulate tissue shape and form[34]. Our laboratory has reported experimental evidence in support of these models, showing that regions of high tensional stress in epithelial monolayers correlate with increased proliferation *in vitro*[35]. Inhibition of myosin-generated tension or disruption of cell–cell contacts relaxes these regions of stress, which leads to the inhibition of proliferation. So, tissue form and forces can in fact feed back to regulate growth[35]. Furthermore, it is not contractility *per se* that directly regulates proliferation, but rather the resultant mechanical stresses that are associated with contractility that can be transmitted through a tissue. In this regard, it is important to note that mechanical feedback-regulated proliferation probably applies to embryogenesis only after the earliest stages of blastocyst formation. Although cell division is necessary to form a blastocyst from the zygote, the shortened cell cycle that controls proliferation at this stage is regulated independently of cell–cell interactions[36,37].

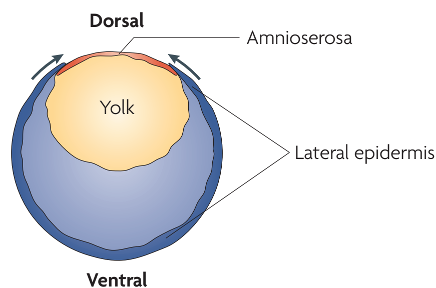

Figure 1 | **Forces that regulate *Drosophila melanogaster* dorsal closure.** A diagram of a cross-section of a *Drosophila melanogaster* embryo in the early stages of dorsal closure. The surface of the embryo (including the lateral epidermis and amnioserosa) is thought to be under tension throughout this stage, partly as a result of the contractile activity of the cells in these tissues[22]. The arrows show the movement of the tissue that results from these forces. Figure is modified, with permission, from REF. 22 © (2000) Rockefeller University Press.

Blastocyst
A structure in early embryogenesis that contains the inner cell mass. The blastocyst gives rise to the embryo.

Cytoskeletal tension regulates cell proliferation. Several lines of evidence implicate cytoskeletal tension as a strong regulator of proliferation. For example, a decrease in proliferation is observed in smooth muscle cells on low stiffness substrates or that have inhibited contractility[38,39]. The small GTPase RhoA regulates contractility[40] and is also required for proliferation[41]. The RhoA effector, Rho kinase (ROCK), induces contractility through the phosphorylation of myosin light chain (MLC; also known as MYL) and MLC phosphatase to increase myosin ATPase activity[42–44]. *In vitro*, inhibition of ROCK in many diverse cell types inhibits proliferation[39,45,46], whereas activation of ROCK is necessary and sufficient to induce G1–S-phase cell-cycle progression[47]. The RhoA–ROCK pathway seems to regulate proliferation, at least in part, through its effects on contractility and force generation, as inhibition of myosin also blocks proliferation *in vitro*[35,48]. Contractile regulation of proliferation is also observed in models of blood vessel mechanotransduction. *In vivo*, blood vessels are subjected to various strains that are created by pulse pressure. *In vitro* models that mimic these forces show that stretch is a potent activator of RhoA signalling and proliferation in endothelial and smooth muscle cells[49,50]. RhoA or ROCK inhibition blocks stretch-dependent proliferation[49], again highlighting the requirement for forces and contractility in proliferation.

Linking proliferation and cell shape changes. Changes in cell shape and morphology are required at most steps of embryogenesis[31]. Although these changes are usually described as being the result of myosin-driven cell movements or upregulation of specific genes[51], cell shape also has

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an important role. Folkman and Moscona were the first to show that cell proliferation could be regulated by changes in cell shape[52]. Using poly(2-hydroxyethyl methacrylate) to modulate cell shape, they found that DNA synthesis increases with cell spreading and flattening against the substrate, which suggests that cell shape has a key — although underappreciated — role in growth regulation[52]. Early studies that used changes in ECM density to control cell shape also reported shape-regulated proliferation[53]. Sophisticated microcontact printing techniques were later used to adjust the extent of cell spreading without changing ECM density to confirm that cell shape *per se* imparts proliferative cues[54]. Mechanistically, it seems that cell shape regulates proliferation in late G1 phase by regulating RhoA and its effector mammalian diaphanous (DIA). Restricting cell spreading blocks RhoA and DIA-dependent SKP2 expression — when expressed, this ubiquitin ligase ubiquitylates the cyclin-dependent kinase (CDK) inhibitor p27^{KIP} and regulates its degradation[55,56]. p27^{KIP} (also known as CDKN1B) is an inhibitor of the cyclin D1–CDK4 complex; p27 degradation releases this inhibition on the complex, which phosphorylates retinoblastoma (RB) and allows cell-cycle progression in spread cells[55,56] (FIG. 2).

Regulation of proliferation by cell shape also seems to be mediated through the effects of cell shape on ROCK-mediated contractility. Restricting cell spreading suppresses RhoA activity and cellular force generation, and constitutively activated RhoA rescues proliferation in unspread cells[57,58]. This suggests a model in which cell shape regulates RhoA–GTP levels to control DIA and ROCK activity, which both contribute to cell proliferation (FIG. 2).

The regulation of proliferation by cell shape and forces is particularly intriguing because there are many events during embryogenesis that involve dramatic changes in cell shape, structure and mechanics (see above). In the adult, it is thought that muscle, skin and other soft tissues in a limb react (by increasing proliferation) not just to soluble cues but also to tensional forces that are generated by the growing long bones. This model is borne out in orthopaedic settings in which the lengthening of a limb bone results in the coordinated growth of all of the surrounding soft tissue[59]. Thus, as in adult tissues, we postulate that the local stresses and shape changes generated during late embryogenesis could provide local proliferative controls that can maintain tissue mass homeostasis.

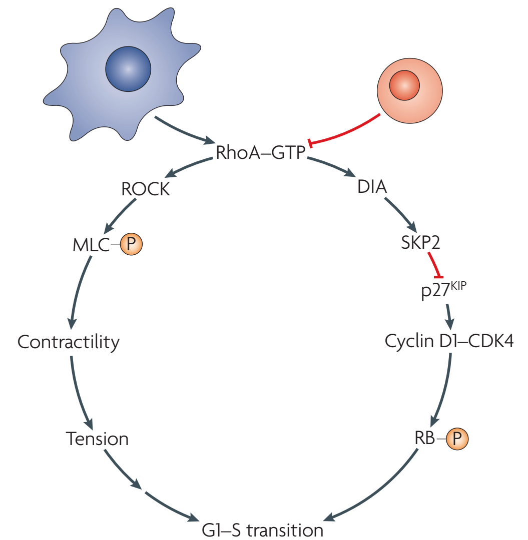

Figure 2 | **Cell shape regulates proliferation through the small GTPase RhoA.** Restricting cell spreading decreases proliferation through the regulation of RhoA activity. RhoA promotes G1–S-phase transition and cell proliferation through two pathways. First, the RhoA effector, Rho kinase (ROCK), increases myosin light chain (MLC; also known as MYL) phosphorylation to generate cellular contractility. This generates the tension in the cell that is required for proliferation[57,58]. Second, the RhoA effector, diaphanous (DIA), activates the ubiquitin ligase SKP2 to inhibit the cyclin-dependent kinase (CDK) p27^{KIP} (also known as CDKN1B). p27^{KIP} can no longer degrade the cyclin D1–CDK4 complex, so this complex phosphorylates retinoblastoma protein (RB), thereby leading to the G1–S-phase transition[55,56]. Restricting cell shape decreases RhoA activity in some cell types, so these two pathways are not activated. Without contractility and tension generation as well as SKP2 activity, G1–S-phase transition is blocked and proliferation is reduced.

Poly(2-hydroxyethyl methacrylate)
(polyHEMA). A hydrophilic polymer that prevents cell attachment and spreading.

Microcontact printing
A method in which an elastomeric stamp with relief features is used to transfer 'inked' molecules (usually self-assembled monolayers or ECM proteins) onto the surface of a substrate through conformal contact.

Germband extension
The process by which the *D. melanogaster* embryo lengthens and narrows during gastrulation.

Apical constriction
Apically localized actomyosin-driven inward bending of tissue to promote invagination.

Mechanotransduction and differentiation

Differentiation is necessary during many stages of development so that differentiated cells can perform their specific functions. Both mechanical forces and cell-generated contractility regulate differentiation *in vitro* and *in vivo*.

***Twist is mechanically regulated* in vivo.** Intriguing evidence from *D. melanogaster* suggests that mechanotransduction might regulate differentiation *in vivo*. During gastrulation, germband extension (GBE) causes an endogenous compression of stomodeal cells, which correlates with an increase in *twist* expression[60,61]. This is one of the genes that controls the formation of the digestive tract[62] and regulates apical constriction during mesoderm or midgut invagination[63]. Uniaxial stretching of *D. melanogaster* embryos upregulates *twist* expression, which suggests that Twist is sensitive to mechanical perturbations during GBE[61] (FIG. 3).

To test the role of mechanical forces on *twist* expression, laser ablation of the *D. melanogaster* dorsal epithelium was used to prevent the deformation of the future digestive track anterior pole cells of the embryo, which normally occurs during gastrulation. Laser ablation inhibited both *twist* expression in these cells and subsequent tissue invagination[61]. Normal levels of stomodeal *twist* expression in laser-ablated embryos can be rescued by mimicking GBE-triggered endogenous deformation, by using either a micromanipulated needle or magnetic tweezers to compress the adjacent ferrofluid-injected tissue[60]. Furthermore, femtosecond laser-pulse-induced ablation and third-harmonic generation microscopy (to visualize both velocity fields and cell movements during *D. melanogaster* GBE) were used to verify that active tissue movements in the ventral side of the embryo

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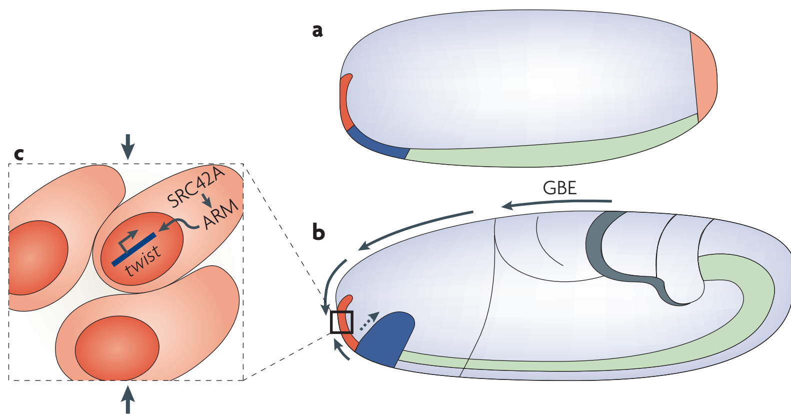

Figure 3 | **Mechanical regulation of *twist* gene expression. a** | A *Drosophila melanogaster* embryo at the beginning of gastrulation. **b** | An embryo during germband extension (GBE). In gastrulation, the tissue-lengthening movements that occur during GBE push the tissue in a posterior direction, causing tissue buckling (black arrows). At the same time, the endoderm (blue) invaginates (dashed arrow). This causes compression of the adjacent stomodeal cells (red). **c** | It is postulated that this compression (black arrows) leads to the SRC42A (a close relative of the vertebrate Src)-dependent nuclear translocation of the Armadillo (ARM; the vertebrate β-catenin) transcription factor, which increases *twist* expression[60,61]. This model places *twist* expression in the appropriate location to regulate midgut differentiation. Figure is modified, with permission, from REF. 61 © (2003) Cell Press.

correlate with *twist* mechanosensitive gene expression[25]. Mechanistically, this compression led to force-dependent nuclear translocation of Armadillo (the fly homologue of β-catenin) to increase *twist* expression in a SRC42A-dependent manner[60,61] (FIG. 3). Together, these data suggest that the compressive strain or the decreased dimensions that are caused by GBE-induced tissue deformations propagate through the dorsal tissue to control *twist* expression during *D. melanogaster* early gastrulation[60,61].

Twist regulation of *D. melanogaster* mesoderm invagination implies that Twist activity might feed back to regulate contractility during apical constriction. How might mechanically activated *twist* expression, in turn, regulate cellular contraction? Twist is a master regulator of cell shape changes during mesoderm invagination. Twist activates the transcription of *folded gastrulation* (*fog*), an apically secreted protein that regulates cell shape changes during gastrulation[64]. These mesoderm cells receive this FOG signal at their apical face, and this causes activation of a RhoA exchange factor, Rho guanine nucleotide-exchange factor 2 (RhoGEF2)[51], through two cooperative mechanisms. RhoGEF2 is released from microtubules and is localized at the apical side of the cells[65]. At the same time, Twist-dependent upregulation of the transmembrane protein T48 is targeted to the apical membrane, where it binds to RhoGEF2 (through its PDZ domain)[66]. This apical localization of RhoGEF2 results in enhanced Rho activity and activation of the Rho effector ROCK. Asymmetrical ROCK activity leads to polarized actin and myosin accumulation; thus, the polarized actomyosin contracts at the apical side, which leads to constriction[51]. Because actin is tethered to adherens junctions, this contraction is postulated to cause the apical localization of these cell–cell contacts in *D. melanogaster*[51]. It is important that contraction is properly regulated during apical constriction. This could be accomplished if the movements created by constriction activate Twist, leading to a positive-feedback loop[67].

Contractility regulates differentiation. Further support that contractile forces are necessary in development is provided by MSC lineage commitment and differentiation studies. A murine genetic knockout of p190B Rho GTPase-activating protein (RhoGAP), which is a negative regulator of RhoA activity, has defects in adipogenesis[68]. p190B RhoGAP-knockout fibroblasts show defective adipogenesis and enhanced myogenesis, which suggests that enhanced RhoA activity inhibits its differentiation into the adipogenic lineage[68]. By investigating the role of RhoA-mediated contractility in *in vitro* lineage commitment and differentiation in human MSCs, it was found that RhoA and ROCK-generated contraction is required for MSC commitment into the osteoblast cell fate. This pathway inhibits MSC adipogenesis[48]. Furthermore, it was previously observed that adipogenesis is inhibited in spread cells[69]. We have confirmed that cell shape acts as a master regulator of this lineage switch[48]. These studies implicated RhoA and ROCK-generated contractility as the mediator of shape-regulated lineage commitment, whereby well-spread cells increased contractility and osteogenesis and unspread cells suppressed contractility to promote adipogenesis[48]. The MSC lineage differentiation effect of substrate stiffness (see above) is also dependent on contractility, as inhibition of non-muscle myosin II blocked differentiation into any of the lineages studied: neuronal, myogenic and osteogenic[15]. Together, these *in vitro* and *in vivo* studies support a central role for contractile forces in differentiation during development.

Contractility also regulates *in vitro* differentiation in adult tissues. More than 20 years ago, it was reported that mammary epithelial cells form differentiated structures only when cultured on floating collagen gels, and not more rigid substrates, such as two-dimensional glass, petri dishes or even on collagen gels that remain attached to the dish[70,71]. This finding was important because mammary cell culture on rigid two-dimensional substrates is different from the *in vivo* environment that cells normally encounter[13]. For mammary epithelial differentiation to occur, contraction of the floating collagen gel is required[72]. This was a seminal observation, as it was later shown that many cell types contract their surrounding matrix during *in vitro* differentiation and morphogenesis[73–76]. Inhibition of myosin-mediated contractility blocks matrix contraction and differentiation, thereby confirming that contractile forces are required for differentiation in many different cell types and contexts *in vitro*[13,15,76,77].

Thus, although GBE-induced Twist regulation in *D. melanogaster* shows the intricate interactions between mechanical forces, gene expression and differentiation in a developmental context, *in vitro* studies suggest that many other factors must be present to orchestrate the many complex movements that are demanded in early

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embryogenesis. The presence of such mechanotransduction mechanisms in adult stem cells and differentiated cells points to a clear gap in our understanding of their relevance to developmental biology.

Spatial organization of cells

The spatial organization of cells is regulated by morphogenetic movements and is crucial for tissue structure and function. There are several examples of how mechanotransduction and contractile forces might regulate embryonic cellular movements that result in proper cell and tissue organization.

Cell-generated force in border-cell migration. Mechanical forces are thought to be important in border-cell migration — in which clusters of *D. melanogaster* follicle cells migrate down the centre of the developing egg chamber towards the nurse cell–oocyte border during oogenesis[78] (FIG. 4a). Filamentous (F)-actin cytoskeletal dynamics control a crucial checkpoint for this migration, as mutants of actin-regulatory proteins disrupt border-cell migration[79]. F-actin dynamics regulate the activity of the transcription factor serum response factor (SRF) and its cofactor MAL (also known as MKL1)[80]. In turn, SRF-mediated transcription regulates the expression of many proteins, including actin-regulatory proteins[81]. Indeed, border cells expressing a MAL mutant show altered cytoskeletal rearrangements and F-actin dynamics that lead to cell fragmentation; this fragmentation prevents productive border-cell migration[79,82].

Mechanical tension is proposed to regulate SRF-dependent gene expression in *D. melanogaster*. Analysis of MAL nuclear localization (which is an indicator of activity) showed that MAL nuclear localization is most apparent in cells that appear stretched[82]. To determine if stretching could directly affect MAL nuclear translocation, *slow border cells* (*slbo*) mutants were analysed for MAL localization. These mutants cannot migrate. However, because cells move as clusters during border-cell migration, and not as individual cells, they can be pulled by other wild-type cells, probably through adhesion complexes[83]. Mutant cells only showed MAL nuclear translocation when pulled by other wild-type cells[82]. Therefore, it was proposed that tension-induced MAL nuclear accumulation allows MAL and SRF to maintain the gene expression that is required for a robust cytoskeleton, which is necessary for efficient migration and cellular differentiation[82] (FIG. 4a). Stretch-induced regulation of SRF also increases differentiation markers *in vitro* in vascular smooth muscle cells[84], which suggests that local stretch, as generated by other cells, might be a common mechanism that has evolved to direct differentiation and morphogenesis in a number of tissues.

Wnt regulates contractility in embryogenesis. Wnts are secreted proteins that are essential regulators of development. They bind the Frizzled (FZ) family of receptors and members of the low-density-lipoprotein receptor-related protein (LRP) family to activate several intracellular signalling cascades that regulate diverse cell behaviours[85]. Wnt signalling is required for the establishment and

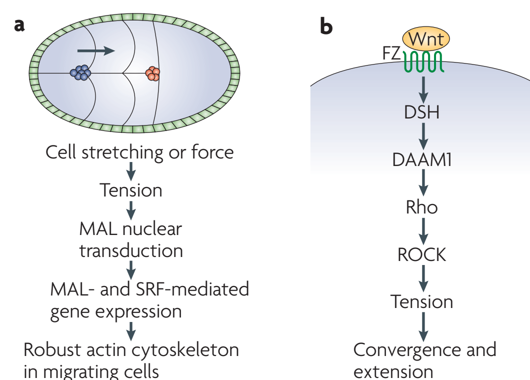

Figure 4 | **Forces regulate the spatial organization of cells.** **a** | During *Drosophila melanogaster* oogenesis, follicle cells migrate down the midline of the egg chamber (blue cells are migrating cells and red cells denote their final position). As cells are stretched or subjected to external force during migration, the tension generated causes the nuclear translocation of the serum response factor (SRF) cofactor MAL. Nuclear MAL and SRF can then regulate the expression of many genes, including the genes that are required for cytoskeletal integrity. This model is proposed to allow cells to assemble and maintain a robust actin cytoskeleton during migration[82]. **b** | The non-canonical Wnt pathway, also known as the planar cell polarity (PCP) pathway, regulates many morphogenetic movements that lead to cell and tissue spatial rearrangements during convergence and extension[89–91]. When Wnt binds to the Frizzled (FZ) receptor, it activates Dishevelled (DSH), which then activates Dishevelled-associated activator of morphogenesis 1 (DAAM1). This leads to Rho (RhoA in mammals) activation and Rho kinase (ROCK)-generated contractility and cellular tension.

maintenance of cell polarity during *C. elegans* gastrulation[86–88]. Besides regulating polarity, Wnt signalling also modulates actin cytoskeletal organization and contractile forces[87]. At the beginning of gastrulation, different progenitor cell types must separate and individual cell types then undergo apical constriction that allows them to internalize, which results in the separation of the nascent germ layers. Wnt signalling leads to the phosphorylation of MLC in the apical cortex, which generates localized contraction during this process[87].

Wnt signalling also regulates convergence and extension movements in *X. laevis* and zebrafish[89–91] (FIG. 4b). During *X. laevis* elongation, Wnt–FZ signalling activates the cytoplasmic scaffolding protein Dishevelled and the formin Dishevelled-associated activator of morphogenesis 1 (DAAM1)[90], which leads to activation of the small GTPase Rho[90,92]. Activation of Rho regulates the cytoskeletal changes that contribute to planar cell polarity (PCP) signalling[90] (also known as the non-canonical Wnt pathway; FIG. 4b). In contrast to the canonical Wnt pathway, the PCP pathway is transcription independent and contains different molecular players[93]. Whether Wnt–Rho signalling leads to changes in contraction remains unclear. However, the PCP pathway signals to Rho and ROCK (REF. 93). ROCK is a potent regulator of contractility and therefore it is possible that the PCP pathway might also regulate cellular contraction.

Formin
A protein that nucleates actin filaments to promote elongation.

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Wnt regulation of Rho and actomyosin contraction has important implications for mechanotransduction during embryogenesis. *In vitro* studies indicate that Wnt signalling might have a role in mechanotransduction. First, in osteoblasts, fluid shear activates Wnt pathways downstream of the early mechanosensitive genes β1 integrin (*IGTB1*) and cyclooxygenase 2 (*COX2*; also known as *PTGS2*), which mediate signalling downstream of shear-regulated osteoblast proliferation[94]. Second, the Wnt co-receptor LRP5 is required for strain-induced mechanotransduction in osteoblasts[95]. Strain also decreases the expression of the Wnt–LRP5 inhibitor sclerostin (which is encoded by *SOST*), which suggests that there are mechanisms to regulate Wnt activity downstream of mechanical cues. It is tempting to speculate that the various mechanical movements in embryogenesis could locally activate or modulate Wnt signalling in a similar fashion to the mechanical induction of *twist* expression[60,61]. Mechanical regulation of Wnt signalling could be a highly efficient way to create mechanical 'checkpoints' that control Wnt signal activation and duration during embryogenesis, thereby ensuring that certain structural criteria are met before the next stages of development are triggered.

Diverse roles for contractility. Contractile force is required for numerous processes during the movements that regulate the spatial organization of cells in embryogenesis. Myosin has recently been implicated in several other processes, which shows that cell-generated force has surprisingly diverse essential roles in embryogenesis. For example, myosin has been implicated in cell sorting at the beginning of gastrulation. One model for how progenitor cell types separate is the differential adhesion hypothesis, which states that cell sorting is a consequence of the different adhesive properties that are inherent to the cells[96]. Krieg has recently revisited this phenomenon in zebrafish and found that differences in actomyosin-dependent cell-cortex tension, and not only differences in adhesion, are necessary and sufficient for progenitor sorting[97]. Such studies show that, in addition to differential adhesion, local regulation of cytoskeletal tension provides another means to alter the interfacial energies that drive cell sorting.

Another unexpected role for myosin was shown during *D. melanogaster* GBE, as cells change position to physically extend and lengthen the tissues during gastrulation. Neither cell shape changes nor regulated cell proliferation seem to regulate GBE[98]. Rather, cell rearrangement is regulated by controlled global adherens junction remodelling that arises from myosin II-mediated junction disassembly and assembly[99]. The presence of myosin II at adherens junctions and its requirement for cell movements in GBE suggests that the local contractile forces between cells (and not external forces) drive *D. melanogaster* intercalation[99]. Although other mechanisms might also control cell rearrangements in other tissues and organisms[31,100], these data suggest that local cell-generated contractile forces are important regulators of cell function during embryogenesis.

Intercalation
The process by which cells rearrange and exchange neighbours to result in one plane of cells. This thins and expands the tissue during epiboly and convergence or extension.

Conclusions and future perspectives

Although mechanotransduction classically refers to the response of cells to applied forces, we have come to appreciate the importance of the forces that cells exert through regulated actomyosin contractility. These contractile forces allow cells to sense and respond to various different mechanical and structural contexts, and seem to be required for many steps in embryogenesis. Because the characterization of forces *in vivo* is a complicated and daunting task, it is important for the field to be able to draw from *in vitro* mechanotransduction studies to help explain complex developmental behaviours *in vivo*. Furthermore, understanding how cells sense and respond to mechanical cues is important not only for our understanding of embryogenesis but also for diseases, such as cancer, in which the mechanical properties of the microenvironment are postulated to regulate tumorigenesis[13] (see the Review by Jaalouk and Lammerding[111] in this issue).

To continue this type of comparative analysis, three main areas warrant further study *in vitro*. First, much of our understanding of cellular forces is based on measurements of those forces when cells are in isolation. Given that embryonic cells are almost always in contact with other cells, it is important that forces are also examined in multicellular contexts and that the forces between cells are characterized, as well as the forces that are exerted on matrices. Second, the role of key developmental patterning genes (such as the Wnts, BMPs and transcriptional regulators) in mechanotransduction should be further defined given their essential role in development. Finally, because we do not understand the constitutive behaviour of embryonic tissues, the field is in dire need of sophisticated *in vitro* three-dimensional experimental and conceptual models that will allow the detailed analysis of single cells embedded in tissues. These models should recapitulate specific aspects of embryogenesis, including physiological ranges of stiffness, to study how both forces and genetics cooperate to regulate cell rearrangements and tissue movements.

In parallel, to better understand how biomechanics controls development, we must have a more complete appreciation of the forces that are generated during embryogenesis. These forces, whether generated by the cell or external to the tissue, should be capable of both mechanically deforming the embryo and causing changes in signal transduction and downstream cellular processes that are required for development. Once these forces are characterized, forces (of physiological magnitude and duration) can then be reapplied to the embryo to determine the physical and biochemical responses[60]. In cases for which contractile forces are distributed throughout the embryo[21,22], this analysis will be crucial to understand how cells locally and globally respond to force. A detailed characterization of these forces, combined with quantitative modelling, will be necessary for the field to determine how mechanotransduction cooperates with other known pathways to regulate development. Therefore, to have a more complete understanding of development, our future challenge is to develop advanced *in vitro* and *in vivo* models to link the biochemical and biomechanical events of embryogenesis.

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Note added in proof

During the final preparation of this article, some important findings were published that corroborate the crucial role of myosin-mediated contractility during embryogenesis. Although it has been accepted that a continuous 'purse-string' contraction drives apical constriction, recent work has revealed that apical constriction in *D. melanogaster* embryos is pulsed[112]. Continuous pulses of contractions, followed by the stabilization of those contractions, lead to apical constriction during gastrulation[112]. Interestingly, in later embryogenesis, contractility during *D. melanogaster* elongation also has a key morphogenetic role. Differential myosin distribution generates anisotropic cortical forces that can drive junctional remodelling and intercalation during elongation[113]. Together, these findings support the key role of mechanical force during development and further our understanding of how these contractile forces are locally controlled.

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