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Mechanical Force Sensing in Tissues

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Abstract

Tissue size, shape, and organization reflect individual cell behaviors such as proliferation, shape change, and movement. Evidence suggests that mechanical signals operate in tandem with biochemical cues to properly coordinate cell behavior and pattern tissues. The objective of this chapter is to present recent evidence demonstrating that forces transmitted between cells act as signals that coordinate cell behavior across tissues. We first briefly summarize molecular and cellular mechanisms by which forces are sensed by cells with an emphasis on forces generated and transmitted by cytoskeletal networks. We then discuss evidence for these mechanisms operating in multicellular contexts to coordinate complex cell and tissue behaviors that occur during embryonic development: specifically growth and morphogenesis.

1. INTRODUCTION: MOLECULAR MECHANISMS OF MULTICELLULAR FORCE SENSING

Tissues are dynamic multicellular structures that are extensively remodeled, particularly during embryonic development when tissues of all different shapes and sizes are formed. Precise control of cell behaviors, such as growth, death, shape change, and movement within a tissue is crucial to generate and maintain the characteristic shape, size, and function of embryos and organs. Thus, understanding tissue organization and function requires knowledge of the mechanisms responsible for coordinating cell behaviors between the different cells. One way for cells to communicate is to exchange biochemical cues such as secreted signaling ligands. In addition to biochemical signals, cells also sense and respond to mechanical cues. Because cells in tissues (e.g., epithelia) are physically coupled to each other through intercellular junctions, forces are transmitted between the cells of a tissue and also between neighboring connected tissues. Such forces can rapidly and globally impact cell behavior in a tissue.¹ Thus, mechanical forces transmitted between cells provide a critical complement to biochemical signals to coordinate multicellular behavior.

Animal cells exert mechanical forces on their environment largely through the action of the actin cytoskeleton. Actin networks that vary in network architecture can generate different types of force, such as protrusive and contractile force. Forces that are transmitted between cells and result in mechanical signals often rely on the contractile activity of actin networks that contain the molecular motor myosin II (Myo-II).^{2,3} Actomyosin networks can be organized into fibers made of bundles of antiparallel actin filaments (F-actin) that are cross-

linked by Myo-II, such as cytoplasmic stress fibers. Alternatively, F-actin and Myo-II can form interconnected two-dimensional contractile meshworks, such as the actomyosin cortex that underlies the plasma membrane. These different network types are coupled to the cell membrane and to neighboring cells and/or the extracellular matrix (ECM) by adhesion complexes, transmitting tension between cells via cell–cell junctions or to the ECM via focal adhesions.³ The magnitude and direction of transmitted forces depend on the connectivity of the network to adhesion complexes.^{4–7}

In addition to actively generating force, actomyosin networks also provide cells with mechanical properties such as elasticity and viscoelasticity,⁸ therefore conferring mechanical resistance to deformation by increasing cell and tissue stiffness.^{9–13} The actin cortex as well as stress fibers resist external forces and exert traction forces at adhesion sites against the surrounding cells or the underlying ECM.^{14,15} Elasticity occurs over short time scales where stretch or compression of actin networks leads to a restoration force that is proportional to the strain. Strains occurring over longer time scales can result in a viscoelastic response due to the turnover (assembly and disassembly) of F-actin within the network and binding/unbinding of F-actin cross-linkers.¹⁶ In addition to resisting external forces, the actin cortex also resists the hydrostatic pressure from the cell cytoplasm (in plant cells, this turgor pressure is resisted by the cell wall). These mechanical properties are important in multicellular contexts for transmitting and sensing mechanical signals.

To effectively use force as a signal to coordinate cell behavior in tissues, cells must sense different types of stress or strain, such as compression, tension, or shear.¹⁷ How do cells sense forces transmitted through a tissue? Transduction of a mechanical signal (mechanotransduction) resembles classical biochemical signal transduction in many ways. A specific mechanical force, which can be distinguished by its magnitude, orientation, and/or frequency, must be recognized by specific mechanosensing machinery. Several molecules or molecular complexes can directly respond to physical stress or strain by changing conformation or macromolecular assemblies. Classic examples are the unfolding or stretching of molecules or the opening of ion channels under mechanical forces that would transduce a signal to downstream-signaling pathways.¹⁸ In addition, rather than a single molecule or molecular complex responding to force, mechanical constraints that alter cell geometry can lead to rearrangements of the cytoskeleton due to the self-organizing properties of such cellular systems.¹⁹ We first describe several molecular- and systems-level mechanisms by which cells respond to forces. We then discuss evidence that suggests roles for these mechanisms of multicellular sensing during tissue growth and morphogenesis.

1.1. Force sensing by adhesion complexes

As cells interact with each other and their physical environment through adhesion complexes, these complexes serve as the cell's front line for receiving mechanical stimuli. The classic example of an adhesive complex serving as a mechanosensor is focal adhesions: complexes of greater than 100 different proteins including integrin receptors that link the cell's actin cytoskeleton and plasma membrane to the underlying ECM.^{20,21} Focal adhesions grow in size and exhibit different protein composition and dynamics in response to tension.^{22,23} Stretching of several focal adhesion proteins (e.g., Talin, Vinculin, and p130-

Cas) can result in the exposure of cryptic-binding sites for cytoskeletal proteins or kinases, resulting in increased adhesion assembly.^{24–26} While focal adhesions connect isolated cells, such as fibroblasts, to the ECM, integrin-containing adhesions can also mediate intercellular connections through an intervening ECM as in the case of muscle–tendon cell attachment.²⁷

More common examples of intercellular adhesions include adherens junctions, tight junctions, and desmosomes. Apical adherens junctions are the principal structures mediating mechanical coupling between the cells of epithelial tissues. Adherens junctions contain adhesion receptors called Cadherins (e.g., E-Cadherin in epithelial cells) that mediate homophilic adhesion between cells in the epithelium. The intracellular domain of E-Cadherin binds to the adaptors β -catenin and α -catenin that link adhesion receptors to the underlying actin cortex.²⁸ β -Catenin forms a stable linkage to the intracellular tail of E-Cadherin, while the actin-binding protein α -catenin binds dynamically to β -catenin.^{29–31} Recent studies have suggested that, like focal adhesions, adherens junctions can respond to mechanical forces. Using microfabricated force sensor arrays on which endothelial cells were plated, Liu *et al.* demonstrated that adherens junctions undergo mechanically induced growth in response to loading.³² Pulling forces exerted by the contractile cytoskeleton on adherens junctions or direct application of mechanical tugging force tend to cluster and develop adhesion complexes, increasing the size of adherens junctions.^{30,32} The mechanism of mechanotransduction is still not clear, but could involve protein stretching that reveals cryptic-binding sites for additional actin-binding proteins, similar to focal adhesions. It has been proposed that force-induced stretching of α -catenin exposes a binding site for the actin-binding protein Vinculin³³ (Fig. 13.1A). Recruitment of Vinculin to adherens junctions seems to play a central role in anchoring adhesions to actin networks as disruption of α -catenin–Vinculin binding can lead to reduced epithelial integrity between cells undergoing force-dependent junction remodeling.^{14,34} Thus, for both focal adhesions and adherens junctions, the primary cellular response to applied tension is to strengthen adhesion complexes that bear tension and thus reinforce cell–cell or cell–matrix adhesion.

1.2. Force sensing by actomyosin networks

Forces transmitted through adhesion receptors are subsequently transmitted to the cytoskeleton. In animal cells, adhesion complexes are coupled to either the actin cytoskeleton or intermediate filaments. While we will discuss later how force can alter intermediate filaments attachment to adhesion sites, we will focus here on how mechanical forces alter the dynamics and force generation by actomyosin networks. Spatial regulation of Myo-II motor activity and F-actin assembly–disassembly are determinants of the magnitude and direction of the contractile forces generated. Evidence suggests that mechanical forces can influence both Myo-II motors and F-actin networks. Myo-II motors convert the energy from ATP hydrolysis into mechanical work, undergoing a conformational “power-stroke” that is linked to the ATP hydrolysis cycle⁴⁰ (Fig. 13.1B). Myo-II motors differ from motors such as Kinesin in that the fraction of the time during the ATP hydrolysis cycle in which they are bound to F-actin (the duty ratio) is small, necessitating that Myo-II motors assemble into antiparallel oligomers called minifilaments to processively move along F-actin. Because myosins are mechanochemical enzymes, the duty ratio is influenced by mechanical force. Single-molecule measurements have shown that load stabilizes myosins, including Myo-II,

in the ADP-bound state, favoring their association with F-actin in a state that sustains tension, with low levels of ATP cycling.^{41–43} Interestingly, Myo-II is ectopically recruited to the cortex in response to micropipette aspiration,^{44,45} potentially due to the force sensitivity of the Myo-II motor. Moreover, Myo-II has a higher affinity for stretched actin filaments, which might be another mechanism stabilizing the F-actin–Myo-II interaction under tension.⁴⁶

In addition to regulating Myo-II motor dynamics, mechanical forces could also impact the activity of proteins involved in F-actin assembly and disassembly (turnover). Mechanical forces modulate the activity of formins that are actin nucleation and elongation factors³⁵ (Fig. 13.1B). Recent results demonstrate that application of force on the cell cortex triggers actin monomer release from filaments. This transient increase in actin monomer concentration in turn stimulates formin-dependent actin polymerization.³⁶ Furthermore, Myo-II in some settings accelerates F-actin turnover, suggesting that intrinsic and possibly extrinsic mechanical forces could modulate actin network depolymerization and remodeling.^{47,48} A potential mechanism for force regulated F-actin disassembly could be that compression of actin filaments results in buckling, where bends in the filament are more prone to severing³⁷ (Fig. 13.1B). Thus, mechanical forces have the potential to influence force generation by altering Myo-II and F-actin dynamics.

1.3. Force sensing by the cell plasma membrane

Because actomyosin networks are linked to the plasma membrane through adhesion sites, intrinsic forces generated by cortical actin networks and their response to extrinsic forces from neighboring cells can be sensed through changes in plasma membrane tension.⁴⁹ Plasma membrane tension can modulate cellular processes and activate signals that would change cell behavior in several different ways, of which we will discuss two. First, membrane tension can induce conformational changes in transmembrane channels called mechanosensitive ion channels.⁵⁰ Mechanosensitive channels can be activated by increased membrane tension, which increases the probability for the channel to adopt an open conformation. Other mechanosensitive channels are linked to the internal cytoskeleton and/or the external ECM, which could allow these channels to be regulated by strain resulting from movement of the cytoskeleton relative to the cell exterior.⁵¹ By mediating flux of a specific ion, opening of mechanosensitive channels can induce a variety of cell responses. For example, signaling through Ca²⁺-stretched-sensitive channels, such as those in the transient receptor potential channel M (TRPM) family, results in actin cytoskeleton remodeling.⁵² Increased plasma membrane tension can also modify plasma membrane removal and insertion by modulating rates or mechanisms of endocytosis and exocytosis. Increased plasma membrane tension counteracts endocytosis, increasing the dependence of internalization on the actin cytoskeleton.⁵³ Conversely, increased membrane tension activates exocytosis.⁵⁴ Modulating the rate of endocytosis and exocytosis will in turn regulate plasma membrane tension and can directly impact on biochemical signaling by modulating the number of secreted ligands and/or transmembrane receptors present at the cell surface.

1.4. Force sensing by change in cell geometry

Cellular structures, such as the cytoskeleton, exhibit self-organization that depends on the geometry of the cell and the arrangement of anchor points at the cell periphery.^{19,55} Because forces transmitted between cells in a tissue are likely to impact cell shape, cells in multicellular tissues could also sense forces via changes in the organization of supramolecular structures that depend on cell geometry.¹⁹ Exposure of confluent layers of endothelial cells to laminar flow (and thus shear stress) leads to cell elongation in the direction of flow and the assembly or maintenance of stress fibers aligned with the long axis of the cell.⁵⁶ There is a positive correlation between the extent of cell spreading (cell-substrate contact area) and stress fiber assembly, such that cells confined to a small ECM substrate contact area display fewer focal adhesions and stress fibers that are also thinner compared to spread cells^{38,57,58} (Fig. 13.1C, upper panels). Recent studies using laser nanopatterning illustrated how dynamically changing cell shape and the geometry of adhesive connections to the substrate changes the organization and orientation of actomyosin stress fibers. Generating new adhesion sites separated by non-adhesive regions promotes actin stress fiber assembly at the expense of other actin network types, suggesting that the distance between adhesive contacts is a critical determinant of actomyosin fiber assembly and contraction³⁹ (Fig. 13.1C, lower panels). Thus, reorientation of actomyosin networks in response to altering cell shape and adhesion geometry will change how these cells generate and resist forces.

Cell shape is also a critical determinant for the orientation of the micro-tubule (MT) apparatus that segregates chromosomes during cell division, the mitotic spindle. Mitotic spindles tend to align with the longest axis of the cell, resulting in a division plane that is perpendicular to the long axis^{59–61} (Fig. 13.1D). The position and orientation of the mitotic spindle are set by forces exerted on astral MTs that extend from the spindle poles toward the cell cortex. Recent studies in sea urchin eggs (nonadherent cells with no external cues to direct spindle orientation) suggested a model where the length-dependent pulling forces exerted on astral MTs link cell geometry to the alignment of the mitotic spindle.⁶² Cell division in adherent cells is linked to the geometry of matrix-adhesion sites, which affect the location of cortical cues that orient the spindle.^{63,64} Interestingly, a recent study demonstrated that forces exerted on adhesive sites can reorient the spindle independently of cell shape.⁶⁵ Thus, the geometry of cells, and also the geometry of externally applied forces, can regulate the organization of cytoskeletal networks, providing a cellular mechanism of responding to mechanical cues during development.^{66,67}

Described above are some of the mechanisms for mechanotransduction that have been documented in cells. An important next step is to determine how molecular mechanisms for mechanosensing operate in tissues to coordinate cell behavior during development. Connecting specific mechanical forces to distinct molecular mechanisms in embryonic tissues presents several challenges. Cells in tissues are exposed to a combination of biochemical and mechanical signals that often cannot be easily disentangled. In addition, tissues experience combinations of compression, tension, and shear stress along different dimensions, making it difficult to determine what type of stress or strain is sensed and what cellular structure(s) are responding. Finally, it is often difficult to find a handle on a tissue to

apply a specific type of force to individual cells or populations of cells. Despite these limitations, research on developmental systems has identified key roles for mechanical forces in cellular coordination. We will discuss evidence for multicellular force sensing in tissues, focusing on tissue growth and morphogenesis.

2. MULTICELLULAR SENSING DURING TISSUE GROWTH

During development and homeostasis, tissue growth is tightly regulated to generate, repair, or renew organs with precise size, structure, and function.^{68,69} Thus, individual cells in a tissue must somehow sense the overall size or growth state of a tissue to prevent overproliferation that would lead to inappropriate tissue size or tumor formation. Much effort has focused on secreted signals that regulate tissue growth, but tissues also experience mechanical stress during growth that could, in principle, serve to globally coordinate cell growth across a tissue. Tissues can grow either by increasing their cell volume or by increasing cell number through cell division/proliferation. In both cases, growth might engender local stress in the context of a tissue where cells exhibit intercellular adhesion and are thus not free to immediately rearrange in a manner that would relieve stress. Here, we discuss evidence that suggests a role for mechanical forces in modulating the magnitude and orientation of cell growth and proliferation within developing tissues.

2.1. Mechanical regulation of tissue growth

Tissue growth and patterning during animal development relies on biochemical signals called morphogens. Morphogens are signaling molecules that diffuse from a localized source and form a concentration gradient across the developing tissue, providing a series of concentration thresholds that control the behavior of surrounding cells depending on their distance from the source.^{70–72} Therefore, morphogen gradients serve as instructive signals that pattern tissues and also serve as growth factors that orchestrate the growth and division of cells that constitute the tissue.^{73,74} One of the clearest examples where morphogen gradients regulate tissue growth is the wing imaginal disc in the fruit fly *Drosophila melanogaster*. Wing imaginal discs are epithelial layers that grow from about 50 cells to 50,000 cells within 5 days during *Drosophila* larval development. The growth of the wing disc is primarily controlled by the morphogens Decapentaplegic (Dpp), which is a bone morphogenetic protein homolog and Wingless (Wg), which is a Wnt homolog. Dpp and Wg are produced in anterior–posterior (AP) and dorsal–ventral (DV) stripes, respectively, which intersect in the middle of the disc (Fig. 13.2A).^{69,73,74,80} Both Dpp and Wg are secreted ligands that are distributed in a graded manner in the disc from their site of production. However, the occurrence of cell divisions is uniform across the disc,^{81–83} indicating that growth rate does not simply follow the concentration of these secreted growth factors. Moreover, divisions cease when the disc reaches its critical size corresponding to the time when the larvae enter pupation. Cessation of growth can be controlled by extrinsic signals such as insulin-like growth hormones, but tissue-intrinsic factors, including signaling by morphogens also play critical role in determining organ final size. While models using changes in morphogen concentration and gradient shape as regulators of tissue size can account for growth regulation, these models do not accurately predict experimental results

where morphogen expression is perturbed.^{69,75,84–86} Therefore, additional signals must spatially and temporally regulate growth in the wing disc.

Mechanical models of tissue growth have predicted that the buildup of compressive stress at the center of the wing disc that results from inhomogeneous growth in the tissue could provide an inhibitory growth signal that stabilizes uniform cell division patterns.⁸⁷ Models of tissue growth combining positive growth signals provided by morphogen concentration in the middle of the disc (represented in Fig. 13.2B by the green gradient) with mechanical signals, whereby compression inhibits and stretching promotes cell proliferation, recapitulates uniform cell division and the cessation of cell division at a critical tissue size^{75,86} (Fig. 13.2B). Quantitative analyses of cell behavior in growing imaginal discs verified several important assumptions of theoretical models predicting a role for forces. First, very few cell rearrangement events (such as neighbor exchanges) have been observed during wing disc growth, suggesting that tissue stress that results from inhomogeneous growth is not immediately dissipated.^{88,89} In addition, cells in the center of the disc exhibit smaller apical areas, consistent with increased compression in the disc center as it grows. In contrast, cells at the periphery of the disc become stretched in the direction tangential to the boundary of the tissue, consistent with peripheral cells serving as a barrier to growth that would result in the compression of more central cells (Fig. 13.2B).^{88,90} Moreover, the distribution of mechanical stress has been measured in the wing disc using photoelasticity, showing that cells are compressed in the center and stretched in the periphery.^{91,92} Although a direct demonstration of inhibition of proliferation by compression remains a technical challenge, it has been recently reported that mechanical stretching increases growth rate in the wing the disc.⁹² An outstanding question is how mechanical signals are sensed by the cells and transduced to influence growth rate.

A possible pathway that could link mechanical forces to growth control is the Hippo pathway: a signaling pathway implicated in tissue growth in *Drosophila* and vertebrates, including a role in imaginal disc growth.^{93–98} The Hippo signaling pathway includes a kinase called Hippo (MST1 and MST2 in mammals), which regulates a downstream kinase called Warts (LATS1 and LATS2 in mammals). Activation of the Hippo pathway suppresses cell proliferation and promotes apoptosis through inhibitory phosphorylation and nuclear exclusion of a transcriptional coactivator called Yorkie (YAP and TAZ in mammals). Inhibition of the Hippo pathway leads to Yorkie/YAP/TAZ nuclear translocation and activation that ultimately induces proliferation and differentiation^{93,94,99,100} (Fig. 13.2C). The signal(s) required for spatial and temporal activation of the Hippo pathway within a tissue remains a debated and controversial question. Several biochemical signals can regulate the activity of the Hippo/MST1/2 or Warts/LATS1/2 kinases, including the protocadherins Fat and Dachshous, the membrane-associated FERM-proteins Merlin and Expanded, the scaffolding protein Salvador or the apical polarity protein Crumbs.¹⁰¹ Recent evidence indicates that mechanical forces may also serve as inputs that regulate YAP/TAZ and Yorkie activity.^{76,102} Studies examining YAP/TAZ nuclear localization and transcriptional activity in mammalian cells cultured on micropatterned substrates have demonstrated that YAP/TAZ activity is positively correlated with ECM stiffness and the area of cell spreading.^{38,76} Importantly, YAP/TAZ activity is induced on polylysine-coated

substrates, suggesting that the signal to YAP/TAZ does not involve focal adhesion signaling, but rather changes in cell geometry/cytoskeletal organization.¹⁰³ Substrate stiffness and cell area are correlated with actomyosin stress fiber formation and traction forces exerted by cells on the substrate.^{38,102} Correspondingly, YAP/TAZ activity and their nuclear localization are inhibited by treatments that disrupt actomyosin stress fiber assembly.^{38,76–78,103,104} Experiments using cultured cells support a model where mechanical forces and/or stress fiber formation activate YAP/TAZ activity. F-actin networks might directly regulate Hippo pathway components, either by inactivating LATS1/2^{38,103} or by sequestering a LATS-independent inhibitor of YAP/TAZ.^{76,78} In accordance with the first model, a recent study has found that the activation of the LATS kinase and its interaction with the plasma membrane-associated protein Merlin is regulated by the status of the actin cytoskeleton, where disruption of actin cytoskeleton enhances Warts/ LATS1/2 activity.¹⁰⁵ While there is not yet consensus on whether regulation of YAP/TAZ by mechanical forces depends on LATS1/2 activity, a LATS1/2-independent pathway, or both, cell culture studies support a link between mechanical forces and resulting changes in cell geometry and cytoskeletal organization to the activity of a key regulator of growth, YAP/TAZ (Fig. 13.2C).

Does mechanical regulation of cell shape and/or cytoskeletal organization adaptively regulate YAP/TAZ activity in a multicellular context? When confluent monolayers of cells are grown in culture, YAP/TAZ becomes excluded from nuclei in dense monolayers where cells have a small contact area with the substrate.⁷⁸ Furthermore, YAP/TAZ nuclear translocation and cell proliferation are induced by locally stretching cultured epithelial monolayers.⁷⁸ Importantly, increasing actin filament assembly and actin stress fiber formation by depleting actin-capping/severing proteins can restore YAP/TAZ activity in dense monolayers, resulting in constitutive cell proliferation. Therefore, uncoupling stress fiber assembly from cell shape can make proliferation independent of tensile or compressive stress.⁷⁸ Experiments in *Drosophila* imaginal discs also support a crucial role for actin network organization in regulating Yorkie activity.^{77,79} Increased actin assembly resulting from either depletion of inhibitors of actin assembly (e.g., capping protein) or by overexpressing the formin *Diaphanous* induces overgrowth in the wing disc and promote Yorkie nuclear accumulation.^{77,79}

While a definitive mechanism for mechanosensing is yet to be established in developing tissues, the current data suggest that mechanical stress in the disc tissue modulates cellular forces and/or cytoskeletal assembly, which feeds back to regulate cell division during wing disc development. Overall, a possible model for the mechanical regulation of cell proliferation is that tissue growth and resulting cell compression at the center of the tissue reduces apical and basal cell area and stress fiber assembly, resulting in cytoplasmic/inactive Yorkie. Conversely, mechanical stretching of peripheral cells could enhance actin fiber formation, by analogy to cell culture, promoting Yorkie nuclear translocation and transcriptional activity. Indeed, actomyosin fibers are observed to form along cell interfaces in stretched peripheral cells⁸⁸ (Fig. 13.3A, lower panels), although it is not yet clear whether induction of cell division by stretching is associated with Yorkie activity and whether these actomyosin fibers are required for nuclear translocation of Yorkie. Tension could induce

formin-dependent actin polymerization or stabilize cortical Myo-II providing possible pathways for tension-sensitive actomyosin fiber assembly.^{35,36,45,107} How these candidate mechanical signals are integrated with biochemical signals to regulate, YAP/TAZ and Yorkie nuclear localization will be a very interesting area of research in the coming years.

2.2. Mechanical regulation of cell growth orientation

In addition to growth rate, the orientation of cell growth and division is a critical determinant of how tissue growth affects tissue mechanics. For instance, oriented cell division along the principal axis of tension has been shown to release tension and might therefore be an adaptive mechanism used in growing epithelia to adapt to their mechanical environment.¹⁰⁸ It is therefore possible that mechanosensitive pathways regulating the orientation of growth and division in a tissue will also impact the growth rate, final size, and shape of tissues.

Different parameters regulate cell division orientation. Biochemical signals can polarize cortical proteins that interact with astral MTs and regulate spindle orientation, as occurs in cells that undergo asymmetric cell divisions.¹⁰⁹ In addition, oriented cell division can occur in the absence of an extrinsic biochemical cue, depending instead on cell geometry and/or mechanical forces.^{62,65} Cell division orientation in the wing disc appears to depend on the interplay between biochemical and mechanical cues. Cell divisions are preferentially aligned radially from the center of the disc to the periphery in the central region and tangential to the tissue boundary in the periphery (Fig. 13.3A). Radially oriented cell divisions depend on the activity of the protocadherin Fat, which acts as a receptor for its ligand Dachsous. Wings in *fat* mutants are less elongated than wild-type wings and the division planes are random during wing disc development in the central region, demonstrating that cell division orientation affects tissue shape.¹¹⁰ In the wing disc, activation of the Fat–Dachsous pathway leads to the planar polarization of the atypical myosin Dachs, which is enriched in tangential junctions (oriented perpendicular to the radial axis of the tissue) and depleted from radial junctions.¹¹¹ Planar polarization of Dachs could localize cortical cues that directly interact with astral MTs to orient the mitotic spindle. However, since Dachs is a myosin, planar polarization of Dachs might indirectly orient the spindle by controlling cell shape. In accordance with this latter view, Dachs has been shown to define local patterns of tension and contraction in tissues,¹¹² and Dachs planar polarization leads to increased tension along the tangential cellular junctions where it accumulates.¹¹⁰

This polarized tension is predicted to cause radial cell elongation, which is correlated with the axis of cell division. Thus, biochemical signals could polarize local patterns of tissue tension to orient cell and tissue growth by altering cell shape.¹¹⁰ The planar polarization of the myosin Dachs does not explain why cell divisions are oriented tangentially in the periphery of the wing disc. In this case, the orientation of cell division may rely on the global pattern of tissue stress that results from tissue growth. Cells at the disc periphery that are elongated exhibit higher tension tangential to the boundary of the tissue.^{88,90} The higher tangential tension is associated with tangential Myo-II fibers at junctions, suggesting that cells at the periphery adapt to balance cell stretching by assembling Myo-II fibers⁸⁸ (Fig. 13.3A, lower panels). Similar to more central cells, peripheral cells also tend to divide along

the axis of elongation.^{88,90} Importantly, local induction of cell growth can reorient cell division plane and Myo-II fibers assembly in surrounding cells (occurring tangentially to the source of growth), suggesting that mechanical stress that results from tissue growth is the cue that reorients cell division, possibly providing a means to dissipate tension at the tissue boundary and allow for further tissue growth. A critical question will be to determine how epithelial cells sense mechanical forces to orient the mitotic spindle: Do they sense cell geometry⁶² or could mechanical forces polarize cortical cues⁶⁵?

During animal development, tissue growth generates and adapts to mechanical forces. In plant tissues, mechanical forces are also thought to guide tissue growth; however, the nature of the forces and mechanotransduction mechanisms are different.¹¹³ In plants, cell and tissue growth is counteracted by stiff extracellular walls that also prevent cell rearrangements. Increase in plant cell volume results from internal turgor pressure that induces tension in the surrounding cell wall. Anisotropy in the cell wall stiffness dictates the direction of cell growth, defined as growth anisotropy: the cell will grow in a preferred direction where it encounters less resistance from the extracellular wall. Because cellulose fibrils are thought to bear most of the cell wall's tensile stress, the orientation of cellulose fibrils is a critical determinant of growth in plants.^{114,115} Importantly, the orientation of parallel MT arrays in the apical cortex of the cells correlates with anisotropic deposition of stiff cellulose microfibrils in the cell wall because MTs guide cellulose synthase.^{114,116–118} Recent studies of the growth at the shoot apical meristem have elucidated mechanisms that allow plant cells to sense and respond to forces to orient plant growth. The shoot apical meristem consists in a population of stem cells that can continuously grow (by increasing cell volume) and divide. The meristem adopts the shape of a dome at the periphery of which flower primordia emerge forming small buds (Fig. 13.3B). Interestingly, cortical MTs are aligned with theoretically predicted directions of principal stress in the different regions of the meristem.¹¹⁹ MTs are poorly aligned in the central region where there is isotropic stress, whereas MTs are arranged into circumferential arrays of parallel bundles at the periphery of the dome where there is highly anisotropic stress (Fig. 13.3B). Artificially changing the pattern of stress in the tissue through laser ablation or tissue compression is sufficient to reorient MT array.¹¹⁹ Thus, MT array orientation and consequently anisotropic growth are responses of mechanical stress in plants.

The mechanotransduction pathway allowing cells of the shoot apical meristem to sense and respond to mechanical forces involves MT dynamics and their self-organization into parallel arrays. Compressive forces tend to induce MT shrinking, whereas tensile forces favor MT elongation.^{120–122} Thus, the direction of maximal tension may bias MT self-organization in a preferred orientation. The MT-severing protein Katanin is an important regulator of MT dynamics and has been implicated in the force-sensing mechanism. *katanin* mutants exhibit more disorganized and less dynamic cortical MTs in the whole meristem. Interestingly, *katanin* mutants are not able to efficiently respond to changes in mechanical stress pattern: cells do not reorient cortical MT arrays after laser ablation or compression.¹⁰⁶ Katanin has been shown to cut MTs where they intersect each other,^{123,124} disfavoring MT crossover, and increasing the number of free MTs amenable to self-organize according to stress directions.¹¹³ This suggests that MT dynamics is required to allow cells to sense and

respond to mechanical forces. In addition, in *katanin* mutants, the meristem adopts an aberrant morphology, which is correlated with a more homogeneous growth rate. Thus, growth orientation that results from mechanical feedback is critical to regulate the morphology of the growing meristem. This feedback has been proposed to be important for emergence of the floral primordial buds at the periphery of the meristem¹⁰⁶ (Fig. 13.3B).

3. MULTICELLULAR SENSING DURING TISSUE MORPHOGENESIS

During tissue morphogenesis, individual cells have to constantly adapt and coordinate cell shape changes and movements relative to their neighbors to maintain tissue integrity and collectively drive coherent movements or deformations of the overall tissue. Forces critical for tissue morphogenesis can be generated within cells changing shape (cell autonomous). Mechanosensitive signaling could potentially impact how cells generate forces, possibly helping coordination between cells or propagation of cell shape changes among cells. In addition, forces extrinsic to a tissue (cell non-autonomous) can influence cell and tissue shape by compressing/stretching a neighboring tissue or serving as a mechanical constraint to deformation. Here, we discuss how multicellular sensing plays a role in shaping tissues.

3.1. Mechanical coordination of actomyosin contractility

Many morphogenetic events that shape embryos and organs take place within epithelial tissues, which undergo highly coordinated movements and deformations.² Actomyosin contractility within epithelial cells can promote tissue remodeling such as tissue bending, elongation, contraction, or tube formation. During morphogenesis, cells in epithelia might coordinate force generation to engender a specific tissue response and the appropriate deformation or movement. The magnitude, direction and timing of cellular forces depend on the organization of the contractile actomyosin meshworks and fibers at the level of the cell and how these networks are connected between cells at the level of the tissue.

Recent live imaging and quantitative analyses of actomyosin contraction and associated cell shape changes have highlighted dynamic behaviors of cortical actomyosin meshworks, where actomyosin contractility is not continuous, but occurs as discrete contractile events or pulses. In these cases, Myo-II structures appear and often coalesce or condense in the apical (and sometime basal) cell cortex, before being remodeled.^{125–131} In epithelia, Myo-II pulses have been shown to correlate with transient fluctuations of cell shape and area. Contraction pulses are not only characterized by a temporal cycle of actomyosin appearance and remodeling but can also exhibit spatial dynamics such as directional flow toward one edge of the cell.^{7,127,132,133} Contraction pulses have been observed for example in the amnioserosa, an epithelial tissue that covers the dorsal surface of the *Drosophila* embryo. During a process called dorsal closure, the amnioserosa cells constrict and eventually disappear inside the embryo, pulling the neighboring tissue, the lateral epidermis, toward the dorsal midline of the embryo in a process that resembles wound healing^{134,135} (Fig. 13.4A). At the beginning of dorsal closure, amnioserosa cells exhibit pulsatile constrictions, where pulses in neighboring cells are predominantly out of phase, resulting in a back-and-forth contraction between neighbors^{127,136} (Fig. 13.4A and B). This begs the question whether amnioserosa cells sense pulses in neighboring cells and whether there is a mechanosensitive mechanism to stagger contraction pulses.

When tension is locally released by laser ablation of a single cell–cell junction or by laser-induced wounding of a single amnioserosa cell, cells surrounding the wound instantaneously stop pulsing.^{136,138,139} In addition, inhibiting contractility in individual cells by driving the expression of dominant negative RhoA not only inhibits contractile pulses cell autonomously but also inhibits contraction pulses and Myo-II accumulation in adjacent cells¹³⁸ (Fig. 13.3C). Inducing the arrest of pulsed contractions by locally wounding a cell is accompanied by a change in Myo-II dynamics in the nearest neighbor of the perturbed cell. Instead of flowing toward the center of the cell apex, Myo-II flows toward the cellular junction with wounded cell.¹³⁸ These data suggest that the transmission of tension between neighboring cells is required for the collective pulsing behavior and that mechanical signals sensed by neighboring cells leads to staggered contraction pulses.¹³⁶

In contrast to the pulsatile contraction of cortical actomyosin mesh-works, other morphogenetic processes are associated with more stable actomyosin structures that resemble stress fibers, such as actomyosin cables that assemble at intercellular junctions. Junctional actomyosin cables are often connected between cells, leading to multicellular actomyosin cables that are critical for tissue morphogenesis such as during axis elongation in *Drosophila* or during neural tube closure in vertebrate.^{137,140,141} The assembly of multicellular actomyosin cables requires coordination to properly link actomyosin structures between cells. An attractive hypothesis is that cells utilize mechanical forces to coordinate the assembly of multicellular actomyosin cables. In the early *Drosophila* embryo, actomyosin cables are assembled to drive directional rearrangement among epithelial cells that leads to the elongation of the embryonic epithelium¹⁴² (Fig. 13.4D). Directed cell–cell intercalation is achieved by the shrinkage and loss of cell–cell interfaces followed by the elongation of new interfaces in the orthogonal direction, leading to convergence and extension of the tissue.^{126,143} Shrinkage is associated with the formation of actomyosin cables along the interface of convergent cells. Actomyosin cables along a single interface bring four cells together; however, interfaces and their associated actomyosin cables can become aligned across multiple cells whose contraction results in the formation of multicellular rosettes, with 5–11 cell converging at a single point¹³⁷ (Fig. 13.3D, lower panel). Evidence suggests that a positive mechanical feedback coordinates actomyosin cable assembly to align multiple interfaces. Analysis of tissue recoil velocity after laser ablation of cell–cell junctions revealed that interfaces that are part of a multicellular actomyosin cables support higher levels of tension than isolated interfaces enriched in Myo-II. In addition, fluorescence recovery after photobleaching (FRAP) experiments demonstrate that Myo-II is more stable in these multicellular cables, indicating that tension is inversely correlated with Myo-II dynamics. Importantly, laser ablation of multicellular actomyosin cables results in an acute loss of Myo-II intensity in adjacent unablated interfaces. Induction of local stress by micropipette aspiration of the apical membrane of these cells induces Myo-II recruitment. Because tension appears to stabilize Myo-II at junctions, cell–cell interfaces under tension might recruit or stabilize Myo-II in neighboring junctions, resulting higher order organization of actomyosin into multicellular cables.¹⁰⁷

While the evidence suggests that both actomyosin meshworks and cables are influenced by mechanical signals transmitted between cells, a future challenge is to determine how cells

sense mechanical signals and transduce these signals to cytoskeletal regulation. Because Myo-II itself is a mechanosensitive motor whose attachment to actin filaments is influenced by force, a possibility is that tension directly modulates the mechanochemical cycle of the Myo-II motor leading to stabilization of actomyosin cables or meshworks.^{41,45} However, tension could also modulate upstream signaling pathways, such as the activation of the small GTPase RhoA.¹⁴⁴ Activation of RhoA leads to the activation of its downstream effector Rho-associated Kinase, which phosphorylates and activates Myo-II. During *Drosophila* gastrulation, RhoA signaling functions to promote apical constriction and tissue invagination.^{5,145,146} Interestingly, mechanical compression of *Drosophila* embryos at this stage can rescue Myo-II accumulation and contraction in mutants that are normally defective in these processes, potentially by mechanically inducing RhoA signaling activity.¹⁴⁷ RhoA signaling could be activated by elevating the activity of upstream G-protein-coupled receptors that localize to the plasma membrane. Increased membrane tension due to mechanical perturbation could inhibit endocytosis of G-protein-coupled receptors, which would increase the signaling activity (more receptors can be activated by an extracellular ligand) and therefore elevate RhoA signaling and Myo-II accumulation. Mechanical compression has also been shown to upregulate expression levels of transcription factors known to activate RhoA signaling, providing a positive transcriptional feedback reinforcing Myo-II accumulation and/or activity.^{5,148–151} Interestingly, this model assumes that morphogenetic movements that profoundly reshape the embryo can in turn regulate developmental genes expression through mechanosensitive pathways. Compressive forces exerted by the extension of the underlying tissue on cells that will form the anterior gut have been shown to induce gene expression further required for gut formation in the *Drosophila* embryo.¹⁴⁹ Moreover, a similar mechanosensitive pathway has recently been described in the zebrafish embryo to induce mesoderm identity in a subset of cells that are compressed by epiboly movements.¹⁵² In both cases, mechanical strains developed by morphogenetic movements induce β -catenin nuclear translocation where it regulates targets genes expression. How mechanical stimulus activates β -catenin pathway remains to be fully understood but evidence supports a model where mechanical forces stimulate β -catenin phosphorylation by the kinase Src, leading to β -catenin nuclear translocation.¹⁵²

Actomyosin activity in cells of a tissue may also be altered by the action of mechanosensitive ion channels. The stress-activated calcium channel TRPM has been implicated in epithelial response to wounding both in *Caenorhabditis elegans* and *Drosophila*.^{153,154} Wounding induces the formation of a supracellular actomyosin cable at the margin of the wound acting as a purse string to close the hole. Interestingly, downregulation of TRPM channel impairs actomyosin cable formation and compromise wound closure. Epithelial wounding results in the stretching of the cells surrounding the wound, which may induce the opening of TRPM. In accordance with this view, an increase of intracellular calcium in cells that surround the wound has been observed in both *Drosophila* and *C. elegans*. Calcium fluxes and several ion channels have also recently been implicated in Myo-II accumulation in unwounded embryos, specifically during dorsal closure.¹⁵⁵ Intracellular calcium signaling might regulate actomyosin remodeling through the activation of Gelsolin, a calcium-activated actin filament-severing protein.¹⁵⁴

3.2. Mechanical reinforcement of junctions

Cell–cell adhesion complexes are critical to transmit tension between cells. The absence of strong adhesion, especially in the presence of tension, can result in catastrophic loss of adhesion and the loss of mechanical integrity in the tissue.^{156–158} Given the importance of maintaining tissue integrity during morphogenesis, there could be mechanisms that strengthen adhesion in response to tension to prevent loss of cell–cell adhesion during morphogenetic events. In addition, cell–cell adhesions are well positioned to sense forces transmitted between cells, which could activate signaling pathways that coordinate cell behavior within a tissue. Although recent cell culture studies have suggested that the size and protein composition of E-Cadherin-containing adherens junctions can be modulated by mechanical forces, evidence for a role of adherens junction strengthening has not been yet observed during tissue morphogenesis. However, there is evidence for mechanotransduction playing a role to reinforce other types of cell–cell adhesion between neighboring tissues during morphogenesis.

Because muscle contraction can generate tensile forces of high magnitude, adhesion sites linking muscles to tendons or to an overlying epithelium need to be tightly regulated and reinforced, such that muscle contraction is transduced to surrounding tissues without impairing tissue integrity. In *Drosophila*, muscle–tendon cell attachment is mediated by an integrin-based intercellular adhesion complex that links the two tissues via an intervening ECM. FRAP experiments have been used to measure the stability of proteins within these intercellular adhesions in response to increased or decreased muscle contraction. Inducing muscle hypercontraction leads to greater stability of integrin and associated proteins at adhesion sites, whereas integrin turnover is increased when muscle contraction is blocked.¹⁵⁹ This property may have important implications in the context of development to maintain muscle–tendon connectivity and integrity of both tissues; the authors show that adhesive complexes become more stable over time from late embryogenesis to larval stages as muscle develop and generate increasing levels of tensile force.

Adhesion reinforcement by mechanical forces is not only important for long-term tissue maintenance but also participates in tissue morphogenesis. In *C. elegans*, mechanical stimuli applied by the muscles on the developing epidermis are required for the full elongation of the worm epidermis. Communication between muscles and the epidermis relies on hemidesmosome-like junctions in the epidermis that attach the epidermis to the muscle at the basal side and connect the epidermis to the exoskeleton at the apical side. Intermediate filaments span the apical-basal attachment sites, ensuring mechanical continuity between muscles and the exoskeleton. A signaling pathway has been identified at these junctions that is activated by mechanical tension and stimulates phosphorylation of intermediate filaments and their recruitment to hemidesmosome junctions, hence strengthening the adhesion sites.¹⁶⁰ In addition, genetic interaction studies suggested that muscle tension also activates Myo-II in the epidermis. Together, these results describe a mechanosensitive pathway that reinforces muscle–epidermis attachment sites and their linkage to the intracellular cytoskeleton. In this context, the mechanotransduction pathway is required for tissue elongation and coordination of epidermal and muscle tissue morphogenesis.¹⁶⁰

In addition to reinforcing stable intercellular junctions, recent studies have demonstrated that forces transmitted through Cadherin-containing junctions can polarize cell migration. Collective cell migration requires maintenance of adhesive contacts between cells and the coordination of cellular protrusions and movements among cells. Cell culture systems of collective cell migration have shown that the speed, persistence, directionality, and coordination of cell movements is increased on stiff substrates, indicating that migrating cells can collectively sense and respond to their mechanical environment.¹⁶¹ Importantly, this response depends on Cadherin-mediated adhesion between migrating cells as reducing cell–cell adhesion decreases directional migration and the polarization of cells in the direction of migration.^{161–163} The importance of cell–cell adhesion in collective cell migration and a potential mechanism for mechanotransduction has been demonstrated during *Xenopus* gastrulation. In *Xenopus*, mesendodermal cells collectively migrate along the ectoderm using the ECM as substrate. Every cell displays leading edge protrusions and a rear retracting edge, but when cells from this tissue are dissociated from one another, they become multipolar, protrude randomly, and fail to effectively migrate toward a source of guidance cues. Strikingly, polarized protrusion in a single cell can be restored when a pulling force is applied to Cadherin adhesion proteins using magnetic tweezers, with protrusions forming on the opposite side of the cell as the pulled bead.¹⁶⁴ The mechanotransduction pathway involves the catenin Plakoglobin (PG) that links the cytoplasmic tail of Cadherin to intermediate filaments. Local forces applied to Cadherin result in PG-dependent recruitment of intermediate filaments and reorganization of the intermediate filament network.¹⁶⁴ PG might then act as a mechanosensor, possibly changing conformation under force as it has been shown for α -catenin.³³ The model posits that intercellular adhesion integrates traction forces arising from every migrating cell, leading to higher cell–cell tension at the rear of migrating cells. This polarized mechanical signal is transduced into polarized localization of intermediate filaments and polarized protrusive activity of cells.¹⁶⁴

3.3. Extrinsic mechanical constraints influencing tissue shape

Morphogenesis is an integrated process and different tissue movements and deformations often occur at the same time. Cells and tissues undergoing shape changes in concert may actively apply forces to each other. In addition, cells and tissues have viscoelastic properties, such that neighboring cells or tissues can give rise to opposing forces that must be overcome to reshape a tissue.¹³ Forces that result from mechanical constraints on tissues could be sensed by cells and impact both cell and tissue shape. A classic example of the impact of surrounding tissues on cell shape change is the apical constriction of bottle cells during *Xenopus* gastrulation. *In vivo*, bottle cells display anisotropic constriction, however, when explanted and cultured *in vitro* bottle cells constrict isotropically.¹⁶⁵ This observation suggests that the mechanics of the surrounding tissue can influence individual cell shape changes.

Apical constriction is also anisotropic during *Drosophila* gastrulation. *Drosophila* gastrulation involves collective apical constriction of cells in an epithelial sheet that forms a furrow along the ventral midline where cells are pulled inside the embryo along the DV axis, but the tissue remains extended along the AP axis¹⁶⁶ (Fig. 13.5A). Because of this

anisotropy in tissue movement, it is not surprising that the underlying cell shape changes are anisotropic: ventral furrow cells undergo anisotropic apical constriction such that cells constrict predominantly in the DV direction and remain longer along the AP axis (Fig. 13.5B). This anisotropy is not a cell autonomous property but appears to rely on the global tissue force pattern. Laser ablation experiments and the resulting recoil of the surrounding tissue have suggested that epithelial tension is highest along the AP axis, the axis along which cells are most elongated. Epithelial tension can be locally released by reducing cell–cell adhesion, which causes a loss in tissue integrity in the form of tissue-wide tearing events where clusters of cells separate. Importantly, individual cells that undergo apical constriction after a neighboring tissue tear constrict isotropically, suggesting that integration of tensile forces across the tissue causes cell anisotropy.¹⁵⁷ The mechanisms that generate this asymmetry in epithelial tension are still not clear. It is possible that there is greater resistance to pulling forces oriented in the AP direction at the poles of the embryo than to pulling lateral cells. Alternatively, a gradient of Myo-II contractility along the DV axis could be responsible for directing movement toward the ventral midline.¹⁶⁸ The importance of this anisotropy for tissue invagination and how this tension might alter the activity of the force generating actomyosin cytoskeleton are future areas of investigation.

The ability of tissue-level forces to impact morphogenesis extends beyond morphogenetic processes that require active force generation by cytoskeletal networks. A series of recent studies of the vertebrate gut have demonstrated that differences in the growth rate and mechanics of connected tissues can sculpt tissues into different shapes. The embryonic gut is initially a straight tube, but rapidly grows and folds in the intestinal cavity forming a looping pattern that is stereotypical for different species of animals. However, gut looping neither does not result from differential cell proliferation within the gut tissue, nor does looping result from the spatial constraint of the body cavity in which it grows. Instead, gut looping results from the coupling of the gut along its entire length to a tissue called the mesentery. The gut tube grows faster than the mesentery, resulting in an elastic stretch of the mesentery and resulting compression and buckling of the gut tube, giving it a wavy shape (Fig. 13.5C–E). Quantitative measurement of the mechanical properties of the gut and mesentery combined with a theoretical model of the gut–mesentery composite was able to accurately predict the looping pattern in several species¹⁶⁷ (Fig. 13.5C–E). Thus, differential growth between coupled tissues can drive morphogenesis.

In addition to looping, the gut tissue forms internal folds that result in villi. Interestingly, the formation of villi can also be explained by mechanical interactions between two tissues, in this case the gut epithelium and surrounding muscle cells.¹⁶⁹ During villification, the gut epithelium first buckles inward to form longitudinal ridges that are parallel to the axis of the gut tube. Longitudinal ridges subsequently buckle to form parallel zigzags and finally bulges arise from the zigzag pattern and individual finger-like villi are produced. Importantly, every step in this morphogenetic process leading to a new geometrical conformation of the epithelium coincides with the differentiation of a new layer of smooth muscle, which are all required for proper villification. Given that the formation of longitudinal ridges can be rescued by replacing muscle cells with a synthetic silk tube, the muscle layer appears to act as a stiff barrier that prevents expansion of the growing epithelium and promotes buckling.¹⁶⁹ Mathematical modeling considering the gut epithelial layer as flat elastic sheet

that is compressed during growth first in the axial then in the longitudinal axes mimic the role of the three muscle layers in the stepwise process of villification. Although the two above models of gut looping and villification both describe purely mechanical interpretations of gut morphogenesis during growth, suggesting that the epithelium fold passively, it is not known whether epithelial cells actively respond to mechanical stress by adjusting their growth rate or by regulating their stiffness through remodeling of the actomyosin cytoskeleton. These examples illustrate how forces across different spatial scales, molecular, cellular to the tissue level can serve as signals in multicellular contexts to coordinate cell and tissue behavior.

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Figure 13.1.

Molecular and cellular mechanisms of mechanosensing. (A) Mechanosensing at intercellular junctions. Cell–cell adhesion in epithelial tissues is mediated by homophilic interaction of E-Cadherin in adjacent cells. E-Cadherin molecules are bound to the actomyosin cytoskeleton through adaptor proteins such as β -catenin and α -catenin. Pulling forces generated by actomyosin contraction can induce conformational changes in adhesion proteins, possibly α -catenin, causing the recruitment of proteins such as Vinculin. Recruitment of additional adaptor proteins and growth of adhesion size can reinforce adhesion complex attachment to actin filaments.^{32–34} (B) Forces in actin filament networks regulate Myo-II dynamics and actin turnover. Tension resisting the myosin power-stroke can decrease the rate of ADP release and cause myosin to persist in an F-actin-bound conformation. In addition, mechanical stimulation can increase actin monomer availability and induce formin-mediated F-actin assembly. Compression of actin filaments during myosin-mediated contraction, and potentially external forces applied to cells, causes filament bucking and increased severing.^{35–37} (C) Response of actomyosin networks to changes in cell geometry. Cell spreading is associated with actomyosin stress fiber assembly (upper panels).³⁸ Reprogramming cell shape by changing the pattern of cell-substrate adhesion (gray pattern) leads to stress fiber assembly between well-separated adhesion sites (lower panels).³⁹ (D) Response of cell division to changes in cell geometry. During cell division, the mitotic spindle preferentially aligns with the longest axis of the cell, resulting in oriented cell division even in absence of extrinsic biochemical polarizing cues.

Figure 13.2.

Mechanical forces influence growth rate. (A) Expression pattern of the morphogens *Dpp* and *Wg* in the developing wing imaginal disc. The central region of the disc (wing pouch, gray) will give rise to the adult wing. (B) Model of wing disc growth regulation by a combination of biochemical and mechanical signals. The secreted morphogens form a concentration gradient from the center to the periphery of the wing pouch (green gradient), providing a positive signal for cell proliferation in the center. Localized growth in the center causes stretching forces (blue) in the periphery promoting proliferation, and compressive forces (red) in the center inhibiting proliferation. Combination of morphogen signaling and mechanical feedback could promote uniform growth in the wing disc.⁷⁵ (C) The signaling activity of the Hippo pathway integrates biochemical and mechanical signals to regulate growth. Biochemical activation of the Hippo kinase leads to activation of the Warts/LATS1/2 kinase, cytoplasmic retention of the transcription factors Yorkie/YAP and TAZ, and inhibition of growth. Yorkie/YAP/TAZ activity is also regulated by mechanical signals. The mechanism linking force sensing to nuclear translocation of Yorkie/YAP/TAZ remains unknown and may or may not involve Warts/LATS1/2 inhibition, but does involve the formation of actin fibers.^{38,76–79}

Figure 13.3.

Mechanical forces influence growth orientation. (A) The pattern of mechanical stress influences cell division orientation in the wing disc possibly by regulating cell shape. Wing disc marked for Myo-II (upper panel) or E-Cadherin and Myo-II (lower panels). Cells are compressed in the center leading to small apical areas, whereas cells are stretched in the periphery. Blue arrows illustrate the orientation of cell divisions, which follows the patterns of stress. Divisions are radial in the center and tangential in the periphery. At the disc periphery, Myo-II forms polarized supracellular fibers lining the direction of maximal stress, which resist further stretching. Scale bar: 5 μm . (B) The pattern of mechanical stress influences growth orientation in the root apical meristem. Apical meristem marked for MTs (green). The red lines indicate the orientation of the MT array for each cell. MTs align with the pattern of stress in the tissue. MT orientation confers greater resistance to cell deformation along the maximal stress axis. Blue arrows indicate orientation bias of growth, toward the center of the meristem. Scales bars: 10 μm (upper panel) and 2.5 μm (lower panel). (A) Adapted from Ref. 88 and (B) adapted from Ref. 106.

Figure 13.4.

Mechanical feedback during tissue morphogenesis. (A–C) Mechanical feedback is required for collective pulsed contraction of amnioserosa cells. (A) Schematic representation of dorsal closure in *Drosophila* embryo, the amnioserosa epithelium is highlighted in orange. Amnioserosa cells display pulsed apical contraction during dorsal closure such that neighboring cells pulse out of phase¹³⁶ (B). (C) Overexpression of a dominant negative version of Rho (Rho-DN) in a single cell abolishes constriction pulses cell autonomously (pink curve) as well as in the nearest neighbor (green dotted curve). Pulsatile constriction is not affected however in distant neighbor (gray dashed curve). (D) Mechanical feedback regulates convergence and extension of the *Drosophila* embryonic epithelium. Schematic representation of the elongation of the embryonic epithelium (blue) in *Drosophila* and multicellular rosette formation.¹³⁷ Myo-II (red) becomes enriched at junctions oriented with the dorsal–ventral axis. Mechanical feedback recruits or stabilizes Myo-II in neighboring

junctions resulting in supracellular cables assembly and alignment of cells interfaces.¹⁰⁷
Contraction of myosin cables leads to rosette formation, which is resolved by the growing of new junction in the orthogonal direction (light blue) and convergence and extension of the tissue. (C) *Adapted from* Ref. 138.

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Figure 13.5.

Extrinsic mechanical constraints influencing tissue shape. (A) Tissue-level tension influences individual cell shape change and regulates tissue invagination. Schematic representation of ventral furrow formation in *Drosophila* embryo. During invagination, tension is highest along the anterior–posterior axis (red arrows).¹⁵⁷ Anisotropic epithelial tension results in anisotropic apical constriction (B) and the formation of a narrow furrow. (C) Gut-looping morphogenesis is driven by mechanical constraints exerted by the connected mesentery to the gut tube. Looping pattern of the gut can be modeled using a stretched rubber sheet (pink, mesentery) attached to a straight, non-stretched rubber tube (blue, gut). Relaxation leads to generation of compression forces and looping of the tube. The rubber model (D) deforms into a structure similar to the chicken gut (E). *Adapted from* Ref. 167.