Generation, Transmission, and Regulation of Mechanical Forces in Embryonic Morphogenesis

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Abstract

Embryonic morphogenesis is a biological process which depicts shape forming of tissues and organs during development. Unveiling the roles of mechanical forces generated, transmitted, and regulated in cells and tissues during these processes is key to understanding the biophysical mechanisms governing morphogenesis. To this end, it is imperative to measure, simulate, and predict the regulation and control of these mechanical forces during morphogenesis. In this article, we aim to provide a comprehensive review of the recent advances on mechanical properties of cells and tissues, generation of mechanical forces in cells and tissues, the transmission processes of these generated forces during cells and tissues, the tools and methods used to measure and predict these mechanical forces *in vivo*, *in vitro*, or *in silico*, and to better understand the corresponding regulation and control of generated forces. Understanding the biomechanics and mechanobiology of morphogenesis will not only shed light on the fundamental physical mechanisms underlying these concerted biological processes during normal development, but also discover new information that will benefit biomedical research in preventing and treating congenital defects or tissue engineering and regeneration.

Box 1: Terminology

Actin/Myosin: Protein complex that is responsible for cell movements. Most prominently known for its role in muscle fibers but plays key roles in nearly all cell/tissue movements such as those described in morphogenesis.

Adherens junctions: protein complexes (a notable example of which are E-cadherin-based adherens junctions) responsible for many cell-cell interactions and on a larger scale play a key role in the ability for cells and tissues to sense and respond to mechanical stimuli and/or biochemical signals.

Agent-based models: Computational models that study a behavior of a system using small, autonomous sub-components referred to as agents. The model looks at the net result that occurs with the action of many small agents, such as a tissue moving as a result of the response of many different single cells acting in a coordinated way.

Atomic force microscopy (AFM): High-resolution scanning microscopy technique known for its sub-nanometer level resolution. Not to be confused with electron microscopy, as AFM does not use beams or lenses.

Cell intercalation: The act of cells exchanging places through different layers of tissue. Often associated with the spreading or growth of tissues during embryonic development.

Cell polarity: In development, describes the orientation of cells along three axes: left/right, dorsal/ventral, and anterior/posterior. In epithelial cells specifically, it describes the sides of the cell and interactions specific to a given side. Common names for the "sides" are:

- Apical membrane: side facing towards the outside of the layer, i.e., the outer edge. Apical constriction in morphogenesis describes when the outer edge of a group of cells on the epithelium contracts.
- Basal membrane: side typically facing towards connective tissue/inner layers, i.e., the inner edge
- Lateral membrane: sides of the cell facing other cells in the epithelial layer (2 per cell).

Epithelium: Outermost layer of a tissue or organ. In the case of hollow structures, also includes the inner lining. In Drosophila a single extraembryonic epithelium develops in the early stages of embryogenesis and is referred to as the amnioserosa.

Epithelial-To-Mesenchymal transition (EMT): Process by which epithelial cells lose adhesion and begin to migrate as part of the process of becoming mesenchymal stem cells, a pluripotent stem cell responsible for differentiating into a wide variety of cell types.

Extracellular matrix (ECM): A complex network of a variety of macromolecules to provide a structurally stable structure that contributes to the mechanical properties of cells and tissues and host growth factors and other biochemical molecules to regulate cellular behaviors such as adhesion, proliferation, migration, and apoptosis.

Förster Resonance Energy Transfer (FRET): A technique in microscopy which allows the detection of interactions between molecules as two different fluorophores (an exciter and an emitter) interact, resulting in emission from the emitter fluorophore.

Gastrulation: An early stage of embryonic development in which a single-layer embryo re-organizes into a structure containing multiple layers of cells (known as the gastrula). These layers are often referred to as "germ layers" of the cell and are described below.

Germ layers of the embryo:

- Endoderm – Inner layer, in animals this differentiates to form the gastrointestinal system, respiratory system, endocrine system, and auditory system
- Ectoderm – Outer layer, in animals this differentiates to form central nervous system and epithelial skin tissues.
- Mesoderm – Middle layer, in animals differentiates to form connective tissues, circulatory system, skeletal system, and kidneys.

Integrin: Transmembrane protein most known for binding the extracellular matrix to the cell membrane.

Left-Right Asymmetry: Embryos typically begin as perfectly symmetrical forms (about their left-right axis); in higher level organisms, this symmetry breaks along the left-right axis as the tissues and organs develop and orient towards their final position in the body.

Mechanobiology: Field which studies the underlying physical mechanisms and forces that drive or result from biological events/ phenomena.

Mechanosensing: The process by which cells detect mechanical stimuli from their environment, such as fluid flow or collision. Cilia are a common mechanosensor in many different types of cells and are known to drive early development of left-right asymmetry in rodents.

Mesendoderm: Also known as the "primitive streak" the cell layer in the very early embryo that will differentiate into the mesoderm and endoderm.

Morphogenesis: The process by which an organism and its respective tissues and organs develop their shape and form.

Nonlinear structured illumination microscopy: (nSIM) Super-resolution fluorescence microscopy technique recently developed known for its (theoretically) unlimited resolution and high speeds obtained by rapidly switching high-contrast illumination patterns to create a time-lapse image.

Traction Force Microscopy: Process by which forces from cells can be measured directly by observing their effects on a synthetic substrate, typically some form of silicone.

Tyrosine kinases: Family of proteins which regulate the integrin-cytoskeleton interaction, making it responsible for the transduction of mechanical signals.

Introduction

Embryonic morphogenesis, the generation of biological form in development, has been described as a series of orchestrated processes in which the physical properties of tissue and the force which act upon them are delicately controlled. The idea that biological form can be viewed as a diagram of forces was initially described by Thompson in his classic book, *On Growth and Form*[1], over a century ago, yet the mechanical processes driving morphogenesis have long been understudied as compared to their molecular and genetic counterpart. Recent findings have attempted to remedy this by aiming to both improve the understanding of the biomechanical mechanisms observed in the embryo as well as relate these back to our understanding of the genetics and biochemistry surrounding morphogenesis[2,3]. Approaching embryonic morphogenesis from a biomechanics perspective leads to several interesting questions which should be addressed. What comprises the researcher's toolkit when conducting experiments, and how does this compare to that used in a biomolecular approach? What role do they play in well-known biological events, such as formation of the left-right axis or the looping of the heart? How are biomechanics and molecular biology interrelated, and how do mechanisms in one drive those in the other? Answering any of these questions will provide valuable new insights into not only embryonic development but also the broader understanding of biomechanics.

Here we review recent advances about the generation of mechanical forces in cells and tissues during embryonic morphogenesis, the transmission of these generated forces, and the tools used to measure and model these conditions via simulations or experiments.

Generation of mechanical forces in cells and tissues

The mechanics of morphogenesis is concerned with forces generated and acting upon cells and/or tissues to cause discernible morphological changes on the level of cells, tissues, and entire organisms. This section will first address the underlying mechanical properties that forces act upon, and then describe the nature of internal and external forces acting on cells and tissues during morphogenesis.

Subcellular mechanics and cell stiffness

Stiffness is a fundamental biomechanical property of cells which is essential in determining a cells' response to mechanical forces. During tissue development, cells sense mechanical cues in the environment and respond with biological changes. These include membrane deformation, nucleus deformation, spreading, cytoskeletal reorganization, and cell bursting/motility[4]. In this context, cell stiffness is reflective of the elasticity of the cells and relevant to the cells' sensing. Furthermore, many physiological processes involve cell-cell interactions. For instance, adjacent cells in a tissue establishing tight connections via surface receptors like cadherin, platelets and leukocytes can attach to endothelial cells during hemostasis and inflammation, while macrophages can adhere to and engulf other somatic cells during phagocytosis. In such processes, the stiffness of one cell serves as a biomechanical cue for the other cell for mechanosensing. As demonstrated in previous work, when an erythrocyte and a macrophage are in contact, increasing the stiffness of the erythrocyte can hyper-activate myosin-II of the macrophage, thereby

overpowering its inhibitory self-signaling for phagocytosis[5]. Cell stiffness is also implicative of a cell's fate and pathological status. Transformed cells, or cancer cells, are oftentimes softer than those non-transformed cells from the same tissue[6], while aging and type II diabetes both cause the stiffening of erythrocytes[7]. Therefore, cell stiffness could potentially be a useful marker for diagnostics of certain diseases, and possibly even a target for disease treatment.

Single-cell stiffness can be measured by multiple experimental approaches including micropipette aspiration, micro-force sensors, cell pokers, optical tweezers, and magnetic tweezers[4,8]. Among these approaches, the most well-developed technique is the atomic force microscopy (AFM) indentation assay[4]. It allows for an elastic AFM cantilever tip to either directly or indirectly (by attaching a microsphere to increase the contact area) compress a single cell that is seeded onto a surface. By measuring the indentation distance and the compressive force (as reflected by the deformation of the cantilever), one can calculate the stiffness of the cell. However, AFM is limited to samples that have open surfaces and can be in direct contact with the AFM tip. Thus, it will not work for *in situ* measurement of cell mechanics in 3D. When measuring cell mechanics in 3D, optical-tweezers have been shown to be a useful tool when compared with AFM [9]. It uses an optical beam to manipulate an endogenous particle or organelle to probe the mechanics of cells, and has been utilized to measure both mechanics of cell interior[10,11], and mechanics of cells inside a growing multicellular system[12].

The stiffness of cells and tissues plays an important role in embryogenesis. This has been observed during *Xenopus laevis* gastrulation, where the mesoderm and notochord need to maintain sufficiently high stiffness to resist buckling and allow converging and extensional movements while the involuting marginal zone actively undergoes stiffening to prevent the collapse or deformation of the tissue[13]. As an important fact, the different cell species a stem cell differentiate into can have highly distinctive stiffness. As measured by AFM single-cell indentation[14], human cortical neurons have a Young's modulus of around 100 pascal (Pa)[15], human lung epithelial cells and chondrocytes have a Young's modulus in the scale of 10^2~10^3 Pa[16,17], human breast and prostate cell lines have a Young's modulus of around 2000 Pa[6], whereas that of human erythrocytes reaches as high as 10^5 Pa[7]. Such distinctions in stiffness imply that different cells sense the mechanical environments very differently.

Notably, cell stiffness can be actively regulated by environmental biomechanical cues. The rigidity of extracellular matrix (ECM) directly affects cell stiffness via a process called 'rigidity sensing'. As an example, transformed cells can respond to the more rigid ECM – caused by the extensive deposition of collagen fibers – and undergo stiffening[18,19]. Similarly, increased ECM rigidity enhances the stiffness of stem cells[20]. The rigidity sensing also determines the direction of stem cell differentiation: with an ECM substrate rigidity of low (0.1-1 kPa; mimicking brain elasticity), intermediate (8-17 kPa; mimicking muscle elasticity) and high (25-40 kPa; mimicking osteoid elasticity), naïve mesenchymal stem cells were shown to respectively specify lineage toward neurons, myoblasts, and osteoblasts[21]. This behavior is considered as an *in vitro* mimicking of natural development of mammalian tissues. Indeed, it opens the door to a variety of stem-cell based bioengineering applications.

Subcellular structures allow force transmission

The stiffness of a cell is primarily maintained by the nucleus and the cytoskeleton, the latter of which consists of three types of biopolymers: actin filament, intermediate filament, and microtubule[8]. Together with the cell membrane (including the cell cortex), these compose the main subcellular structures that allow force transmission on and inside a cell. Depending on the type of forces generated in and applied to the cell, which can be either extracellular tensile, intracellular tensile or extracellular compressive, the mode of force transmission is different.

Extracellular tensile force is usually sensed via receptor-ligand interactions, wherein transmembrane receptors expressed on the cell surface receive forces from the ligands as they bind to them (Fig. 1A). These ligands are immobilized on an opposing surface, which could be either the ECM or an interacting cell[22]. The tensile force is first propagated along the receptor and across the cell membrane, later

transmitting along the cytoplasmic linkage of the receptor towards the cytoskeleton. As an example, the cytoplasmic tail of integrin is sequentially connected to talin, vinculin and the actin/myosin network. Once force reaches the integrin cytoplasmic tail, it will be transmitted to the whole cytoskeletal structure, and potentially reach the nucleus and mediate nuclear mechanotransduction[23]. In addition, for cells that have contact with fluidic environments, extracellular tensile forces can also be generated by fluid flow (Fig. 1B). In these cases, a molecular receptor on the cell surface is still needed to 'sense' the force, but no ligand is necessarily presented.

Intracellular tensile force is another type of mechanical force experienced by a cell, which mediates critical cellular functions such as ECM rigidity sensing[24] (Fig. 1C). Unlike extracellular tensile forces, where the direction of force propagation is outside-in, intracellular tensile forces are first generated inside the cell via actomyosin contractile activities, and then propagate bi-directionally, i.e., both towards the nucleus and the cell membrane. Even so, the structures responsible for force transmission are identical. At the cell surface, receptor-ligand interactions specifically must act as the 'anchoring point' to allow for force accumulation; otherwise, the force will be easily dissipated. Furthermore, it has been recently shown that cytoskeletal intermediate filaments play an important role in the transduction of intracellular tensile forces[11].

Extracellular compressive forces are normally received during cell compression against the ECM or another cell[22] (Fig. 1D). Therefore, unlike tensile force, compressive force does not require receptors for mechanoreception but instead is directly exerted to the cell membrane. This causes membrane deformation and in turn the rearrangement of the actin/myosin cytoskeleton and the deformation of the nucleus.

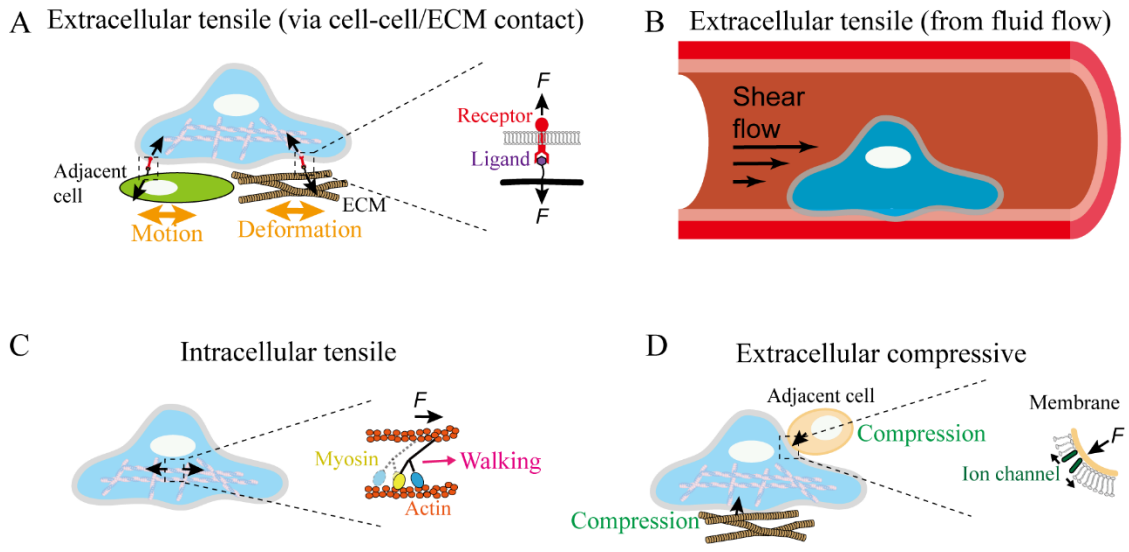

Figure 1: Mechanisms of force development on and within a single cell. (A) Extracellular tensile force can be generated due to relative motion of adjacent cells or the deformation of ECM. The force is received by the cell via receptor-ligand interaction. (B) For cells in contact with fluid, the fluid flow generates mechanical force that will be sensed by the cell. (C) Energy-driven myosin walking on the actin filament can cause the rearrangement of the cytoskeletal structure and generate intracellular tensile forces. (D) Collision of adjacent cells or compression of ECM can generate extracellular compressive forces, which will cause the deformation of the cell membrane, and in certain cases, the opening of mechanosensitive ion channels.

Mechanosensing is intrinsic to morphogenesis as forces can develop on and inside a cell via either extracellular force reception or intracellular force generation[22,25]. Extracellular force reception requires a cell to receive forces from the ECM or other cells in contact, or from the environmental fluid flow. By contrast, intracellular force generation primarily relies on the development of cytoskeletal forces via actomyosin contractile activities. When a force is generated and transmitted throughout a cell body, that cell undergoes

the process of ‘mechanosensing’, described as the mechanism by which the cell senses and reacts to the biomechanical stimulations. Mechanosensing is an important process throughout all stages of embryogenesis. In many cases, the force transmission and reception could go across multiple scales, starting from the macroscopic organism level, down to the microscopic cellular and molecular levels; whereas the molecular activities resulting from mechanosensing can in turn regulate cell fate and eventually contribute to morphogenesis at the organism level (see Fig. 2 as an example). Here we review both extracellular and intracellular mechanosensors and their role in the development of the embryo.

Extracellular force generation and mechanosensing in embryonic morphogenesis

Extracellular tensile and compressive forces can both originate from the relative movement of its adjacent cells or interacting ECM. This is common in embryonic development since an embryo is constantly undergoing physical expansion, structural rearrangement and morphological change[26]. For instance, the germ-band extension in Drosophila embryos causes tissue deformation to compress anterior stomodeal cells. Such compression triggers the nuclear translocation of β-catenin and upregulates the expression of the transcription factor *Twist*[27,28]. In this case, the mechanical force goes through a scale-down process: it is first generated at the organism level, then transmitted at the cellular level through cell-cell junctions, and eventually received at the molecular level by cadherin/β-catenin complex for mechanosensing to trigger downstream signaling (Fig. 2). On the other hand, the signaling regulation goes through a scale-up process: the resulting upregulation of *Twist* (molecular level) regulates cell differentiation (cellular level), which in turn contributes to the midgut development (organism level). Similarly, in mouse embryos, morphogen induced compression of mesenchymal cells underneath the dental epithelium results in tooth-specific cell fate switching, which also involves the scale-down of force signals and the scale-up of resulting regulatory signals[29]. As another example, intrauterine breathing causes the stretching of bronchial epithelial cells, which facilitates the development of lung smooth muscles[30].

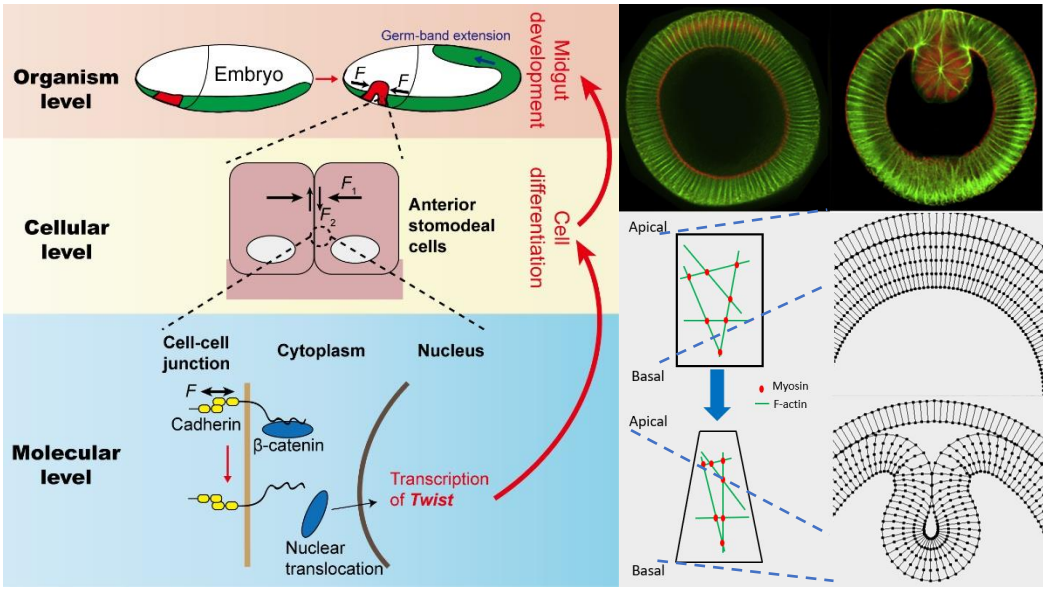

Figure 2: Left: Multi-scale force transmission and signal transduction during Drosophila embryo germ-band extension. Force is transmitted from tissue deformation and leads to the mechanosensing of anterior stomodeal cells, which results in the nuclear translocation of β-catenin and the upregulation of *Twist* transcription. The resulting signals then regulates cell differentiation and in turn contributes to midgut development. Right: (top) Seen here in Drosophila (reprinted from Polyakov et al.[31] with permission), apical constriction results in ventral furrow formation also illustrated by (bottom) diagram and simulated model of an embryo.

Cilia provide a well-known example of mechanosensors; it has been well documented that they respond to fluid shear stress in cells such as epithelial cells of the kidney [32,33]. This ability of cilia to respond to fluid flow is also valuable during embryonic development. Embryos across all species must develop in a fluidic environment, which provides fluid forces to cells on the embryo surface. During gastrulation, fluid

flow at the microscopic scale already starts to regulate cell differentiation and embryonic patterning. In mouse embryos, it has been shown that a subset of cilia on the ventral node undergoes vortical motion to generate a leftward extracellular fluid flow, which triggers the mechanosensing activities of another subset of cilia and in turn the asymmetrical signaling for left-right determination[34,35]. This is also discussed in the "Left-Right Asymmetry" sub-section.

Certain organs within an embryo contain fluidic components such as blood and lymph, which are in constant flow and therefore exert shear forces onto the contacting cells. The extracellular force resulting from such fluid flows is crucial to the development of these organs. Blood flow is also important to the maturation of the vascular system. Shear forces from blood flow can be detected by vascular endothelial cells and drive cardiogenesis[36] and vascular remodeling (angiogenesis)[37], as well as arteriovenous differentiation[38,39]. The same shear force also stimulates endothelial cells to produce nitric oxide (NO), which upregulates the production and release of hematopoietic stem cells into the circulatory system[40,41]. Similarly, the lymph flow can be sensed by lymphatic endothelial cells[42] to mediate lymphatic vascular remodeling[43], lymphatic valve formation[44] and the expansion and maturation of lymph node[45]. Also, ventricles formed in the embryonic brain contain cerebrospinal fluid (CSF) secreted by the choroid plexuses. The CSF microfluidic flow is sensed by ependymal cells and radial glia cells, which is important to the development of the brain ventricular system and spinal cord[46,47].

Intracellular force generation and mechanosensing in embryonic morphogenesis

During embryogenesis, cells can generate internal forces through the contraction of non-muscular myosin II in the cytoskeletal network, resulting in the converging and extending movement of tissues[13,48]. During this process, two regulatory molecules, the small GTPase RhoA and the Rho kinase (ROCK), play central roles in triggering the force generation[13]. Internal forces are transmitted towards both the nucleus and the cell surface via the anchorage of extracellular receptor-ligand interactions, which thereby allows the cell to sense extracellular environment. For example, in the abovementioned "ECM rigidity sensing" process, stem cells use integrins as the anchoring points and exert internal forces to sense the ECM rigidity to determine the direction of their differentiation[24,49]. p190B RhoGAP-knockout fibroblasts showed weakened adipogenesis but reinforced myogenesis, indicating that RhoA-mediated cell contraction initiates signaling that affects the fibroblasts' choice of differentiation between the two directions[50]. Cell-generated internal forces are also important to cell spatial organization and proliferation. In *Drosophila melanogaster* oogenesis, cell contractility maintains the border-cell migration of the follicle cells down the midline of the egg chamber[13]. Smooth muscle cells would have impaired proliferation on soft substrates or with inhibited contractility[51,52], whereas the transfection of an active form of RhoA resulted in the abnormal proliferation of unspread endothelial cells[53]. Furthermore, it has been shown in Drosophila embryos that cells undergoing apoptosis would release apoptotic signaling molecules, which trigger intensified contractile forces in neighboring cells to extrude the apoptotic cells out of the monolayer, facilitating dorsal closure[54].

Cell-generated internal forces bear another critical role beyond mechanosensing; they actively transport molecules across the cytoplasm. In *Caenorhabditis elegans* zygotes, actomyosin contraction was shown to generate cellular level cortical flows, which transport anterior and posterior PAR proteins respectively to the anterior and posterior half of the cell, leading to stable polarized subcellular patterning[55]. When the embryo divides to contain four cells, its myosin further generates torques and twists the actin cortical layer, driving the skewing of the spindle orientation and facilitating chiral symmetry breaking[56]. In addition, various intracellular force generations also result in a randomized force fluctuation in the cytoplasm, which significantly enhances "diffusive like" transport of both organelles and molecules[57].

Molecular mechanisms of mechanosensing

Mechanosensing is an important mechanism in regulating embryonic morphogenesis, cell movement, and tissue deformation. Generally, a cell fulfills a mechanosensing process in four steps: mechanopresentation, mechanoreception, mechanotransmission and mechanotransduction[58]. Depending on the force being tensile or compressive, the detailed mechanisms of the first two steps are different. For receptor-mediated tensile force mechanosensing, mechanopresentation represents the presentation of immobilized ligands for cell adhesion and the exertion of extracellular force pulling, while mechanoreception represents the binding of the receptor to the ligand and the reception of the force signal. By contrast, for mechanosensing of compressive force, mechanopresentation is realized by cell-cell interaction, whereas mechanoreception is performed by the cell membrane; in other words, both steps are achieved at the cellular, instead of molecular level[22]. Often regarded as the most mysterious step of mechanosensing, mechanotransduction is responsible for the conversion of the biomechanical signal into a biochemical signal. It is perceived that mechanotransduction can be achieved by force-induced conformational changes of certain mechanosensitive molecules, thereby exposing encrypted domains for chemical reactions[22,58]. For more details of mechanosensing mechanisms, the audience can refer to our previous reviews[22,58].

As mentioned previously, the mechanosensing of tensile force signals is primarily mediated by adhesion receptors at the cell surface. Currently, four cell adhesion receptor families have been identified: cadherins, selectins, integrins, and immunoglobulins. Among them, cadherins primarily modulate cell-to-cell junctional adhesion. Tensile forces pulling on cadherin molecules lead to both whole-tissue morphogenesis as well as single cell rearrangements[59]. In wound healing, increased stiffness of the substrate enhances the speed and polarity of epithelial cell migration through the improved coupling of contractile forces between neighboring cells through cadherin-mediated adhesion[60]. In *Xenopus* embryos, the force pulling on C-cadherin was shown to cause polarization in migratory cells[61], whereas the force pulling on E-cadherin was also shown to be required for cranial neural crest migration[62]. In the zebrafish neural tube, cadherin-mediated adhesion allows relative cell movements to sort and organize the neural progenitor cells to form strictly demarcated geometric patterns[63]. Integrins are another family of cell receptor that play versatile roles in embryonic morphogenesis. Previous works[64,65] have summarized the key roles of specific integrins in the development of stems cells respectively in the three primary germ layers: endoderm (α_1, α_V, α_5, β_1), mesoderm (α_{6A}, β_8, β_4, β_5, $\alpha_5\beta_1$ and $\alpha_6\beta_1$) and ectoderm (α_V, β_1). Of these, only the mechanosensing function of several integrins has been verified. In the vascular development, it was demonstrated that blood shear stress activates $\alpha_V\beta_3$ to mediate cytoskeletal alignment of endothelial cells[66]. In the rigidity sensing of stem cells, β_3 and α_2 integrins respectively mediate the myogenic and osteogenesis differentiation of MSCs (mesenchymal stem cells) on substrates with medium and high stiffness. By contrast, much less mechanosensory functions have been identified on selectins and immunoglobulin receptors in embryonic morphogenesis.

Notably, in special cases tensile forces can trigger mechanosensing via certain structures on the cell surface independent of receptor-ligand interaction. Typically cells use bristles, hair bundles, and other “professional components” to sense the physical environment[67], all of which play a critical role in the left–right patterning of embryos[34,35]. The only known exception to this is observed in the glycocalyx (also known as the pericellular matrix), a collection of a transmembrane glycoprotein and glycolipid which coats animal cell surfaces at a very high expression level[68]. Using its long extracellular domain, glycocalyx on endothelial cells can directly sense the shear force of the bloodstream independent of ligand association[69]. Engineered glycocalyx was found to regulate the differentiation of embryonic stem cells, indicating its role in embryonic morphogenesis[70]. It nevertheless remains unclear whether this requires the mechanosensing function of glycocalyx or just its biochemical functions.

By comparison, the mechanosensing of compressive force signals are primarily initiated by membrane deformation, which controls the closing/opening of mechanosensitive ion channels in the cell membrane to trigger downstream chemical signaling. As an example, PIEZO1 can be activated by traction forces to trigger intracellular Ca^{2+} fluxes in a substrate rigidity-dependent manner, which was demonstrated to guide the differentiation of human neural stem cells[71], the proliferation of mouse embryonic stem cells[72], the development of mouse vascular and lymphatic systems[73,74] as well as epithelial development in multiple organs[75]. Many other mechanosensitive ion channels, like the PKD, TRPC, TRPM and TRPV families, can also direct stem cell differentiation[76]. Directly supporting embryonic development, mechanosensitive ion channels were found to transduce mechanical cues during chick joint development[77]. In this study, a stretch-

activated type of calcium ion channel was inhibited in both static and dynamic cultures for hindlimbs of explanted chick embryos. In the dynamic culture which provides mechanical stimulations to the cells, inhibiting the ion channels significantly modified the configurations of the developing knee joints, including condyles separation and tibial plateau and crest formation, which became similar to chicken knee joints grown under static culture. In addition, the mechanosensitive ion channel TRPV4 was also found to regulate lung development and stabilize pulmonary vasculature[78]. Recently, it has also been discovered that volumetric compression can increase the degree of molecular crowding in cells, that promotes Wnt/β-catenin signaling[79], which can be used to regulate development of mouse intestinal organoids[79], as well as de-differentiation of human and mouse adipocytes[80]. Similar effect has also been observed on YAP/TAZ signaling[81], suggesting that this physical mechanism may have a more general regulatory role in development.

Since the cytoskeletal structure allows force transmission intracellularly, mechanosensing does not necessarily occur at the membrane surface or juxtamembrane level but can also be fulfilled by cytoskeletal/nuclear proteins. Actomyosin meshworks are shown to operate as mechanosensors. During *Drosophila* gastrulation, contraction of actomyosin in the ventral cells produces epithelial furrows along which actomyosin fibers and tension spread. Chanet and colleagues used both genetic and mechanical perturbations to modify the tissue geometry, and revealed that geometrical and mechanical factors assist orienting the cytoskeleton and tension in the formed ventral furrows[82]. In four scenarios, they showed that cytoskeleton structured itself into rings in the middle of apical domain with isotropic force pattern. However, in the wild-type (naturally formed) ventral furrow, the actomyosin meshwork formed nodes other than rings, and both fibers and force are clearly guided along the direction of the a-p axis. Their results showed that cellular force in a tissue is not always regulated by genetic factors, but also depends on the geometrical factors of the tissue. In fact, the geometry of tissues provides useful information for cells to produce forces and generate the needed tissue structure from actomyosin mechanosensing. The cell nucleus contains multiple mechanosensitive molecules that can fulfill the task of mechanotransduction. For instance, nuclear pore complexes (NPCs) can open under nuclear membrane stretch, allowing the nuclear import of signaling molecules[83,84]. Nuclear membrane stretch also expands the densely packed nuclear membrane phospholipid bilayers, enabling the insertion of hydrophobic protein residues[85]. Mechanical forces also induce the unfolding of nuclear proteins (e.g., Lamin A/C and Emerin) and the re-organization of chromatin, which expose protein domains for phosphorylation and cause DNA and histone modifications of the chromatin, leading to changes in gene expression[23]. In mouse embryonic stem cells, both extracellular tensile and compressive forces in the nucleus regulate cell differentiation and reprogramming[86]. In this process, nuclear deformation modifies the spatial accessibility of chromatin to transcriptional regulators, and therefore results in transcriptional changes[87].

Experimental and computational methods for measuring and predicting forces

At present, there are several mature measurement techniques to study/measure different aspects of cellular and tissue/cell forces[88,89]. Typically, a sensor probe consisting of materials with well-known properties is used to acquire responses to applied forces, and deformations of the probe in response to tissue forces is converted into measured forces[90]. In addition, some measurements are also used to investigate the material properties of tissues *in vitro*, *in situ*, and *in vivo*.

Many groups have used the combination of experimental and computational methods (such as microscopy, physical tools, quantitative modeling, and mathematical models) to characterize forces generated in embryonic morphogenesis. For example, atomic force microscopy (AFM) was employed to measure adhesion forces at the single cells' level, from the gastrula of zebrafish embryos to substrates with fibronectin[91], and to quantify the de-adhesion forces necessary to separate mesendodermal cells from the substrate[92]. Similarly, laser microsurgery and quantitative modeling were used to investigate dorsal closure by analyzing forces and processes[93]. Förster resonance energy transfer (FRET) has been shown to be an effective way to quantify interactions between proteins[94–96], a good candidate to monitor forces acting on

cells as protein complexes responsible for sensing and the signal transduction interact. This technique has also been used to study signaling pathways[97]. For example, an encoded Src (a type of tyrosine kinase) reporter was developed for quantitative assessment of mechanotransduction activities *in vivo*[98]. Another recently developed method, nonlinear stress inference microscopy (NSIM), can perform extremely high resolution (in both space and time) analysis of tissues and is also effective in three-dimensions[99]. This allows for the study and quantification of mechanisms that were previously not possible; for example it has been used to quantify cell generated contractile forces in the surrounding extracellular matrix by utilizing the extracellular matrix's nonlinear stiffening property[100]. In events where measurements of forces *in vivo* are difficult, computational methods are often used to provide useful information that will shed light on mechanisms of morphogenesis.

In this section we review commonly used techniques for measuring tissue forces in embryonic morphogenesis, using laser microsurgery and quantitative modeling, droplets, traction force microscopy, and FRET sensors as examples, followed by discussion of computational modeling for measuring and predicting tissue forces.

Laser microsurgery and quantitative modeling

Laser ablation is also extensively used to determine cell and tissue mechanics both *in vivo* and *in situ*[101]. To this end, a nano-pulsed laser ablates a portion of either tissue or single cells in the system, which generates a mechanical perturbation resulting in a measurable response[90,102–104], and has been used to illustrate mechanisms of embryogenesis and wound healing.

Hutson et al.[93] also quantified the forces driving morphogenesis during dorsal closure through a combination of laser ablation and computational modeling. Here these techniques were applied to identify the biomechanical factors that drive dorsal closure in Drosophila embryos. To study both "native" (normal shape) and "perturbed" (laser-ablated) embryos using this technique both the laser hardware and image processing tools were optimized (snapshots from the video of the embryos are shown in Fig. 3). This resulting in high-resolution images provided the researchers with the measurements required to design a model of embryo deformation during dorsal closure. Applying this model to a mutant with a β_{PS} integrin deficiency (Fig. 3E) a significant decrease in zipping was identified as a main cause of dorsal closure failure. The study illustrated the effectiveness of combining experimental methods and computational modeling, as both were used in tandem to identify the origin of contractile forces responsible for dorsal closure. The findings shed light on the mechanical and molecular origins of morphogenesis.

Laser ablation was also used to measure and predict cortical tensions in different dimensional directions in Drosophila elongation and dorsal closure. For example, Kiehart et al.[105] studied the distribution of stiffness and tension during dorsal closure in Drosophila based on responses of the various tissues dissected by an ultraviolet (UV) laser. They demonstrated that during dorsal closure stages, all lateral and dorsal sides of the embryo's surface were in tension. As time-lapsed imaging revealed that laser-wounded tissues healed rapidly, long-term effects from laser-ablated wounds on the embryo's health condition were negligible.

Hutson et al.[106] further combined experimental and computational methods to study force generation in embryonic morphogenesis. They succeeded in using laser microsurgery to quantify cell-level mechanics in the dorsal epithelium of fruit fly embryos, verifying predictions derived from a finite-element model[106]. After designated areas of the embryonic epithelium underwent laser ablation, it was determined that the surrounding cells recoiled from the wound site having a high level of initial recoil velocities. Fig. 4(a) showed their experiments of post-ablation changes in cell-level regions. The ablated cells grew by approximately 40% in the early stage, with adjacent cells keeping a nearly constant area. Over a larger time scale, the adjacent cells would see increases or decreases in area, though there were no significant differences from changes in area observed prior to ablation. The experimental observations supported and justified their modeling of initial recoil using two dimensions. They simulated the changes stimulated by laser hole-drilling using cellular-level finite element (FE) models (see Fig. 4(b)). In the basic model, there are three mechanical contributions from each cell: (1) a uniform tension on the interface, γ; (2) a system of internal dashpots which can be treated as a uniform viscosity, μ; (3) an area constraint formed from an isotropic, in-plane cell stress, σ. The initial recoil was well reproduced by the FE model. In conclusion, all the observations and data of hole-drilling experiments can be reproduced with 2D FE model by considering tensions, viscosity, cell stress, externally applied stress, and a viscoelastic rods' network inside of a cell.

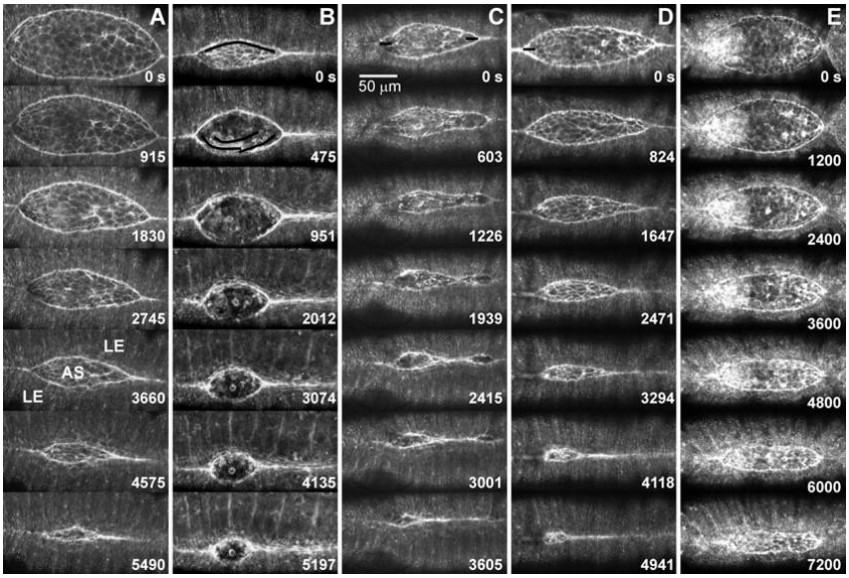

Figure 3. Confocal fluorescent images representing native embryos and embryos with different postprocessing steps (AS refers to the amnioserosa and LE refers to the lateral epidermis). (A) Native embryos; (B) a set of four slices to fully anatomize the amnioserosa; (C) inhibition of zipping through laser microsurgery at two flanks of lateral epidermis of the embryo (canthi); (D) inhibition of zipping through laser microsurgery at one canthus; and (E) Embryos with *myospheroid* mutation. Reproduced from Hutson et al. [93], with permission.

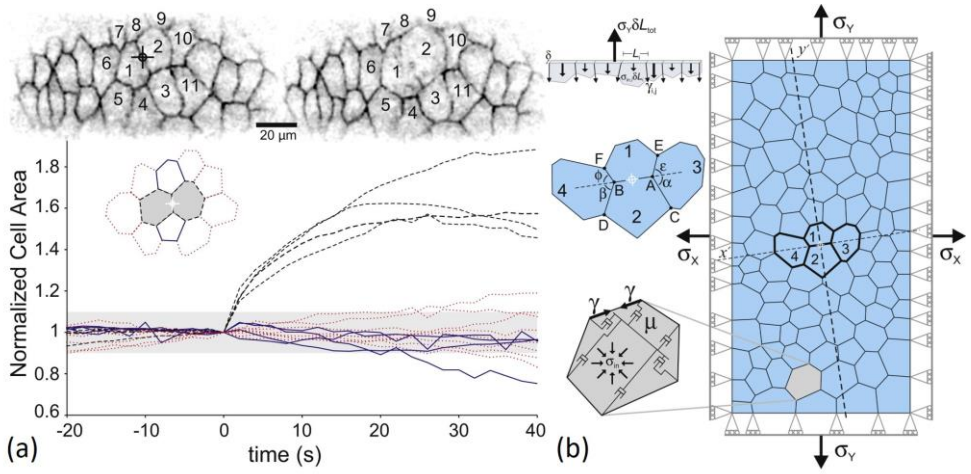

Figure 4. (a) Laser hole-drilling simulations; (b) Example of epithelium constructed by the finite-element model. Reprinted from Hutson et al.[106] with permission of Elsevier.

A similar technique was implemented by Rauzi et al.[107] in which they used laser nano-dissection and quantitative modeling to quantify mechanical properties of morphogenesis in the *Drosophila melanogaster* embryo. An epifluorescence inverted microscope was coupled with a 50-MHz 1030-nm fs laser to enable local ablation synchro fluorescence imaging. They used quantitative modeling of cell intercalation and compared predictions with *in vivo* data. The model characterized steady states involving nature, magnitude, and spatial distribution of forces, and addressed how local forces influence the tissue embryogenesis. It illustrates that increased levels of tension appear to be generated by actomyosin cables in the Drosophila embryo's intercalating cells. Moreover, multicellular contractile structures maintain increased tension[108]. The findings revealed that tension forces can stabilize myosin-II localization in a fashion that results in a more cohesive, higher-level organization by actomyosin cables and efficient tissue elongation.

Another important development is the establishment of an experimental approach to detect the dynamics of cells and tissues by combining laser dissection with genetic manipulation[109]. For instance, Wells et al.[110]

applied laser dissection approaches to investigate the requirement for intact canthi during closure. Their experiments confirmed the robustness of the dorsal closure process and supported the existence of emergent properties related to changing the purse string/actomyosin geometry of cable to achieve successful closure process. Machado et al.[111] successfully measured mechanical properties of endothelial cells during morphogenesis by measuring fluorescence. Combining their findings with laser ablation experiments, both apicomedial stress and junctional stress tend to increase as time goes by and reaches twice of relative increasing in apicomedial stress compared with other obtained measures. Saias et al.[112]used the laser microsurgery technique to illustrate that the tissue contraction leading to shape changes of the amnioserosa during Drosophila dorsal closure was correlated with decreases in cell volume. To evaluate forces which contribute to the kinetics of the closure, they quantified and qualified myosin levels. Additionally, they conducted laser dissection experiments in the amnioserosa, actin cables, and epidermis. This identified a contraction mechanism that the dorsal closure was mediated by two mechanisms working together: the contraction of actin cables surrounding the tissue as well as the cell volume decrease.

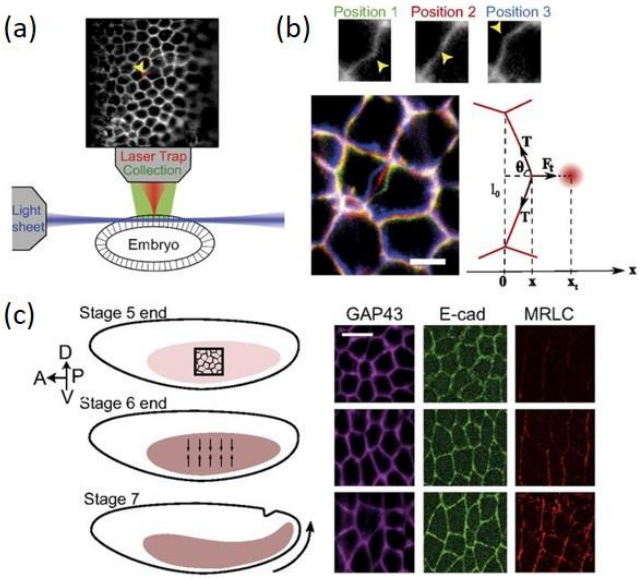

Figure 5. Characterization of optical tweezers-induced deflection of cell–cell interfaces. (A) Experimental setup, and (B) Three deflection interfaces I. (C) Three stages of tissue elongation with corresponding images. Stage 5, before tissue elongation; stage 6, the beginning of Myo-II accumulation at cell junctions and stage 7, during tissue elongation process. Different colors show the cell interface for various stages: purple for GAP43::mcherry, green for E-cadherin, and red for Myo-II. Reproduced from Bambardekar et al[113] with permission.

Despite these advances, laser techniques are relatively not straightforward to measure forces *in vivo* to help understand the mechanics of morphogenesis, since they only provided the relative magnitude of stresses with directions from cell and tissue shape changes[113]. The absolute values of tension forces were not measured, thus the mechanical forces between cells *in vivo* remaining unknown. To address this issue, Bambardekar et al.[113] developed a novel approach to apply forces to localized areas on cells using laser manipulation in the early stage of Drosophila embryo, using optical tweezers associated with light-sheet microscopy (shown in Fig. 5(a)). This enabled a high sample rate for imaging tissue dynamics while manipulating objects *in vivo*, making the method very reliable. The researchers were able to directly manipulate contacts between cells without the need of an external glass or polystyrene probe, possibly due to the difference in refractive index between the cell boundary and cell interior (see Fig. 5(b)). Using this method, they were able to determine how tension in the germ band layer changes over time, in particular between stages 5 and 7 (Fig. 5(c)). The experiment demonstrated an overall increase in tension occurred between these stages. They later examined the mechanical response of cells to forced deflection at different time and space domains and developed a predictive model of cell contacts[113].

Droplets

The use of oil droplets as a model was initially proposed by Boukellal et al.[114] as a method to demonstrate mechanisms of force production resulting from elastic stresses induced by actin networks on the surface of bacteria. Furthermore, droplets act as a more appropriate representation of the cell membrane compared to beads or *Listeria* as the fluid surfaces of droplets better mimic the cell membrane[115] and the deformation of droplets can provide critical information for the calculation of exerted forces[116]. Oil droplets were first used to study how Vasodilator-stimulated phosphoprotein (VASP) affected Arp2/3 complex-catalyzed actin filament formation[115]. This technique was later adapted to measure mechanical stresses within live tissues[90]. By using oil microdroplets that were both fluorescent and of approximate size to cells, Campàs et al.[116] were able to refine existing methods to more accurately measure cell-generated stresses within live embryonic tissues. These refinements allowed for the use of fluorescence microscopy techniques and image analysis, which allowed for the resulting deformations from cell-droplet interactions to be easily visible. Oil microdroplets were used to apply forces to cells within live embryonic tissue (Fig. 6(a)). At equilibrium, an ideal microdroplet will be perfectly spherical (Fig. 6(b)) but will become deformed if stresses from cells exceed the resistance force of the droplet's interfacial tension (Fig. 6(a)). When these microdroplets were embedded in embryonic tissue, the pressures of local tissues were able to be measured by monitoring the hydro-static pressure of the droplet. This technique can also quantify stresses from a single cell or cells within a monolayer culture. Combined with confocal imaging techniques, it was demonstrated that epithelial and mesenchymal cells can evoke limited droplet deformations (example in Fig. 6(c) and (d)). Additionally, cell-generated forces within the embryo were able to be directly quantified by these droplets, shown in Fig. 6(e). They measured the intercellular stresses generated by tooth mesenchymal cells in live tooth mandible explants (Fig. 6(f)). Based on these experiments, cells can generate and resist shear stresses in the entire stage of morphogenesis and can even change behaviors related to these stresses.

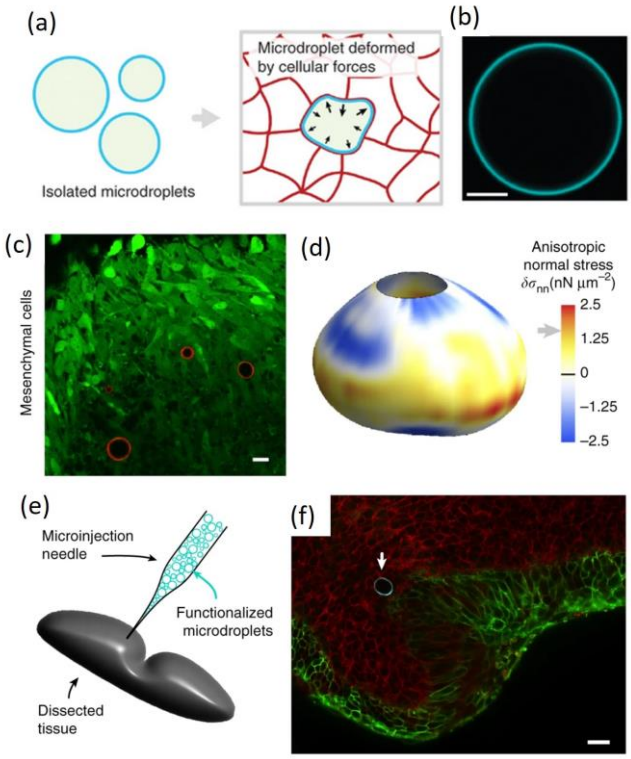

Figure 6. (a) Design of oil microdroplets for force measurement: isolated oil droplets in emulsion (left) and deformed at the interface of embryonic tissue cells (right). (b) A functionalized fluorocarbon oil at equilibrium. (c) Fluorocarbon droplets (red) embedded in a tooth mesenchymal cell aggregate (green). (d) 3D surface stress distribution of a droplet in a cellular aggregate showing anisotropy. (e) Microinjection of oil droplet in a dissected mandible. (f) A fluorocarbon droplet (cyan) is inserted into dental mesenchyme cells. Adapted from Campàs et al.[116] with permission of Nature Publishing Group.

Lucio, Ingber, and Campàs[117] further developed techniques using droplets so they could better measure mechanical stresses acting on both cells and tissues. The droplets were used to apply forces to cells *in/ex vivo*, and *in vitro* to quantify these mechanical stresses. The use of droplets can help clarify how forces influence essential cellular behaviors, including cell migration, proliferation, and differentiation within live embryonic cells and tissues. Surface/interfacial tension of embryonic tissues were measured by droplet-based sensor[118,119], and the viscoelastic behavior or mechanical properties of living embryonic tissues were also modeled using droplets[120–123].

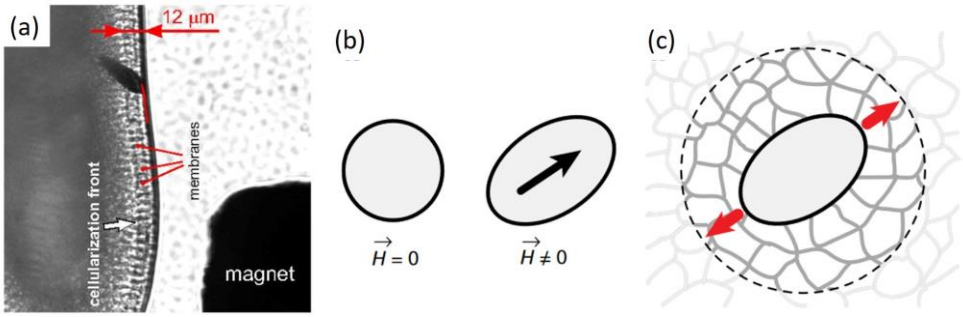

Figure 7. (a) A ferrofluid droplet is pulled by an external magnet to be prolate (black spot on the right). The droplet elongates toward the cortex along the axis pointed by the redline. Reprinted from Ref.[120] with permission. (b) Ferrofluid oil droplet in the absence (left) or presence (right) of an unform magnetic field. (c) Deformation of a ferrofluid oil droplet when applied to a uniform magnetic field. A local force dipole is generated as the red arrows indicate force direction/ magnitude. (b) and (c) are from Serwane et al.[121], reprinted with permission from Nature Publishing Group.

Magnetic (often referred to as ferrofluid) droplets are also extensively applied to mechanical properties of embryos. For example, Doubrovinski et al.[120] utilized this technique to measure the physical properties of internal components of the fruit fly embryo. In their study, a single ferrofluid droplet was micro-injected into individual embryos to study their response to external stimuli. (Fig. 7(a)). This is done by applying a magnetic field to the tissue sample with embedded ferrofluid droplet, which caused the ferrofluid to deform due to its electromagnetic properties. Bright-field imaging was used to track where the droplet is located throughout the duration of the experiment, in particular its ability of returning to its original position after the magnetic field was applied. The droplet never fully returned to its original position; this “incomplete recoil” is a result of viscoelastic properties. Thus, this was used to measure both viscosity of the cytoplasm and cortical elasticity and later was used to compare against cells affected by microtubule-destabilizing drugs. The latter study indicated that while the elastic properties of the embryonic cortex are dependent on an intact cytoskeleton (the actin cortex) they are not dependent on microtubules. Another example of using ferrofluid droplets to study cell mechanics is seen in Serwane et al.[121], whereby they serve as micro-actuators within developing embryos. Ferrofluid oil droplets can generate a localized force dipole in the cells after being actuated via a uniform magnetic field (Fig. 7(b) and (c)). The mechanical properties and applied magnetic fields of the ferrofluid droplet were then extracted, and thus surrounding the droplet, the corresponding performance of embryonic cells/tissues are obtained. In addition, Zhu et al.[124] applied a 3D magnetic device, to quantify tissue stiffness distribution in a vertebrate, organ-stage embryo under live conditions. Ferrofluid droplet techniques provide a non-invasive, remotely controllable way to probe cell mechanics since the droplets can be manipulated even if they are located deep within tissue.

Traction force microscopy

Traction force microscopy (2D TFM) is one of the most widely used techniques for measuring cell force[125]. To measure cellular forces, cells are placed onto an elastic substrate which will deform as the cells exert force on them. Synthetic (e.g. silicone rubber) substrates are typically used, as their slower degradation time ensures accuracy by removing fluctuations due to changes in the properties of the environment[126,127]. Both in-plane forces and contractile forces produced by cells can also generate traction forces perpendicular to substrate surface. TFM techniques evolved from the use of 2D silicone membranes and bioinert polyacrylamide (PAA) gels, where fluorescent beads are incorporated into the PAA substrata to monitor and show the changes caused by cellular traction forces. Gjorevski and Nelson subsequently summarized that TFM can investigate both cellular behavior exerted by external force and measurement

of internal forces, and can produce force maps at high resolution at the same time[128]. TFM methods have been extended to track bead deformation in 3D domain to deeply characterize 3D traction force field of a cell[129,130]. Three-dimensional traction forces induced by cells were visualized and quantified using atomic force microscopy and confocal reflectance, and were used to reveal and characterize mechanical stress of the neighboring matrix[128,131]. The 3D traction force microscopy helped directly measure cellular stresses and pressures generated by 3D tissues and control parameters related to cell force generation. Nerger et al.[132] discussed the differences of traction forces in different dimensional systems. Compared with 3D system, 2D based microfabricated tissues are easier to produce and characterize, nonetheless, 3D tissues provide a more accurate model of biological systems[133]. We should note that calculating stress and strain fields in a 3D biopolymer network remains highly challenging, due to the complex mechanical behavior of biopolymer networks; compared to PA gel, biopolymer networks are nonlinear elastic and viscoelastic. Recent studies have focused on obtaining the cell-generated displacement field as a proxy of cell contractility[134]. Han et al. recently observed that cell contractility can stiffen their surrounding extracellular matrix (ECM) by a factor of 100 to 1000; this highlights the difficulty of translating cell induced displacement to stress field in a physiologically relevant 3D environments, which can be easily done on a 2D elastic material[100]. These authors also offer an alternative method, Nonlinear Stress Inference Microscopy (NSIM), which directly infers local cell-induced stresses from the degree of ECM stiffening, using the nonlinear stiffening behavior of the ECM[100].

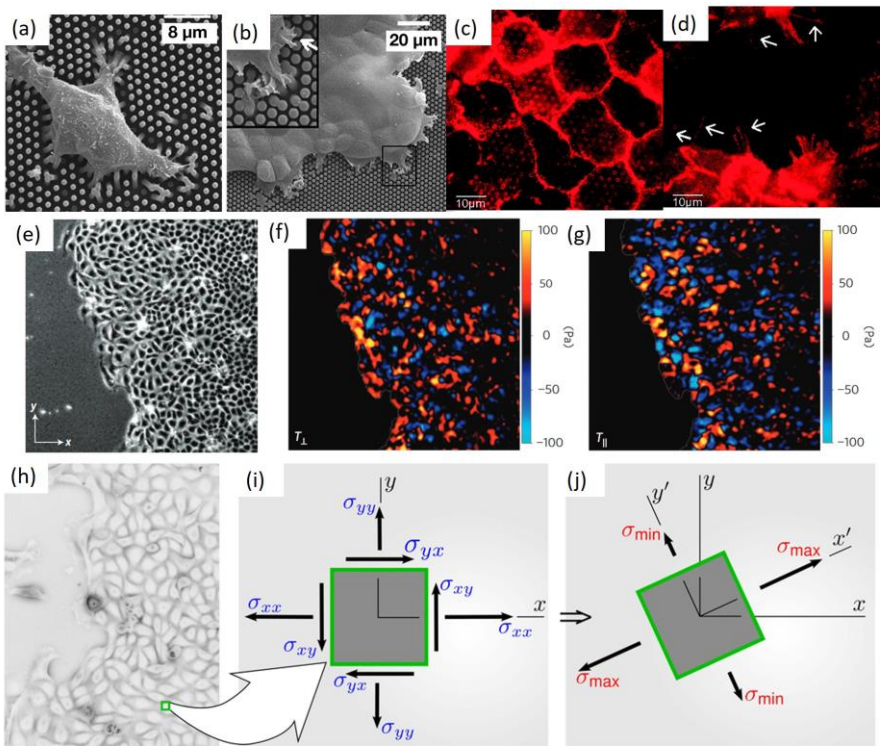

Figure 8. Scanning electron microscope (SEM) images of: (a) Individual cells lying on μFSA, and (b) a cell monolayer. (c) Immunofluorescence staining of actin: Partitioning of actin within an unaltered cell monolayer and (d) for a monolayer altered by hepatocyte growth factor (HGF) for 6 hours. (a)—(d) are reprinted with permission from Du Roure et al.[135], Copyright (2005) National Academy of Sciences, U.S.A. Traction force maps of a collectively migrating cell sheet: (e) phase contrast image, (f) traction forces that are normal to the edge of the cell sheet, and (g) traction forces that are parallel to the edge of the cell sheet. (e)—(g) are reprinted from Trepat et al[136], with permission from Nature Publishing Group. At any point in the interior of the monolayer (h), the intercellular stresses (i), can be rotated locally to obtain the principal stresses, (j) as, shear stresses vanish. (h)—(j) are from Tambe et al.[137], reproduced with permission from Nature Publishing Group.

TFM has been extensively employed in the study of mechanobiology of cell migration and tissue morphogenesis[132]. For instance, Du Roure et al.[135] measured dynamic traction forces generated during epithelial cells migration. Traction forces were extracted from the pillars' bending and amended by actin

localization using fluorescence microscopy. Since cell–surface interactions occur only at the top of the micropillars, and therefore, cell traction forces on the substrate are proportional to the deflection of the posts. No extra tractions are exerted by elastomer compliances between the pillars (Fig. 8(a) and (b)). The forces were identified by drawing a vector on each pillar with its length being proportional to the force intensity. Traction stresses were calculated with the assumption that forces were only transmitted through the pillars. In addition, they analyzed the migratory dynamics of individual epithelial cells by correlating traction tresses with spatial localization of protein and treating cells with hepatocyte growth factor (HGF) /scatter factor (Fig. 8(c) and (d)). Trepat et al.[136] were the first to directly measure traction forces of migrating cells to show that the driving forces primarily stem from many cell rows trailing the leading cells. By mapping out the traction forces they were also able to assess the locus of traction forces near the leading edge (see Fig. 8(e)--(g)). To their surprise, high traction forces were also found to have multiple cell rows away from the leading edge[137]. Tambe et al.[137] developed monolayer stress microscopy to generate a map of traction forces and used the force balance relationship to calculate cell-cell interaction forces. The stresses are calculated following the definition (i.e., force per unit area) (Fig. 8(h)) and the force balance equation implies that the gradient of the stress is equal to the traction force per unit volume. By using a local coordinate system (depicted in Fig. 8(i)) the principal stresses can be identified (Fig. 8(j)). This method was then applied to examine the stress distribution in monolayers comprised of Madin-Darby canine kidney (MDCK) cells and of well-established breast-cancer cell lines, respectively. This technology enabled them to find that the direction of local cellular migration followed that of the maximum principal stresses.

Several morphogenetic processes are also likely influenced by traction forces. The physical mechanism of how mechanical stresses influence cell growth and tissue geometry has been described in literature[138–140]. Unfortunately, the mechanisms of traction forces inducing changes in morphogenesis are not fully developed due to the difficulty of accurately quantifying these forces *in vivo*. To address this, TFM has recently been applied to measure the traction forces, tissue elasticity and endogenous mechanical stresses generated by tissue morphogenesis. Gjorevski and Nelson[141] studied and analyzed technological procedures to measure tissue elasticity and internal stresses during real-time organ development. And they discussed current approaches of manipulating forces in intact embryos. At present, the most achievable methods to determine mechanical forces or stresses during morphogenesis *in vivo* are laser microsurgery[93,106,107] and injected microdroplets[116,117,120]. Quantifying traction forces *in vivo* or *in situ* is still a crucial challenge and should be fully addressed to understand embryonic and tissue morphogenesis.

Förster resonance energy transfer (FRET) sensor

A Förster resonance energy transfer (FRET) sensor consists of an excite-electronic-state donor fluorophore and an acceptor fluorophore. The donor, regarded as an oscillating dipole, may transfer its energy to the nearby acceptor, which has a similar resonance frequency. The strength of the FRET signal is dependent on the distance from donors to acceptors, reflective of the extended length of the inserted molecular spring, and provides readable values of the tension between two ends of the sensor[90]. FRET sensors were originally used to measure tension between molecules in living cells[142,143], and have since been applied to measure adhesion forces in cell–cell[144], cell–substrate[145], and cell surface[146]. Unlike laser microsurgery and microdroplets, which are invasive imaging measuring techniques that use mechanical sensors, FRET sensors are molecular sensors that are capable of non-invasively detecting sub-cellular mechanical forces.

In recent years, FRET sensors have been widely used to characterize the intracellular and intercellular forces in a variety of biological systems. For example, Wang et al.[98] developed an encoded Src reporter using FRET to image and quantify spatio-temporal activation of Src in various mouse embryonic fibroblasts. Furthermore, it was designed to monitor mechanotransduction in living cells with respect to both space and time. The Src reporter was then used in experiments with human umbilical vein endothelial cells (HUVECs). In this example, beads coated in fibronectin have been implanted HUVECs, to allow the laser tweezers to act on these cells. The pulling force of the tweezers on the HUVEC membrane resulted in a directional FRET response (Fig. 8(a)). These results indicated that a localized mechanical stimulus can cause a directional Src-activation wave which can lead to long range transduction of signals to distant spatial locations. This led the researchers to conclude that mechanically-induced transmission of Src could vary greatly dependent on its location, and thus could use the cytoskeletal network as a method of signaling to

different locations within the cell. Using transgenic flies which expressed an optical tension sensor module, Cai et al.[147] were able to study mechanical tension and adhesion through epithelial cadherin (E-cadherin) in the margin cells of the ovary during cell migration. The mechanical tension across the E-cadherin molecule was measured via FRET. Examples of FRET processing images of examining tension distribution on E-cadherin in margin cell clusters are shown in Fig. 9(b). These images demonstrate that Rac take effects on the margin cells of the migrating cells and increase tensile forces on E-cadherin-mediated adhesions. They concluded that several significant functions for E-cadherin may contribute to the diversity of cell migrations *in vivo*. These included a pair of polar cells located in the middle of the cells and surrounded by multiple migratory cells, increase of the leading edge, the feedback loop of the RTKs, E-cadherin, and Rac, increase of a border cell and cell junction, and expansion of junction of a border cell and polar cell (shown in Fig. 9(c)). Meng et al.[143] designed and used a FRET-based stress probe (Fig. 9(d)) to investigate the spatio-temporal distribution of molecular stress *in vivo* in living cells. To do this, the group designed a FRET cassette, which they called stFRET. As a cassette protein, the sensor could be inserted into several different structural proteins, allowing for observation of proteins such as actinin, filamin, and spectrin. Initial experiments indicated this technique could be used to observe stresses in endothelial cells *in situ*[142]; It was further demonstrated that stFRET is sensitive enough to detect stresses in real time in live cells, tissues, and animals .

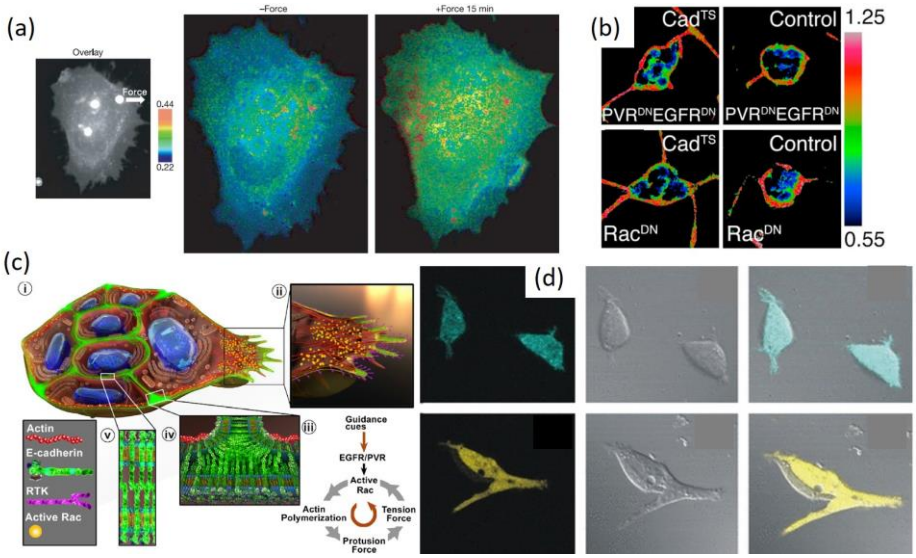

Figure 9. (a) Directional and long-range transduction of Src generated by intercellular forces: laser-tweezer force on the bead at the top right of the cell-induced FRET responses. Reprinted with permission from Wang et al.[98]. (b) FRET images of CadTS and the tension-insensitive control at the edge of cell clusters creating PVRDN and EGFRDN or creating RacDN for guiding receptors to inhibit signaling. (c) Multiple functions of E-cadherin at the edge of cell cluster. (b) and (c) are reproduced from Cai et al[147] with permission from Elsevier. (d) Efficient FRET images are generated by stFRET in HEK-293 cells. Reference images are Cerulea and Venus. Adapted from Meng et al.[143] and permitted form Wiley.

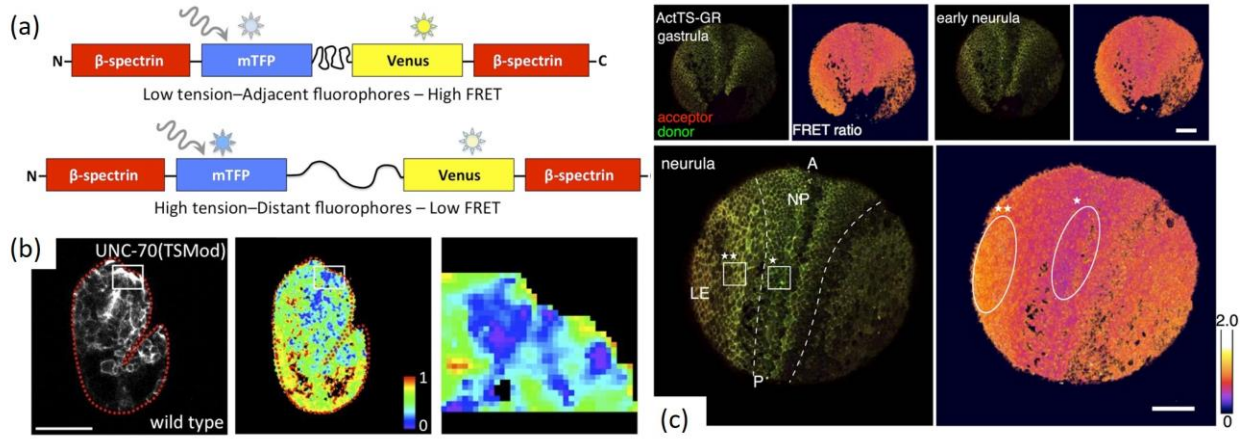

Figure 10. (a) Schematic for how UNC-70 (TSMod) detects tension for A FRET-based TSMod. (b) Images of wild-type strains that express UNC-70(TSMod) with FRET measurements. (a) and (b) are reprinted with permission from Kelley et al.[148]. (c) Time-lapse images (left) and corresponding FRET ratio images (right) of tension during morphogenesis for a ActTS-GR cell at the stages of gastrula, early neurula, and neurula. Reprinted from Yamashita et al[149] with permission from Nature Publishing Group.

FRET-based molecular tension microscopy enables the measurement of forces acting upon cells and thus furthers the way to characterize the mechanical properties of cells and tissues. More recently, FRET has been developed to measure molecular tension during embryonic morphogenesis. A FRET-based tension sensor (an N-terminal/ C-terminal tagged sensor) combining genetic and molecular methods was used to characterize a pulling force induced by the elongating pharynx on anterior epidermis during C. elegans embryogenesis, while the sensors in β-spectrin exhibit tension differences across tissues during C. elegans embryonic development[148,150]. As another example, Kelley et al.[148] used strains of *C. elegans* containing a tension sensor module (TSMod) embedded within the coding sequence of the unc-70 gene (representative images of strains that express UNC-70 (TSMod) are shown in Fig. 10(b)). The TSMod sensor, consisting of a donor (mTFP) and acceptor (Venus) fluorophore, acts as an entropic nanospring. As stretching forces are applied to this spring, the two FRET fluorophores are pulled apart and lead to an observable energy transfer. As a result, the FRET index is inversely proportional to tension, i.e., a high index of FRET indicates low or no mechanical tension whereas a low index indicates high mechanical tension (see Fig. 10(a)). Here the FRET tension sensor serves as a new means to quantifying mechanical stresses to complement the model they proposed, which shows that the foregut exerts large mechanical forces on the anterior epidermis. Yamashita et al.[149] used FRET-based sensors to measure tissue tension in *Xenopus laevis* embryos. The researchers noted this method's usefulness due to its ability to measure across several tissue regions simultaneously while maintaining a high enough spatial resolution to be able to determine significance. Using this sensor, the group successfully characterized the spatio-temporal distribution of the tension of the differentiating ectoderm. The results indicated that morphogenetic events (specifically neurulation and gastrulation) in Xenopus development were causally linked with the differential distribution of tension in the neural ectoderm and the epidermal ectoderm (see Fig. 10(c)).

FRET sensors were also used by Vuong-Brender et al.[151] in an attempt to address how mechanical forces are integrated by the adherens junctions during morphogenesis in the spatio-temporal domain. The biosensors were applied within HMP-1/α-catenin of C. elegans. Based on imaging and biophysical approaches, they revealed that HMP-1/α-catenin is tension-sensitive as indicated by a decrease in tension on HMP-1 that is dependent on actomyosin activities. A new finding of ERK regulation and function in morphogenesis and tissue homeostasis in Drosophila was reported by Hayashi and Ogura[152]. They integrated FRET into extracellular-signal-regulated kinases (ERK, a MAP kinase family member) activity and were able to successfully detect it with a probe. Their most significant contributions involve that of the pre-gastrulation stage, ERK played a role of on/off switch, while in the late embryonic stage, ERK changed to function regulation of morphogenesis and tissue quality control.

Computational modeling

Advances in computational power and tools have allowed *in silico* models to provide new insights connected with the mechanical process of embryonic morphogenesis. The ability of predicting mechanical behaviors using these models can help uncover causal relations and enable new understanding of fundamental biomechanical mechanism of embryogenesis[153]. The growing field of artificial intelligence has led to the development of agent-based models, which examine the behavior of many small, autonomous agents and the resulting behavior over the entire system is[154]. A relatively early use of agent-based models as they apply to cell migration and mechanics is a study on cell dedifferentiation and epithelial-mesenchymal transition as it applied to cancers[155]. Since then, agent-based models have been used to study a variety of topics within the fields of mechanobiology and embryonic development including single-cell biomechanical behaviors[156,157], multi-cellular interactions [158], and multi-cell phenomena at the tissue level[159]. Recently, an agent-based model was introduced to replicate epithelial and tissue tectonics in zebrafish early embryogenesis[160]. This model is named MecaGen, which is the abbreviation of the biomechanical (Meca-) and biomolecular/genetic (-Gen) components of the model. The Meca-side (Fig. 11(a)) includes a discrete-element method applicable to ellipsoidal cells. The method yields the equation of motion, $\lambda\,\vec{v}=\vec{F}$, with an overdamped term, where λ is the viscous coefficient and $\vec{v}$ is the cell's velocity. The biomolecular side of the model (Fig. 11(b)) accounts for chemical signaling and GRNs. These mechanical and biomolecular/genetic components of the model interact with each other based on built-in relationships between their sub-components. MecaGen establishes a spatio-temporal based model to study embryonic tissue transformations, which results in calculating their quantitative differences combining biological data. Agent-based models (such as cellular Potts model[161,162]) and lattice-based models[163] are both capable of integrating heterogeneous processes and exploring corresponding dynamics in the circumstance of spatio-temporal phenomena[164]. Advantages and technical challenges of agent-based models to investigate various tissue morphogenesis process at multi-levels were elaborated by Glen et al.[164]

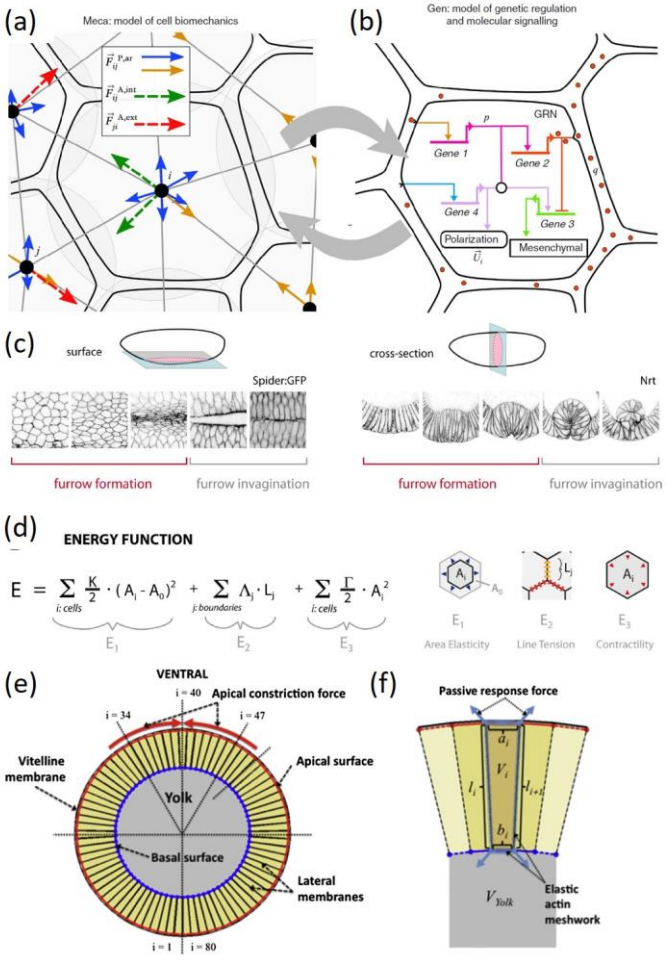

Figure 11. Schematic view of the MecaGen model (a) Meca: cell biomechanics model, and (b) Gen: model of molecular signaling and genetic regulation. (a) and (b) are reprinted with permission from Delile et al[160]. Surface view of ventral epithelium of the Drosophila embryo during the ventral furrow formation using 2D vertex model. (c) Surface sections (left) and cross-sections (right). (d) Surface-view of energy contributions of the vertex model of the epithelium. (c) and (d) are adapted from Spahn and Reuter[165] with permission from PLOS. (e) Schematic of the cross-section of the vertex model at the end of cellularization of the Drosophila embryo. (f) The embryo cell is defined as a unit of cytoplasmic polygon volume, consisting of the apical, basal, and lateral membrane surfaces. (e) and (f) are reproduced from Polyakov et al.[31] with permission from Elsevier.

These agent-based models, nevertheless, poorly capture the changes of complex cell morphology. Vertex models are proposed to fill this gap with more details than agent-based models on cell interfaces. Vertex models can also capture and visualize topological changes in the cell environment[153]. Yu and Fernandez-Gonzalez[166] investigated the introduction of vertex models into epithelial morphogenesis. Cells are configured as polygons and are composed of edges and nodes in the vertex model. They then reviewed the application of investigating dorsal closure of Drosophila during embryonic development and wound repair more specifically. The investigation is a combination of diverse types of cells behaviors, molecular dynamics, and its corresponding variations in tissue architecture. Drosophila ventral furrow formation was previously simulated using a 2D vertex model by Spahn and Reuter[165]. In that 2D model, the terms 'furrow formation' and 'furrow invagination' are defined separately when the folds happen to mesoderm inside the embryo (see Fig. 11(c)). A vertex model can track the change of the potential energy during the cell changes in the process of furrow formation. The involved potential energy is generated from line tension, elasticity of area, and contractility, shown in Fig. 11(d). This model described the cell sheet of the Drosophila ventral epithelium and was further used to explore the reasons for the ventral furrow formation from the epithelium. The results illustrated that when random compression occurs in ventral cells, a ventral furrow can be completely reproduced. Polyakov et al.[31] also studied Drosophila ventral furrow formation, while they focused on passive mechanical forces, which are generated to adjust furrow formation and deformation in cells due to apical contraction during Drosophila gastrulation. Their vertex model only considered the active force, i.e., spatially patterned stress. The cells are confined by the outer vitelline membrane and enclosed by the inner yolk (Fig. 10(e)). Each cell is approximated as a polygon wherein the sides represent the apical, basal, and lateral faces of that cell (Fig. 10(f)). The energy considered in their model is accounting for the active stress generated by combinations of the vertex contraction, cell cortex elastic response, and the incompressible cytoplasm and yolk. More applications of tissue morphogenesis using vertex models, including 2D and/or 3D apical model, 2D lateral model, and mechanical inference, were reviewed and discussed by Alt et al.[167] There are other computational modeling approaches to address concerns and difficulties of embryonic morphogenesis, such as hydrodynamic models[168,169] and viscoelastic models[170,171], which combine knowledge from mathematics and physics with powerful computation tools. Comparisons of different computational modeling approaches for epithelial tissue morphogenesis has been discussed by Fletcher et al.[172] Computational models provide a possibility to integrate experimental findings at different spatial and temporal scales. Consequently, computational modeling provides several useful auxiliary methods for force measurement and prediction.

Many cells detect external mechanical forces and use them as signals to guide tissue or organ morphogenesis, functionality regulation, and gene expression. It is demonstrated experimentally that when the cell substrates are under pre-stretch, the cells align themselves accordingly[173]. The mechanosensing process for cells is complicated and not completely understood so far. Many theories are proposed to explain the mechanism, e.g., strain or stress signaling[174] and dynamic or static sensing[175]. Baek et al. further implemented a computational and theoretical-based approach to study how the anisotropy of the cell substrate under its pre-stretch using a small-on-large linearization technique[173]. Cells then can detect such anisotropy to regulate their cellular events. It is assumed that initially the extracellular matrix is compressible and isotropic, and then a uniaxial or biaxial loading is imposed on the matrix. According to assumptions above, they computed a linearized elastic tensor under such pre-stretch, and then obtained the directional (effective) elastic moduli of the substrate by taking the penalty parameter of the energy function to be infinity. They also concluded that the directional elastic moduli, different in different directions, are the actual moduli that are sensed by the cells. In the uniaxial pre-stretch of the substrate, the directional elastic moduli along the pre-stretch direction and the other orthogonal directions are, respectively, $E_1 = \frac{3\mu+2\lambda_1 T_1}{\lambda_1}$, and $E_2 = E_3 = \frac{6\mu^2+4\lambda_1 T_1}{\lambda_1(2\mu+\lambda_1 T_1)}$, where μ is the original shear modulus of the substrate, λ_1 is the stretch

ratio in the prestretch direction, and T_1 is the Cauchy stress in the pre-stretch direction. In particular, the simulated results indicate that the directional moduli depend on stress caused by pre-stretch in the original substrate material. Thus, the force environment in the extracellular matrix is sensed by cells through such force-dependent elastic moduli, a novel approach to explain how cells sense the forces in the extracellular matrix for morphogenesis or other cellular activities.

Regulation and control of mechanical forces in embryonic morphogenesis

During embryonic development, organs begin development as a series of tubes and begin folding and expanding to create their final structures. These events can be described as resulting from a combination of forces (constriction, bending, etc.) and their biomechanical interactions with tissue. Recent experiments have illustrated either *in vivo* or *in situ* the mechanisms behind morphogenesis. Here we will discuss the biomechanical basis of the formation of the left-right axis and chirality, bending, twisting, and elongation. Many of these processes are dependent on mechanosensitive feedback, which will be discussed in detail following the previous sections.

Invagination

The invagination of the ventral furrow (VF), an indentation on the ventral side of the blastoderm of the early embryo, is one of the primary steps in early morphogenesis. This early step is critical in the development of the gastrula containing multiple layers of cells (known as gastrulation, the establishment of multiple germ layers of the embryo) and has been well characterized in *Drosophila*[165,176]. This event is induced by the apical constriction of mesodermal cells (Fig. 2)[171,176]. In turn, this is driven by the formation of myosin-II filaments/ fibers at their apical cortex[177]. These structures are dynamic, resulting in pulsed actin-myosin flows, which likewise cause pulsed apical constrictions of the mesodermal cells, which over time create a distinct ventral furrow[178]. The forces driving constriction have been largely unknown but have recently been the focus of several studies aiming to understand the nature of what forces drive constriction. Apical constriction has been shown to drive constriction by deforming non-compressible cytoplasm, allowing the tissue to change shape while retaining the same overall volume[179]. Models of VF formation have implied that apical constriction cannot be the only force applied to drive constriction, and it has been shown in a model that a transition in the rigidity of basal membrane surfaces may provide the remaining driving force behind gastrulation[31].

Left-Right Asymmetry

Mammalian embryos often exhibit asymmetry regarding the positioning of organs throughout the body. For development to be successful, the asymmetric positionings of these organs must be near perfect. It has been hypothesized that the chirality of certain molecules arranged along the anterior-posterior and dorsal-ventral axes drives the direction of the left-right (L-R) axis. This is known as the “F-molecule” hypothesis[180]. The F-molecule hypothesis has been supported by evidence in mouse models indicating that left-right asymmetry is largely mediated by cellular flow forces, in which motile cilia (present in nearly all vertebrate cells) act as chemical and mechanical sensors during developmental processes. This leads to a process known as nodal flow, where the movement of cilia in a small pit on the ventral side of the early embryo known as the node induces a leftward movement of extracellular fluid by rotating clockwise[181,182]. This has been further supported by work illustrating mechanosensory proteins (e.g. Trpp2) are linked to left-right asymmetry/ patterning[182,183]. The mechanical response of left-right asymmetry has been linked to several molecular mechanisms, and it has been hypothesized that the asymmetric distribution of signaling molecules leads to initiation of left-right asymmetry[35,184]. It has recently been shown that calcium oscillations in motile cilia have been shown to initiate left-right patterning[183].

Some vertebrate species do not rely on cellular flow forces, but rather upon asymmetric patterns of cell migration or division, both of which can lead to the initial break in left-right symmetry[185]. However, the mechanosensitive properties of cells still play a major role, as it has been demonstrated via cell culture that cell migration directionality is mechanosensitive[186]. These processes are heavily reliant on the actin-myosin pathways to properly function. In zebrafish, it has been demonstrated that regulators of the actomyosin complex play a role as mediator in the zebrafish left-right organizer (L-RO)[187], eventually controlling

symmetry-breaking events. Further, it has been shown that impairing the myosin-II pathway (critical for cell motility by forming actomyosin complexes) prevents the designed leftward asymmetrical development of the chick embryo during development[185].

In addition to the mechanical response to molecular chirality and mechanisms providing a source for left-right asymmetry, the shape and form of the cells themselves can also drive left-right asymmetry during morphogenesis. Specifically, recent studies have shown that cells can form an intrinsic chirality based on the structure which can be critical in driving L-R asymmetry[188]. There is a genetic basis for this; Drosophila mutants in the *Myosin31DF* gene have been shown to have the left-right symmetry of entire organs reversed[189].

Bending and Twisting

Complex movements of tissues are observed to occur to drive proper asymmetry and organization in the embryo during morphogenesis. There are two notable types of complex movements that are commonly observed, described as flexure (bending) and torsion (twisting). Key examples of tissue movements in the early embryo included that of the outer epithelium[172,178], neural plate,[190] and the heart tube[141].

A notable example of coordinated bending in organogenesis occurs at the neuroepithelium to form the anterior brain and spinal cord (the posterior end of the spinal cord is generated from loosely connected cells)[191]. The biomechanical aspects of bending prove strikingly similar to invagination; Williams et al.[192] describes the formation of the neural plate requires both apical constriction and basolateral forces. These are the same types of biomechanical forces commonly associated with ventral furrow formation observed at the start of gastrulation[31,169]. Other tissues play a key role in neural plate formation. The mesoderm, long suspected of playing a role in the movement of cells in the neural plate during morphogenesis, has been recently shown to be instrumental in maintaining coordinated cell movements as the zebrafish neural keel is formed[193]. Due to the interconnecting nature of many tissues at the early embryo stage, the bending and twisting of various organs in the embryo are hypothesized to be interconnected. For example, it has been hypothesized that the twisting of the early brain is directly influenced by the rightward looping of the heart (which occurs later at stages 11-15), which has been supported with work done in chick embryos[194]. The correlation between brain and heart morphogenesis was further shown by perturbing the bending of the brain. This perturbation also influenced proper twisting of the brain and the looping in the heart, showing definitively that these structures are not wholly independent of each other and that mechanical stresses play a critical role in these morphogenetic events.

The looping of the heart is a common example of a twisting event and is often described as the first major morphological feature of the breaking of left-right symmetry in vertebrate embryos[195]. This process is quite complex as it requires the action of multiple forces and interactions between surfaces to properly form the early heart. Several specific forces have been implicated in the proper folding and looping of structures during development and can result in different phases of bending and twisting. For instance, the heart initially twists in a phase known as c-looping which is followed by s-looping[196]. This looping has been attributed to the asymmetric buildup of cells on the splanchnopleure membrane in the embryo[197,198]. This has been shown both through *in vivo* experiments as well as computational (finite-element methods) and physical models (a rubber tube with a metal rod inserted to add rigidity). The physical models were able to illustrate the looping of the heart is bound by physical constraints which help drive its shape[198], proving the effectiveness of a “hybrid” approach to studying morphogenesis. This motif is also seen in gut formation, where an asymmetric buildup of cells in the dorsal mesentery drives symmetry and folding. The role of mechanical forces in gut looping has been elegantly illustrated by a simple theoretical scaling analysis and a physical model made of silicone rubber, which shows that differential growth between tissues and the forces therein induce the formation and evolution of hemihelical pattern[199]. Although the forces are difficult to measure *in vivo*, the physical model was able to reproduce patterns that are almost identical to those observed in avian embryos, thus addressing the challenges in quantifying the actual forces and their roles in morphogenesis.

Mechanics of Body Axis Elongation

Closely following and transitioning from gastrulation, vertebrate embryos undergo a major reorganization of three germ layers to form the rudimentary animal body plan. The highlight of this process is the extension of the body axis along the anterior-posterior (head to tail) direction. Unlike directional tissue extension driven by cell proliferation and growth, such as that fueled by the meristem in plants, in many vertebrates a major portion of the body elongates by a few folds (net growth of a few millimeters) within the time scale of hours and a few days. Because cell proliferation is rate-limited by fundamental constraints on cell metabolism, it cannot account for this drastic speed of body axis elongation. Therefore, embryonic tissues must reorganize, or be "molded", to support the materials needed for elongation. This requires genetic programs that control cell behaviors to generate tissue level stresses that are anisotropic, and to regulate tissue soft matter properties with specific spatial patterns. The investigation of these mechanisms has been limited by the inability to directly measure stresses and mechanical properties *in vivo*, and the unclear links between genetic signals and tissue mechanics. Recent work in several model systems starts to overcome some of these limitations and reveal intricate principles that have broader implications in our understanding of tissue morphogenesis and applications in tissue engineering.

Cell intercalation and tissue convergence

Cell intercalation is a cellular mechanism by which cell exchange neighbors in a tissue, via the breakage and formation of protrusions and junctions. Cell intercalation is the primary contributor of body axis elongation in vertebrate embryos as compared to cell proliferation or deformation[200]. This is particularly the case in the anterior body[200]. In the posterior body, the contribution of cell intercalation to axis elongation is less significant and is driven in part by the extrinsic forces generated by random cell movements[201,202]. To produce a directional change on the tissue level, the intercalations cannot be random but must have a spatial preference. This is achieved through cell polarity signaling. In particular, the Planar Cell Polarity (PCP) pathway is closely associated with the body axis tissues undergoing convergence and extension[203,204]. For example, cells in the neural ectoderm as an epithelial sheet exhibit asymmetrically distributed cellular components in the plane of the sheet. These components are regulated by Wnt5/11 signaling that forms a posterior to anterior gradient[205]. Different components of the PCP pathway interact biochemically within and between the cells to stabilize their cortex location. Like small magnets lining up, these polarized cells establish a tissue-wide polarity. It has been noted that this polarity is also regulated by tissue forces[206,207] in addition to secreted signals. Following this, several cellular mechanical maneuvers need to take place to complete the intercalation following the PCP bias without disrupting tissue integrity. These are carried out in different scenarios, but all require the spatiotemporally regulated activity of actomyosin and adhesion. In mesoderm such as the early amphibian notochord, cells show migratory behavior during intercalation. Cells extend along the direction of intercalation (and shrink along the elongation direction) and single cell labeling reveals lamellipodia-like protrusions. Contacts are established or broken by these protrusions before stabilization[208,209]. These protrusions are actin-rich and controlled by cortical actomyosin contractions[210]. In the epithelium however, migratory behavior and protrusions are not as prominent and the intercalation is achieved through "T1 transitions" where the junction between two original neighboring cells shrinks and breaks, merging two tricellular junctions to form a junction between two new neighbors that then expands[211]. The junctional changes are driven by the same cellular mechanical network of actomyosin. These two modes are not exclusive and may be different turnouts of the same biophysical process for different cellular cortex states[192]. Finally, the drivers of intercalation are not all tissue-intrinsic. Recent studies suggest that intercalation can be promoted by extrinsic forces from neighboring tissues, such as the notochord being compressed by the adjacent presomitic mesoderm[201]. The outcome of these cellular and tissue level mechanical actions is a reorganization of materials along a new direction. In the context of the body axis, this results in stresses driving tissue lengthening along the anterior-posterior direction.

Axial elongation stress.

Another major contributor to body axis elongation is the volumetric growth of certain tissues. The most prominent example is the axial notochord in amphibians and fish, which establishes a strong sheath of extracellular matrix while its cells undergo swelling through a process called vacuolization, when cells absorb large quantities of water[208,212–214]. The resulting increases in stiffness and volume generate a strong pushing force onto the tail end of the axis. In amniote embryos, the notochord tissue contributes to a smaller part of the whole axis and undergoes vacuolization later rather than simultaneously with body axis elongation[215]. Despite that the amniote notochord may contribute less forces than the anamniote counterpart, the total stress produced by the axial tissues is still rather strong and estimated to be in the order of ~100 Pa in avian embryos[201]. For the posterior body axis, the contribution of volumetric growth to elongation is even more variable depending on the model system and the stage. Such variability could be explained by the developmental characteristics of the model. For example, animals that require fast body axis formation and early use such as zebrafish mainly rely on cell intercalation driven rearrangement to complete elongation, whereas a mouse embryo extends its posterior body slowly by cell proliferation and growth[216]. The sum of these processes leads to significant tissue movement towards the posterior, and a net stress on the progenitor domain at the tail end of the body axis.

Tissue melting and flow.

The other part of the equation of body axis elongation is the tissue mechanical properties. Under axial stress, the posterior progenitor zone, and the newly formed tissues at the end of the axis undergo distinct shape changes to facilitate elongation. In particular, the mesodermal tissues show a solid-to-fluid transition driven by epithelial-to-mesenchymal transition (EMT). During EMT, cells change adhesion and delaminate from the epithelial tissue and start to move and mix at a higher rate[217,218]. In chicken embryos, the newly formed presomitic mesoderm is composed of these highly motile cells and the tissue is predicted to be more deformable[202]. A similar process can be observed in zebrafish as cells flow from the dorsal marginal zone to the progenitor zone that feeds the paraxial mesoderm; under these conditions cell movement coherence decreases[219]. This results in the tailbud area to lower yield stress, the stress limit before tissue starts to flow[122]. These changes effectively “melt” the tissue enabling their deformation in the form of flow under the axial stress. The flow is not only important for axis elongation but also progenitor dispersion into different tissues. For example, mesodermal progenitors require the axial stress driven flow to effectively enter the posterior presomitic mesoderm in chicken embryos[201].

Mechanical coupling of different elongating tissues.

The body axis is a complex structure composed of tissues from three germ layers. These layers are organized in a highly conserved and packed pattern in the cross-section, opening possibilities of inter-tissue mechanical interactions. For example, the ectoderm is mechanically required for the connected neural plate to complete convergence[190]. It was recently found that the presomitic mesoderm assists the elongation of the neural tube and notochord. In return, the axial tissues drive mesodermal progenitors away from the midline to join the forming presomitic mesoderm, forming a mechanical feedback loop on the tissue level[201]. These results show that tissues affect each other during morphogenesis and vertebrate body axis elongation should be taken as a mechanically coordinated group process. Many questions remain on this front: how cell level mechanical behaviors (such as modulating adhesion and actomyosin contractility) lead to tissue level forces still requiring a formal physical model and accurate *in vivo* measurements. The role of the extracellular matrix in defining tissue rheology and inter-tissue connections and frictions remains largely unexplored in the developing embryo. The cellular responses to the tissue mechanics in the coupled system and the mechanisms carrying out such responses will be of importance in future studies.

Discussions and Summary

This article reviews several important aspects for embryonic morphogenesis including theoretical mechanical foundations, experimental explorations, modeling and computational simulations, and regulation and control of mechanical forces. All these approaches together contribute to a comprehensive

understanding of embryonic morphogenesis development. We highlight and summarize the main review results in this article as follows.

It is found that during embryonic morphogenesis, forces can develop inside and outside of cells by either intracellular force generation or extracellular force reception[22,25]. Intracellularly, cells generate forces through the contraction of myosin II in the cytoskeletal network through regulatory molecules including the small GTPase RhoA and the Rho kinase[13], resulting in the converging and extending movement of tissues[13,48]. Internal forces are transmitted both towards the nucleus and the cell surface by the anchorage of extracellular receptor-ligand interactions, allowing the cell to sense extracellular environment. The stiffness of a cell is primarily maintained by the nucleus and the cytoskeleton, the latter of which is composed of three types of micro-filaments, actin filaments, intermediate filaments, and microtubules[8]. Depending on the type of forces generated in and applied to the cell (extracellular tensile, intracellular tensile or extracellular compressive), the mode of force transmission is different. In particular, different types of cells can use distinct compositions of the cytoskeletal networks to adapt and respond to external cues uniquely; yet how this is done in a complex multicellular tissue remains mysterious.

Under the internal and external mechanical environment of cells, mechanosensing serves as a critical mechanism to regulate cell movement, cell deformation, and tissue deformation, and hence morphogenesis. Such mechanism is realized through involving various internal cellular parts such as ion channels and actomyosin network. A general mechanosensing process is formed in four steps consisting of mechanopresentation, mechanoreception, mechanotransmission and mechanotransduction[22] Extracellular cellular forces can be generated by the relative movement of adjacent cells or interacting ECM, particularly common in embryonic development in which an embryo is constantly undergoing physical expansion (growth), structural rearrangement and morphological changes[26]. During development, cells sense mechanical cues in the environment and respond with biological changes in the form of membrane deformation, nucleus deformation, spreading, cytoskeletal reorganization, and cell bursting/motility[4].

The mechanical properties of the cells are measured and quantified with multiple experimental methods. For instance, cell mechanics can be studied via various methods[88,89] such as micropipette aspiration, micro-force sensors, cell pokers, optical tweezers, magnetic tweezers[4,8], and atomic force microscopy[4]. Atomic force microscopy was also used to measure adhesion forces of single cells from the zebrafish gastrula embryos to substrates with fibronectin[91], and to measure the specific de-adhesion forces which are generated to split single mesendodermal cells from substrates[92]. Fluorescent resonance energy transfer can quantify activation of Src in both space and time domains in live cells[98]. Nonlinear Stress Inference Microscopy can quantify cell generated contractile forces in the surrounding ECM utilizing the nonlinear stiffening property of the ECM. Additionally, laser microsurgery is used to investigate dorsal closure to quantify the forces in relation to the evolving geometry[93]. These methods measure different aspects of mechanics and forces in a living system; a current major challenge is how to use a combination of some of these methods to reveal the spatial and temporal evolution of cellular properties during development, which would be the foundation to understand the evolution of these complex systems.

Computational modeling employs *in silico* models to provide new insights for the mechanical basis of embryonic morphogenesis, and to create predictive capacity to help reveal causal links and contribute to the understanding of embryogenesis[153]. Agent-based models are typically utilized to explore single-cell biomechanical impact[156,157], multi-cellular interactions[158], and multi-cell phenomena at the tissue scale[159]. However, agent-based models fail in adequately capturing the changes of complex cell morphology. By contrast, vertex models are capable of providing more information about cell interfaces and could analyze topological changes in the cell environment[138]. Nevertheless, vertex models are only suited for simulating the dynamics of confluent cell clusters, hence the hybrid model has been developed to bridge the gap[204],

which bears the hope to become a universal platform for simulating collective cell migration in morphogenesis, would healing, and cancer progression[205]. More emphasis should be put on integrating modeling and experiments: experiments generate inputs for the computational models, which in turn helps improve the understanding of the fundamental biomechanical mechanisms at work during morphogenesis and in some cases can yield predictions that have yet to be verified through experiments. When these methods are used in tandem, they serve as powerful tools to test important hypotheses about the mechanobiology of morphogenesis. Models help us better understand results obtained through experiments, and they provide a means to testing whether a given hypothesis is consistent with physical law. Biomechanical models use continuum-mechanics theory to connect strain, stress, and deformation associated with morphogenesis, which greatly facilitate understanding the internal mechanism of the altered organs from mechanical, causal perspectives. Other advanced hyperelasticity theories such as morphoelasticity or growth theory can help interpret how mass addition and volume changes regulate morphogenesis. As the mechanics theory advances, advanced models with an increasing number of physical parameters will emerge to provide deeper interpretation of various phenomena in morphogenesis. Nevertheless, simple theoretical models with reasonable assumptions can sometimes get us closer to the mechanistic understanding of morphogenesis, for example, with the proper non-dimensionalization and scaling analysis. Experiments are often needed to provide estimates of stresses, strains, and material properties that are used in the models, or to validate theoretical predictions from the models. More often than not, there are quantities difficult to examine in vivo, or the measurement itself could influence the values being measured. Computational models will bridge the gap and provide useful information to shed light on the biomechanical mechanisms underlying the morphogenetic processes being investigated and recent studies have shown that creative use of physical models can serve the purpose as well.

Another characteristic in embryonic morphogenesis is the regulation and control of mechanical forces. During development, organs start as a series of formed or unformed tubes, and then gradually fold and expand to create more mature organ structures. Many phenomena are discovered during this process. Invagination and ventral furrow formation were observed to occur in the early stages of the development of several organs[165,176]. Mammalian embryos were found to exhibit asymmetry in positioning their organs throughout the bodies, and complex tissue movements were observed to drive asymmetry in the embryo morphogenesis with two types of common complex movements including flexure and torsion. Furthermore, vertebrate embryos undergo major reorganizations of three germ layers to form the rudimentary animal bodies, e.g., the extension of the body axis along the head to tail direction. Cell intercalation is used to describe cells exchange with neighbors and insert themselves among surrounding cells, and is the main mode of body axis elongation in vertebrate embryos[200]. Cell intercalation is not the only producer of the stress driving body axis elongation as another major contributor is the volumetric growth of certain tissues.

In summary, deciphering embryonic morphogenesis requires cross-disciplinary collaborations. Understanding the roles of mechanical forces is important to comprehend the formation and evolution of structures of various organs and the body during development. More significantly, this research will facilitate the understanding of the development of developmental defects and other pathological processes, and thus laying the foundation for innovative prevention or treatment strategies. Given the complexity and the plethora of factors involved, multiscale analysis and techniques are needed to elucidate the fundamental mechanisms underlying morphogenesis. We also need to acknowledge the fact that the spatio-temporal embryonic morphogenesis is a complicated process where mechanical and chemical controls have to synergistically modulate the pluripotency, cell fate, and tissue pattern formation[222]. Theoretical and experimental studies have together contributed to the advances in our understanding of the biomechanics and mechanobiology of morphogenesis. The work is far from complete, however, with the rapidly expanding toolkit available to researchers today, many research topics that were once impossible to study are starting to be investigated. For instance, the roles of gravitational forces and buoyancy in development have been long overlooked, but now with the possibility to study embryonic development at the International Space Station, it becomes feasible to examine how embryonic organs can develop differently than on the earth and what the roles of the body forces are. With still many secrets awaiting to be unveiled about morphogenesis, close collaboration between experimentalists and theorists from multiple disciplines

including, but are not limited to, biology, physics, chemistry, applied mathematics, and engineering mechanics, would be needed to shed light on the driving forces behind these concerted biological events and ultimately the intricate interplay between biomechanical, biochemical, and molecular genetics factors therein.

Conflicts of interest:

The authors declare that they have no conflicts of interest.

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