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REVIEW

The Mechanics of Development: Models and Methods for Tissue Morphogenesis

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Embryonic development is a physical process during which groups of cells are sculpted into functional organs. The mechanical properties of tissues and the forces exerted on them serve as epigenetic regulators of morphogenesis. Understanding these mechanobiological effects in the embryo requires new experimental approaches. Here we focus on branching of the lung airways and bending of the heart tube to describe examples of mechanical and physical cues that guide cell fate decisions and organogenesis. We highlight recent technological advances to measure tissue elasticity and endogenous mechanical stresses in real time during organ development. We also discuss recent progress in manipulating forces in intact embryos. **Birth Defects Research (Part C) 90:193–202, 2010.** © 2010 Wiley-Liss, Inc.

Key words: mechanobiology; tension; cytoskeleton; traction force microscopy

INTRODUCTION

Embryonic development follows instructions prescribed by the genetic material of the embryo and maternally-derived cues deposited in the egg before fertilization. Analysis of the spatial and temporal expression of genes has enabled developmental biologists to describe the choreographed motions of embryogenesis as gene regulatory networks–blueprints of the chemical signaling interactions required for tissue specification (Li and Davidson, 2009). However, development is also a physical process wherein tissues bend, fold, and twist into the elaborate structures of mature organs, and the mechanical stresses associated with these alterations have long been recognized as potentially conveying information to the developing tissues (His, 1874; Beloussov and Grabovsky, 2006; Mammoto and Ingber, 2010). Mechanical properties relevant to developing embryos include isometric tension (i.e., contraction of the cytoskeleton), stiffness (elasticity of the cells and their surrounding substratum), pressure (stress perpendicular to a surface resulting from differences in fluid volume), and shear (stress parallel to a surface by friction from fluid flow). Here we review how mechanical stresses instruct organogenesis, and describe novel strategies to measure and manipulate these physical properties in the embryo.

How Mechanical Stresses Influence Basic Cellular Processes

Endogenous cytoskeletal tension and exogenous mechanical stresses regulate many cellular behaviors, including cell turnover (proliferation and death) and various modes of differentiation. Tension transmitted from the extracellular matrix (ECM) through transmembrane integrin receptors to the cytoskeleton cooperates with cyclins to control transit through the G1 phase of the cell cycle (Chen et al., 1997; Huang et al., 1998; Huang and Ingber, 2002; Mammoto et al., 2004; Assoian and Klein, 2008; Klein et al., 2009). Endogenous mechanical stress is mediated in part by signaling through the small GTPase Rho, its effectors Rho kinase (ROCK) and mDia, and subsequent effects on myosin phosphorylation, actin polymerization, and actomyosin contraction. Tension-mediated increases in RhoA activity lead to ubiquitin-mediated degradation of the cyclin-dependent kinase inhibitor $p27^{kip1}$, which then permits G1 phase progression (Mammoto et al., 2004). As described below, tension is thought to play a key role in regulating cell proliferation during development of the lung airway epithelium. Endogenous mechanical stresses also regulate programmed cell death. When the ECM scaffold is depleted or removed such that cells no longer have sufficient resistive adhesive contacts, the resulting decrease in

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cytoskeletal tension and cell rounding leads to apoptotic cell death (Chen et al., 1997). Such death is commonly seen concomitant with the loss of basement membrane during physiological involution of the mammary gland (Wicha et al., 1980; Lund et al., 1996) and tissue remodeling during amphibian metamorphosis (Mathew et al., 2010).

In addition to increases in cell number and localized apoptosis, embryonic development requires refinement of phenotypes as multipotent stem and progenitor cells differentiate down the various lineages that comprise the mature organism. Recent studies of mesenchymal stem cells (MSCs) have revealed a strict regulation of differentiation by the mechanical properties of the microenvironment and endogenous cytoskeletal tension (McBeath et al., 2004; Engler et al., 2006). Indeed, it appears that the differentiation of multipotent MSCs is tuned by the stiffness of the surrounding ECM: a soft microenvironment promotes neuronal differentiation, whereas a stiff microenvironment promotes osteogenic differentiation (Engler et al., 2006). Differentiated function is also regulated by cytoskeletal tension, as the rhythmic contractility of embryonic cardiomyocytes is optimal on ECM with mechanical properties matching that of the heart in vivo (Engler et al., 2008).

Differentiation is not always a terminal process, however. Epithelial-mesenchymal transition (EMT) is a phenotypic transformation by which epithelial cells detach from their neighbors and become motile and invasive. These phenotypic changes are driven by alterations in gene expression: downregulation of epithelial markers including E-cadherin and epithelial keratins, and upregulation of mesenchymal markers including N-cadherin, vimentin, and α-smooth muscle actin (αSMA) (Kalluri and Neilson, 2003). Embryonic EMTs are responsible for gastrulation and creation of the neural crest; pathological EMTs are thought to be involved in cancer progression (Thiery et al., 2009). Depending on the cell type and environmental conditions, EMT can be induced by many soluble stimuli, including cytokines and proteases. In transgenic mice, overexpression of matrix metalloproteinase (MMP)-3 (also known as stromelysin-1) causes EMT, fibrosis, and eventual formation of frank tumors in the mammary gland (Sternlicht et al., 1999). In culture, treating mouse mammary epithelial cells with MMP3 leads to EMT through induced expression of Rac1b, a highly active splice variant of Rac1 (Radisky et al., 2005). Here, the EMT process is accompanied by striking changes in cellular morphology, specifically an increase in cell spreading against the underlying substratum. This increased cell spreading is necessary for MMP3-induced alterations in gene expression, as forced cell rounding prevents MMP3-induced EMT (Nelson et al., 2008b). These observations suggest that endogenous mechanical stresses may regulate EMT signaling. Indeed, in two-dimensional (2D) epithelial tissues in culture, transforming growth factor-β (TGFβ)-mediated EMT is restricted to regions of high endogenous mechanical stress (Gomez et al., 2010). Elevated mechanical stress causes enhanced nuclear localization of the serum response factor (SRF) cofactor, myocardin-related transcription factor (MRTF)-A, which itself regulates the transcription of a wide variety of genes associated with proliferation and actin cytoskeletal dynamics (Olson and Nordheim, 2010). As will be covered below, mechanical stresses accompany regions of EMT during heart valve formation, further suggesting that embryonic EMTs may be regulated by mechanical stress.

Tissue Mechanics During Branching Morphogenesis and Development of the Lung

The adult human lung is comprised of a volume-filling tree of $\sim$17 million branches that conduct air to 300 million alveolar gas-exchange units with a total surface area of 70 m^2. Lung development proceeds via two sequential processes: branching morphogenesis of the epithelial tree during the pseudoglandular and canalicular stages (approximately 5–24 weeks in human; embryonic days 12.5–17.5 in mouse), followed by development of the air sacs and alveoli during the saccular and alveolar stages ($\sim$24 weeks to postnatal in human; embryonic days 17.5-postnatal in mouse) (Hilfer, 1996). The development of the larger bronchial airways of the mouse has been mapped using immunofluorescence analysis of fixed specimens, and was found to be identical between individuals, suggesting that early branching of this organ is stereotyped (Metzger et al., 2008). The airway epithelium is separated from the lung mesenchyme by a basement membrane layer containing laminins and type IV collagen. Electron microscopy analysis (Bluemink et al., 1976) and immunostaining (Mollard and Dziadek, 1998; Moore et al., 2005) of embryonic lungs revealed that the basement membrane thins out adjacent to growing buds, suggesting that the ECM is either physically stretched or proteolytically degraded at these regions.

Cellular contractility is critical for branching morphogenesis of the lung. Inhibiting actomyosin-mediated contractility in whole lung explants decreases branching (Moore et al., 2005), whereas activating contractility increases branching (Moore et al., 2002). On a larger scale, lung development depends on mechanical distention, which is controlled in part by fetal breathing movements and fluid secretion into the lumen of the airways. These processes may cause local distentions at growing buds which stretch out the surrounding basement membrane. Several MMPs are expressed during lung development, and treatment with a broad-spectrum MMP inhibitor blocks airway branching (Kheradmand et al., 2002). Furthermore, knockout of tissue inhibitor of metalloprotei-

nase (TIMP)-3 function disrupts lung branching morphogenesis (Gill et al., 2003). However, the mechanism of action is unclear as a large majority of MMP knockout mice develop normally without any apparent lung phenotype (Parks and Shapiro, 2001). An exception is the membrane type (MT)1-MMP/MMP14 knockout mouse, which shows normal lung branching morphogenesis (Oblander et al., 2005) but has defective alveolar development (Atkinson et al., 2005).

Defects in lung development can result in a hypoplastic lung (with too few branches) or abnormal alveolar structures, both of which reduce the surface area for oxygen transport and thereby disrupt lung function. Many of these defects arise from alterations in the mechanical environment of the thoracic cavity. Normal development of the lung requires fetal breathing movements, an adequate volume of space within the chest cavity, and sufficient volumes of fluid within and surrounding the growing lungs. Transecting the spinal cord at the level of the phrenic nerve prohibits fetal breathing and disrupts lung development in rabbits and sheep (Wigglesworth and Desai, 1979; Fewell et al., 1981). During fetal breathing, the diaphragm contracts and increases the volume of the thoracic cavity, which stretches the developing lungs; this stretching may influence lung development by increasing cell proliferation (Fewell et al., 1981; Liu et al., 1992). Combined with upper airway resistance, fetal breathing movements maintain transpulmonary pressure by controlling the volume of fluid within the lungs. Decreasing fluid or pressure leads to pulmonary hypoplasia, whereas increasing fluid leads to hyperplasia (Alcorn et al., 1977; Moessinger et al., 1990; Kizilcan et al., 1995).

In humans, there are several conditions that can lead to fetal pulmonary hypoplasia, a major cause of respiratory insufficiency of the newborn (Harding and Hooper, 1996). Congenital diaphragmatic hernia (CDH) is a malformation of the thoracic diaphragm which permits organs of the abdominal cavity to bulge into the chest cavity. The herniated organs are thought to compress the developing lung. CDH affects approximately one in every 2500 births, with mortality rates of 10 to 35% for live births and possibly higher when accounting for fetal demise (van den Hout et al., 2009). Patients with CDH present with pulmonary hypertension shortly after birth. Fetal pulmonary hypoplasia can also result from abnormally low levels of amniotic fluid (oligohydramnios) resulting from premature rupture of membranes (PROM). Conversely, fetal defects characterized by accumulation of fluid, including pleural effusions or fetal hydrops, result in pulmonary hypoplasia as well, presumably from the increased pressure on the growing lungs.

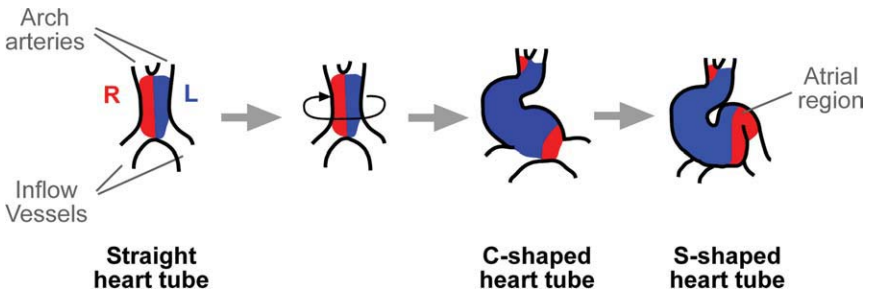

Figure 1. Morphological changes during looping of the heart tube. The straight heart tube bends and twists dextrally to form a C-shaped tube. The tube then shortens and twists into an S-shaped tube.

Tissue Mechanics during Development of the Heart

The cardiovascular system is the major transport system of the body, conducting oxygen, cells, nutrients, and waste products to their final destinations. The heart is the first organ to develop; severe defects in heart development are one major cause of embryonic lethality. In the human embryo, the heart begins beating at ~21 days postconception. The heart pumps throughout its morphogenesis, and its constituent cells are exposed to multiple mechanical stimuli, including wall shear stress, transmural pressure, and stretch. The development of the heart proceeds via sequential processes of cardiomyocyte specification (day 15 in the human embryo; HH stages 5 to 9 in the chicken; Hamburger and Hamilton, 1951), formation and looping of the heart tube (days 20–28 in the human; HH stages 10–24 in the chicken), development and growth of the chambers (days 28–32 in the human; HH stages 19–24 in the chicken), and development of cardiac cushions, valves, and septae (days 32-birth in the human; HH stages 25–34 in the chicken) (Bruneau, 2008; Manner, 2009).

The mechanical forces accompanying heart development have been most closely examined in chicken and zebrafish embryos. Cardiac looping morphogenesis in the chicken closely resembles that in the human embryo (Kirby, 2007). During looping, the initially straight heart tube undergoes bending and torsion to form the basic topographical pattern of the multi-chambered heart (Fig. 1). During the first stage of looping, C-looping, the heart tube bends ventrally and twists toward the right (dextrally) to form a C-shaped tube. During S-looping, the C-shaped tube shortens and twists into an S-shaped tube. Bending can occur normally even in embryonic chicken hearts isolated in vitro (Manning and McLachlan, 1990), and is thought to result from actomyosin-mediated changes in cell shape (Latacha et al., 2005). As the heart tube bends, myocardial cells flatten at

the outer convex curvature, whereas others elongate and thicken at the inner concave curvature (Manasek et al., 1972). Inhibiting actin polymerization blocks bending in vivo and in isolated hearts (Latacha et al., 2005), consistent with a cell shape-driven process.

Changes in cell shape also appear to be responsible for the ballooning process that sculpts the chambers of the heart. Ballooning involves expansion and bulging of the linear walls of the looped heart tube into bean-shaped chambers, again with a convex outer curvature and a concave inner curvature. High-resolution imaging of the developing zebrafish heart revealed that cells of the outer curvature flatten and elongate relative to those of the inner curvature, which remain cuboidal (Auman et al., 2007). Physical forces resulting from normal blood flow were shown to be required for cell shape change, as disrupting flow either genetically or pharmacologically inhibited the flattening and elongation of cells in the outer curvature (Auman et al., 2007). It is still unclear how the cells are sensing these forces and transducing them into alterations in cell shape.

Forces from blood flow also regulate development of the heart valves. After looping is completed in the zebrafish embryo, cardiac cushions form at the atrioventricular (AV) boundary, the separation between the ventricle and atrium chambers (Eisenberg and Markwald, 1995). Communication between the myocardial and endocardial layers at the AV boundary leads to an EMT of the endocardial cells, forming cushions that then differentiate into the flaps of the AV valve. Quantitative in vivo imaging of hemodynamics in the developing zebrafish heart revealed the presence of high-shear (>1 dyn/cm^2) vortical flow during and after valvulogenesis (Hove et al., 2003). Valves fail to form in mutant embryos that lack a heartbeat (Bartman et al., 2004), and mechanically blocking blood flow disrupted looping of the heart tube and valve formation (Hove et al., 2003). Fraser's group recently showed that disturbed flow patterns, rather than shear stress per se, are responsible for activating the flow-responsive genes that direct valvulogenesis (Vermot et al., 2009). Specifically, reversing flows activated Kruppel-like factor (Klf)-2a in the endothelium in valve-forming regions, and morpholino-mediated knockdown of Klf2a disrupted heart valve formation (Vermot et al., 2009). How endocardial cells sense and respond to disturbed flow is unclear, but extensive analysis of hemodynamic signaling in the endothelium of adult vessels yields several promising clues. Oscillatory flow patterns with recirculation tend to form at branch points within the vascular bed and distal to stenoses, and these hemodynamic forces have been implicated in the local development of atherosclerosis (White and Frangos, 2007). Endothelial cells in regions of disturbed flow in vivo and in culture exhibit increased proliferation, apoptosis, junctional permeability, and oxidative stress (Hahn and Schwartz, 2008) resulting from sustained production of reactive oxygen species (ROS), which themselves act as mediators of EMT (Radisky et al., 2005). It remains to be determined whether and how disturbed flows signal to endocardium through ROS.

Formation of the heart valves proceeds similarly in amniotes. In the chicken embryo, the AV and outflow tract cushions start to emerge as expansions of cardiac jelly, the ECM in between the myocardium and endocardium that is rich in hyaluronan and chondroitin sulfate proteoglycans. The myocardium then induces an EMT of the endocardium, causing the latter cell population to migrate into the cushions and form the mitral and tricuspid valves, as well as the atrial and ventricular septae (Martinsen, 2005). The Rho GTPase effector, ROCK, is expressed in the migrating population and treatment with the ROCK inhibitor, Y27632, blocks valve formation in the chicken embryonic heart (Sakabe et al., 2006). Furthermore, the basic helix-loop-helix (bHLH) transcription factor Twist1 is expressed in the mesenchyme of the developing heart cushions (Ma et al., 2005), and induces proliferation and migration of endocardial cushion cells (Shelton and Yutzey, 2008). Mechanical stress activates ROCK (Wozniak and Chen, 2008; Chiquet et al., 2009) and induces expression of Twist in *Drosophila* embryos (Farge, 2003; Desprat et al., 2008a), suggesting that hemodynamic forces may induce EMT and heart valve formation through Rho GTPases and Twist.

Congenital heart defects affect $\sim$1% of newborn infants (Hoffman, 1995a), making them the most common type of birth defect and a major cause of infant mortality in the first year of life. Abnormalities in the early stages of heart development result in embryonic lethality (Hoffman, 1995a), so the majority of heart defects in infants that make it to term result from abnormal development of the valves and septae (Hoffman, 1995b). Members of the Notch family have emerged as key regulators of valve development, and disruptions in Notch or Jagged signaling can lead to bicuspid aortic valve (BAV) (Garg et al., 2005), a defect in which the aortic valve has two leaflets instead of three. Notch is thought to act by promoting EMT in the cushions during valve formation (Timmerman et al., 2004). It will be interesting to determine the links, if any, between mechanical stress, Notch, and EMT in cardiac morphogenesis.

Novel Techniques to Study Developmental Mechanobiology

The study of mechanobiology requires understanding of the three main aspects of cell and tissue mechanics: rheological properties, endogenous generation of force, and response to exogenous mechanical perturbations. As a consequence of the dynamic nature of cells and tissues, however, these three aspects are often coupled (Bissell et al., 1982; Ingber, 2003a,b; Davidson et al., 2009), which poses a great challenge in precisely isolating the mechanical stimuli which cells sense

and to which they are able to respond. To rigorously define the role of mechanics in developmental processes, the existing techniques must be refined and new sophisticated techniques introduced.

The vast majority of our current knowledge in developmental mechanobiology comes from studies performed in culture. Cell-generated mechanical forces were visualized for the first time nearly three decades ago, when Harris and coworkers (1980) demonstrated that fibroblasts locomoting on 2D silicone membranes pull on their substratum, creating wrinkles. Monitoring the distribution and size of the wrinkles allowed semi-quantitative characterization of endogenous cellular forces. Approximately two decades later, Pelham and Wang (1998) introduced the use of bioinert polyacrylamide (PAA) gels with tunable stiffnesses to study the effect of external mechanical perturbations on cellular behavior. These two techniques later evolved into traction force microscopy (TFM), which incorporates fluorescent beads into the PAA substrata to visualize displacements due to cell-generated traction forces. TFM allows study of both cellular response to external mechanical changes and measurement of endogenous forces, producing force maps with subcellular resolution (Dembo and Wang, 1999; Pelham and Wang, 1999). An alternative technique involves plating cells on top of an array of elastomeric micrometer-scale posts. Here, the cell-generated forces are computed from the deflection of the elastic posts, which reduces the mathematical complexity of TFM (Tan et al., 2003).

Sophisticated lithography-based microfabrication methods developed in the past decade have enabled researchers to control the magnitudes of mechanical stress experienced by isolated single cells by restricting cell spreading (Fig. 2) (Chen et al., 1997). Furthermore, these methods were critical in demonstrating that tissue geometry controls the distribution of mechanical stress over cellular monolayers

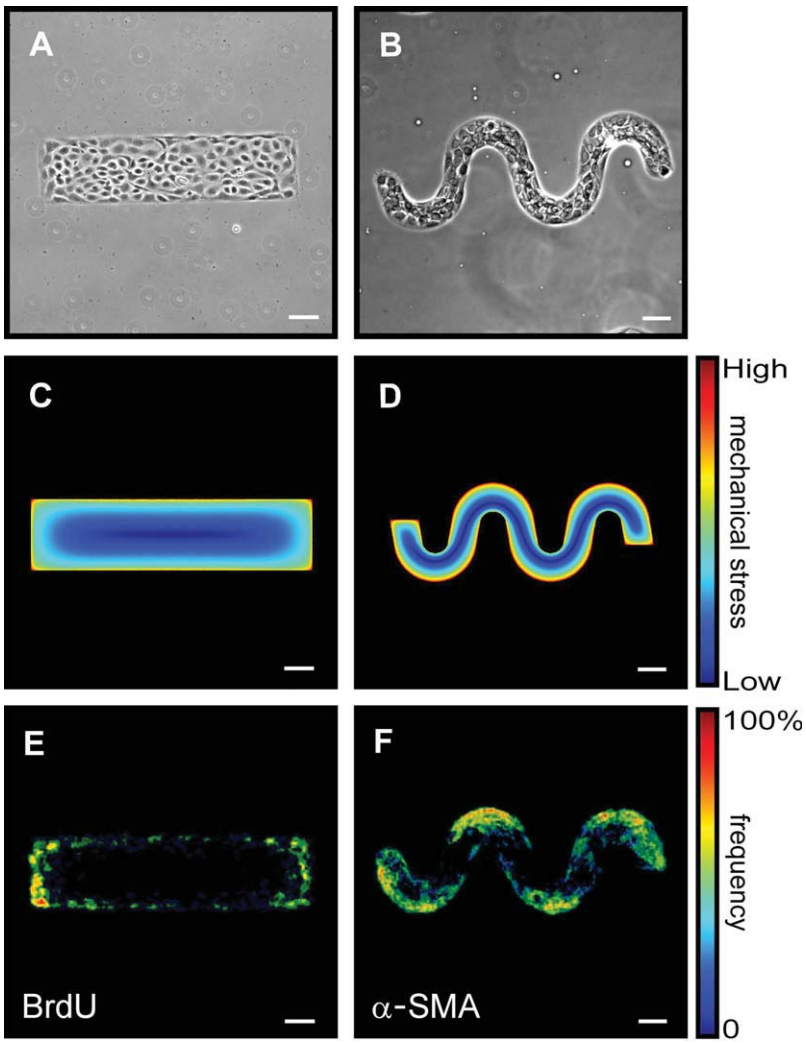

Figure 2. Spatial control of mechanical gradients through microfabrication. Monolayers of different geometry microfabricated from (**A**) endothelial and (**B**) epithelial cells. (**C, D**) The geometry of the monolayer dictates the spatial distribution of mechanical stress. Mechanical stress spatially patterns (**E**) proliferation, as measured by incorporation of BrdU, and (**F**) EMT, as measured by expression of αSMA. (C,D,F) reproduced with permission from Gomez et al., *J Cell Biochem*, 2010, 110, 44-51, Wiley InterScience©. (E) reproduced with permission from Nelson et al., *Proc Natl Acad Sci USA* 2005, 102, 11594-11599, NAS©.

(Nelson et al., 2005). This discovery inspired the use of microfabrication as a means to precisely control tissue geometry, and consequently spatial patterns of stress within cell monolayers, allowing further insight into the role of mechanics as a potential regulator of biological processes. Indeed, microfabrication was instrumental in demonstrating that mechanical stress spatially patterns important developmental processes, such as apoptosis (Chen et al., 1997), proliferation (Nelson et al., 2005), differentiation (McBeath et al., 2004; Kilian et al., 2010), and epithelial-mesenchymal transition (Nelson et al., 2008b; Gomez et al., 2010), and unveiling some of the underlying mechanisms of mechanotransduction (Gomez et al., 2010).

Several recently-developed techniques for microfabricating three-dimensional (3D) epithelial tissues hold promise in addressing questions in developmental mechanobiology with a higher level of physiological and histological realism (Nelson and Tien, 2006). We recently developed an approach that relies upon replica molding of

TABLE 1. Mechanical Stresses in Culture and In Vivo

Parameter	Magnitude	Cell/tissue type	Context	Reference
Endogenous mechanical stress	120–3.6 $\times$ 10^4 Pa	Fibroblast	2D PAA gel	Dembo and Wang, 1999; Munevar et al., 2001; Paszek et al., 2005; Maskarinec et al., 2009
Endogenous traction force	50–100 μN	Endothelial cells	2D glass and PAA gels	Nelson et al., 2005; Ghosh et al., 2008
Mechanical stress during lumen formation	0.03 Pa, max	MDCK cells	3D collagen culture	Zeng et al., 2006
Shear stress (induces changes in gene expression)	$<$0.1 Pa	Endothelial cells	Glass capillary tubes	Olesen et al., 1988
Shear stress (induces cytoskeletal rearrangements)	0.5–1.5 Pa	Endothelial cells	2D glass	Davies et al., 1986
Wall shear stress	0.3 Pa	Human aorta (normal)	In vivo	Barker et al., 2010
Wall shear stress	0.2 Pa	Human aorta (bicuspid aortic valve)	In vivo	Barker et al., 2010
Wall shear stress	$>$0.1 Pa	Zebrafish embryonic heart	In vivo	Hove et al., 2003

biomimetic hydrogels around elastomeric stamps to generate cavities of micrometer-precision size and geometry (Nelson et al., 2006; Nelson et al., 2008a). Cells subsequently inserted into the cavities self-organize into 3D tissues. The main advantages of engineered 3D epithelial tissues include the accessibility and ease with which mechanical modulations can be performed and endogenous mechanical characteristics interrogated quantitatively. The concentration of the hydrogels or the extent of crosslinking can be varied to modulate matrix stiffness, thereby altering the mechanical signals originating outside the cells. Conversely, molecular or pharmacological approaches can be taken to perturb mechanical elements within the cells, including integrins, intercellular adhesion, and cytoskeletal proteins. In a fashion similar to the microfabricated 2D tissues, the patterns of mechanical stress can be controlled by the 3D geometry, which would in this case allow researchers to draw correlative and causative links between the spatial profile and magnitudes of mechanical forces, on one hand, and sites and extents of 3D morphogenetic processes within the tissues, on the other. Furthermore, matrix deformations due to tissue morphogenesis can be readily measured and used to quantify morphogenetic forces in a spatially and temporally resolved manner.

Although studies in culture have elucidated the mechanical control of basic cellular processes, a comprehensive understanding of developmental mechanobiology will not be secured until the contribution of mechanics is confirmed in vivo. A first step in that direction could be to confirm that mechanical forces of magnitudes corresponding to those required for cellular response in culture also exist in vivo. Such an effort, nevertheless, requires precisely defined lower and upper limits of force which cells can sense, and information of that nature is generally lacking in the field. The typical approaches in mechanobiology modulate the magnitude of endogenous cellular force by varying the compliance of the substratum (Wozniak et al., 2003; Paszek et al., 2005; Engler et al., 2006; Alcaraz et al., 2008) or the geometry of the tissue (Nelson et al., 2005; Nelson et al., 2008b; Gomez et al., 2010). The magnitudes of the forces that accompany or are needed to elicit various cellular responses are summarized in Table 1, where available. Future work should focus on defining the range in which these forces have developmental relevance in culture, and determining whether such forces and the corresponding biological effects are also present in vivo.

Techniques to Study Mechanobiology In Vivo

As argued above, a comprehensive understanding of the mechanical control of development requires techniques to apply and measure mechanical force in vivo. The large plastic deformations, fragility, and inaccessibility of developing tissues, however, further complicate the already challenging task of studying developmental mechanobiology in culture. In the following sections we describe some of the techniques used to measure and perturb the mechanical properties of and forces generated within embryos and embryonic tissues.

Methods for Measuring Mechanical Properties of Embryos and Embryonic Tissues

The mechanical properties of cells and tissues are determined by the properties of individual molecules within the cells, their organization into subcellular struc-

tures, and the organization of cells and the ECM (Davidson et al., 2009). Measuring the mechanical properties of materials generally involves applying a known force and assessing the resulting deformation. Microindentation techniques, which rely on the same fundamental principles as atomic force microscopy, apply nano-Newton force via a thin cantilever beam on small fragments of embryonic tissue and measure the resulting tissue displacement. Such methods have been successfully used to measure the mechanical properties of chicken embryonic heart tissues (Zamir and Taber, 2004a,b). More specifically, Taber and coworkers fitted force-displacement and surface displacement experimental data to the strain-energy density function to determine the material parameters of the myocardium and cardiac jelly of the chicken heart during cardiac looping at the C-shaped stage. In addition, they found that the myocardium at this stage experiences substantial residual stresses, which may be of morphogenetic relevance.

Microaspiration methods are nondestructive approaches which apply known negative suction pressure to aspirate small portions of embryonic tissue into microchannels. The elastic modulus of the tissue is calculated from its displacement into the channel, the radius of the channel, and the applied pressure (von Dassow and Davidson, 2009). Microaspiration techniques have been used to monitor changes in stiffness during the gastrulation of whole *Xenopus laevis* embryos (von Dassow and Davidson, 2009). Embryonic tissue stiffness has also been extracted from uniaxial and parallel plate compression studies. One such study measured the resistive force that arises upon a given compressive deformation and uses the force, the compressive strain, and the cross-sectional area of the spherical explant to compute the stiffness of the *Xenopus laevis* embryo during key stages of its development (Zhou et al., 2009). Similar studies have described the mechanical properties of cellular aggregates from chicken and sea urchin blastula (Forgacs et al., 1998; Davidson et al., 1999). The main limitation of these methods is the requirement of direct contact between the cells and the physical probe at the time of measurement. However, in their physiological environment cells are typically inaccessible to physical probes, as they are often embedded in ECM or surrounded by other tissues. Microrheological approaches circumvent these problems by injecting nanoparticles into individual cells of interest and tracking the particles as the embryo develops. The Brownian motions recorded are informative of the micromechanical environment surrounding the particles, allowing quantification of the viscoelastic properties of the cells with high spatial and temporal resolution (Daniels et al., 2006).

Methods for Measuring Force and Tension in Embryos and Embryonic Tissues

Defining the spatial and temporal profiles of mechanical forces within the developing embryo and correlating them with the spatiotemporal dynamics of developmental processes arising therein would likely shed light on the role of mechanical signals in development. In addition, quantitative knowledge of embryonic forces would help confirm the in vivo relevance of mechanically-controlled biological phenomena reported by culture studies. Therefore, methods to precisely quantify mechanical forces in vivo are required.

An indirect method to examine the mechanical profile of developing embryos is to visualize molecular markers of mechanical tension. Researchers have commonly monitored the spatiotemporal distribution of ROCK (Daley et al., 2009), phosphorylated myosin light chain kinase (Wang and Riechmann, 2007; Daley et al., 2009), and myosin II (Martin et al., 2009), all associated with cellular contraction, as means to map the mechanical forces within the embryo. A more direct method to estimate mechanical force in vivo is laser ablation. Here, a tightly focused laser beam is used to disrupt presumably tensed subcellular structures (Hutson et al., 2003; Rauzi et al., 2008). Shortly after the ablation, the structures of interest and their surroundings relax and displace at a given rate. The relaxation rates of distinct locations within the embryo provide a relative measure of the mechanical tension present at each location before the ablation. The main limitation of this technique is the fact that, while allowing comparison of mechanical force between different spatial and temporal points in the embryo, it does not furnish an absolute measure of force, which necessitates knowledge of the generally elusive local mechanical properties. Optical coherence tomography (OCT) is a technique that measures backscattered light as a function of depth, allowing 3D subsurface imaging of biological samples with high resolution and sensitivity (Filas et al., 2007). Filas et al. (2007) have used OCT to determine the spatial and temporal variations of myocardial strain of the embryonic chicken heart during the first phase of looping, that is, as it forms a C-shaped tube. More specifically, microbeads placed on the myocardium were tracked during the process and their displacements used to calculate the longitudinal, circumferential, and shear strains in seven different regions of the heart. The study, however, does not calculate the corresponding forces, most likely due to the complex geometries and constitutive relations of the morphogenetic tissue.

Measurement of hemodynamic forces in vivo has been considerably more successful. Characterization of hemodynamic stress generally involves determination of the velocity profile of blood within the vessel of interest, and subsequent calculation of the corresponding shear stress by using Newtonian fluid mechanics theory (Friedman and Giddens, 2005).

Researchers have developed methods for rapid (440–1000 frames s^{-1}) 3D imaging of the embryonic mouse and zebrafish heart using laser slit-scanning and line-scanning confocal microscopy (Hove et al., 2003; Jones et al., 2004; Liebling et al., 2006). These techniques have been instrumental in demonstrating that both the early (37 hours postfertilization) and later (4.5 days postfertilization) embryonic zebrafish heart experiences shear forces above the threshold necessary to induce gene expression and cytoskeletal changes in cultured endothelial cells (Davies et al., 1986; Olesen et al., 1988; Hove et al., 2003). The optical inaccessibility of large blood vessels makes confocal imaging approaches unfeasible for hemodynamic measurements in adult animals and humans. The blood velocity profile here is either directly measured using phase-contrast magnetic resonance imaging (MRI) (Barker et al.; Hollnagel et al., 2007) or is modeled numerically by performing computational fluid dynamics on vessel geometries reconstructed from MRI or ultrasound images (reviewed in Steinman, 2002).

Techniques for Mechanical Perturbation of Embryos

In addition to methods to measure mechanical force, establishing causative links between the mechanical environment and developmental processes in vivo requires methods for mechanical manipulations. A simple approach to impose mechanical stress globally is uniaxial compression. Submitting the *Drosophila* embryo to a 10% lateral uniaxial deformation has been used to demonstrate mechanically-driven expression of Twist, an important morphogenetic protein (Farge, 2003). More localized application of mechanical stress is possible with microinjection of magnetic nanoparticles in specific regions of the embryo. The particles are manipulated by magnetic tweezers, causing them to move and impart mechanical stress on the host cells (Desprat et al., 2008b). Endogeneous mechanical stresses within embryos and stress arising due to morphogenetic movements are routinely ablated with subcellular resolution using laser microsurgery (Supatto et al., 2005; Desprat et al., 2008b).

CONCLUSION

Understanding how developing embryos generate and respond to force, as well as their mechanical properties will undoubtedly shed light on the mechanisms whereby physical cues regulate developmental processes. Comprehensive description of embryo mechanics, however, is hindered by several technical limitations, but perhaps most strongly by the dynamic nature of living cells and tissues, which are both influenced by the environment, and in turn themselves influence their surroundings. Therefore, developing sophisticated methods that address questions in mechanobiology in a targeted and controlled manner is of great importance.

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