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Sculpting Organs: Mechanical Regulation of Tissue Development

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Abstract

The ramified architectures of organs such as the mammary gland and lung are generated through branching morphogenesis, a developmental process through which individual cells bud and pinch off of pre-existing epithelial sheets. Although specified by signaling programs, organ development requires integration of all aspects of the microenvironment. We describe the essential role of endogenous cellular contractility in the formation of branching tubes. We also highlight the role of exogenous forces in normal and aberrant branching.

Contents

INTRODUCTION

Morphogenesis, the process by which cells and tissues organize into structures, is a physical phenomenon. Morphogenesis is driven by cell- and tissue-scale processes that integrate genetic programs with mechanical, chemical, and electrical stimuli from the local microenvironment. These factors pattern tissue movements and geometry. The formation, branching, and overall architecture of tubular structures are essential for the development, growth, and function of most multicellular organisms. Tubes give rise to several organs and organ systems, including the gut and gastrointestinal tract, the heart and vessels of the circulatory system, and the reproductive organs including the uterus and fallopian tubes. Ducts and vessels serve as the primordial architecture for organs that undergo further morphogenesis and as the fundamental building blocks for other organs including the lung, kidney, and salivary gland. For these ramified organs, proper function is defined by their branched architecture. Aberrant branching and disruptions in multicellular morphology cause tissue dysfunction and play a major role in multiple pathologies including cancer. Polycystic kidney disease, a life-threatening condition in which nephron tubules and collecting ducts dilate and form cysts (1, 2), disrupts proper function and causes chronic hypertension, infections, kidney stones, and pain for the individual (3). An underdeveloped airway tree from hypoplastic branching of the lung is a common cause of neonatal mortality (4). As such, initiation and regulation of tubulogenesis and branching morphogenesis are essential in organ development.

This review seeks to lay out some of the fundamental aspects of how the mechanical environment shapes the development of branched organs. We acknowledge that a complete picture of branching morphogenesis can be achieved only by considering signals from the entire microenvironment and gene regulatory networks; however, it is difficult to separate the effects of biochemical, mechanical, and electrical gradients. Cell survival, proliferation, differentiation, and migration are all influenced by these gradients (5–9). Morphogen gradients that encode spatial and functional cues, both directly and indirectly through electrical gradients (10, 11), are sculpted

Microenvironment: the local cellular environment that consists of the extracellular matrix and neighboring cells as well as local mechanical, chemical, and electrical signals

Morphogen: a chemical signal that diffuses into gradients, which provide developmental instructions to cells and tissues

by tissue geometry and physical laws. Furthermore, mechanical force has been implicated in the activation of latent molecules by cleavage from their proisoforms, as in the case of transforming growth factor beta (TGFβ) (12). As there are clear consequences to modifying the mechanical environment, we choose to highlight these effects and their contributions to branching morphogenesis. Although we include examples from a variety of branched organs, we focus primarily on epithelia. We include a deeper discussion of branching in two organs that represent the major classifications of ramified geometries: the mammary gland, which has a stochastic branching pattern, and the lung, which has a stereotyped branching pattern that is conserved across individuals.

TGF: transforming growth factor

Stochastic: not stereotyped; determined primarily by environmental cues

Stereotyped: highly reproducible between individuals; conventionally thought to be hardwired by genetic cues

EGF: epidermal growth factor

GENETIC BLUEPRINTS OF BRANCHING

The functional anatomy of many organ systems relies on networks of tubes. Although their final morphologies are different, the genetic programs activated during branching morphogenesis are surprisingly similar across organs and phyla (13). At its core, branching morphogenesis is directed by the interplay between an inductive stimulus and its counterbalancing inhibitor (14). These opposing molecular forces instruct large-scale cellular rearrangements that drive branches forward (15–17).

Milk of Life: Branching of the Mammary Gland

The mammary gland distinguishes mammals from all other classes of vertebrates. The mammary duct is a bilayered tree-like structure comprised of an inner layer of lumenal epithelial cells and an outer layer of myoepithelial cells, surrounded by a basement membrane. The epithelial duct is embedded in a complex stromal compartment that contains various quantities of adipose and fibrous tissues depending on the species. Similarly, the gross architecture of the gland also varies between species. The molecular and genetic details of mammary branching have been discerned primarily for the mouse, which has served as the major model system for studies of mammary gland development (8).

Unlike most other organs, the branching morphogenesis that builds the mammary epithelial tree begins during puberty. Ovarian estrogens bind to receptors in the stromal compartment of the gland, thereby signaling the production of hepatocyte growth factor (18). When secreted by the stroma, this growth factor binds to its cognate receptor, Met, expressed in the epithelium to induce the branching process. Estrogen also instructs epithelial cells to produce and secrete a member of the epidermal growth factor (EGF) family, amphiregulin, which binds to the EGF receptor in the stromal compartment (19, 20). What happens next is unclear, but branching morphogenesis does not proceed in the absence of stromal expression of EGF receptor (21).

These signals highlight a major feature of branching morphogenesis across organs: Sculpting of the epithelial tree requires concerted and reciprocal signaling between the epithelium and its surrounding stroma or mesenchyme. This truism of organ development is made strikingly clear by tissue-recombination experiments—cut-and-paste approaches in which living pieces of tissue from various regions of the developing animal are combined and monitored. Combining mammary epithelium with mammary mesenchyme results in the development of a mammary tree, but recombination with mesenchyme taken from the salivary gland causes the mammary epithelium to form a structure reminiscent of the salivary epithelial tree (22, 23). These and other studies revealed that the mesenchyme plays a major role in specifying the pattern of branching of the epithelium.

Nonetheless, in the mammary gland, much of the information that specifies patterning comes from the epithelium itself. Mammary epithelial cells synthesize and secrete TGFβ, which acts in an autocrine fashion and thereby serves as a repulsive cue during development of the tree

(24, 25). High concentrations of TGFβ prevent branching in culture and in vivo. The signaling downstream of TGFβ is still unclear, but part of its effects appear to result from the induction of a second inhibitory morphogen, Wnt5a, which phenocopies TGFβ signaling in mice and in branching assays in culture (26, 27). These molecular cues allow developing branches to avoid one another and thus result in a tree with an open architecture at the end of puberty.

Monopodial: a mode of branching in which buds branch laterally off a main stem

Dichotomous: a mode of branching in which the tip of a stem divides to generate two equivalent buds

FGF: fibroblast growth factor

Breath of Life: Branching Morphogenesis of the Lung

The evolution of the lung can be traced back to the ancestors of modern reptiles, which moved from water to land approximately 300 Mya and thus committed to acquiring oxygen from air. Lungs are an adaptation to provide a large, internally protected surface area with a thin barrier that promotes the exchange of gases with the environment while preventing loss of water from the body. One can envision several anatomic configurations that could meet these basic design constraints; indeed, several have evolved to meet the varied oxygen and energetic needs of vertebrates. Although we focus exclusively on the morphogenesis and structure of the mammalian lungs, these are relatively inefficient gas-exchange apparatuses (28). In fact, the lungs of birds, which must meet the high energy demands of flight, are the most efficient of all vertebrates; avian lungs consist of cross-current gas exchangers that are ventilated with a continuous and unidirectional supply of fresh air, akin to the design of a heat exchanger in the radiator of a car. Our understanding of the signals that regulate the development of avian lungs is relatively poor, but it will be interesting to determine the similarities and differences between these pinnacle gas exchangers and those of mice or humans.

The mature mammalian lung contains ∼12–24 generations of airways, depending on the species, which terminate in blind-ended cavities for gas exchange known as alveoli. Because of its blind-ended design, the mammalian lung is ventilated with air via bidirectional, or tidal, flow, which is driven by contraction of the diaphragm, a large muscular sheet at the bottom of the chest cavity. This contraction increases the volume of the chest cavity and decreases its pressure, causing air to flow from the nasal passages into the trachea and airways. The larger airways, including the main bronchi, which bifurcate off the trachea, primarily serve as conduits for the flow of air, whereas gas exchange is limited to the terminal bronchioles and alveoli.

Much of our understanding of the development of the mammalian lung has been gleaned from observations and experimental manipulations of the mouse (29). The murine lung begins as an outpouching of the foregut epithelium on embryonic day (E) 9.5, splitting to form the two main bronchi that penetrate the left and right lungs. These undergo successive rounds of monopodial and dichotomous branching during the pseudoglandular (E9.5–E16.5) and canalicular (E16.5–E17.5) stages of development. Detailed mapping of the branches as a function of time revealed that the murine lung follows an exquisitely stereotyped branching program, with each new branch forming at a precise time and location relative to the other branches of the epithelial tree (30). Monopodial branching was designated as "domain branching," whereas dichotomous branching was designated as "planar bifurcation" or "orthogonal bifurcation," depending on the orientation relative to the parent airway. These branching strategies are thought to be controlled by genetically encoded subroutines, used reiteratively during lung development.

The initiation of branches is determined in part by coordinated signaling between the developing airway epithelium and its surrounding mesenchyme, and it relies principally on fibroblast growth factor 10 (FGF10). FGF10 is expressed focally in the mesenchyme and acts as a chemotactic cue by binding to its cognate receptor FGFR2 in the epithelium (31–33). Mice with partial loss of FGF10 expression develop hypoplastic lungs (34), whereas total knockouts fail to form distal airways (35). Binding of FGF10 to its receptor initiates MAPK signaling, which is necessary for

branching (36). FGF10 signaling is regulated in part by an inducible inhibitor, Sprouty-2, which is expressed in the distal tips of branching epithelium in response to FGF10, thus forming a negative feedback loop (37, 38). Wnt5a is expressed in the mesenchyme in a pattern complementary to that of FGF10 and induces the expression of bone morphogenetic protein 4 in the adjacent epithelium, which acts in a concentration-dependent manner to restrict branching to the nascent buds (39, 40).

The branching of individual airways has been examined using time-lapse analysis and follows a predictable sequence of events: The branch extends and then arrests; afterward, the tip of the branch bifurcates into two daughter branches, which repeat the process (41). The rate of extension determines the distance between branches in the tree, and this rate is currently thought to be controlled by a clock mechanism mediated by signaling through FGF10 and Sprouty (41). As we discuss below, this hypothesis is difficult to reconcile with data on the mechanics of lung development. Nonetheless, the entire program as currently inferred relies on pre-existing foci of FGF10 in the surrounding stroma. How these patterns of FGF10 expression are templated in the mesenchyme is unknown.

ECM: extracellular matrix

Mechanotransducer: a cellular structure that produces biochemical signals in response to external forces applied to the cell

FA: focal adhesion

AJ: adherens junction

MECHANOTRANSDUCTION: HOW FORCE IS TRANSDUCED INTO BIOCHEMICAL AND FUNCTIONAL RESPONSES

Cells exist and interact in a complex microenvironment that contains a plethora of chemical, mechanical, and electrical signals (**Figure 1**). These signals instruct cellular phenotype. Conversely, cells can modify their own microenvironment by remodeling the extracellular matrix (ECM) or their interactions with neighbors. This dialogue, whereby the microenvironment defines cellular responses that in turn regulate the microenvironment, is termed dynamic reciprocity (42).

So how does a cell sense and modulate its mechanical microenvironment? Although the complete picture of cellular mechanosensing is still being unveiled, some mechanisms are known. Several subcellular structures transduce extracellular forces into biochemical and physical changes within the cell. Some of the most noted mechanotransducers include stretch-activated ion channels (43), primary cilia (44, 45), focal adhesions (FAs) (46, 47), and adherens junctions (AJs) (48). These molecular-scale interpreters change conformation in response to an applied force, thereby triggering a cascade of biochemical events inside the cell (**Figure 1**). Strain within the plasma membrane opens stretch-activated ion channels to regulate flux across the membrane (43). Primary cilia bend in response to shear stress resulting from the flow of interstitial or lumenal fluid, which induces uptake of calcium ions and calcium-induced signaling (44, 45). FAs and AJs grow and mature, reinforcing themselves as force increases (48). Furthermore, these junctional mechanosensors provide a physical link to the cytoskeleton and mechanically couple the extracellular world to the intracellular machinery. This feature not only enables FAs and AJs to serve as mechanosensors and mechanotransducers, but also permits the transmission of intracellular forces to the ECM and neighboring cells, thereby modulating the microenvironment. In lieu of an exhaustive description of all mechanosensory machinery (see other more detailed reviews, 49, 50), we focus on the junctional structures that enable the bidirectional transmission of force. These cell-ECM and cell-cell junctions regulate several fundamental cellular behaviors that are necessary for branching morphogenesis.

Focal Adhesions and Adherens Junctions: Touchy-Feely Spot Welds

The formation of cellular adhesions requires protein machinery that both couples to the ECM and links to the cytoskeleton. Integrins are heterodimeric transmembrane proteins with extracellular domains that bind to amino acid sequences found in ECM proteins including collagen, fibronectin, vitronectin, and laminin (46). Extracellular engagement induces the recruitment of

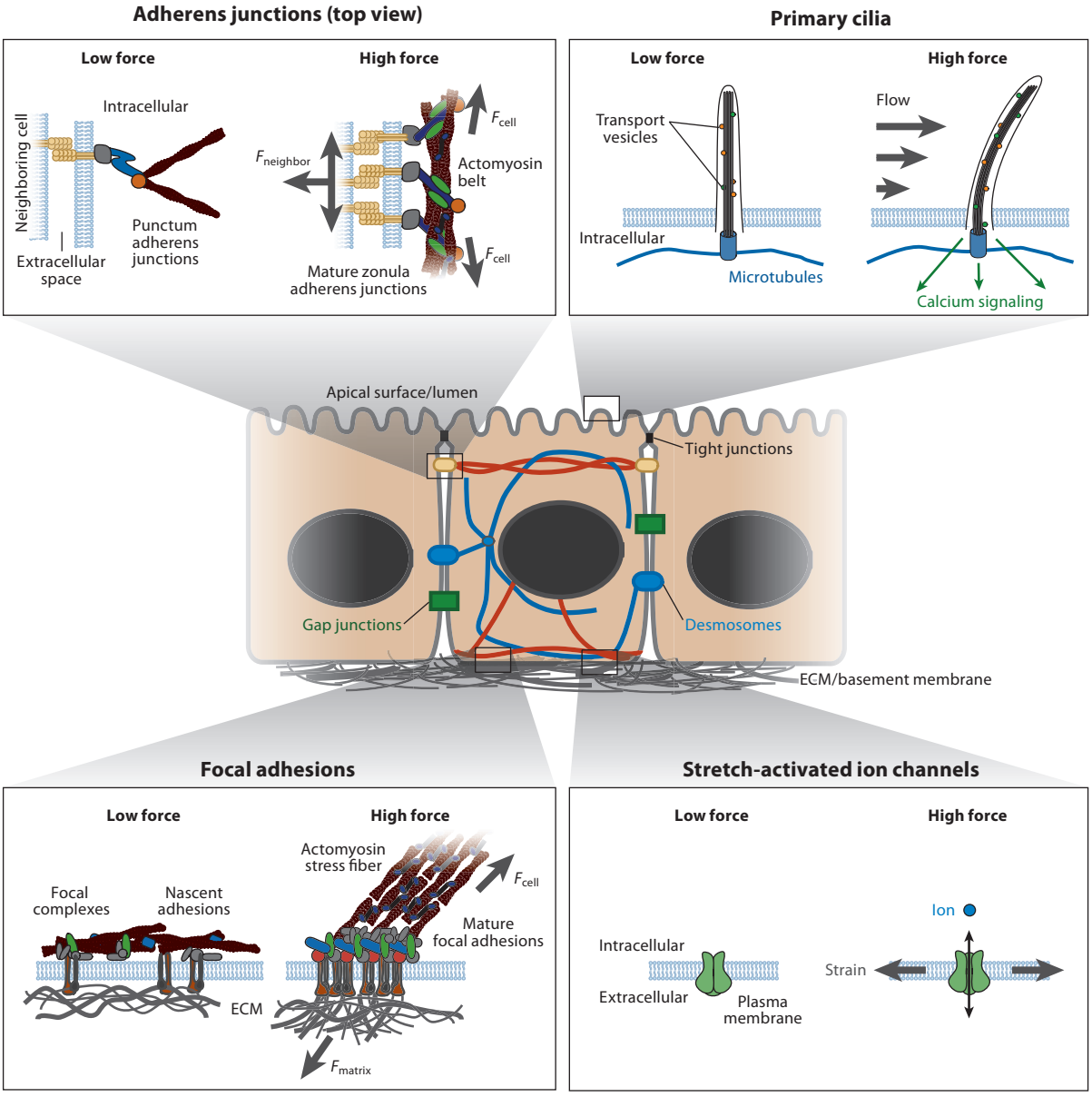

Figure 1

Epithelial cells exist in complex microenvironments with many connections to the outside world. (*Inset boxes*) Some of the well-characterized cellular mechanotransducers that convert mechanical force to structural and biochemical events. Abbreviation: ECM, extracellular matrix.

additional integrins and several cytoplasmic proteins that link the adhesion complex to the actin cytoskeleton. The integrin-binding scaffolding proteins paxillin and talin recruit focal adhesion kinase, vinculin, filamin, and α-actinin to the complex and link to F-actin (47). Recruitment of vinculin is force dependent, and stabilization of the FA requires transmission of force (51). The recruitment and phosphorylation of additional proteins in the complex, including Src-family

kinases, bind additional F-actin bundles to create an FA. Cell-generated forces transmitted through FAs have magnitudes on the order of 5–100 nN per FA (52, 53).

Whereas FAs are the primary cellular connection to the ECM and are found in all cells, AJs are most prominent in epithelial tissues and represent one type of intercellular adhesion. AJs are located in mammalian cells on the lateral plasma membrane, immediately basal to the tight junctions. Both FAs and AJs contain a transmembrane linker: integrins in the former, cadherins in the latter. Cadherins are calcium-dependent single-pass transmembrane proteins that form homophilic bonds with identical proteins expressed in the neighboring cells. The cytoplasmic domain of cadherin binds directly to p120-catenin and β-catenin, which form a complex with α-catenin to link the adhesion to the actin cytoskeleton (54, 55). As with cell-ECM adhesions, AJs grow and mature in response to force. In particular, α-catenin has been proposed to undergo a conformational change and bind vinculin in response to tension (56). This force-dependent recruitment of vinculin reinforces the AJ by permitting additional binding to F-actin, thus enabling the transmission of greater forces. As the force across the membrane is increased, either exogenously from tension applied to the cell or endogenously from contraction of the actin cytoskeleton, the adhesion complexes mature, they become reinforced, and the AJ straightens (57, 58). As the AJ matures, α-catenin recruits and binds to the actin-stabilizing EPLIN. The actin cytoskeletal filaments change their orientation, from an initial alignment perpendicular to the plasma membrane in punctum AJs to a bundled alignment parallel to the plasma membrane in zonula AJs (59). These mature zonula AJs form an actomyosin band (or "belt") encircling the apical end of the cell, with the retention of EPLIN and the stabilization of AJs dependent on junctional tension (60).

GAP: GTPase-activating protein

GEF: guanine nucleotide exchange factor

In addition to the intracellular protein conformation, phosphorylation, and binding events that occur upon extracellular engagement of integrins and cadherins, several molecular signals are initiated that both mature and regulate these junctional complexes. In particular, the Rho family of small GTPases, which includes RhoA, Rac1, and Cdc42, are essential in organizing cytoskeletal structure (61). GTPases are active when bound to GTP and inactive when bound to GDP, thus enabling regulation through guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GEFs catalyze the dissociation of GDP and enable activation, whereas GAPs inactivate GTPases by increasing the rate of GTP hydrolysis (62, 63, 64). Local activation of RhoA occurs concomitantly with early adhesion formation resulting from engagement of either integrin or cadherin (65, 66). Activation of RhoA induces several signals that are necessary for nascent contacts to mature and stabilize into FAs and zonula AJs. Additionally, RhoA increases actomyosin contractility by activating its effector, Rho-associated protein kinase, leading to increased phosphorylation of myosin light chain that results in increased binding between actin and myosin II (67, 68). Local control of Rho activation is critical for cell function. For instance, upon formation of cell-cell junctions, RhoA is activated locally while being downregulated in other parts of the cell, which results in reduced cell spreading and inhibition of proliferation (69).

Functional Consequences of the Mechanical Microenvironment

External forces applied to the cell cause local deformations (see sidebar, The Language of Mechanics) that result in physical alterations to intracellular machinery or changes in cell shape. Pulling on individual integrins using magnetic microbeads moves organelles, changes nuclear shape, and rearranges the nucleolus and cytoskeleton (70). At a larger scale, when uniaxial stretch is applied to cells cultured on two-dimensional flexible substrata, a rapid and sustained activation of RhoA and myosin leads to the development of oriented stress fibers (71). Additionally, uniaxial stretch and fluid shear stress can also polarize cells (72, 73).

THE LANGUAGE OF MECHANICS

The physiological environment exposes cells to a wide variety of mechanical stimuli including hydrostatic pressure, tension, compression, and shear. A force is any influence on an object that causes a change in speed, direction, or shape. External forces can cause movement or stress if the object is immobilized. Stress is a measure of the internal reaction forces within the object resulting from external forces. In addition to experiencing stress, objects can deform. Force and stress are related but not interchangeable parameters. Whereas a constant force can be applied to a deformable object, the stress at a specific point will change as the object deforms.

The geometry and composition of the object significantly affect its resulting behavior. Normalized parameters are used to compare behaviors of different objects. Strain is the normalized deformation that occurs under load, whereas a modulus, such as Young's modulus, refers to geometry-independent material stiffness. The terms stiffness and modulus have been used interchangeably, but an important distinction must be made between the fundamental material property (modulus) and the calculated geometry-dependent spring stiffness. Compliance and elasticity, commonly but imprecisely used interchangeably, are the inverse of the spring stiffness: Less stiff substrata are more compliant or elastic.

It is readily apparent that external application of force will cause cells to deform and will result in changes in cellular behavior. However, it is also important to recognize the role of endogenously generated cellular forces in influencing cell phenotype. The global shape of the cell can be modulated by controlling integrin attachment sites, and thus the pattern of traction forces, as demonstrated using micropatterned islands of ECM proteins (5). Cell shape influences apoptosis, growth, proliferation, and differentiation (5, 6, 74, 75). The arrangement of FAs also determines the distribution of stress fibers within the cell (76, 77) and guides the orientation of the cell-division axis (78).

The physical properties and composition of the ECM collectively determine cell shape and behavior by modulating both external and intracellular forces. Increasing ECM stiffness activates RhoA, leading to an increase in stress fibers and actomyosin contractility (79). Matrix compliance modulates integrin recruitment to regulate the formation of FAs as well as to induce cell motility and differentiation (80–82). Gradients in substratum stiffness result in cell polarization (7) and durotaxis (7, 79), guiding cells to migrate up the stiffness gradient. In addition, abnormally high ECM stiffness can disrupt AJs by reducing localization of β-catenin, resulting in a loss of tissue architecture (9).

Global properties of the ECM clearly influence cell behavior, and the constant local remodeling of the matrix produces microenvironmental changes that also affect neighboring cell function. Cell-generated forces align and deform the ECM, which can alter the topology of the microenvironment experienced by neighboring cells (83). Fibroblasts embedded within uniform gels of type I collagen compress collagen fibrils and stiffen the microenvironment (84); the mechanical properties of these cultured tissues vary with fibroblast density (85). In addition, many protein constituents of the ECM exhibit a nonlinear elastic behavior that results in strain stiffening (86) and leads to local changes in matrix compliance. Such alterations in the local stiffness influence the motility of neighboring endothelial cells scattered on a soft substratum (87). An additional consequence of cell-generated matrix deformation is the reorienting and unfolding of ECM proteins to reveal cryptic binding sites (88), thus enabling additional cellular binding and interaction with the ECM. In this manner, cell-ECM adhesions create a self-reinforcing feedback loop, which is regulated by cell contractility.

Durotaxis: cell migration guided by gradients in substratum stiffness

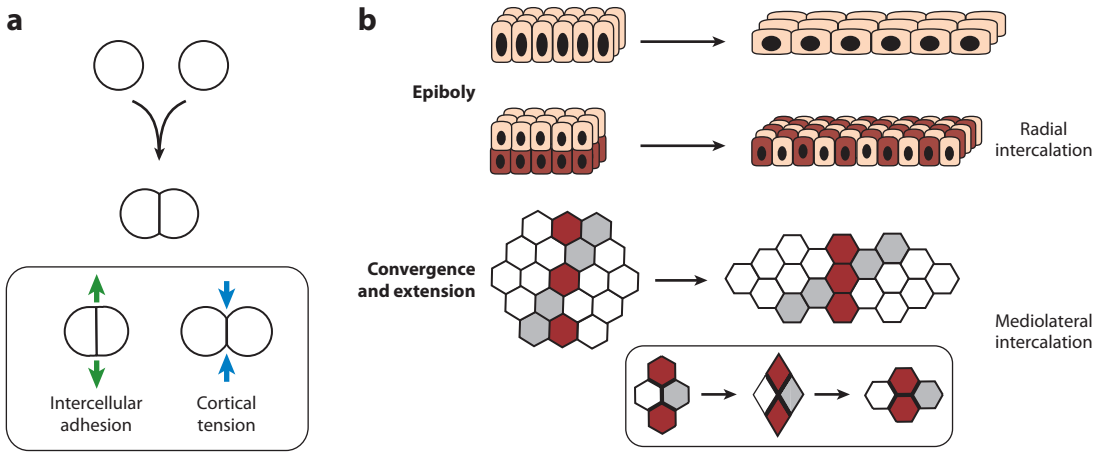

Figure 2

(*a*) Illustration of the line tension that arises from intercellular interactions as a balance between intercellular adhesion and cortical tension. (*b*) Epithelial tissues change their morphologies with controlled cell-shape changes and rearrangements.

EPITHELIAL TISSUES: EMERGENT MECHANICAL ENVIRONMENTS FROM COLLECTIVE BEHAVIORS

A unique and complex mechanical environment arises as a consequence of the multicellularity of tissues. The structure of a given tissue naturally emerges from the self-assembly and mechanical properties of its constituent cells. Intercellular interactions and mechanics define cell shape and behavior. When coupled with cell-ECM interactions, these parameters generate geometry-dependent gradients in the mechanical microenvironment that may span several cell diameters (89, 90). The mechanical integration of neighboring cells is critical not only for development, but also for tissue homeostasis as is evidenced by potentially fatal autoimmune diseases that result in rupture and blistering of the skin (91). Blisters and subsequent open sores result from autoantibodies that attack desmosomal cadherins and destroy the connections between neighboring cells (91). Similarly, blistering and erosions of the skin and mucosa are hallmarks of epidermolysis bullosa simplex, a group of congenital conditions caused by mutations in genes that encode for structural proteins including keratin-5, keratin-14, laminin-332, and the α5 and β4 integrin subunits (91, 92). These pathologies are but a few examples of the need for mechanical regulation, as the physical interactions the cell has with the outside world sculpt the microenvironment, drive tissue morphogenesis, and regulate tissue stability.

Moving and Dividing Cells to Change Tissue Shape

The balance between contractility and adhesion governs cell shape. For two adherent and contacting epithelial cells, E-cadherin-mediated adhesion increases the area of contact between the cells (93) and reduces the intercellular surface tension at the interface (**Figure 2*a***). Conversely, cortical tension, arising from contraction of the actin cytoskeleton (94), reduces the extent of cell-cell contact. Therefore, the balance between these two opposing forces, cortical tension and intercellular adhesion, defines an intercellular surface tension (also referred to as line tension) that modulates cell shape. When many cells are in contact, as in an epithelial monolayer, a cobblestone

Cortical tension: tension generated in the cell cortex from actomyosin contraction that attempts to pull the cell into a spherical shape

Line tension: the resulting interfacial tension between contacting epithelial cells defined by the balance between intercellular adhesion and cortical tension

morphology arises in which the steady-state geometry of individual cells optimizes packing and minimizes surface energy (95, 96).

Intercalation: the insertion of cells between other cells

Tissue shape is defined by the position and morphology of the constituent cells. Epithelial cells are continually remodeling their intercellular contacts throughout morphogenesis, which significantly rearranges cells within the epithelial sheet. These positional changes, referred to as intercalation, transform the overall geometry of the tissue. There are two main classes of movement that promote a change in the shape of an epithelial sheet: epiboly and convergence/extension (**Figure 2*b***). During epiboly, epithelial tissues spread by thinning. In a monolayer, individual cells change their shape and decrease their apical-basal dimension. In multilayered epithelia, radial intercalation causes layers of cells to merge into each other, thinning the tissue and expanding it bilaterally. In contrast, in convergent extension, mediolateral intercalation causes two or more rows of cells to move in between one another perpendicular to the axis of extension. Mediolateral intercalation results in a preferential decrease in length (convergence) in one direction with a concomitant increase in length (extension) in the other direction, all with no change in tissue thickness. Thus, radial intercalation thins the tissue and scales up its area, whereas mediolateral intercalation causes the epithelial sheet to elongate.

As demonstrated in the *Drosophila* embryo, asymmetries in line tension cause cells to intercalate (97, 98). Mediolateral intercalation results in germ-band elongation along the anterior-posterior axis with convergence along the dorsal-ventral axis. To accomplish this highly coordinated morphogenetic process, a series of exchanges among cell neighbors must occur (**Figure 2*b***). Intercellular contacts along the dorsal-ventral axis shrink as a result of local upregulation of nonmuscle myosin II and increased cortical tension at the interface (98, 99). This process transforms the cell from predominantly hexagonal in shape to pentagonal or quadrilateral. Upon forming intercellular contacts with new neighbors, cells return to a hexagonal shape, which results in a global change in tissue morphology (97, 100)

In addition to oriented exchange of neighbors, tissue shape is also influenced by cell proliferation. Whether proliferation is a cause or consequence of morphogenetic movements is unclear, but controlled proliferation is necessary for the morphogenesis of many tissues. In the *Drosophila* wing imaginal disc, the shape of the wing is controlled by the orientation of the axis of cell division (101, 102). The wing grows preferentially along its proximal-distal axis with a majority of cells orienting their metaphase plates approximately perpendicular to this plane. In mutant clones wherein the division axis is oriented randomly, the resulting wing loses the ability to extend along the proximal-distal axis and takes on a more rounded morphology, thus preventing functional flight.

Endogenous Tissue-Scale Mechanics Spatially Regulate Cell Function

Epithelia resist deformation and transmit force across the tissue, depending on the balance between cell-generated forces and the mechanical properties of the local microenvironment. The aggregate isometric endogenous forces generated from within the tissue generate geometry-dependent stress fields with gradients on the mesoscale that define spatial patterns of cellular behavior (89, 103, 104) (**Figure 3*a***). For example, uniform actomyosin-based cell contractility coupled with the shape of the tissue define regions of high traction stress that correspond to areas of increased proliferation (89). With inhibition of cellular contractility or disruption of intercellular connectivity, global patterns of proliferation are lost. These sites of proliferation are determined by the magnitude of the stress, with proliferation occurring above a threshold traction stress in culture (105). In vivo, compressive stress within the epithelial sheet is thought to inhibit decapentaplegic morphogen-induced proliferation in the *Drosophila* wing disc, thereby negatively influencing the size of this tissue (103).

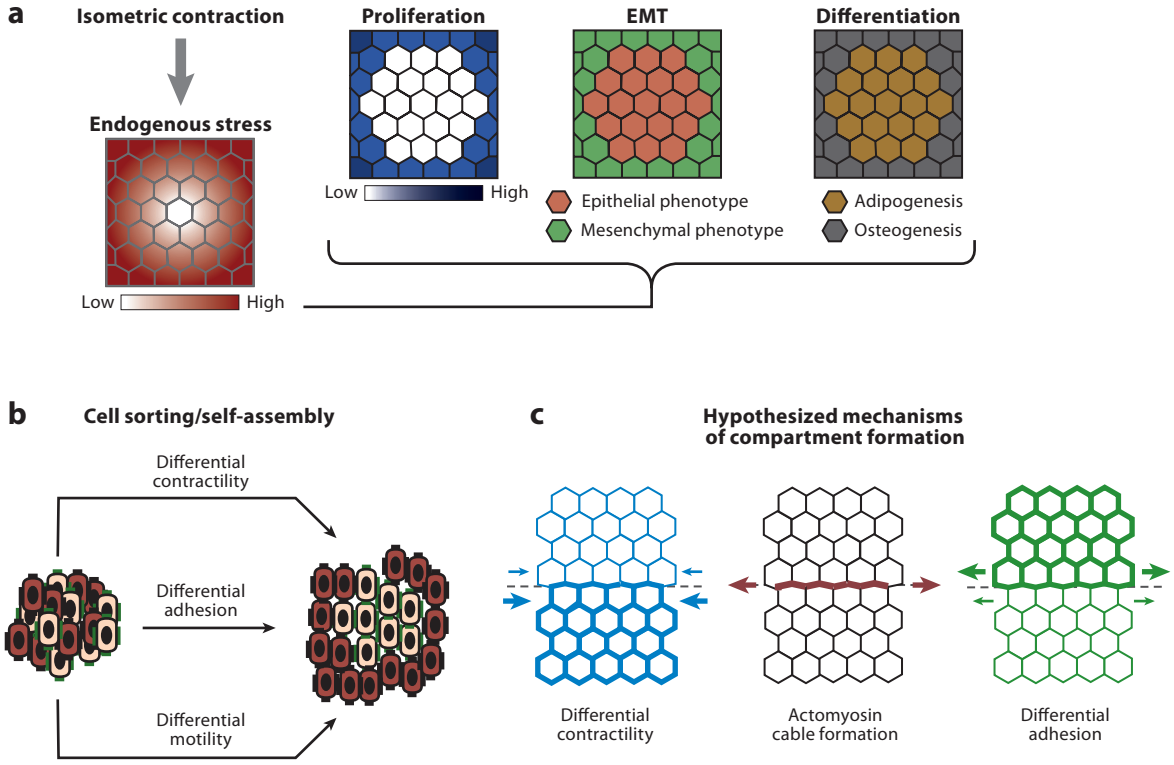

Figure 3

(*a*) Isometric cellular contraction generates geometry-dependent gradients in tissue stress that serve to spatially regulate cellular behavior. (*b*) Differences in contractility, adhesion, and motility between cell populations permit cells to sort and self-assemble both in culture and in vivo. (*c*) Similarly, differences in contractility and adhesion are two potential mechanisms for compartment formation observed in vivo. Recently, a third mechanism was proposed on the basis of in vivo observations whereby interfacial cells increase actomyosin activity at the boundary, thereby forming a continuous actomyosin cable. Abbreviation: EMT, epithelial-mesenchymal transition.

The mechanical environment can also affect the polarity of the intracellular machinery. In studies of single epithelial cells cultured on micropatterned ECM, the shape and distribution of adhesive contacts directed the orientation of the axes of cell polarity (106). In scrape-wound assays and micropatterned epithelial tissues, cells along the free edge orient the centrosome and intracellular motility machinery away from intercellular contacts (107). This directional polarization is not observed in cells isolated from their neighbors and is abrogated when engagement of E-cadherin is disrupted in epithelial tissues.

Experiments using cells cultured on micropatterned ECM have also established that spatial patterns of endogenous stress regulate cellular differentiation programs including epithelial-mesenchymal transition (EMT). EMT is a shift in cellular phenotype whereby epithelial cells lose both their intercellular contacts and apicobasal polarity and activate migratory machinery, thereby acquiring mesenchymal attributes. EMT is critical for gastrulation and formation of the neural crest and heart valves (108). In regions of high endogenous stress, epithelial cells exposed to TGFβ undergo EMT, whereas cells in areas of low stress retain their epithelial phenotype (109). Likewise, differentiation, another cellular program fundamental to development, exhibits spatial variation in cell-lineage commitment. Mesenchymal stem cells cultured on micropatterned

ECM commit to an osteogenic lineage in regions of high endogenous stress and to adipogenesis in regions of low stress (110). As both EMT and lineage commitment require changes in the expression of specific genes, tissue-scale mechanics must induce signaling pathways that activate transcription factors, thereby connecting to cell phenotype.

EMT: epithelial-mesenchymal transition

Dynamic Motion Within Epithelial Tissues Organizes Populations of Cells

As evidenced thus far, an epithelial tissue is very dynamic. In addition to the continuous coordinated cellular rearrangements that modulate tissue geometry, cells also move individually and collectively throughout the tissue. These movements are governed, at least in part, by differences in intercellular adhesion (111), which direct tissue sorting and segregation. Several studies have demonstrated the sorting of cell populations both in culture (112–114) and in vivo (115, 116) (**Figure 3*b***). Cells transfected to express different quantities of cadherins reveal that the surface tension of a group of cells is proportional to the concentration of membrane-associated cadherin (117). The resulting difference in surface tension forces the less cohesive group of cells to envelop the more cohesive population, thus producing self-sorted tissues.

However, differential adhesion is not the only mechanism by which cells can sort themselves. Differential contraction is also consistent with the envelopment phenomenon that results in cell sorting (118). Experimental and numerical studies have shown that differences in cortical tension (contractility) may contribute to cell sorting in zebrafish embryos (119, 120). Furthermore, differences in cell motility promote sorting behavior in engineered tissues (121). Motile populations of mammary epithelial cells that express varying levels of the membrane-anchored matrix metalloproteinase-14 had differential directional persistence times resulting in spatial patterns of cell sorting in engineered tubules.

The mechanisms to collect or segment cell populations appear critical for proper organ morphogenesis. Cells from adjacent tissue compartments do not intermingle, thus forming smooth boundaries. The classic examples of cell compartments are found in the *Drosophila* embryo: The wing disc is separated into anterior-posterior and dorsal-ventral compartments, whereas the body segments have anterior-posterior compartments. The mechanism used to keep compartments separate is unclear; however, in addition to differential adhesion and contractility, experimental observations have pointed toward an interfacial actomyosin cable that acts like a "fence" to prevent cells from moving between compartments (122) (**Figure 3*c***). Filamentous actin and nonmuscle myosin II are enriched at the interfaces between compartments (122), forming cable-like structures that may increase tension along compartment boundaries and create a physical barrier that prevents mixing of cells.

In addition to the dynamic processes of self-assembly, sorting, and compartmentalization that have been discussed thus far, migration—specifically for this discussion, collective cell migration—also occurs within the tissue. During collective migration, cells maintain junctions with their neighbors and move as multicellular groups, often as clusters, sheets, or strands. Collective migration has been observed in multiple tissues in vivo, including neural crest, *Drosophila* border cells, and epithelium of the gut mucosa (123). Cell-cell communication is crucial for directed cell migration; however, the precise mechanisms that control collective motility are still being elucidated. In addition to chemokine gradients and tugging forces from neighboring cells, communication through the ECM may direct collective migration (124). When cultured on soft substrata, epithelial cell–generated deformations precede and correlate with collective migration (125, 126), thereby implicating an additional mechanism by which microenvironmental mechanics may regulate tissue movements. Collective cell migration and the fundamental tissue-level morphogenetic

movements discussed herein establish the basis for tubulogenesis and branching morphogenesis, the underlying processes needed to build ramified organs.

TISSUE ORIGAMI: SCULPTING A TUBULAR ARCHITECTURE

Placode: a thickened region of an epithelium that develops when cells change from a cuboidal to a columnar shape

Circulatory, respiratory, and secretory organs are all built from networks of tubes. Thus far, we have discussed tissue-level mechanics and dynamics in the context of epithelial sheets, as these provide simpler models, both in culture and in vivo, to study the integration between forces, cellular interactions, and morphogenetic movements. However, morphogenesis of ramified organs requires that epithelia bend and fold to create three-dimensional (3D) structures. The formation of the head fold in the chicken embryo (127), formation of the ventral furrow in the *Drosophila* embryo (128), and closure of the neural tube in the *Xenopus* embryo (129) are all examples where endogenous cellular forces drive the development of 3D structure through one or more physical mechanisms including differential growth, convergence and extension, cell wedging from coordinated apical constriction, and local epithelial contraction and stiffening. These physical mechanisms that drive the initial morphogenetic movements in the embryo are also required for the formation of tubes.

Despite the diversity and complexity of the epithelial tubular structures throughout an organism, the lumen of the mature duct is always defined by polarized cells. Apicobasal polarity is generated and maintained by polarity complexes including PAR, Crumbs, and Scribble (130). These complexes organize and modulate the microtubule cytoskeleton and membrane trafficking, in part, by regulating Rho GTPase through GAPs and GEFs (130). The apical surfaces of the epithelial cells, stitched together by tight junctions, form the wall of the lumen. In mammals, tight junctions sit apical to AJs and are formed by transmembrane proteins, primarily claudins and occludins, that physically join neighboring cells (131). These intercellular junctions impart the barrier function to the epithelium and serve to segregate the apical and basolateral portions of the plasma membrane.

Rolling, Budding, and Hollowing Epithelia to Make Tubes

Whereas tubular organs differ vastly in shape and function, only a handful of strategies to make tubes have been revealed (132–134) (**Figure 4*a***). Wrapping and budding act to form tubes from polarized epithelial cells. In both cases, a localized placode induces the epithelium to form a trough or cup-like invagination, the edges of which come together, seal, and separate from the original epithelial sheet. In general, invagination results from coordinated apical constrictions that pinch cells into a wedge-like shape (128, 135) and locally bend the epithelial tissue. In contrast, budding, which can be thought of as lateral branching, forms a tube roughly orthogonal to the original epithelial sheet while maintaining a continuous lumen. Budding is observed in the *Drosophila* primary tracheal sacs (136) and salivary glands (137) as well as in the vertebrate lung (138), kidney (139), and pancreas (140).

Nonpolarized cells use different processes to form tubes and simultaneously establish polarity. Cavitation forms lumina in the mammary (141) and salivary glands (142) in vivo and in mammary acini in 3D culture models (143). During cavitation, cells in the center of a thickened cluster undergo selective apoptosis, whereas those contacting the matrix become polarized, resulting in a patent epithelial tube. In contrast, chord hollowing relies on cell polarization and apical secretion to form microlumina between cells to generate tubes in the absence of cell death. Nonpolarized cells migrate out from a polarized epithelium to form a chord-like structure measuring a few cells in width. These cells then polarize and form microlumina that coalesce into a central lumen.

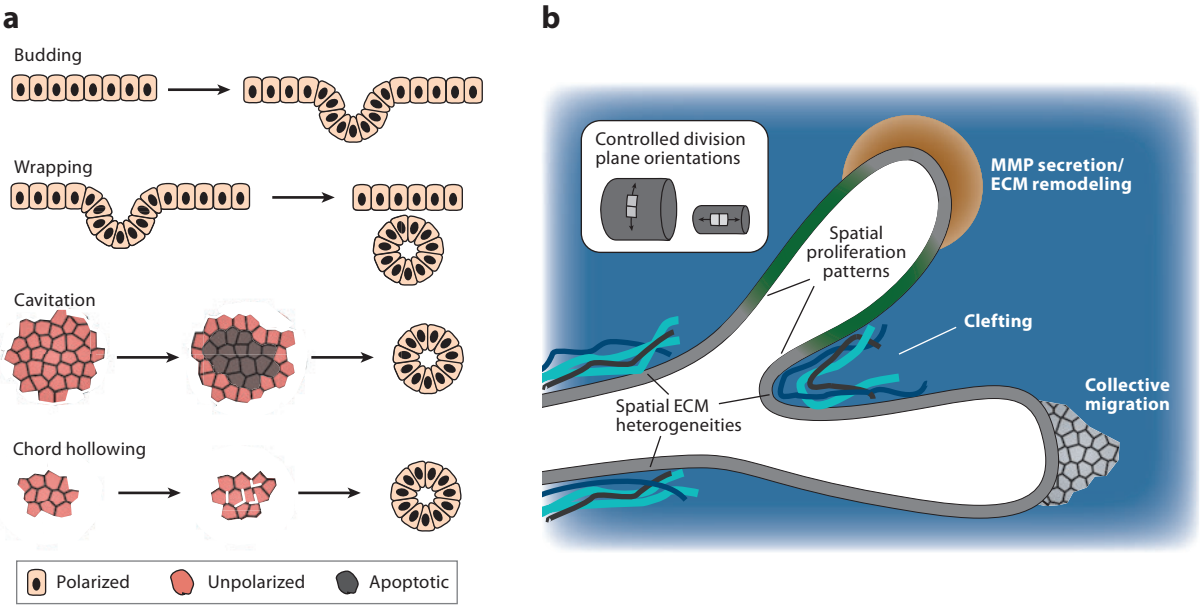

Figure 4

(*a*) Mechanisms of tube and lumen formation. (*b*) Some of the physical mechanisms that have been identified in branching morphogenesis. Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase.

Chord hollowing has been observed in the zebrafish gut (144), *Caenorhabditis elegans* gut (145), *Drosophila* heart (146), and MDCK (Madin-Darby canine kidney) cells in culture (147). Tubes can also form via intracellular hollowing, as in *Drosophila* tracheoles (148), whereby vesicles coalesce within a single cell to form a long vesicle that spans the cell and eventually connects to the lumen of neighboring cells.

Defects in the regulation of lumen size often result in dysmorphic branching and loss of organ function. Tube stenosis in the circulatory system can lead to ischemia (149), whereas tube dilation and cyst formation are hallmarks of polycystic kidney disease (150). One of the main physical mechanisms by which the epithelium regulates lumen size is apical secretion. Lumenal fluid maintenance by regulated apical ion transport and vesicle-mediated endo- and exocytosis (151–153) is a key function of epithelial cells lining the lumen. In addition to modulating the biochemical composition of the lumenal fluid, the barrier function of the epithelium, provided by tight junctions, coupled with apical transport imparts the ability to control transmural pressure.

Fluid regulation is integral to the ultimate function of the tube; however, fluid transport is also a key regulator of lumen size (152, 153). In the zebrafish gut, secretion into microlumina is required for condensation into a central lumen during chord hollowing (144). Conversely, secretory pulses followed by a rapid fluid endocytosis drive the expansion and maturation of the *Drosophila* trachea (151). In addition to ion and fluid transport, the *Drosophila* tracheal epithelium secretes and remodels several proteins including chitin that act as an apical ECM (154, 155). This lumenal polysaccharide-rich ECM guides the diameter of the final trachea; mutations in genes involved in the synthesis, secretion, or remodeling of chitin cause dilation or constriction of the trachea (156). Although a role for an apical ECM has been demonstrated in *Drosophila* (157, 158), no equivalent structure has been found in vertebrates.

Mechanical Regulation of Branch Initiation

Tube extension and branching occur through a few distinct mechanisms including cell-shape change, rearrangements, migration, and spatially patterned or oriented proliferation. Organs such as the *Drosophila* trachea and salivary gland arise from cell elongation and migration because proliferation is completed in these tissues prior to branching (137). Conversely, the *Drosophila* hindgut and vertebrate neural tube extend through convergent extension, elongating the tube while decreasing its diameter (159, 160). Differential proliferation is observed in the murine ureteric bud (161) and lung (162), with increased cell division at nascent buds compared with nonbudding regions of the proximal ramified network. Furthermore, controlled orientation of the cell division plane drives branch extension in the murine kidney and gut (163, 164) and the chick neural tube (165); it also influences the diameter and length of murine airways (166).

Confirming expectations, the most dynamic processes occur at the extending or budding regions of the tube (**Figure 4*b***). Polarity, motility, and matrix remodeling are coordinated during branching and tube extension. Cell polarity is regulated dynamically throughout the branching process. In some organs, such as the *Drosophila* salivary gland and both the murine and zebrafish kidneys, the epithelium remains polarized throughout morphogenesis (167); however, in other systems, such as the murine mammary gland (15) and MDCK cells in culture (147), a partial to complete loss of polarity occurs in cells located in the distal tip. For the end bud to migrate outward during branch formation and extension, the cells reorganize the ECM in part by locally secreting matrix metalloproteinases, as observed at the invasive fronts of mammary and ureteric buds (168, 169). Collective cell migration is commonly employed to extend branches into the surrounding matrix (15, 167); however, clefting drives branching in organs such as the murine salivary gland (170, 171). Spatial assembly of fibronectin determines sites of clefting and, thus, patterns branching of the salivary gland. Similar heterogeneities are found in the distribution of ECM proteins surrounding the murine lung and kidney epithelium: Dense accumulations of fibronectin are found in the clefts and along the main branches of the airways (170). The exact mechanism that guides the direction of branching is unknown; however, it is likely that the morphology of the branched structure is influenced by soluble signals as well as the mechanical and material properties of the ECM.

Developing a Branched Architecture

One of the central questions in branching morphogenesis remains: How are these branching patterns and ultimate branching architecture generated? As described above, the primary focus has been on genetic and molecular regulation of branching; however, computational and experimental studies highlight the importance of the stroma in defining the branching architecture. Mathematical and mechanical models have been used to describe the branching patterns found in vivo. In secretory organs with stochastic branching, such as the mammary gland, branching patterns are well described by space-filling mechanisms regulated by repulsion caused by gradients of inhibitory morphogens (24). However, in other ramified organs in which the branching architecture is controlled more tightly, such as in the vertebrate lung and kidney, the mechanisms that determine the highly regular stereotyped branching patterns are not well understood. In stereotyped organs such as the vertebrate lung, the iterative use of a few basic branching patterns produces complex self-similar geometries that form patterns with a fractal dimension of 1.6 ($y \propto x^{1.6}$) (172). Mechanical models that have been used to describe branching morphogenesis and the resulting architecture have focused, in large part, on tissue-scale interactions between the epithelium and the mesenchyme or stroma. These continuum models describe the aggregate behavior of

many cells, as opposed to tracking individual cells. Due to the long timescales of branching, the short-term elastic components of the viscoelastic tissues are ignored and the tissues are modeled as fluids, having a viscosity and surface tension. These mechanical models that attempt to capture the dynamics of tissue morphogenesis have provided new interpretations for the role of the mesenchyme as a physical sculptor of branching architecture. In one such model (173), viscous fingering phenomena, which occur when a less viscous fluid (epithelium) is pushed into a more viscous fluid (mesenchyme) in a Hele-Shaw flow, have generated branching architectures that occur on the same time and length scales as branched embryonic organs. The differential surface tensions at the fluid-fluid interface coupled with the pressure gradient resulting from one fluid being pushed into the other generates a near-equilibrium instability that results in self-similar branching patterns reminiscent of the airways of the lung. Although these mechanical models do not prescribe a specific mechanism for branching morphogenesis or pattern formation per se, they do demonstrate the possibility of a mechanical function for the mesenchyme and the importance of differential mechanical properties between tissues in organ morphogenesis.

The formation of branching architectures observed in vivo requires a mesenchyme to surround the epithelium. Branching does occur in ex vivo culture of murine lung and kidney explants from which the mesenchyme has been removed (174, 175); however, the stereotyped branching architecture is lost. Enzymatic removal of collagen from the ECM of the lung, salivary gland, and ureteric bud causes dysmorphic branching (176, 177), whereas treatment of explant cultures with collagenase inhibitors enhances branching of the murine salivary gland (178). Studies using explanted murine lungs and kidneys (179, 180) as well as organotypic models of the mammary ducts (90) have identified cell contractility as one modulator of branching morphogenesis. Disrupting actomyosin contractility inhibits branching of explants in a dose-dependent manner, whereas accelerated hyperplastic branching arises from activation of RhoA. Similarly, in 3D cultures of engineered mammary epithelial tubules, actomyosin contractility is required for branching, and an increase in contractility generates higher endogenous stress gradients and increases branching (90). Furthermore, sites of branching in the lung are coincident with regions of basement membrane thinning (180), whereas regions of low inhibitory morphogen concentration and high traction stress, resulting from the epithelium pulling on the stroma, establish the sites of branching in engineered mammary epithelial tubules (90). Importantly, these studies implicate actomyosin contractility coupled with the physical presence and geometry of the stroma as potential modulators of branching morphogenesis.

Much of our understanding of the physical mechanisms of tubulogenesis and branching morphogenesis has focused on endogenously generated forces and stress fields. However, exogenous forces that result from fluid pressure and flow also act as physical regulators of organ development. Decreasing fluid pressure and shear stress by blocking fluid flow prevents prescribed looping of the heart tube and valve formation in the developing zebrafish heart (181). It is not surprising that the physical forces from blood flow would contribute to cardiac morphogenesis; however, less intuitive is the critical need for pressure and intraluminal flow during development of the lung and kidney (**Figure 5**). In the developing embryonic lung, the airway epithelium secretes fluid into the lumen (4), and the trachea is normally obstructed because the larynx is closed (4). Combined, these features create a transmural pressure on the order of 200–400 Pa (1.5–3 mm Hg) in several animal models (182, 183), maintenance of which is required for normal branching. Cyclic changes in pressure and fluid flow from peristaltic movements of smooth muscle around the airways as well as fetal breathing movements at later stages of development affect the elaboration and growth of the airway epithelial tree (184, 185) as well as differentiation of alveolar cells and production of surfactant (183). The pressure waves from fluid flow cause cyclic strain in the distal tips of the airways (182) and generate geometry-dependent spatial gradients of force on

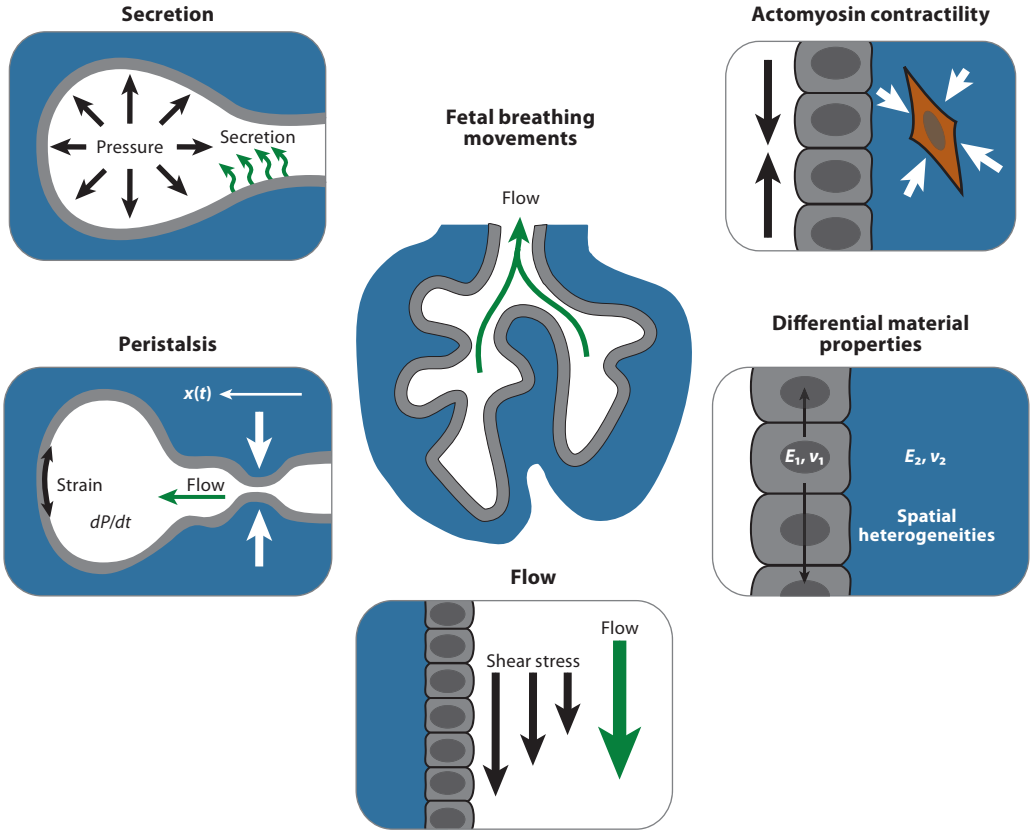

Figure 5

Description of the mechanical environment during lung development. Fetal breathing movements decrease transmural pressure by enabling flow to leave the lungs, whereas pressure is globally increased by epithelial secretion. Local distention and pressure gradients are generated by cyclic peristaltic waves, and the epithelia are exposed to fluid shear stress resulting from mechanical perturbations causing fluid flow. Additionally, tissue-dependent material properties including the modulus (E) and Poisson ratio (ν) shape the nonuniform contractility of the epithelia and mesenchyme to produce gradients in solid stress throughout the developing lung.

the developing tissue. Experimentally decreasing the transmural pressure (186), inhibiting fetal breathing movements (187), or preventing smooth muscle peristalsis (188), and thus flow, results in hypoplastic branching of the lung, whereas increasing the pressure or the rate of peristalsis (41, 188) produces hyperplastic branching. Similarly, fluid flow within the pronephron of the zebrafish kidney is critical for migration of epithelial cells to define nephron segment boundaries and for the convolution of the proximal nephron tubule (167). Blocking flow within the pronephron prevents both cell migration and convolution. As additional data are collected that establish a role for exogenous forces from fluid flow as modulators of branching morphogenesis, we may hypothesize that organisms use fluid flow to regulate morphogenesis of highly stereotyped branching architectures, such as those found in the lung, kidney, and circulatory system.

Indeed, the critical effects of external forces on branching morphogenesis of the mammalian lung are at odds with the current genetic-clock model of stereotyped development. As described above, signaling through FGF10 and Sprouty are thought to control the rate of branch extension, and thus branch spacing, in the iterative extension-arrest-bifurcation program that is observed

during branching morphogenesis of the murine airways. If a genetic clock served as the master regulator, then mechanical manipulations would be expected to have no effect on the final branching pattern. Clearly this is not the case, and developing ramified tissues integrate mechanical forces with biochemical signals as they sculpt their final forms.

FUTURE DIRECTIONS

The precise mechanisms used to build epithelial trees remain enigmatic. Do common strategies or signals used to generate the diverse networks of tubes exist within an individual organism? Clearly, one such set of signals arises from the mechanical environment. However, the signals that instruct the ultimate tissue architecture are likely to be complex, and "appropriate" cellular responses needed for morphogenesis require integrating multiple types of environmental cues. As such, the microenvironment provides instructions—physical, chemical, electrical, and spatial—that enable self-assembly, collective behaviors, and the forces that drive morphogenesis. Understanding the rules by which cells interpret and react to their environment from these integrated stimuli across multiple length scales—cell, tissue, and organ—will be the key to unraveling the mystery of how multicellular organisms develop complex ramified networks necessary to support life.

SUMMARY POINTS

1. Isometric tension generated from actomyosin-mediated contractility regulates cell behaviors.
2. The structure of epithelial tissues generates gradients in endogenous mechanical stress that feed back to sculpt the tissue form.
3. Cellular contractility regulates tube formation and branching.
4. Exogenous forces are essential in the development of branched organs.

DISCLOSURE STATEMENT

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