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Fluid mechanics as a driver of tissue-scale mechanical signaling in organogenesis

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Abstract

Purpose of Review—Organogenesis is the process during development by which cells self-assemble into complex, multi-scale tissues. Whereas significant focus and research effort has demonstrated the importance of solid mechanics in organogenesis, less attention has been given to the fluid forces that provide mechanical cues over tissue length scales.

Recent Findings—Fluid motion and pressure is capable of creating spatial gradients of forces acting on cells, thus eliciting distinct and localized signaling patterns essential for proper organ formation. Understanding the multi-scale nature of the mechanics is critically important to decipher how mechanical signals sculpt developing organs.

Summary—This review outlines various mechanisms by which tissues generate, regulate, and sense fluid forces and highlights the impact of these forces and mechanisms in case studies of normal and pathological development.

Keywords

morphogenesis; ion channels; mechanotransduction; birth defects; mechanics of development

Introduction

Morphogenesis, the process of cellular self-assembly into tissues and organs, is a series of dynamic and coordinated events that are influenced not only by genetic programs but also physical and chemical microenvironmental cues. Importantly, the morphogenesis of organs, termed organogenesis, is a complex, multi-scale process whereby local changes in cell behavior or gene expression induce spatial patterns in global tissue architecture essential for subsequent function. Tissues bend, buckle, branch, and fold to create astoundingly complex architectures necessary for proper organ function, and dysregulation of this process is the basis of several birth defects and account for morbidity well into adulthood [1]. As such, much effort has been dedicated to understand the regulators of tissue geometry and spatial

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Conflict of Interest

Rachel Gilbert, Joshua Morgan, Elizabeth Marcin, and Jason Gleghorn declare that they have no conflict of interest.

Compliance with Ethical Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

patterning in organogenesis; i.e. why do cells in one region differentially proliferate, change shape, or migrate whereas others a few cell diameters away remain quiescent?

Decades of innovative work have identified numerous molecular regulators of gene transcription, intracellular signaling, and cell and tissue crosstalk. However, organogenesis is a physical process with cells moving, pulling, and rearranging to enable organ growth and progression to a final architecture. As tools from the physical sciences have advanced and been applied to these biological questions [2], a greater appreciation of the role of mechanical forces in organogenesis has emerged. Several excellent reviews have been written on this topic [3–5]; however, the focus of these reviews and much of the research in general is centered on contributions of solid mechanics to organogenesis, e.g. cell contractility, tension within epithelial tubes, and tissue stiffness. Our focus in this review is on the importance of understanding the multi-scale nature of the mechanics that sculpt developing organs, and highlighting the less appreciated role of fluid mechanics as regulators of organogenesis.

Multiscale mechanics and mechanotransduction

Understanding the genetic changes that affect molecular signaling has been at the forefront of research in the developmental biology field. The expression of key autocrine and paracrine molecules such as fibroblast growth factor (FGF), Notch, β -catenin/Wnt, sonic hedgehog (SHH), and transforming growth factor beta (TGF- β) have been implicated in controlling zygote asymmetry, cell-cell communication, and cell fate, polarity, adhesion, and migration [6–8]. However, during organogenesis, mechanical changes have been shown to act as developmental cues that regulate patterning and differentiation [1,4,9].

To fully appreciate how mechanical forces sculpt tissue and organ morphogenesis, we must consider the origin and regulation of these mechanical forces and subsequent tissue deformations within the developing multicellular organ, and how those forces are subsequently transduced into altered cellular function. Much of the progress in our understanding has centered on the latter aspect – cellular mechanotransduction. Several cellular mechanosensors have been identified that transduce extracellular forces into intracellular molecular signaling cascades and gene transcription events [1]. For example, significant work has, in part, uncovered mechanisms of integrin engagement and downstream signaling in cell-extracellular matrix (ECM) interactions that regulate cell contractility, adhesion, spreading, and migration [10–13]. Extracellular domains of transmembrane integrin proteins are physically bound to ECM ligands and transmit forces that are applied to the cell. The intracellular integrin domains are physically connected via a series of protein complexes to the cellular cytoskeleton and the cell dynamically modulates and responds to the cytoskeletal tension. One mechanism by which cytoskeletal tension is then coupled to gene expression changes occurs through the linker of nucleoskeletal and cytoskeletal (LINC) complex and nuclear lamins [14]. However, this outside-in mechanotransduction is only part of the story as it is well understood that cells also use these same physical connections to apply forces to their surroundings.

Cells are able to push and pull on surrounding matrix or on neighboring cells to produce different force patterns within a tissue. Cell-matrix connections and cell-cell junctions enable the transmission of force over multi-cell length scales and geometry-dependent spatial patterns of endogenous mechanical stress emerge from the multicellular system [2,15–17]. For example, cardiac looping of the heart tube during early embryogenesis has been shown to be driven by global forces within the heart tube itself [18]. Cardiac looping represents the first major left-right symmetry breaking in vertebrates and first involves bending and subsequent twisting of the heart tube into position [18], driven by differential internal forces within the heart itself and external forces from neighboring tissues, respectively [19–21]. Cardiac looping is but one example of relatively simple mechanical cues guiding the emergence of complex and functional architectures. Increasingly, research into the development of different organs have revealed numerous parallels, and repeated use of a few mechanisms to generate, sense, and regulate the fluid mechanics within the embryo.

Generating, sensing, and regulation of fluid mechanics

As deceiving as their final complex and integrated structures may be, many organs originate from single tubular structures. In addition to the primitive heart tube example above, other organs, such as the lung and kidney, likewise originate as a simple tubular structure that undergoes repeated patterns of branching to define the highly branched architecture of the resulting organs. Despite their diverse functions, it is instructive to understand the structural commonalities that these organs share with other organs throughout our body such as the mammary gland, prostate, pancreas, and vasculature. Typically, there is a highly interconnected lumen lined by a continuous polarized epithelial or endothelial layer supported by a stromal cell population. The cellular and structural makeup of these tubular systems varies widely in accordance with organ function, but this fundamental structure allows for distinction of intra- and extra-luminal fluid pressures and composition. Further, this architecture allows for coordinated action of a tissue to generate, sense, and regulate luminal fluid forces. Polarized epithelial or endothelial cells act as a barrier between the lumen and the surrounding tissue, guiding the motion of fluid, ions, and macromolecules into and out of the tubular network. This barrier function can perhaps be most intuitively understood in adult organs such as the kidney, where regulated secretion into the collecting ducts allows for the removal of waste, or in the lung, where the epithelial barrier must restrict liquid to facilitate gas exchange. In these cases, we can readily associate compromised organ function with failure of the barrier function; e.g. pulmonary edema resulting from liquid leaking across the airway epithelium into the lumen.

As the lumen spaces in developing organs are entirely liquid filled, proper partitioning of fluid across lumens is increasingly appreciated to be an essential component of development. This partitioning allows for different external versus internal fluid mechanics, such as fluid pressures and flows, that can create spatial patterns to influence local growth behaviors. Here, we introduce the general mechanisms by which luminal fluid composition and movement is regulated, how this regulation influences tissue mechanics, and the manner by which these mechanics are sensed at the cellular and tissue levels.

Regulation of Tonic Pressure via Solute Secretion

Fluids are secreted to pressurize and ‘inflate’ the developing lumens. Fluid secretion is driven by osmotic gradients that are produced following ion or other solute flux [22]. This gradient is often created and maintained by polarized epithelium through the active secretion of ions or other osmolytes, such as proteoglycans, into the lumen. Water is then drawn into the lumen through paracellular or transcellular pathways, swelling the organ. This increased fluid will exert force outwards on the developing organ which produces a static tissue strain from the tonic luminal pressure (Figure 1A). Importantly, this luminal pressure can influence large-scale organ development as this force and resulting strain acts on the tissue level, rather than single cell length scale.

Regulating Fluid Motion via Motile Cilia

In addition to facilitating the fluid accumulation that induces static pressures, epithelial cells can induce large-scale fluid motion using cilia. New interest in cilia was sparked with recent investigations relating their function to intracellular signal transmission, growth regulation, and fluid motility during fetal development [23]. Cilia are specialized, small, hair-like organelles with a structure of microtubules that protrude from the epithelium into the lumen [23]. Motor proteins within the cilia allow the cilia to actively ‘beat’ to drive fluid motion. When cilia movements are coordinated across the entire epithelium, large scale flows can be generated (Figure 1B). A prime example of this in adult tissues is the clearance of mucus from the airways, which is essential for clearing foreign pathogens and chemicals from the lungs. Additionally, research within past few decades has identified the important role that larger scale fluid flows, generated by coordinated cilia rotations, have during development including left-right (LR) symmetry breaking and tissue specification [24,25]. In the earliest stages of mouse embryogenesis, motile cilia rotate clockwise to create and monitor a leftward fluid flow across a medial fissure, called the node [26]. Peripherally located crown cells have non-motile cilia that act as mechanosensors by detecting slight differences in forces created by the fluid flow and triggering differential expression of specialized proteins and signaling pathways, eventually leading to LR symmetry breaking [26,27].

Regulation of Dynamic Pressure via Smooth Muscle Contraction

In addition to epithelial cell involvement, fluid pressure and motion can also be mediated by mesenchymal cells surrounding the lumen via active contraction. In many developing organs (e.g. lung, gut, ureter), smooth muscle wraps circumferentially around the epithelial-lined lumen. Coordinated peristaltic contraction of the smooth muscle propels fluid within the lumen, inducing locally altered fluid pressure and tissue distention. As this distention is produced by a directional peristalsis, this allows for patterning of tissue strain to cells in specific regions, enabling spatially organized activation of mechanosensitive pathways (Figure 1C).

Sensing Forces via Mechanosensitive Ion Channels

To regulate fluid forces, cells within the developing organ must be able to sense changes to their mechanical environment and alter their behavior (e.g. epithelial secretion) to achieve proper force balances. Several mechanotransduction pathways are known, and some of the

most prominent and well-studied mechanosensitive molecules are ion channels. Mechanically active ion channels (MAIC) are transmembrane proteins that change conductivity (i.e. modulate ion flux through the membrane) in response to mechanical forces (Figure 1D). Changing the balance of ions inside the cell activates a host of downstream pathways that can regulate proliferation, apoptosis, metabolism, and differentiation. MAIC are found in a wide range of cell types [28], although lack consistent homology. Of the identified MAIC, there is a wide range of tertiary structures, with anywhere from 2 to 38 transmembrane domains, no known shared homology among all genes, and a range of dimerization and oligomerization behaviors [28]. Despite these knowledge gaps, a growing number of studies (discussed in a later section) using genetic knockouts have highlighted the essential role of these molecules in proper development, emphasizing the role of mechanotransduction in the formation of complex tissues.

Dysregulation of Fluid Pressures and Partitioning Impairs Development

Highlighting the importance of fluid mechanics during development, improper *in utero* fluid pressures can cause a variety of congenital abnormalities. During gestation, the embryo is entirely liquid filled, and global fluid pressure irregularities during development are associated with a variety of issues. Hydrops fetalis, which involves fluid accumulation in organ spaces such as the heart, lungs, or abdominal cavity, is often associated with polyhydramnios, or too much amniotic fluid. These conditions can cause problems including premature birth, placental abruption, and stillbirth. Conversely, too little amniotic fluid, termed oligohydramnios, is also problematic resulting in a variety of birth defects and can cause miscarriage, still birth, or premature birth. How these gross changes in fluid partitioning are transduced to tissue and organ abnormalities remains unclear and these are likely coupled to changes in the balance of fluid forces on, and within, developing organs.

Examples of fluid mechanical regulation of organogenesis

Cardiovascular Morphogenesis and Remodeling

Cardiovascular morphogenesis is a well-established example demonstrating the relevance of fluid mechanical forces during morphogenesis and remodeling. Fluid forces are often thought about in the context of the heart and vasculature, which are known for their ability to induce and tolerate large amounts of fluid flow and pressure in both the adult and fetal systems. Besides moving blood to supply oxygen to the developing tissues, these flows can also define local cellular gene expression patterns as is the case with site and initial formation of the heart valves [29–31]. The primitive valveless heart behaves as a dynamic suction pump whereby local contraction of cardiac muscle propagates a bi-directional contractile wave that reflects back to create negative pressure gradients that will “suction” blood to create flow [32]. These oscillatory blood flows were recently shown to control the essential valvulogenesis gene *klf2a*, as sensed by the MAICs TRPV4 and TRPP2 [30]. These blood flows also influenced the expression of miRNA-21 [33], another molecule essential for valvulogenesis. Several computational and physical models support the role of hemodynamic forces driving valve formation [30,34,35].

Though not fully understood, cilia also play a complex role as mechanosensors in the morphogenesis of the developing heart. In the first stages of murine heart development, around embryonic day (E)8, cilia primarily function to generate flow and influence heart LR symmetry breaking [36]. Mutations that affect ciliary motion thus present clinically as LR malformations, and not as direct cardium-ciliary defects [36]. Between E9–E12, however, cilia motility is less pertinent to heart morphogenesis and cilia switch to primarily function as mediators of cell signaling pathways [36,37]. However, they are suspected to still have a mechanotransduction role to sense contraction-induced blood flow through the developing heart [36]. A study of fetal mouse hearts with mutations in the genes *Kif3a* (causes a loss of cilia) or the ciliary-localized *MAIC Pkd2* (prevents cilia from sensing flow), exhibited hearts with little to no mesenchymal cells in the developing endocardial cushions, eventually leading to severe structural defects of the AV valve. The dysfunctional cilia associated with this low number of mesenchymal cells suggests that cilia mechanosensation of blood flow plays a vital role in the proliferation of mesenchymal cells, and is therefore integral to heart organogenesis [36]. Further research has associated atrial-ventricular septal defects with mutations that result in deformed tubules and limited motility in cilia, marking cilia directed fluid flow and mechanosensation as a fundamental aspect of proper heart development [37].

In normal mice, the vascular system differentiates from surrounding mesenchyme in each organ, that is remodeled into a mature, organized network [38–41]. Much remains to be understood about this remodeling process, but it is thought that mechanical forces produced by active circulation play a critical role [40,42]. Consistent with this, the *MAIC Piezo1* was identified to play a distinct role in vascular morphogenesis [43,44]. Mice lacking *Piezo1* did not undergo normal vascular remodeling and experienced embryonic lethality [43,44]. However, it remains unclear exactly how *Piezo* channels are mechanically regulated as their function is controlled in part by a number of accessory proteins and the composition of the lipid membrane [45–47]. Given the importance of *Piezo1* in vasculature development, there is a widely unexplored area of the role of *MAICs* in other lumenized developing structures that warrant further investigation.

Lung Morphogenesis

Studies of embryonic lung development highlight a critical role for mechanical forces in regulating branching of the airways. A tonic positive pressure within the developing airways is created due to fluid secretion from the epithelium and a closed epiglottis. Initially, chloride (Cl^-) is actively pumped into the luminal space by CFTR and other channels [48–53]. The Cl^- gradient then drives a neutralizing ion flow of sodium and water through leaky cell junctions into the lumen [52,54,55]. Emphasizing the importance of transmural fluid force balance and regulation, in cases of oligohydramnios, or less than usual amniotic fluid, the external pressures acting on the organs are decreased and the resulting lungs are hyperplastic [56]. In addition to luminal pressures generated via secretion, dynamic pressures are generated from smooth muscle that is circumferentially wrapped around the proximal airways and absent at the actively growing distal tips [57–59]. Co-ordinated proximo-distal contraction of the airway smooth muscle moves the luminal lung fluid through the airways towards the closed distal tips in a peristaltic-like wave [60–62] (Figure 1C). Airway smooth muscle, once termed vestigial [63,64], is now thought responsible for

triggering local growth events by mechanically stretching the distal airway epithelium to pattern growth to these regions, leading to further airway branching [1,6,65–67]. These contractions are driven by calcium flux [68–71] and *ex vivo* lung culture has shown that alteration of contraction frequency is directly correlated to growth [67,72], with higher contraction frequencies producing an increased number of airways.

The importance of lung luminal pressure in fetal development has been leveraged clinically. Chemical disruption of the Cl^- flux [48,73–75] or draining of the lung fluid [76,77], decreases lung pressure, tissue distension, and results in an under branched lung, termed lung hypoplasia. Conversely, artificially increasing luminal pressures has shown to rescue lung hypoplasia in animal models. These results have been used clinically to develop a procedure called fetal endoscopic tracheal occlusion (FETO) [78]. FETO uses a small balloon inflated within the intubated fetal trachea to block fluid efflux out of the lung and increase the fluid pressure within the developing lung. FETO and strategies used to increase transepithelial Cl^- flux have been attempted in utero to increase the luminal fluid pressure with modest success to date in humans [78–80]. As such, it is clear that the in utero mechanical environment is complex and additional study is needed to understand how pressure and/or local dynamic pressures and flows from peristaltic smooth muscle contractions spatially induce cellular mechanotransduction pathways that regulate lung morphogenesis.

Renal Morphogenesis

A large range of diseases fall into the category of congenital abnormalities of the kidney and urinary tract, otherwise known as CAKUT. Several of these diseases arise due to dysregulated fluid forces leading to improper cellular signaling, organ malformation, and disease. As in the lung, fluid pressure must be balanced for proper kidney organogenesis. In the kidney, there is evidence that ion channel secretion is a key aspect of the CAKUT polycystic kidney disease (PKD). In PKD, increased fluid secretion, mediated by Cl^- flux, drives the formation of cysts [81]. Additionally, cysts were shown to form in cases where of a blockage in urine flow out of the kidney [82–85]. These findings suggest that maintenance of fluid pressure is important in normal kidney development, with an imbalance between secretion and urine flow driving cyst formation.

During the development of the renal system, multiple organs develop distinct layers of smooth muscle including the bladder, the renal pelvis, and the ureter. The CAKUTs encompassing congenital upper urinary tract disorders, including ureteropelvic junction (UPJ) obstruction and hydronephrosis, have demonstrated roots in the failure of proper smooth muscle formation around the ureter [86–93]. Immediately after separation from the placenta, urine must be systematically moved via peristalsis of the ureteral smooth muscle from the kidney to the bladder [89,94,95]. In neonates, dysregulated or absent ureter peristalsis due to disorganized or absent smooth muscle leads to build up of intrarenal pressure causing hydronephrosis and improper post-natal development of the lower urinary tract [92,94,96,97].

Further emphasizing the importance of pressure balance in kidney development, maternal rabbits with increased intra-abdominal pressure have fetuses with increased intra-amniotic

pressure [98]. This produces higher external kidney pressures, and these fetuses have decreased ureteral smooth muscle development, potentially due to the lack of proper pressures and tissue distention required to induce smooth muscle development [98]. This decreased smooth muscle may allow for larger pressures inside the kidney to maintain the appropriate pressure difference between the two compartments.

Interestingly, the hallmark renal pelvis and ureter malformations in congenital UPJ obstruction can be visible in the embryonic stage, which is prior to the peristalsis required for the movement of urine after birth [88,91–93]. This indicates that these malformations are not due to lack of urine flow. Peristalsis of the ureteral smooth muscle begins around E15.5 in mice, and aberrant smooth muscle formation indeed affects the embryonic ureteral peristalsis [88,97]. However, the potential mechanical function of the peristalsis at this early time point has not been thoroughly investigated and, similar to the lung, may be important for proper pressure regulation during early renal organogenesis.

Neural Tube Morphogenesis

The neural tube is the precursor structure that eventually becomes the various components of the central nervous system. It is lined by the neural ectoderm, which is a polarized epithelium that creates the necessary fluid pressure within this structure by controlling the internal lumen fluid composition. The ectoderm will actively transport Na^+ into the ventricular lumen through the action of ion pumps such as Na^+/K^+ ATPase [99]. Additionally, the epithelium secretes chondroitin sulphate proteoglycans to retain water within the lumen. Indeed, increasing or decreasing the presence of these osmolytes dramatically changed neural tube swelling, confirming the role of an osmotic gradient [100]. The positive pressures generated in the neural tube are important for proper morphogenesis and neural tube closure as dysregulated pressures lead to a variety of neural tube defects (NTDs) [101].

In addition to pressure dysregulation, some of the most severe NTDs are linked to altered fluid flows from malformed or immotile cilia. Similar to LR symmetry breaking, cilia in the neural tube and embryonic brain beat in a synchronized motion to regulate cerebrospinal fluid flow through the neural tube and across the ventricles of the brain [102,103]. As embryonic brain development progresses, the cilia-induced cerebral spinal fluid flow is necessary for intracellular signaling, neuronal migration, and subsequent morphogenesis [102]. It has been suggested that this flow-induced pressure in the neural tube is a necessary factor for the original differentiation and enlargement of the brain in early stages of embryogenesis [102]. New evidence shows that mutations affecting proteins necessary for ciliogenesis or ciliar motility, like *Celsr2*, *Celsr3*, and *MDnah-5*, decrease flow velocity and persistence, leading to neural malformations [103]. Neural deformities resulting from such mutations depend on the age of the embryo as well as the location and number of defective cilia [104]. Furthermore, decreased flow across the developing midbrain results in later stenosis of the cerebral aqueduct, which increases pressure in the ventricles, leading to varying degrees of cerebrospinal fluid build-up and hydrocephaly [105].

Conclusions/Future Directions

The specific cases of organogenesis of the heart, lung, kidney, and neural tube demonstrate the importance of fluid forces in regulating proper organ formation. Fluid forces are not widely studied in organ formation and yet, as exemplified in this review, play a critical role in organ development. These large-scale fluid forces create spatial patterns of mechanical forces that are sensed and integrated into signaling pathways at the single cell level. When the global mechanics are altered, cell-level gene expression is affected, and proper organ formation is disrupted leading to disease and congenital malformations. Increased understanding of these systems presents a unique opportunity for future clinical studies. By understanding the specific impacts of tissue-scale mechanics, as well as the molecular mechanisms by which cells transduce them, we can reveal novel therapeutic targets that may be exploited to mitigate or treat birth defects.

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Figure 1. During organogenesis, a variety of methods are used to move fluid around throughout the luminal compartments of diverse organs

A) Ion channels allow for fluid movement across cell layers. For example, in lung epithelium, the CFTR channel will actively transport chloride ions across the apical side of the epithelium. The elevated chloride concentration draws neutralizing sodium currents and water through the leaky cell junctions, providing a tonic pressure within the lumen. A variety of diseases can occur due to defective ion channels, which cause either too much, or not enough, fluid in organ lumen structures. **B)** Cilia are small microstructures found on the apical side of epithelium. They will beat in a uniform, circumferential motion to drive fluid flows. Other cells sense these different fluid flows and will change their growth accordingly. Cilia-based fluid flows are a major driver of symmetry breaking during development and organogenesis. **C)** Smooth muscle is found in a variety of organs during development such

as the ureter and lung. Smooth muscle can contract in a peristaltic wave that moves fluid in the same direction and can influence growth patterns by regulating local fluid pressures within specific regions of the lumen. **D)** Mechanosensitive ion channels are able to respond to tissue scale mechanical forces such as fluid pressure or fluid flow and convert these forces into cellular signals. For example, the calcium channel TRPV4 can respond to fluid flow by allowing the influx of extracellular calcium ions that produce a variety of downstream molecular and genetic effects within the cell.

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