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REVIEW

Integration of luminal pressure and signalling in tissue self-organization

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ABSTRACT

Many developmental processes involve the emergence of intercellular fluid-filled lumina. This process of luminogenesis results in a build up of hydrostatic pressure and signalling molecules in the lumen. However, the potential roles of lumina in cellular functions, tissue morphogenesis and patterning have yet to be fully explored. In this Review, we discuss recent findings that describe how pressurized fluid expansion can provide both mechanical and biochemical cues to influence cell proliferation, migration and differentiation. We also review emerging techniques that allow for precise quantification of fluid pressure *in vivo* and *in situ*. Finally, we discuss the intricate interplay between luminogenesis, tissue mechanics and signalling, which provide a new dimension for understanding the principles governing tissue self-organization in embryonic development.

KEY WORDS: Luminal pressure, Luminogenesis, Signalling, Cell fate, Tissue patterning, Tissue mechanics

Introduction

In many epithelial tissues, cells secrete intercellular fluid that leads to the *de novo* formation of the lumen (see Glossary, Box 1). Typically, lumina form on the apical domain of epithelia and are sealed by tight junctions between the cells (Alvers et al., 2014; Bryant et al., 2014; Vasquez et al., 2019 preprint). Indeed, the molecular mechanisms of *de novo* lumen formation have been studied primarily in this context (reviewed by Blasky et al., 2015; Datta et al., 2011; Sigurbjörnsdóttir et al., 2014). However, in early animal development, fluid lumina also form at basolateral compartments (Chan et al., 2019; Petridou et al., 2019; reviewed by Schliffka and Maître, 2019). The establishment of fluid lumina typically results from the release of macromolecules and solutes into the intercellular space, which creates an osmotic gradient to trigger the formation of nascent lumina. This is accompanied by the action of ion pumps, such as Na^+/K^+ ATPase, which create an inward flux of fluid and leads to lumen resolution and expansion (Bagnat et al., 2007; Navis et al., 2013; Ryan et al., 2019).

Although fluid-filled lumina exist abundantly in many epithelial and endothelial tissues, the function(s) of these lumina during development is not completely understood. In contrast to the well-established roles of actomyosin contractility and cell-cell adhesion in generating and transmitting forces within the tissue (Heisenberg and Bellaïche, 2013; Lecuit and Lenne, 2007), the hydraulic (see Glossary, Box 1) aspect of the lumen in morphogenesis has only begun to be appreciated (Chan et al., 2019; Dumortier et al., 2019; Mosaliganti et al., 2019; Navis and Bagnat, 2015; Ruiz-Herrero et al., 2017). Fluid expansion can exert substantial hydrostatic pressure (see Glossary, Box 1) to stretch the overlying tissue (Adams et al., 1990), while providing a signalling source to direct tissue patterning (Durdu et al., 2014). A fluid lumen, owing to its incompressibility, can transmit fast, long-range hydraulic force to coordinate cellular functions at the scale of an organ or whole embryo. In particular, isotropic stress (see Glossary, Box 1) exerted by hydrostatic pressure can change the tissue geometry and lead to a build up of supracellular tension. These global changes in tissue mechanics and morphology can modify cell behaviour and fate, often through the process of mechanotransduction (see Glossary, Box 1; Chan et al., 2017a; Hannezo and Heisenberg, 2019).

In this Review, we first discuss the roles of tissue mechanics in regulating luminogenesis (see Glossary, Box 1). We next survey the biophysical techniques used to quantify luminal pressure, and discuss their applicability and limitations. We then highlight recent findings revealing that the lumen may act as a spatial niche to integrate mechanical and biochemical cues and influence cell-fate specification and embryonic patterning. We conclude with a discussion of the technical and conceptual challenges, and offer future perspectives with the hope of inspiring new research in understanding the role of luminogenesis in tissue self-organization.

Biomechanical control of luminogenesis

There is growing evidence that tissue mechanics, such as that provided by the extracellular matrix (ECM), can play a key role in guiding luminogenesis. For example, an *in vitro* study in liver canaliculi has shown that ECM adhesion can cause tension asymmetry in the lumen, breaking the symmetry of the lumen shape and leading to the formation of anisotropically tubular lumina (Li et al., 2016). In the *Drosophila* tracheal tube, the ECM is anchored to the apical membrane. The growth of the apical membrane generates an expansion force, which is balanced by the elastic force from the underlying apical ECM (Dong et al., 2014). As tube elongation is constrained during development, apical membrane growth induces tissue buckling, as observed in mutants with excessive apical membrane biogenesis. This example also highlights that apical membrane synthesis can shape luminogenesis (Tsarouhas et al., 2007), in addition to fluid flux and luminal pressure. This is consistent with a recent *in vitro* study of Madin-Darby canine kidney (MDCK) cysts, which showed that the growth and stabilization of nascent lumina are controlled by apical membrane remodelling (Vasquez et al., 2019 preprint).

As the ECM is a mix of elastic networks that are swollen by aqueous solvent, they behave as poroelastic (see Glossary, Box 1) materials. The porous nature of ECM generates significant hydraulic stress that can fracture cell-cell junctions, as shown in epithelial monolayers *in vitro* (Casares et al., 2015). In spheroids and tumours, the ability of the ECM to retain fluid also contributes to high

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Development (2020) 147, dev181297. doi:10.1242/dev.181297

Box 1. Glossary

Archenteron. Hollow cavity of the digestive tract (primary gut) that forms during gastrulation in a developing embryo.

Blastocoel. A fluid-filled cavity that is formed during early development of amphibian and echinoderm embryos.

Coalescence. The process by which two or more lumina fuse to form a single lumen.

Hydraulics. A field of science related to the practical application of fluids to generate force and mechanical work. In biology, hydraulics involves pressures that are orders of magnitude smaller than the atmospheric pressure.

Hydrostatic pressure. The pressure exerted by a static fluid at equilibrium. Often it is the hydrostatic pressure difference between the inside (e.g. cytoplasm or lumen) and the outside (e.g. interstitial fluid or medium) that is important when considering lumen mechanics.

Lumen. Intercellular fluid-filled cavity, typically surrounded by epithelial cells with tight junctions that seal the fluid within the cavity.

Luminogenesis. The emergence of a lumen, which typically involves the initial phase of nucleation, followed by coalescence and expansion.

Mechanotransduction. Processes through which cells respond to mechanical cues by converting them to biochemical signals that trigger specific cellular functions.

Nucleation. The initial phase of lumen formation, characterized by the emergence of fluid-filled microlumina at cell-cell junctions.

Osmolarity. A measure of solute (osmolyte) concentration, defined as the total number of osmoles of solute per litre.

Osmolytes. Low-molecular weight molecules in fluid-filled lumina (extracellular) or cytosol (intracellular). The concentration of these solutes can affect cell volume and functions.

Osmotic pressure. The pressure required to prevent an inward flow of solvent across a semipermeable membrane, which depends on the amount of osmolytes in the fluid. At equilibrium, the osmotic pressure difference is equal to the hydrostatic pressure difference across the membrane.

Poroelasticity. The material property of a fluid-filled porous solid. Fluid pressure in the pores of the matrix can contribute to the total stress in the medium, thereby straining the porous matrix. Conversely, deformation of the porous solid can drive inward or outward flow of the fluid, similar to that of a fluid-saturated sponge.

Signalling niche. Specific regions in a tissue that provide a signalling source to trigger cellular response in the neighbouring cells.

Stiffness. The ability of a material to resist deformation under an applied stress. Physically defined as the amount of stress required to generate a certain degree of deformation (strain).

Stress. The amount of internal forces per unit area developed in an object when it is subjected to an external force. Anisotropic stress from forces acting parallel to the area of the object generates shear stress, whereas forces acting normal to the area of the object generates normal stress or pressure.

Young-Laplace Law. Physical law that states that at mechanical equilibrium, a deformable interface under tension can be curved owing to the pressure difference between the materials it separates. For the case of a simple spherical lumen, $P=2\gamma/R$, where P is the luminal pressure, γ is the interfacial tension and R is the radius of the lumen.

interstitial fluid pressure that can impact cellular migration and proliferation (Dolega et al., 2018 preprint; Stylianopoulos et al., 2018). Remarkably, an *in vitro* study of human pluripotent stem cells showed that three-dimensional ECM, together with a soft gel substrate, can induce the formation of a fluid-filled amniotic cavity surrounded by epithelial tissue (Shao et al., 2016). This study demonstrates that ECM dimensionality and matrix rigidity can control amniogenesis, a key morphological event during mammalian implantation.

In cases where stiffness (see Glossary, Box 1) dominates, the ECM imposes a mechanical constraint that leads to a build up of luminal pressure, which can be crucial for morphogenesis. For example, in mutant fish lacking vacuolated cells that make up the notochord, embryos have a shorter anterior-posterior axis (Garcia et al., 2017). On the other hand, ECM degradation leads to a shorter and kinked axis (Gansner et al., 2007), suggesting that crosstalk between ECM mechanics and intra-notochord pressure is crucial for body elongation. Indeed, similar mechanisms have also been reported for *Xenopus* notochord elongation (Adams et al., 1990). During mouse blastocyst development, mechanical confinement by the zona pellucida (a glycoprotein shell that surrounds the embryo) leads to a higher luminal pressure compared with those without the zona pellucida (Chan et al., 2019; Leonavicius et al., 2018).

Cell contractility and adhesion also impact *de novo* lumen formation. During the initial phase of mouse blastocoel (see Glossary, Box 1) formation, pressurized fluid is injected into intercellular space, possibly through a combination of cytoplasmic vesicle release and fluid inflow from outside the embryo. This causes a separation of the cell membranes and displacement of cell-cell adhesion molecules (Dumortier et al., 2019; Ryan et al., 2019), a process known as hydraulic fracturing. Furthermore, as luminal surface tension is coupled to the hydrostatic pressure of the lumen through the Young-Laplace Law (see Glossary, Box 1), cell contractility can influence the size, coalescence (see Glossary, Box 1) dynamics and the final positioning of lumen within the tissue (Chan et al., 2019; Dumortier et al., 2019). Finally, actomyosin contractility can also mediate resealing of adherens and tight junctions following impaired epithelial barrier function, which is crucial for tissue homeostasis (Casares et al., 2015; Stephenson et al., 2019).

Although we are beginning to appreciate the mechanisms of lumen formation, much less is known about the mechanisms regulating lumen deflation, which may be important for controlling pressure and preserving tissue integrity. One mechanism may involve the transient rupture of tight junctions through mitosis, as in the case of mouse blastocysts (Chan et al., 2019; Leonavicius et al., 2018). Excessive pressure can also lead to a breach of epithelial permeability and pressure relief, as shown in the endolymphatic sac of the inner ear (Swinburne et al., 2018). Other mechanisms may include the active regulation of ion pumping activity and endo- or exocytosis to modulate the lumen volume and pressure, as shown during zebrafish inner ear formation (Hoijman et al., 2015; Mosaliganti et al., 2019).

Techniques to quantify luminal pressure

Broadly speaking, the current techniques to measure luminal pressure *in vivo* and *in vitro* can be divided into direct and indirect approaches (Table 1; Fig. 1). Direct approaches involve the use of a measuring probe to provide a direct readout of luminal pressure, whereas indirect approaches rely on the numerical extraction of pressure from a model based on the mechanical response of a cell or luminal tissue under external force application.

Direct approaches

Micropressure probe (servo-null method)

One of the earliest techniques to directly probe cytoplasmic or luminal pressure is the servo-null method, which is based on the use of a small electrolyte-filled microelectrode (0.5-1 µm) (Petrie and Koo, 2014). Upon insertion of the microelectrode into a cell or lumen, the intracellular or luminal pressure pushes the electrolyte into the micropipette and changes the resistance of the circuit. The system then compensates for this change by exerting positive pressure to restore the electrolyte to its original position. The

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Table 1. Summary of techniques to measure luminal pressure in living tissues

Technique	Estimated pressure range*	Direct?	Strengths	Limitations	Reference
Micropressure probe (servo-null)	0.1-10 kPa	Yes	*In vivo*/*in situ* measurement. No specific sample geometry or preparation. Regional measurement possible.	Invasive; may induce leakage and clogging. Time-lapse measurement difficult. Low throughput.	Kelly and Macklem, 1991; Tomos and Leigh, 1999; Franks 2003; Knoblauch et al., 2014; Petrie and Koo, 2014; Petrie et al., 2014; Chan et al., 2019; Mosaliganti et al., 2019
Traction microscopy	0.1-1 kPa	Yes	Non-invasive. Time-lapse measurement. Potentially high throughput.	*In vitro* measurement so far; limited to cell lines that form a lumen. Specific sample preparation required.	Latorre et al., 2018
Gel deformation assay	0.1-10 kPa	Yes	Non-invasive. Time-lapse measurement possible.	*In vivo*/*in situ* measurement difficult. Measures only global pressure. Confinement may induce active cellular behaviour and tissue restructuring.	Alessandri et al., 2013; Leonavicius et al., 2018; Trushko et al., 2019
Pressure sensors	0.1 kPa-1 GPa (optical sensors) 0.1-10 kPa (hydrogel beads)	Yes	Time-lapse measurement possible. Potentially high throughput. *In vivo*/*in situ* measurement possible.	Potentially invasive, viability needs to be checked. May lack sensitivity and dynamic range.	Gómez-Martínez et al., 2013; Lay et al., 2017, 2019; Shen et al., 2008; Dolega et al., 2017; Mohagheghian et al., 2018; Träber et al., 2019; Lee et al., 2019
Atomic force microscopy	0.1 kPa-1 MPa	No	Non-invasive. *In vivo*/*in situ* measurement. Time-lapse measurement possible.	Model assumption required. Low throughput.	Stewart et al., 2011; Fischer-Friedrich et al., 2014; Beauzamy et al., 2015; Lamiré et al., 2018
Magnetic tweezer	0.1-1 kPa	No	*In vivo*/*in situ* possible. No specific sample geometry required.	Potentially invasive. Time-lapse measurement difficult. Model assumption required. Low throughput.	Wang et al., 2018

*Owing to a lack of sufficient data in the literature, the pressure range presented here is only estimated values, with the lower bounds likely to be overstated.

compensation pressure is then equal to the cytoplasmic or luminal pressure of the tissue (Fig. 1A). Servo-null method has been used extensively in the past to study pressure in whole animal organs (Avila et al., 2001; Desmond et al., 2005; Park et al., 2012). Recently, this technique has been adapted to study compartmentalized pressure in migrating cells (Petrie et al., 2014) and the blastocoel pressure as a mechanoregulator during mouse blastocyst development (Chan et al., 2019). Variations of this pressure probe have also been used to measure turgor pressure in plants (Franks, 2003; Knoblauch et al., 2014; Tomos and Leigh, 1999) and otic vesicular pressure in the zebrafish inner ear (Mosaliganti et al., 2019). The main advantage of the micropressure probe is that it allows for direct measurement of pressure *in vivo* and *in situ*, including those surrounded by ECM (Chan et al., 2019; Petrie et al., 2014). Furthermore, it allows for spatial mapping of pressure in different regions of a cell (Petrie et al., 2014) and potentially in a luminal tissue (single lumen and interstitial fluid), with reasonably good resolution (∼10 Pa). However, microinjection often incurs fluid leakage or clogs the probe tip, rendering time-lapse measurements challenging and experimental throughput low.

Gel deformation assays

A less invasive approach to quantify luminal pressure is to encapsulate the luminal tissue within a viscoelastic shell. Luminal expansion will exert compressive stress on the confining shell, and the pressure can be inferred from changes in the shell thickness, the size of the lumen and the elasticity of the shell. Using elastic microcapsules, this technique has been used to study the role of tissue pressure in tumour progression and physical buckling in cyst-like spheroids (Alessandri et al., 2013; Trushko et al., 2019 preprint). Another study using hydrogel encapsulation allows direct measurement of mouse blastocyst pressure (Fig. 1B) (Leonavicius et al., 2018), with reported values similar to those measured by the servo-null technique (Chan et al., 2019). The main advantage of this approach, compared with the servo-null technique, is that it allows for stable, long-term measurement of pressure. One caveat with this approach is that confinement itself may induce active cellular

Development (2020) 147, dev181297. doi:10.1242/dev.181297

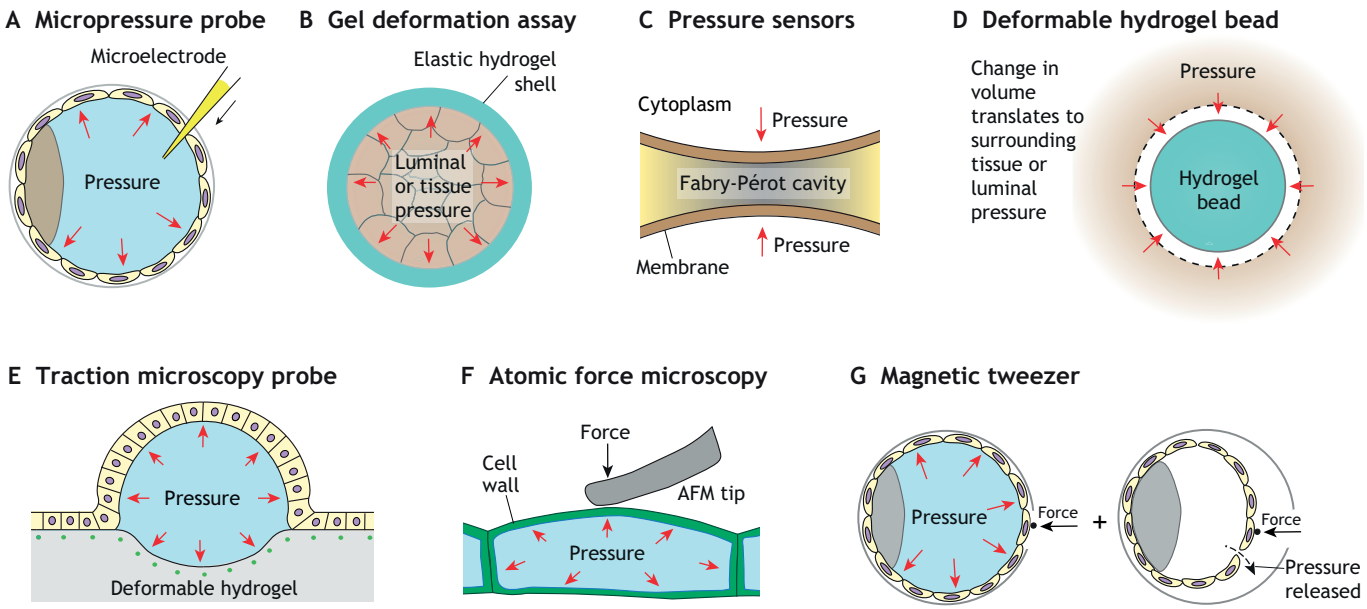

Fig. 1. Schematics of lumen quantification techniques referred to in Table 1. (A) Micropressure probe (servo-null) measures the luminal pressure of a mouse blastocyst by direct insertion of a microcapillary probe into the blastocyst cavity. (B) In a gel deformation assay, the luminal pressure can be inferred from the lumen size, elasticity of the shell and a change in the shell thickness. (C) Pressure sensors based on changes in the optical properties of the probe upon external pressure. One example is a silicon chip consisting of the Fabry-Pérot cavity. (D) Another class of pressure sensors is the deformable hydrogel bead, which provides a direct read-out of the local stress or pressure around it. (E) Traction microscopy probes the luminal pressure of *in vitro* cell lines seeded onto micropatterned substrates. The deformation of the substrate informs the normal stress (pressure) acting on it. (F) In atomic force microscopy (AFM), a probe is indented onto a sample and the deflection force is measured. By fitting the force-displacement curve to a mechanical model that takes into account the material properties of the cell wall and the underlying cytoplasmic pressure, the latter can be extracted. (G) Complementary to AFM, a magnetic tweezer can also be used to indent tissues, such as a mouse blastocyst. By measuring the deformation profiles before and after pressure release, the trophectoderm stiffness and luminal pressure can be assessed.

reorganization and tissue restructuring (Alessandri et al., 2013; Trushko et al., 2019 preprint) and generate artificially higher pressure that complicates data interpretation.

Pressure sensors

A novel approach is the use of pressure sensors, which can be inserted into living tissues to provide a direct read-out of luminal pressure or tissue stress. One such device is a nanomechanical chip containing a Fabry-Pérot cavity (Fig. 1C), which consists of deformable membranes that act as reflecting mirrors to trap light. Upon compression by external pressure, the distance between the membranes decreases, which changes the intensity of the light and gives a read-out for the pressure. This device has been shown to detect changes in cytoplasmic pressure when the cells are subjected to osmotic shock (Gómez-Martínez et al., 2013). Similarly, metallic nanoparticles have been developed to measure forces in the nano- to micro-Newton range (Lay et al., 2017, 2019; Shen et al., 2008). These particles change their crystal structures in response to high pressure, leading to a shift in fluorescence emission intensity that can be calibrated to infer the pressure. However, whether this technique is viable and of sufficient sensitivity to accurately measure luminal pressure in living tissues remains to be tested.

Another class of pressure sensors relies on the use of deformable hydrogel beads of known stiffness and compressibility. Under isotropic pressure, these beads can be compressed, and the changes in volume fraction give a direct read-out of the compressive stress (Fig. 1D). Such force transducers have been used to quantify tissue pressure in multicellular spheroids and in living embryos (Dolega et al., 2017; Lee et al., 2019; Mohagheghian et al., 2018; Träber et al., 2019). However, it is so far not feasible to quantify luminal pressure with these beads because they are porous hydrogel structures that will not deform in response to changes in hydrostatic pressure.

Traction microscopy

Recently, traction force microscopy has been applied to quantify luminal pressure in three-dimensional epithelia (Fig. 1E; Latorre et al., 2018). In this case, MDCK cells were seeded onto micropatterned soft polymer substrates, and the luminal pressure was inferred by direct measurement of the normal stress acting on the substrate using traction microscopy. This method allows for time-lapse measurement of pressure, and can be scaled up to become high throughput. However, this technique is limited to only cell lines that are able to form lumen spontaneously. The sample preparation (i.e. seeding of cells onto a substrate) may also induce changes to apico-basal polarity and tight junction activities, thereby introducing potential artefacts that may limit its biological implications *in vivo*.

Indirect approaches

Indirect approaches to quantify luminal pressure involve the study of cell or tissue deformation under an applied force. Based on mechanical models that describe the mechanical properties of the tissue and the underlying fluid pressure, one can infer the pressure from the deformation profile. For example, in atomic force microscopy (Fig. 1F), a probe is used to indent the cells. By recording the applied force which is proportional to the cantilever deflection, and the distance travelled by the probe, the force-displacement curve can be analyzed to reveal the rounding pressure of mitotic cells (Fischer-Friedrich et al., 2014; Stewart et al., 2011).

Development (2020) 147, dev181297. doi:10.1242/dev.181297

Based on inflated shell models that take into account the stiffness and curvature of cells, this approach also allows the deduction of turgor or cytoplasmic pressure in plants or animals (Beauzamy et al., 2015; Lamiré et al., 2018 preprint). Although this tool has been extensively used to study intracellular pressure, in principle it can be adapted to study luminal pressure in multicellular contexts (e.g. cysts and body cavities).

Recently, magnetic tweezers have also been used to quantify the luminal pressure during mouse blastocyst development (Wang et al., 2018). Here, a constant magnetic force is applied to a magnetic bead in contact with the trophectoderm cells lining the cavity (Fig. 1G). The applied indentation force is balanced by the underlying hydrostatic pressure and the elastic force from the trophectoderm. By measuring the force-displacement profiles before and after pressure release by laser ablation, the elasticity of the trophectoderm cells and luminal pressure can be independently assessed. Their results yielded a pressure in the range of hundreds of pascals, consistent with the values reported in other studies, when the zona pellucida is removed (Chan et al., 2019; Dumortier et al., 2019). Although this technique may be easily adapted to study other systems with a single lumen, it is invasive and does not support temporal tracking of luminal pressure.

The lumen as an integral player of tissue self-organization

An attractive hypothesis is that single lumen formation, perhaps the most direct and universal process driven by fluid pressure, can provide a spatial niche to induce mechanical and biochemical responses in cells. Below, we discuss how mechanical cues from the lumen can polarize the cytoskeleton and trigger cell migration and proliferation. Next, we evaluate how the lumen can provide signalling cues to guide cell-fate specification and tissue patterning. Finally, we review examples showing how the integration of mechanical and biochemical cues influences tissue architecture and morphogenesis during development.

Mechanical cues

Luminal pressure can mechanically induce passive and active cellular responses to modify tissue material properties. For example, fluid-filled epithelial domes *in vitro* have shown that a lumen expansion leads to strain softening and re-stiffening of epithelial cells at extreme stretch, mediated by active cytoskeletal remodelling (Latorre et al., 2018). Increased actomyosin tension and tissue elasticity due to lumen-induced cell stretching has also been observed in zebrafish inner ear formation (Mosaliganti et al., 2019). During mouse blastocyst development, increased luminal pressure leads to epithelial stretching and increased tissue stress in the trophectoderm cells that line the cavity (Chan et al., 2019). This further triggers active contractile tension and tissue stiffening due to increased actomyosin activity, which in turn leads to tight junction maturation required for proper blastocoel development (Fig. 2A) (Chan et al., 2019; Zenker et al., 2018).

Luminal pressure also plays a role in collective cell migration or cellular rearrangement. For example, during gastrulation in *Xenopus*, hydrostatic pressure within the blastocoel generates tissue tension, which in turn polarizes the fibronectin matrix assembly on the blastocoel roof, enabling cell migration to proceed (Dzamba et al., 2009). Interestingly, as the fluid-filled archenteron (see Glossary, Box 1) starts to displace the blastocoel, the increased cell density causes stiffening of the head mesoderm and triggers collective neural crest migration in the overlying tissue (Barriga et al., 2018). An open question here is whether a mechanical crosstalk exists between the hydrostatic pressure in the archenteron and the tissue tension at the epidermis that may underlie neural crest migration and differentiation. In mouse embryos, forces exerted on the epiblast by the expansion of the amniotic cavity can drive convergent-extension of cells during notochord morphogenesis (Imuta et al., 2014). In addition, in tumour cell aggregates, interstitial fluid pressure can trigger epithelial-to-mesenchymal transition and promote collective invasion (Piotrowski-Daspit et al., 2016). Finally, increased transmural pressure decreases smooth muscle contractions and accelerates the branching development of the airway epithelium in mouse embryonic lungs (Nelson et al., 2017).

Fluid pressure has been shown to influence cell proliferation and division. For example, following mouse liver resection, induced bile acid overload leads to elevated osmotic pressure (see Glossary, Box 1) and cortical tension of bile canaliculi, which drives nuclear translocation of YAP and induction of cell proliferation and liver regeneration (Meyer et al., 2019 preprint). Similarly, in the chick embryonic brain, luminal pressure induces neuroepithelial cell proliferation along the tangential plane via symmetric cell division (Desmond and Jacobson, 1977; Desmond et al., 2005; Garcia et al., 2019).

Recently, PIEZO1 has been identified as a key mechanosensor in controlling epithelial proliferation during homeostasis (Eisenhoffer et al., 2012; Gudipaty et al., 2017). Another study has shown that mouse immune cells grown *in vitro* can trigger inflammatory response upon activation of PIEZO1 by cyclical hydrostatic pressure in the range of 1 kPa (Solis et al., 2019). Given that this value is similar to the physiological range in developing embryos (Chan et al., 2019; Leonavicius et al., 2018), it would be worthwhile to investigate the mechanosensitive roles of PIEZO1 in luminal tissues further.

Biochemical signalling

The lumen can also act as a signalling source to direct tissue patterning. For example, during zebrafish lateral line formation, the lateral line primordium migrates from the anterior to the posterior end of the developing embryo. The lateral line primordium is made up of mesenchymal leader cells, followed by epithelia that apically constrict to generate a rosette-like structure. The epithelial rosettes enclose a shared microlumen that traps the fibroblast growth factor (FGF) secreted by the local environment. FGF positively feeds back onto the microlumen by enhancing the epithelial nature of the responding cells, thereby leading to the robust formation of mechanosensory organs (Fig. 2C; Durdu et al., 2014). A recent study showed that luminal signalling can also influence epiblast-primitive endoderm lineage segregation within the inner cell mass during mouse blastocyst development (Fig. 2D; Ryan et al., 2019). In this study, it was shown that inhibition of FGF4 in the lumen impairs cell-fate specification and sorting, whereas luminal deposition of FGF4 expedites the segregation process. This suggests that luminal FGF4 may act as a local chemo-attractant to drive cell-fate changes, which has also been implicated to guide *Drosophila* tracheal branching morphogenesis and patterning (Ghabrial and Krasnow, 2006). On the other hand, differential reception of cells to the same luminal cue can induce distinct cell fates. This is clearly illustrated in a recent *in vitro* study on human embryonic stem cells (Etoc et al., 2016), which showed that transforming growth factor β (TGF-β) receptor localization can spatially modulate the bone morphogenetic protein 4 (BMP4) signal response across the tissue and lead to distinct tissue patterning. This highlights that receptor localization, in addition to the local concentration of signalling molecules within the lumen, is another crucial factor in controlling luminal signalling.

Development (2020) 147, dev181297. doi:10.1242/dev.181297

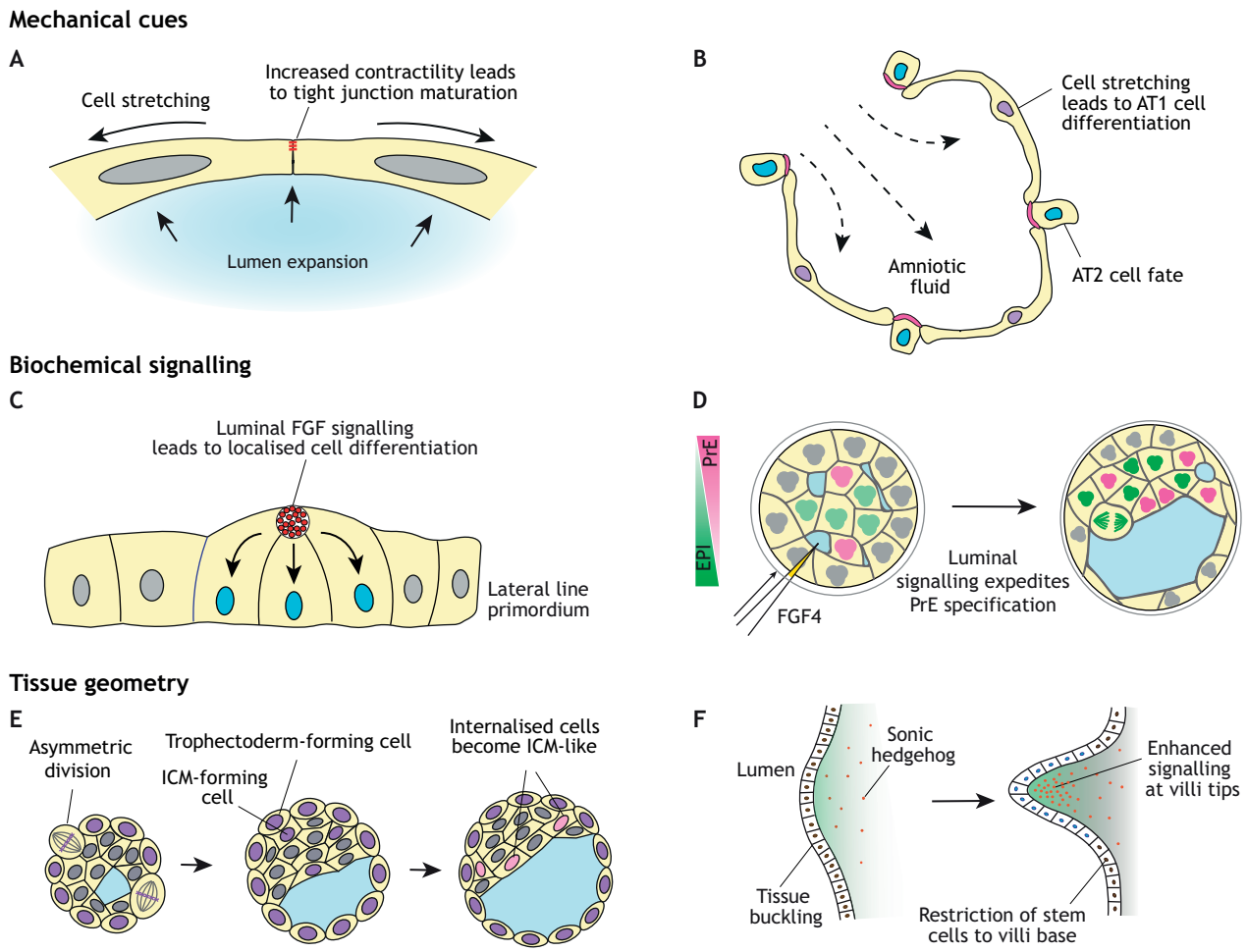

Fig. 2. Examples of lumen-mediated mechanotransduction and biochemical signalling during embryo development. (A,B) The lumen can exert long-range hydraulic force to modulate cellular functions. In mouse blastocysts, increased hydrostatic pressure leads to lumen expansion and stretching of the trophectoderm cells lining the lumen (A). This triggers an increase in cell contractility that helps to reinforce tight junction maturation, allowing the blastocyst to accommodate lumen growth. During branching morphogenesis in early lung development (B), amniotic fluid flow leads to cell stretching and differentiation into AT1 cells, whereas others that are apically constricted do not undergo stretching and instead differentiate into AT2 cells. (C,D) Luminal signalling can impact on cell-fate specification in multicellular contexts. In zebrafish lateral line formation (C), locally trapped fibroblast growth factor (FGF) within the microlumen leads to enhanced signalling and restricted cellular differentiation of the neighbouring cells. Luminal FGF signalling (D) expedites the epiblast and primitive endoderm fate specification in the inner cell mass during mouse blastocyst development. (E,F) Presence of a lumen can induce cellular differentiation through changes in tissue geometry. In mouse blastocysts, reduced lumen expansion leads to more outer cells dividing asymmetrically to generate a trophectoderm cell (purple nuclei) and an inner cell mass-forming cell (E); the latter eventually downregulates its outer cell fate (pink nuclei) and acquires the inner cell mass fate (grey nuclei). In the mouse gut, growth-induced tissue buckling leads to a higher concentration of sonic hedgehog signals in the villi (signalling concentration indicated by green) (F). This leads to the suppression of the progenitor cell fate at the villus tips (blue nuclei) and restriction of the progenitor cells to the base of these structures (grey nuclei).

In addition to growth factors, cell-adhesion modifying molecules secreted into the extracellular fluid can influence tissue morphogenesis and patterning. For example, cytoplasmic release of vesicles containing sialic acids of glycoproteins, which are highly negatively charged, can induce the repulsion of apical membranes and cause cavity formation. This leads to the proper formation of lumenized blood vessels during vascular development in mice (Strilić et al., 2010). Similarly, during early stages of mouse postimplantation development, sialomucin, a negatively charged apical protein, regulates amniotic cavity formation by a charge repulsion mechanism (Shahbazi et al., 2017). In cell culture, podocalyxin (another major sialoprotein), serves as an anti-adhesion molecule to regulate cell aggregation (Takeda et al., 2000). Collectively, these studies demonstrate that electrostatic repulsion may be a generic mechanism in initiating and maintaining luminal structures during development. Incomplete coalescence of lumina can lead to multi-luminal phenotypes and altered ratios of cell types within the tissue, which can disrupt tissue functions (Bagnat et al., 2007; Chou et al., 2016; Kesavan et al., 2009). Osmolytes (see Glossary, Box 1), such as ions and macromolecules in fluid lumina, also generate osmotic stress, which has been shown to regulate cell volume and impact gene expressions *in vitro* (Box 2). The potential implications for the lumen as an osmoregulator for tissue patterning signify exciting new directions for future studies.

Tissue size, geometry and morphogenesis

On a global scale, fluid pressure helps to control the size of lumenized embryos and organs, independently of regulation by cell proliferation. For example, mouse blastocysts undergo a series of lumen expansion and collapse regulated by increased luminal pressure and junctional leakage of epithelial cells (Chan et al., 2019). This hydraulically

Development (2020) 147, dev181297. doi:10.1242/dev.181297

Box 2. Osmotic stress as a potent mediator of mechanotransduction

Interstitial fluid osmolarity has been shown to regulate tissue surface tension, which can impact cell sorting during development, such as during zebrafish gastrulation (Krens et al., 2017). Furthermore, changes in extracellular osmolarity can impact cell differentiation. For example, a recent *in vitro* study demonstrated that osmotic compression triggers intracellular water efflux and reduced cell volume, leading to increased molecular crowding within the cytoplasm that affects mesenchymal stem cell lineage specification (Guo et al., 2017). Gene regulation may be accomplished by changes in the cytosol ribosome concentration, which can tune the cytoplasmic viscosity to impact transcription and post-translational modifications (Delarue et al., 2018). Alternatively, increased macromolecular crowding in the cytoplasm can osmotically challenge the nucleus and change its size, therefore affecting chromatin compaction state and nucleo-cytoplasmic transport directly (Finan and Guilak, 2010; Martins et al., 2012). Nuclear size is also sensitive to changes in the cytosolic pH and salt concentrations (Chan et al., 2017b; Dahl et al., 2005), which may in turn be mediated by extracellular osmolytes. Although the *in vivo* implication of these studies awaits further investigation, these findings raise the interesting possibility that active osmotic regulation by the lumen may control cell fate and tissue patterning during development.

gated oscillation sets a steady-state size of the blastocysts, and the generic mechanism may be applicable to other systems, as shown in *Hydra* and cysts that undergo similar size oscillations (Kücken et al., 2008; Ruiz-Herrero et al., 2017). Remarkably, during zebrafish inner ear formation, loss of fluid from the otic vesicle by puncture leads to a transient increase in fluid flux, which eventually restores the ear size (Mosaliganti et al., 2019). This reveals that luminal pressure negatively regulates fluid influx, which could help to buffer size variations during otic vesicle development. Although the molecular mechanism driving this catch-up growth remains unknown, such negative coupling between pressure and fluid flux may operate in other developmental processes to achieve robust size control.

A lumen could also mediate the crosstalk between tissue mechanics, geometry and cell-fate specification during morphogenesis. An example that unites all these aspects is alveolar morphogenesis in the mouse lung where, during development, mechanical stress arises from the inhalation of amniotic fluid and causes alveolar cell stretching. During this process, some cells are stretched more than the others because of differences in cell-cell contact strength. The degree of cell stretching, in turn, triggers gene expression changes that generate the different alveolar cell types required for proper growth and functions of lungs (Fig. 2B) (Li et al., 2018). Although this study provides evidence for the role of luminal pressure in mechanotransduction, future work will need to reconcile this hypothesis with the recent observation that single cell Wnt signalling niches (see Glossary, Box 1) can independently regulate alveolar cell-fate specification (Nabhan et al., 2018). The influence of lumen formation on embryo size and cell-fate specification is further observed in mouse blastocysts, in which reduced luminal pressure and cavity size promote asymmetric division of outer trophectoderm cells, which gives rise to daughter cells that are allocated to the interior of the blastocyst (Fig. 2E) (Chan et al., 2019). These internalized cells eventually acquire the fate of inner cell mass cells, potentially because of a lack of the apical domain and differential YAP signalling (Korotkevich et al., 2017; Nishioka et al., 2009).

Luminal growth may also indirectly influence cell-fate specification through changes in tissue architecture and morphogenic fields. During mouse gut development (Fig. 2F), intestinal stem cell progenitors are initially distributed throughout the epithelium (Shyer et al., 2015). As the lumen surface grows, it generates compressive forces due to restriction from the surrounding tissue and thus causes the lumen surface to buckle. This leads to a local increase in secreted sonic hedgehog concentration at the villi tips and induces further signalling by BMPs within the cluster to restrict stem cell proliferation to the base of the villi. Lumen expansion may also distance a region of a tissue away from a signalling source, leading to tissue compartmentalization. For example, during mouse blastocyst expansion, the outer trophectoderm differentiates into both the mural trophectoderm (that lines the cavity) and the polar trophectoderm (that is in contact with the inner cell mass). This process of lineage segregation potentially occurs through differential FGF signalling from the inner cell mass (Christodoulou et al., 2019), although there is also evidence showing that filopodia extending from the mural trophectoderm to the inner cell mass can mediate such a process (Salas-vidal and Lomelí, 2004).

Conclusions and future perspectives

Evidently, understanding the intricate interplay between mechanics and biochemical signalling in tissue morphogenesis and patterning (Fig. 3) would not be possible without the advancement of technologies to better characterize the physical and biochemical properties of lumen *in vivo*. In particular, we currently lack tools to

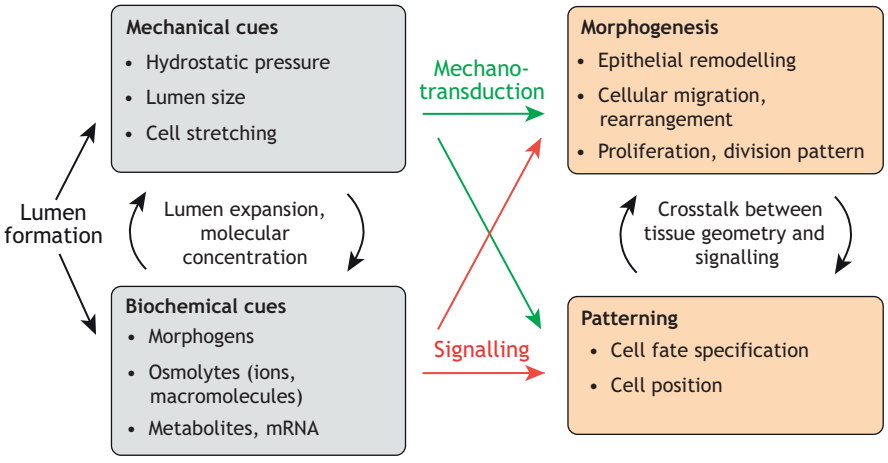

Fig. 3. Multiple crosstalk between mechanical and biochemical signalling during lumen formation leads to robust tissue morphogenesis and patterning. Feedback between luminal mechanics and signalling can drive early luminogenesis. For example, secretion and deposition of osmolytes and polarity proteins into the nascent lumina may lead to fluid expansion through osmotic pressure and apical membrane remodelling. The change in lumen size can, in turn, modify the signalling landscape in the tissue. At the level of tissue self-organization, lumen formation, through its hydraulic action and biochemical signalling cues, can trigger tissue remodelling and collective cell behaviour, such as migration and proliferation, and influence cell-fate specification.

quantify luminal osmolarity (see Glossary, Box 1) *in vivo*, although a recent study demonstrated the use of nano-osmometer in measuring interstitial fluid osmolarity in zebrafish explants (Krens et al., 2017). Polymeric sensors that measure the luminal pH will also reveal how tissues respond to the changing electrochemical environment in the lumen (Cerchiari et al., 2016). To further understand the roles of signalling, development of advanced proteomics to identify the molecules in lumina of small volume (~10 nl) will be essential. Although we have discussed a few emerging techniques for quantifying hydrostatic pressure, *in vivo* application still presents a challenge in many contexts. Sensors that can be introduced into the lumen for direct measurement of hydrostatic pressure may be a promising approach for this purpose. Finally, equally important is the development of tools to precisely control pressure that would allow for testing model predictions.

Cell-fate specification can arise from the combined effect of mechanotransduction and biochemical signalling, both inducible by lumen formation. To disentangle the impact of one from the other, it will be instructive to devise tools that allow us to independently manipulate hydrostatic pressure or molecular concentration in the lumen. This may involve either targeted injection/quenching of the signalling molecules within the lumen (Ryan et al., 2019), or mechanical inflation/deflation of the lumen during short timescales to assess the crosstalk between luminal pressure and tissue mechanics (Chan et al., 2019; Dzamba et al., 2009; Mosaliganti et al., 2019). Rescue experiments involving the replacement of luminal fluid with or without the signalling molecules (Ryan et al., 2019) would also provide insights in this direction.

To date, a number of theoretical frameworks have been proposed to model lumen growth, focusing either on the early phase of lumen nucleation (see Glossary, Box 1) and coalescence (Dasgupta et al., 2018; Duclut et al., 2019; Dumortier et al., 2019), or on the later phase when the singly resolved lumen undergoes size oscillations (Ruiz-Herrero et al., 2017). A general assumption of these models is that lumen growth is governed by water intake resulting from inward ion transport (for example by sodium-potassium pumps). However, given recent evidence that the secretion of cytoplasmic vesicles into intercellular space also contributes to microlumina formation (Ryan et al., 2019; Vasquez et al., 2019 preprint), such a mechanism should also be incorporated into future theoretical models. Interestingly, recent theoretical work has shown that ion flows in the fluid can lead to the build up of an electric field across the tissue, the shape of which can, in turn, influence lumen nucleation dynamics and size (Duclut et al., 2019), although this warrants further experimental investigation. Finally, to understand the role of lumen in tissue patterning, future theoretical frameworks must incorporate various feedback interactions between luminal signalling, tissue mechanics and cell fate across multiple spatial and temporal scales. A promising study provides a first step towards such an integrative understanding, in which the coupling of extracellular fluid flows to intracellular signalling and cell mechanics can generate robust, non-Turing-like spatial patterns of morphogens (Recho et al., 2019).

Although lumina are widespread in many tissues, their roles have so far been studied in limited contexts. The roles of the lumen (Chan et al., 2019; Dumortier et al., 2019; Ryan et al., 2019), interstitial fluid (Petridou et al., 2019) and yolk (Shamipour et al., 2019) in early development require further exploration. For example, in early marine embryos with a hydroskeleton shell around a fluid-filled body cavity (such as sea anemone), fluid pressure is known to be required for body movement (Kier, 2012); however, the interplay between pressure and signalling in embryo morphogenesis and patterning is less well understood. Notably, studies have highlighted the importance of mechanochemical feedback loops in guiding axial formation of *Hydra* (Hobmayer et al., 2000; Mercker et al., 2015). Finally, given that organoids recapitulate some aspects of the organ formation, and typically start their growth with a central lumen, they may provide pertinent and suitable *ex vivo* systems to study the roles of luminal mechanics and signalling in organ development (Serra et al., 2019).

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Competing interests
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