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Developing pressures: fluid forces driving morphogenesis

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Over several decades genetic studies have unraveled many molecular mechanisms that underlie the signaling networks guiding morphogenesis, but the mechanical forces at work remain much less well understood. Accumulation of fluid within a luminal space can generate outward hydrostatic pressure capable of shaping morphogenesis at several scales, ranging from individual organs to the entire vertebrate body-plan. Here, we focus on recent work that uncovered mechanical roles for fluid secretion during morphogenesis. Identifying the roles and regulation of fluid secretion will be instrumental for understanding the mechanics of morphogenesis as well as many human diseases of complex genetic and environmental origin including secretory diarrheas and scoliosis.

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Introduction

The generation of a complete body-plan from a single cell during embryonic development depends on complex interactions between molecular signals and morphogenetic forces. Genetic analyses have identified key molecular mechanisms controlling morphogenesis, but how molecular functions translate to specific morphogenetic movements is still poorly understood. Research into the mechanics of development has been focused largely on short range forces generated at the cell cortex by actin contractility. However, it has become increasingly clear that more broadly acting forces like fluid flow and hydrostatic pressure play substantial roles during morphogenesis. These 'fluid forces' are particularly important during tubulogenesis, a key process underlying the structural organization of most organs. Here, we review the role of hydrostatic pressure during morphogenesis in metazoans; from tubulogenesis to axis elongation and spine formation, and the pathological implications of misregulated fluid secretion.

Fluid secretion 101

The control of water transport is an essential physiological function for all organisms. Even though water accounts for most of the mass and volume of an organism, cells are unable to directly control its movement. Instead, they rely on ionic and osmotic gradients to power fluid transport. The incompressible nature of liquids allows fluid secreted into luminal spaces to generate considerable hydrostatic pressure that is capable of acting at great distances (Figure 1a).

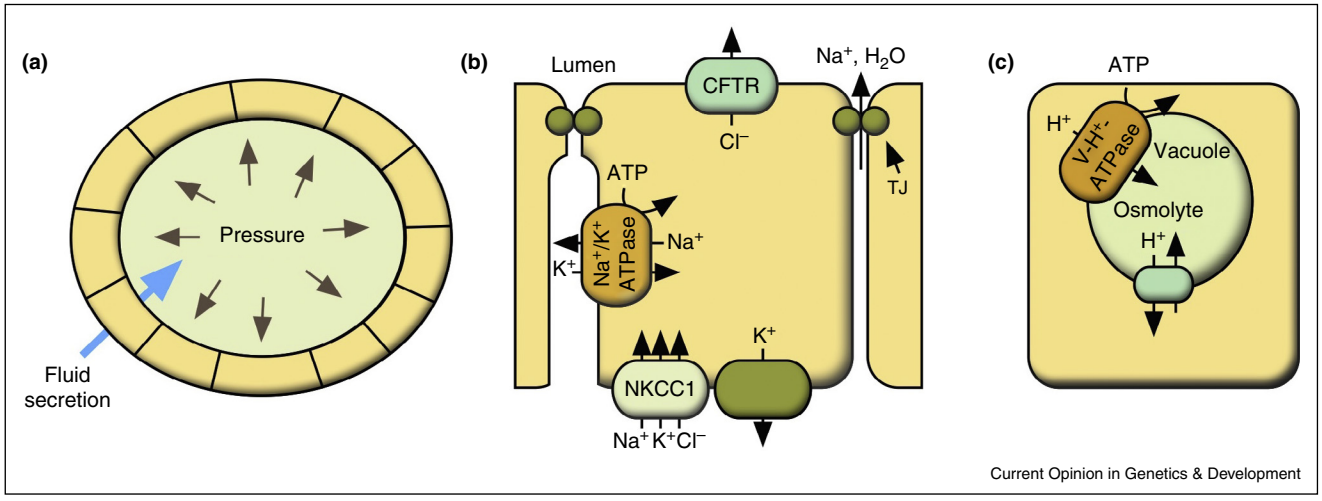
The osmotic gradients that drive fluid secretion across epithelial cells are powered by ATP hydrolysis by the Na^+/K^+-ATPase, which generates a sodium gradient [1–5]. The extracellular sodium is then used to import chloride through symporters like NKCC1. Chloride can then be transported into the lumen through channels like CFTR, bestrophins, or TMEM16a to generate a luminal electrochemical gradient, which draws sodium and water into the lumen (Figure 1b). Fluid can also accumulate within intracellular organelles powered by H^+ transport through the V-H^+-ATPase (Figure 1c).

Fluid secretion across epithelia also depends on the paracellular barrier formed by membrane proteins of the claudin (cldn) and occludin families [6]. These proteins interact across cell boundaries and also laterally within the same cell forming the tight junction (TJ) arrays, which can form ion-selective pores that help regulate fluid secretion [7] (Figure 1b).

The organogenesis bubble: opening and resolution of the lumen

Tubes are a basic structural element underlying the morphology of most organs. Although the specific morphogenetic strategies may vary between organs and across species, tubulogenesis invariably presents two fundamental elements: firstly, all mature tubes contain a single lumen; secondly, lumen opening requires its filling. Typically, one or more lumens initially form through cellular rearrangements to establish a restricted space. Then, lumens expand through fluid accumulation, common in vertebrates, nematodes, and tunicates, or through matrix secretion, as observed in insects. Although fluid and

Figure 1



Mechanics of fluid secretion. **(a)** Fluid accumulation within an enclosed area generates an outward pressure capable of inflating a luminal space. **(b)** Schematic representation of a simplified model of fluid secretion across epithelia through coordinated activity of ion channels that establish electrochemical and osmotic gradients. These gradients drive water into the lumen. **(c)** The V-H^+-ATPase powers H^+ transport into a vacuolar space, which helps transport an unknown osmolyte into the lumen to draw water into the organelle.

matrix secretion occur through different mechanisms, they function in the same way to support and power luminal expansion.

Fluid secretion driving lumen opening

The use of fluid secretion during lumen expansion is a key, conserved process in vertebrate organogenesis. In many tubes that undergo cord hollowing, fluid secretion drives expansion of nascent lumens to form a single tube. In zebrafish, the gut tube forms from a solid rod of cells in which TJs are first assembled at multiple actin-rich foci [8]. Then, the activity of the Na^+/K^+-ATPase drives the opening of several lumens that begin to coalesce as they expand [9•]. Also essential for single lumen formation is the TJ protein Cldn15la, which forms ion-selective pores that allow the accumulation of fluid [9•]. Lumen expansion and coalescence are tightly regulated at the transcriptional level by Hnf1b, which controls the expression of Cldn15la and the Na^+/K^+-ATPase [9•]. Similarly, during development of the zebrafish brain, Claudin5a is required for the establishment of a tight barrier that facilitates fluid accumulation and ventricle inflation [10]. The importance of TJs in lumen opening has also been shown in mammals where they play key roles early in embryogenesis as shown for the mouse blastocyst where Cldn4 and 6 are required for blastocoel opening [11].

A key driver of vertebrate fluid secretion is the chloride channel CFTR. This channel regulates fluid secretion in several organs from morphogenesis throughout life and its loss of function causes cystic fibrosis [12]. Perhaps the clearest example of how CFTR-dependent fluid secretion functions during lumen opening comes from Kupffer's vesicle (KV), the zebrafish organ of laterality [13]. The KV lumen opens through a process of cord hollowing from several small lumens that expand and merge into a single lumen [14,15••]. In KV, loss of Cftr function blocks lumen expansion and organ function [15••]. The loss of fluid secretion leads to a complete absence of the luminal space, even though the surrounding epithelial cells develop normally and exhibit proper apicobasal polarity [15••] (Figure 2a,b). Interestingly, the lack of a luminal space does not impair the formation of normal motile cilia, indicating that fluid secretion and flow are specified independently. CFTR is similarly required for lumen expansion in the mammalian salivary gland [16•].

Fluid-driven lumen expansion has also been observed in other organs. In the zebrafish brain, Na^+/K^+-ATPase is essential for ventricle inflation, independent of the circulation required for later stages of expansion [17•,18]. Similarly, fluid secretion is required to inflate the zebrafish otic vesicle (see review by T. Whitfield). Hydrostatic pressure is also required for the formation of lumens that traverse single cells. During tube formation in the *Ciona intestinalis* notochord, Slc26-dependent fluid secretion is required for the development of the lumen along the length of the notochord rod [19]. A similar process also occurs during the formation of the *Caenorhabditis elegans* excretory canal. In this organ, aquaporins facilitate the movement of water required for unicellular tube extension [20•,21•].

Matrix secretion during lumen formation

Lumen expansion driven by hydrostatic pressure is crucial for lumen expansion in vertebrates, but this is not the