Limiting lumens: a new role for Cdc42

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The formation of a single lumen is a necessary step in the formation of biological tubes. Different tissues have developed diverse ways to form their lumens. In this issue, Jaffe et al. (Jaffe, A.B., N. Kaji, J. Durgan, and A. Hall. 2008. *J. Cell Biol.* 183:625–633) report the development of an in vitro system for studying lumen formation that is driven by fluid transport, recapitulating intestinal lumen formation. Effective ion and fluid transport requires both cell polarity and proper tissue organization. Surprisingly, polarization of cells in this three-dimensional system does not require Cdc42. Instead, Cdc42 prevents formation of multiple lumens by orienting cell divisions and directing apical membrane biogenesis.

A new system for lumen formation in vitro

The mechanisms underlying lumen formation are diverse. In mammary gland development, apoptosis and autophagy drive de novo lumen formation (Debnath et al., 2002; Mills et al., 2004). In the fly trachea and retina, secreted proteins draw fluid into the apical space (Husain et al., 2006; Tsarouhas et al., 2007). Endothelial cells are thought to extend and fuse vacuoles to form a central lumen (Kamei et al., 2006).

Understanding the mechanisms underlying lumen formation has been greatly aided by in vitro three-dimensional cultures that mimic their development. In general, this involves plating isolated cells in three-dimensional matrices such as Matrigel. Under these conditions, cells divide and form hollow spheres by different mechanisms. Mammary epithelial cells grown in these conditions form a hollow ball of cells resembling the mammary gland. Formation of this structure proceeds by apoptosis and autophagy, mimicking the in vivo process (Mailleux et al., 2008).

Recently, it was shown that fluid flow driven by ion transport is responsible for formation of the single intestinal lumen in zebrafish (Bagnat et al., 2007). Although genetic and cell biological experiments in zebrafish have begun to dissect this process, the establishment of an in vitro system would be a great boon (Bagnat et al., 2007). To date, a robust three-dimensional cell culture system that models intestinal lumen formation has not been described. Jaffe et al. (see p. 625 of this issue) now show that intestinal epithelial–derived Caco-2 cells form cysts in three-dimensional culture. Cells grown without additives form few and small lumens. Upon addition of cholera toxin, lumens greatly expand in size. This is likely caused by the downstream activation of an apically localized cystic fibrosis transmembrane receptor chloride channel, although future studies will be needed to confirm this hypothesis. Blocking Na^+/K^+-ATPase activity completely abrogates lumen formation, which is consistent with a role for ion flow in lumen enlargement. As this culture system is amenable to RNAi, transfection, and pharmacologic perturbation, it should greatly aid in the understanding of intestinal lumen biogenesis.

Apical membrane establishment and cell adhesion

Apical identity in the three-dimensional Caco-2 cultures is established at the two-cell stage. After the first cell division, the site of the cytokinetic furrow is marked by atypical PKC, which localizes to the apical domain in mature polarized epithelia (Fig. 1). This intriguing observation suggests that cell division acts as a symmetry-breaking event, allowing the establishment of cell polarity. What is perhaps most surprising is that the site of cell–cell contact, usually occupied by adhesion molecules, may not be a homogeneously adhesive domain. Although not examined in detail in this study, it will be interesting to determine whether tight junctions and adherens junctions encircle a nonadhesive apical core region at this polarized two-cell stage. This is not without precedent, as, after the first division of the *Xenopus laevis* embryo, tight junctions form at the lateral margins of the cell contact zone (Cardellini et al., 1996). In the Caco-2 three-dimensional cultures, it is not yet known whether polarized localization of transmembrane proteins occurs at the two-cell stage. Thus, whether these cells are functionally polarized for secretion at this early time point remains to be determined.

It is noteworthy to point out that in other model systems, a functionally opposite approach is used to generate cell polarity. In the *Caenorhabditis elegans* embryo, as cells divide, the sites of cell–cell contact exclude markers of the apical domain (such as Par6), which become restricted to the peripheral plasma membrane (Hung and Kemphues, 1999; Anderson et al., 2008). An explanation for these differences is not clear at present, although it will be important to determine whether integrin/ECM-based signals coming from the matrix surrounding the Caco-2

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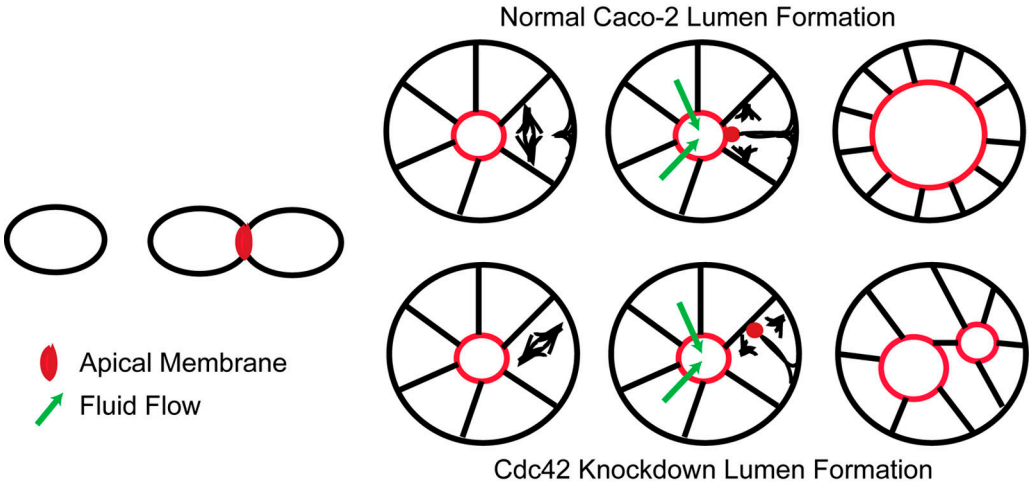

Figure 1. **Model for polarity establishment and lumen formation in Caco-2 cells.** In untreated cells, the mitotic spindle aligns itself perpendicular to the apical–basal axis. Fluid flow increases lumen size. In Cdc42-deficient cells, mitotic spindles do not localize correctly, resulting in formation of additional lumens.

cells are necessary for correct polarization of these cells and collaborate with cell division to set up this polarity.

Cdc42 controls division orientation but not cell polarity

To begin to understand how cells polarize and organize themselves into a hollow sphere, Jaffe et al. (2008) turned to loss of function experiments on Cdc42. This small GTPase is required for cell polarity in many contexts, including in some epithelial cells (Etienne-Manneville, 2004; Wu et al., 2007). However, Cdc42 is not required for all forms of cell polarization. For example, Cdc42-null cells can still form a leading edge and migrate (Czuchra et al., 2005).

In epithelial three-dimensional culture, knockdown of Cdc42 in MDCK cells results in a significant loss of cell polarity and an inability to form cysts (Martin-Belmonte et al., 2007). Caco-2 cells show a dramatically different phenotype caused by loss of Cdc42. Individual cells polarize normally and have intact cell junctions, but the entire cyst lacks proper organization, with two or more lumens forming. The cell biological defect that gives rise to these multiple lumens appears to involve misorientation of the mitotic spindle. In simple epithelia, the spindle normally aligns itself perpendicular to the polarity axis and parallel to the underlying basal lamina (Jinguji and Ishikawa, 1992; Reinsch and Karsenti, 1994; Fleming et al., 2007). In Caco-2 cultures, this same organization of cell division was seen. However, in Cdc42 knockdown cells, spindle orientation was effectively randomized (Fig. 1). Because the site of cell division becomes the apical membrane in these cells, this establishes a new apical membrane region, which is disconnected from the primary lumen. Although Cdc42 has been implicated in spindle orientation previously, this was thought to be secondary to its effects on polarity (Gotta et al., 2001). In this case, the two can apparently be separated. In the future, it will be important to determine not only the downstream effectors and pathways that Cdc42 uses to influence spindle orientation but also how division sites specify apical membrane biogenesis in these cells. Finally, it will be very exciting to determine whether Cdc42 functions to maintain spindle orientation and single lumen formation in an in vivo context.

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