Review

CellPress

# Mechanisms of apical–basal axis orientation and epithelial lumen positioning

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**In epithelial cells, the polarized orientation of the apical–basal axis determines the position of the apical lumen and, thereby, the collective tubular tissue architecture. From recent studies employing 3D cell cultures, animal models, and patient material, a model is emerging in which the orientation and positioning of the apical surface and lumen is controlled by the relationships between the extracellular matrix (ECM), Rho family GTPase signaling, recycling endosome dynamics, and cell division. Different epithelial cells adjust these relationships to establish their specific cell polarity orientation and lumen positioning, according to physiologic need. We provide an overview of the molecular mechanisms required to construct and orient the apical lumen.**

## The orientation of the apical–basal polarity axis

The establishment and orientation of an apical–basal polarity axis is instrumental for the functional shaping of a lumen in a tube-forming epithelial cell mass. Columnar epithelial cells that are arranged in monolayers typically position their apical domain and lumen opposite their basal domain. While the ectopic position of apical lumens at the lateral surface gives rise to defects in columnar epithelium architecture, hepatocytes deliberately position their apical lumens amidst their lateral surfaces to give rise to a canalicular network. Further, while apical lumens in the cytoplasm of epithelial cells are associated with cancer and a fatal disorder (microvillus inclusion disease), other cells deliberately develop apical lumens in their cytoplasm to establish their unique tubular architecture (Figure 1). Much of our understanding of the mechanisms that control the orientation of apical–basal polarity in epithelial cells and the spatial positioning of *de novo*-formed lumens comes from studies with cultured epithelial cells. These include – but are not limited to – the simple epithelial Madin–Darby canine kidney (MDCK) cell line, intestinal epithelial Caco-2, and mammary epithelial cell lines (MEC), embedded in 3D matrices [1–7], as well as hepatocellular HepG2, WIF-B9, Can-10 cells [8,9], and primary hepatocytes [8,10–13]. With the exception of primary hepatocytes, these culture systems allow one dividing cell to give rise to a solitary central lumen-forming cyst (the structural unit of exocrine glandular epithelia *in vivo*) or, in the case of hepatic cell lines, to a multiple lumen-forming cell mass. More recent work has shown that some, although not all, of these mechanisms are conserved in different cell types and *in vivo* during early stages of embryogenesis [5,14]. We examine data from different model systems – supplemented with data from animal models and patients – to identify core molecular mechanisms and key players that determine the spatial orientation of the apical domain and positioning of the apical lumen.

## Signaling at the cell–ECM interface controls the orientation of the apical–basal axis

Single MDCK or Caco-2 cells, embedded in an isotropic 3D ECM, randomly distribute apical and basolateral proteins at their plasma membrane. When these cells enter mitosis, at least two transmembrane proteins that play an important role in apical domain and lumen development in kidney epithelial cells, crumbs-3a and podocalyxin [15,16], are internalized into Rab11a-positive recycling endosomes [7], which concentrate around mitotic spindle poles [7,17]. Following the first cell division, basolateral proteins such as E-cadherin and Na/K-ATPase are sequestered at the lateral surfaces between daughter cells. By contrast, crumbs-3a and podocalyxin accumulate at surface domains facing the ECM [1,2]. High signaling activity by the small GTPase RhoA and its effector Rho kinase-associated protein kinase (ROCK)I at the ECM-facing cell surface promotes the phosphorylation and, thereby, activation of ezrin at this domain [2,18]. Activated ezrin stabilizes a complex consisting of podocalyxin, NHERF1/EBP50, and ezrin at the ECM-facing surface by linking the complex to the F-actin cytoskeleton. Other ezrin-binding apical proteins may similarly be stabilized. Thus, following the first cell division and before the formation of a lumen, the daughter cells establish RhoA activity-mediated apical–basal cell surface polarity with their apical domains facing the ECM.

For the *de novo* generation of an apical lumen in between the cells, a subsequent reorientation of the apical–basal

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*Keywords:* cell polarity; apical lumen; extracellular matrix; Rab11; recycling endosome.
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0962-8924/
 http://dx.doi.org/10.1016/j.tcb.2015.04.002

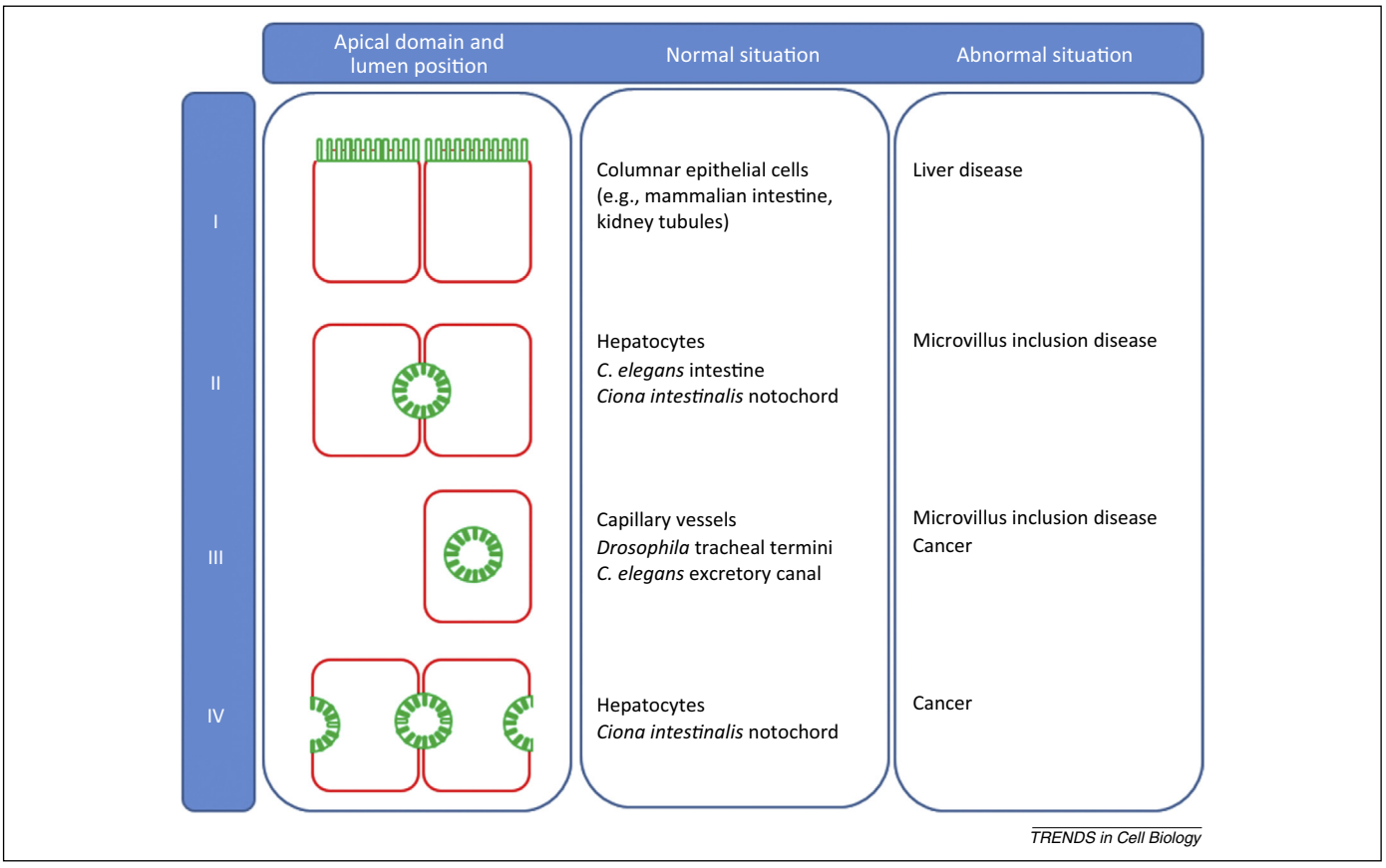


**Figure 1**. Four examples (I–IV) of distinct apical lumen positioning phenotypes in normal and abnormal settings. It should be noted that, in situation II, enterocytes of microvillus inclusion disease patients do show lateral microvilli that are normally only found at the apical domain, but the apical identity of these microvilli requires further investigation. Apical membranes are denoted by green lines; basolateral membranes are denoted by red lines.

polarity axis is required. This occurs via a mechanism that may involve quorum sensing of the ECM by integrin receptors. At the ECM interface, activated β1-integrin receptors form complexes with α2- and α3-integrin pairs [2,19–21]. The ECM, likely via α2β1-integrins, phosphatidylinositol (PI)3 kinase and its subunit p110δ, and/or Arf6, promotes the activation of the GTPase Rac1 [20–25] (Figure 2). Rac1 activity then promotes the assembly of laminin, possibly via α3β1-integrins, at the ECM-facing cell surface [20–22]. Indeed, the expression of a dominant-negative mutant of Rac1 in collagen type I-embedded MDCK cells inhibits laminin assembly, and this results in the formation of cysts that maintain inverted apical polarity (i.e., apical domains facing the ECM [21]) and that cannot establish a central apical lumen. The downstream mechanism via which Rac1 promotes laminin assembly is not clear, and Rac1 is dispensable for polarity orientation in some cells [14], suggesting that Rac1 may have a tissue-specific function [14]. Laminin assembly and the formation of a basal lamina at the ECM-facing cell surface require the polarized secretion of laminin and the polarized delivery of laminin-binding receptors to the cell surface [26]. The intracellular polarity protein Par1b is required for the polarized localization of the laminin-binding dystroglycan complex to the basal cell surface of MDCK cell [27]. In MECs, Par1b regulates the basolateral localization of laminin-111-binding integrins via the phosphorylation of the E3 ubiquitin ligase RNF41 [28]. Consistent with the role of dystroglycans and integrins in ECM remodeling, Par1b regulates focal adhesions [29] and extracellular laminin assembly [27]. The knockdown of Par1b or RNF41 results in cysts with perturbed apical polarity and inhibits ECM-directed central apical lumen formation [30]. Par1b also regulates polarized basal lamina assembly in 3D cultured mouse submandibular salivary glands to coordinate tissue polarity [31]. Interestingly, Par1b can also phosphorylate the insulin receptor substrate p53 (IRSp53). In its non-phosphorylated state, IRSp53 binds to GTP-bound Rac1 and Cdc42, and serves as an adaptor to recruit additional proteins [29]. Phosphorylation of IRSp53 by Par1b recruits 14-3-3 proteins and inactivates IRSp53. IRSp53-depleted MDCK cysts are defective in the assembly of laminin [29], akin to MDCK cysts that overexpress Par1b [29] or a dominant-negative Rac1 mutant [21]. Possibly, Par1b activity may need to be kept within limits to promote the assembly of laminin at the ECM-facing surface and the subsequent development of a central apical lumen.

The assembly of laminin at the ECM-abutting cell surface attenuates RhoA-ROCKI activity (Figure 2). In MDCK cells, activated β1-integrins reduce RhoA activity through the focal adhesion kinase (FAK)-dependent recruitment of a GTP-activating protein (GAP) for RhoA, p190A-RhoGAP [2,18]. Reduced RhoA-ROCKI activity reduces the phosphorylation status, and thereby the activity, of ezrin at the cell–ECM interface. This allows the phosphorylation of the podocalyxin–NHERF1/EBP50–ezrin complex by