

MECHANOBIOLOGY

The mechanics of crypt morphogenesis

Intestinal organoids have been used to model development of the crypt-villus axis and uncover signalling pathways that govern the stem-cell niche and induce differentiation. Two studies now take advantage of intestinal organoids to measure the mechanical forces that drive morphogenesis of the crypt and fate specification of its resident cells.

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The intestine simultaneously serves as a barrier to the outside world and enables the digestion and uptake of nutrients. The epithelial cells that line the lumen of the intestine accomplish these vital functions through an elaborate three-dimensional (3D) tissue morphology that is compartmentalized into villi and crypts. Villi are finger-like extensions lined by non-dividing enterocytes, tuft cells, and goblet cells, which protrude into the lumen to increase the surface area available for nutrient uptake (Fig. 1a). The base of each villus is surrounded by epithelial invaginations known as crypts, which house and physically protect both the stem cells of the tissue located in the valleys and the transit-amplifying cells that migrate up the walls as they proliferate¹. Stem cells in the valleys of the crypts are flanked by Paneth cells, which provide a permissive and supportive niche. Isolated intestinal stem cells can generate organoids in culture that mimic the crypt-villus architecture and contain all of the resident epithelial cell types² (Fig. 1b). These organoids have been instrumental in uncovering the biochemical signalling that promotes epithelial compartmentalization into villi and crypts, drives formation and maintenance of the stem-cell niche, and defines differentiation into the variety of cell types that comprise the functional barrier of the intestinal epithelium³. In this issue of *Nature Cell Biology*, Perez-Gonzalez et al.⁴ and Yang et al.⁵ use intestinal organoids to uncover the mechanical forces that promote crypt morphogenesis. Remarkably, the findings from these studies suggest that the epithelium can generate sufficient forces to bend itself into a crypt in the absence of a surrounding mesenchyme.

The intestinal epithelium is derived from the midgut endoderm, an initially smooth cylinder of tissue that is surrounded by multiple layers of mesenchyme in the developing embryo. The processes that fold this smooth epithelium into the undulating surface of the mature intestine have been investigated for decades^{6,7}. In

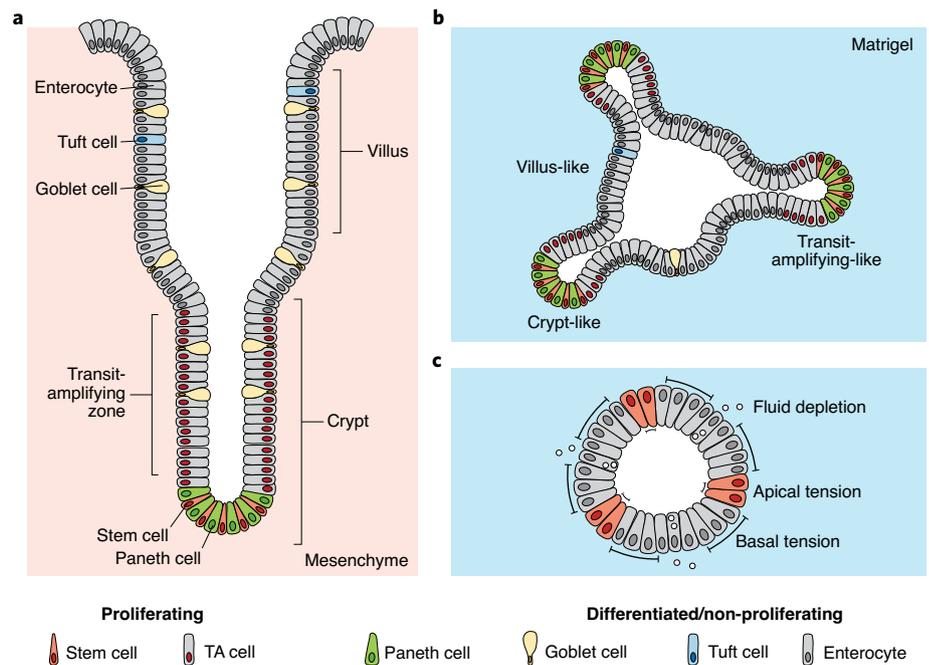


Fig. 1 | Anatomy of intestinal epithelium in vivo and in organoids. a, The large surface area of the intestine results from the generation of villi that protrude into the lumen and crypts that extend into the mesenchyme. Stem cells reside in a niche at the bottom of the crypts. Daughter cells proliferate as they migrate up the transit-amplifying zone and then become quiescent, differentiated cells in the villi. Inspired by ref. ¹. **b**, Stem cells form into 3D intestinal organoids that contain villus-like regions of differentiated cells, crypt-like regions of stem cells, and transit-amplifying-like regions of proliferating cells. **c**, The geometry of the crypt emerges from tension gradients that result from apical contraction of the stem cells and basal contraction of the proliferating cells. Invagination also requires depletion of luminal fluid, which is driven by transport across enterocytes.

the embryonic chicken, the sequential differentiation of circumferential and longitudinal layers of smooth muscle establishes compressive forces that induce the epithelium to passively buckle into villi⁸. In the embryonic mouse, however, villus formation occurs independently of smooth muscle differentiation. Instead, hedgehog signalling from the epithelium activates planar cell polarity genes to promote the aggregation of periodic clusters of mesenchymal cells⁹, which migrate towards the epithelium and locally induce

the formation of villi by signalling through bone morphogenetic protein (Bmp)¹⁰. These local signals cause the epithelium to change shape, from tall-and-thin to short-and-wide, which is thought to alter the mechanical properties of the tissue and promote its folding into villi. Crypts only begin to emerge in the small intestine after villus morphogenesis is complete. Epithelial cells located in the inter-villus regions undergo myosin-II-driven apical constriction, which promotes initial invagination of the developing crypt into the surrounding

mesenchyme¹¹. Epithelial cells located in the regions in between the crypts and villi then undergo a basal constriction and expand apically to generate wedge-shaped cells that form a hinge between the two compartments¹¹. The relative roles of epithelial and mesenchymal forces, as well as crosstalk between these tissues during formation of the mouse intestinal crypt remain a mystery.

Intestinal organoids are perhaps ideally suited to shed light on the physical forces that govern crypt morphogenesis. To address this issue, Yang et al.⁵ combined light-sheet microscopy, quantitative imaging analysis, laser cutting, and micropipette aspiration to measure the physical forces exerted by mouse intestinal organoids as they fold themselves into crypt domains. Their data showed that the volume of fluid within the lumen of the organoid decreases as the crypt begins to form. Local actomyosin contraction led to an increase in apical tension in the crypt region, which was accompanied by basal contraction in the villus region. These differential tensions resulted in an asymmetry of forces that were sufficient to change the local curvature of the epithelium and promote the formation of the crypt if the lumen was deflated (Fig. 1c). Quantitative modelling showed that actomyosin contraction is insufficient to fold the epithelium in the absence of deflation, suggesting that regulation of luminal fluid volume is essential for overcoming the balance of forces in this 3D tissue mimetic. Yang et al. also found that the decrease in luminal fluid volume is regulated by a sodium/glucose cotransporter expressed in the enterocytes that populate the villus region, directly coupling differentiation in one compartment of the organoid to morphogenesis of the other.

To measure the forces of crypt morphogenesis in mouse intestinal organoids at subcellular resolution, Perez-Gonzalez et al. isolated the crypt regions after they had already formed and cultured them on soft, two-dimensional (2D) hydrogels⁴. They found that the 2D crypt organoids spread into a continuous monolayer that maintains the pattern of cell types observed in the 3D system: a central region of stem cells and Paneth cells, surrounded by a highly proliferative inner ring of transit-amplifying-like cells, followed by an outer ring of differentiated villus-like cells. Remarkably, cells moved

from one region of the monolayer to the next, in a series of steps similar to that observed in crypts *in vivo*: stem cells divide but remain in the central region, and daughter cells migrate radially into the highly proliferative zone until they reach the differentiated villus-like ring, where they die and are extruded. By culturing 2D crypt organoids on hydrogels of decreasing stiffness, coupled with traction force microscopy and quantitative imaging analysis, Perez-Gonzalez et al. showed that the central stem cell region exerts compressive (pushing) forces on its underlying substratum and undergoes an apical–basal tilt to bend itself downward as the stem cells—but not the Paneth cells—undergo apical constriction. Similarly to observations of the villus regions of 3D organoids from Yang et al.⁵ and of the hinge region in the mouse intestinal epithelium *in vivo*¹¹, Perez-Gonzalez et al.⁴ found that cells in the highly proliferative region exert tensile (pulling) forces on the underlying substratum as they undergo basal constriction. The transition from apical to basal constriction generates a radial gradient of increasing tension, which causes cells at the outer ring of the 2D monolayer to pull their neighbours out of the organoid in a form of collective migration.

Combined, these two studies reveal the physical forces that are essential for the generation of the crypt, as well as for collective motions of cells out of the crypt and towards the villus. Importantly, it is the combination of forces exerted by cells in the villus and in the nascent crypt that drives the morphogenesis of the latter. The observation that enterocytes promote luminal fluid depletion, which is required for crypt invagination, is consistent with the fact that morphogenesis of the crypt follows that of the villus in the small intestine *in vivo*. Uncovering the factors that regulate expression and activation of the sodium/glucose transporter will help elucidate the relative roles of luminal fluid pressure and villus swelling during crypt morphogenesis. The observation that stem cells actively undergo apical constriction to locally bend the epithelium is consistent with the fact that morphogenesis of organoids in 3D requires soft matrices¹². This mode of morphogenesis could also help to explain the heterogeneity observed in intestinal organoids: the ratio of stem cells to other cell types would be expected to alter the balance of forces and

thus the final morphology of the organoid. Future work is needed to uncover the molecular signalling that activates apical constriction specifically in the stem cells and prevents its activation in neighbouring Paneth cells.

The findings from Yang et al. and Perez-Gonzalez et al. suggest that the stem cells that will eventually reside in the valley of the crypt actively generate the forces necessary to fold this tissue compartment. However, it remains unclear whether the force gradients observed in culture exist in the developing intestine in the embryo and if such forces would be sufficient to induce crypt morphogenesis. Indeed, biochemical signals and mechanical forces from the surrounding mesenchyme are essential for villus morphogenesis in the mouse intestine *in vivo*, and similar mesenchymal populations were recently uncovered in the developing human intestine¹³. Nonetheless, both mouse and human intestinal organoids are capable of forming villus-like compartments in the absence of mesenchyme. Such discrepancies between development in culture and in organoids highlights the need for caution in interpreting physical mechanisms – nature has devised many routes for tissue folding. □

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Competing interests

The author declares no competing interests.