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Digesting the mechanobiology of the intestinal epithelium

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The dizzying life of the homeostatic intestinal epithelium is governed by a complex interplay between fate, form, force and function. This interplay is beginning to be elucidated thanks to advances in intravital and *ex vivo* imaging, organoid culture, and biomechanical measurements. Recent discoveries have untangled the intricate organization of the forces that fold the monolayer into crypts and villi, compartmentalize cell types, direct cell migration, and regulate cell identity, proliferation and death. These findings revealed that the dynamic equilibrium of the healthy intestinal epithelium relies on its ability to precisely coordinate tractions and tensions in space and time. In this review, we discuss recent findings in intestinal mechanobiology, and highlight some of the many fascinating questions that remain to be addressed in this emerging field.

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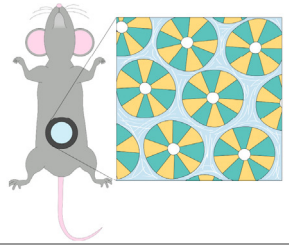
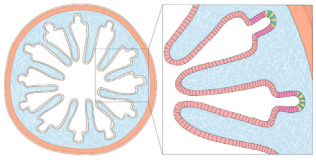
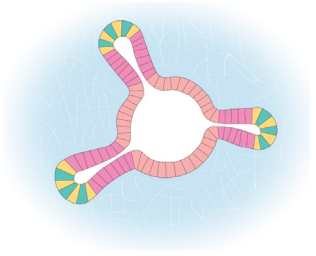
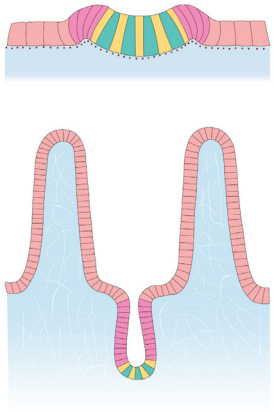
Introduction

The intestinal epithelium is a monolayer of cells that lines the internal surface of the gut. This monolayer constitutes the first physical barrier against the external environment and, at the same time, absorbs food-derived water, ions and nutrients. It is composed of apicobasally polarized columnar cells adhered to a thin basement membrane, rich in collagen IV and laminin. In the mammalian small intestine, the monolayer folds, giving rise to two functional compartments – finger-like protrusions into the lumen called villi, which increase surface area to maximize absorption, and invaginations called crypts, which protect specific cell populations from the potentially aggressive lumen content. Stem cells reside at the bottom of the crypt, where they are exposed to biochemical signals that sustain their self-renewal [1,2]. Some of these signalling molecules are secreted by the stroma [1,3] whereas others are provided by Paneth cells that are intermingled with stem cells in a checkerboard pattern [4]. Stem cells constantly divide, exit the stem cell niche and enter in the crypt neck, where they proliferate faster as transit amplifying cells (TA) [5]. After two days, TA cells reach the villus and differentiate either into absorptive (enterocytes) or secretory (enteroendocrine cells, goblet cells, tuft cells) lineages [5]. Differentiated cells further mature as they migrate towards the tip of the villus, where they are extruded and die only 3–5 days after initially leaving the stem cell niche, making the intestinal epithelium the fastest self-renewing tissue in adult mammals [5].

To sustain homeostasis, the intestinal epithelium must perform multiple mechanical tasks: maintenance of tissue folding, remodelling by crypt fission and fusion, segregation of cell types, migration between tissue compartments and preservation of barrier integrity despite the constant absorption of luminal content, and the frequent cell proliferation and extrusion. Furthermore, the epithelium constantly withstands external forces such as shear stress produced by the flow of luminal content, as well as stretch and compression caused by peristaltic movements. Alterations in the ability to generate or resist forces may lead to pathological conditions such as infections, inflammatory diseases and cancer. Yet, we are only starting to uncover how the intestinal epithelium coordinates forces to robustly perform its functions.

Different model systems now allow researchers to interrogate intestinal tissue mechanics (for in depth review of concepts and techniques about tissue mechanics, we refer to Ref. [6]). *In vivo* and *ex vivo* imaging provides physiologically relevant but quantitatively limited mechanical measurements (Box 1). Intestinal organoids, on the other hand, are simplified models but they enable a more accurate measurement and manipulation of tissue forces

Box 1 Experimental model systems to study the mammalian intestinal epithelium and summary of their key advantages, disadvantages and force measurement techniques available.

Model	Advantages	Disadvantages	Techniques	Refs
In vivo 	Highest physiological relevance	Difficult experimental access. Need of expertise with optical windows for intravital imaging. Limited optical access to the villus due to light scattering	Force inference* Laser ablation* Molecular force sensors*	[25,57]
Ex vivo 	Physiologically relevant information Better experimental access and control than *in vivo* (imaging, drug treatments)	Short term imaging (24h) due to tissue death Challenging experimentally	Force inference* Laser ablation Molecular force sensors* Rheology and atomic force microscopy* Apical surface pipette aspiration*	[56••]
Closed-lumen organoid 	Control of the mechanochemical microenvironment Use of human material	Lack of native tissue microenvironment Short-term culture Pressurized lumen (not necessarily physiological) Unphysiological accumulation of debris in the lumen. Cell migration not properly recapitulated Low resolution traction force measurements	Force inference* Laser ablation Molecular force sensors* Atomic force microscopy Basal surface pipette aspiration Inflation 3D traction force microscopy Stretch	[23••,24,26•, 34,48–50]
Open-lumen organoid 	Control of the mechanochemical microenvironment Use of human material Long term culture (up to 3 weeks) Open lumen allows cell shedding Accessible imaging and traction force measurements Active cell migration Possibility to recapitulate crypt-villus topology	Lack of native tissue microenvironment Lack of substrate remodelling and degradability in polyacrylamide gels. Lack of a well-developed brush border and apicobasal polarization in some systems. Small TA zone on flat substrates compared to *in vivo*	Force inference* Laser ablation Molecular force sensors* Atomic force microscopy* Apical surface pipette aspiration* High resolution 2D and 3D traction force microscopy Stretch	[8–10,11••, 12,13•,14••, 15,21]

*These techniques are theoretically applicable but have not been used in the intestinal epithelium to our knowledge.

(Box 1). Organoids recapitulate many features of the native intestinal epithelium, such as the development of crypt-like and villus-like domains containing most of the cell types observed *in vivo* and their compartment-specific self-renewing functions like proliferation, migration, differentiation and death [7]. Traditional organoids are curved monolayers enclosing a pressurized lumen fully surrounded by ECM [7]. Recent advances enable to grow organoids on flat or curved adherent surfaces, where they form self-organized epithelial monolayers with an exposed apical surface [8–10,11••,12,13•,14••,15], better recapitulating the open-lumen organization *in vivo* (Box 1). Mechanical measurements in the intestinal epithelium unravelled that each of its functional compartments has a distinct mechanical identity, characterized by stereotypical actomyosin apicobasal distributions, dynamic formation and disassembly of cell–substrate and cell–cell adhesions, and polarized protrusive activity to generate active migratory tractions (Figure 1). In turn, the epithelium senses and responds to this mechanical microenvironment by adapting cell compartmentalization, identity and proliferation (Figure 1). In this review, we discuss the latest findings on the forces that orchestrate the different homeostatic tasks of the small intestinal epithelium. We do not discuss the colon epithelium, which lacks villi, compartmentalization is more diffuse [5], and its mechanics are largely unexplored.

Folding

During embryonic development, the epithelium passively folds into villi due to the remodelling of the underlying ECM by other cell types. In chicken, sequential differentiation of the gut muscle layers generates compressive stresses that buckle the tissue to shape villi [16]. In mice, instead, villification is controlled by subepithelial fibroblast condensates [17,18]. *In vitro* experiments show that mouse intestinal fibroblasts can contract and buckle the extracellular matrix, controlling the folding patterns of the intestinal epithelium [19]. Interestingly, villus-like structures can also form *in vitro* in the absence of stromal cells if intestinal epithelial cells are exposed to cyclic stretch [20] or shear stress [21].

In contrast to villi, crypt morphogenesis occurs postnatally. Progenitor cells at intervillar regions undergo actomyosin-driven apical constriction, generating forces that bend the epithelium towards the underlying matrix to drive crypt folding [22•] (Figure 1). As an epithelial-intrinsic property, crypt folding is recapitulated by intestinal organoids [7]. Organoid progenitor monolayers seeded on flat soft gels self-organize into crypt and villus-like regions [14••]. Crypt progenitors progressively accumulate apical actomyosin and exert increasing pushing forces on the substrate to fold, in a process reminiscent of *in vivo* crypt formation. Similarly, organoids cultured in 3D Matrigel form nearly spherical cysts that spontaneously break symmetry and generate crypt-like structures through apical constriction [23••,24]. In both experimental models, inhibition of actomyosin contraction disrupts forces and leads to crypt unfolding. A 3D vertex model imposing cell surface tensions that follow cortical actomyosin distribution recapitulates crypt folding and single cell morphology, supporting apical constriction of stem cells as the driving mechanism for crypt folding [14••,23••]. Even after morphogenesis, adult crypt cells keep an apical actomyosin enrichment [14••]. This suggests that the epithelium actively maintains crypt shape and might contribute to its remodelling during crypt fission and fusion [25]. Whether the underlying stromal cells also contribute to crypt folding *in vivo* remains an open question.

In organoids enclosing a lumen, luminal hydraulic pressure constitutes an additional morphogenetic force [23••]. Newly differentiated enterocytes absorb pressurized luminal fluid, triggering a decrease in luminal volume that relaxes monolayer tension and facilitates crypt folding [23••]. Collapse after inflation of the organoid crypt also triggers crypt fission mediated by Piezo1 mechanotransduction [26•]. Still, it remains to be investigated whether crypts experience significant hydrostatic pressure *in vivo*.

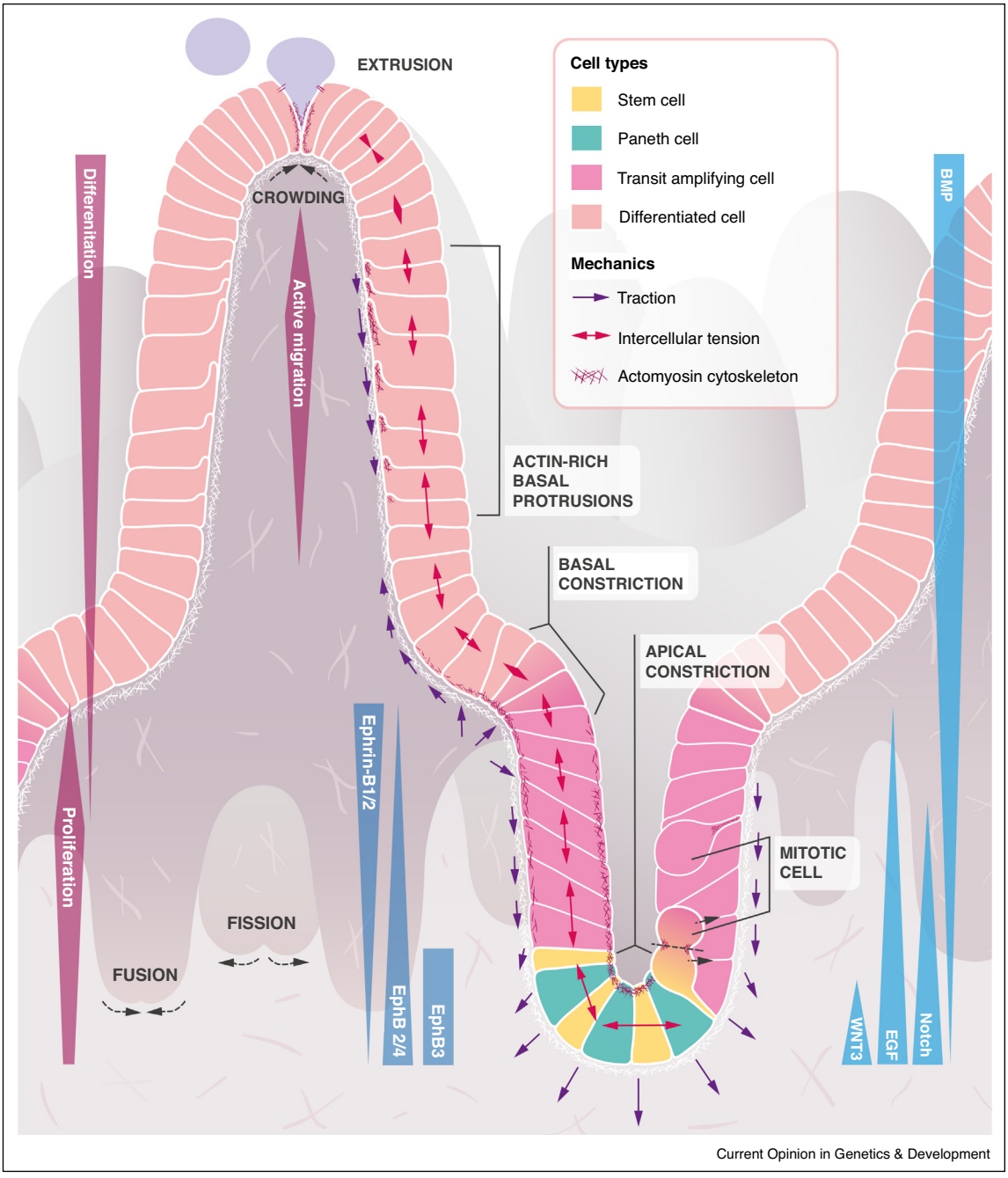
Crypt-villus compartmentalization

Gradients in Eph/Ephrin signalling are master controllers of cell sorting and compartmentalization into crypt and villus [27–30]. Expression of EphB2/4 receptors exhibits an increasing gradient towards the bottom of the crypt, while EphB3 is restricted to the stem cell niche. EphrinB1/2 ligands are expressed in a counter-gradient, with maximal levels at the villus. Differential adhesion between EphB/EphrinB expressing cells is proposed to control cell sorting and compartmentalization in the intestine. At the contact between EphB/EphrinB expressing cells, activation of the metalloproteinase ADAM10 triggers E-cadherin shedding and boundary formation [27].

Other mechanisms such as increased cortical tension may contribute to intestinal compartmentalization [14••,22•,23••,31]. Wedge-shaped cells located at the boundary between the crypt and villus compartments exhibit actomyosin-driven basal constriction [14••,23••] (Figure 1), which may be facilitated by Rac1 activation and downregulation of hemidesmosomal adhesions [22•]. Of note, Rac1 inhibition alters Eph/Ephrin mediated sorting in other systems [32], indicating a possible link between Eph/Ephrin and basal constriction at the crypt-villus boundary.

In organoids, crypt and villus-like regions appear as independent mechanical compartments characterized by tractions forces pointing in opposite directions [14••]. Interestingly, a similar traction pattern is observed

Figure 1



Mechanics of the homeostatic intestinal epithelium. The spatial distribution of the actomyosin cytoskeleton results in patterned tractions (forces transmitted to the extracellular matrix) and intercellular tension (pulling force transmitted through the epithelium by the cell cytoskeleton and cell–cell junctions). These forces were measured or inferred in Refs. [14••,23••,56••]. Cellular forces drive apical constriction to fold the crypt, basal constriction to compartmentalize crypt and villus, cytokinetic contraction for cell division, active migration through basal protrusions, and basolateral contraction to trigger cell extrusion. Signalling pathways at the stem cell niche and the villus regulate cell identity and proliferation, while Eph-Ephrin gradients induce the segregation of different cell types. Upon proliferation at the crypt, sister cells often separate before reintegrating in the monolayer, which may facilitate the exit of the stem cell niche. Crypts are dynamic and undergo fission and fusion.

in colliding monolayers of MDCK cells expressing EphB2 or EphrinB1 [33], further supporting Eph/Ephrin-driven compartmentalization in the intestine.

Cell identity

Different biochemical factors regulate intestinal cell identity along the crypt-villus axis (Figure 1). Wnt, Notch and EGF signalling at the bottom of the crypt induce and maintain intestinal stem cells, while BMP signalling at the villus induces differentiation [5]. Some of these signalling pathways can be activated by mechanical cues in cell lines and organoids. For instance, Wnt signalling is enhanced by tissue compression, which induces intracellular molecular crowding and stabilizes the LRP6 signalosome [34]. By contrast, pulling forces are required to cleave Notch receptor and activate its downstream signalling [35].

The mechanosensitive transcriptional regulator YAP also regulates intestinal cell identity [36–40]. During organoid morphogenesis, symmetry breaking and crypt budding rely on transient cytoplasmic shuttling of YAP [41,42]. Cells that retain nuclear YAP differentiate into Paneth cells, defining the localization of the nascent crypt [41]. Like in other systems [43], YAP signalling is influenced by substrate stiffness in the intestinal epithelium [13•,42]. Synthetic matrices of 1.3 kPa in stiffness favour cyst formation through nuclear YAP translocation. However, matrix softening is required for intestinal stem cell differentiation and organoid budding [42]. Recent reports on open-lumen organoids show that softening of the matrix results in larger crypts with increasing numbers of stem cells [13•,14••]. Both in organoids and *in vivo*, YAP activation triggers differentiation of intestinal stem cells into goblet cells [13•,40], although opposite effects have been also suggested [44]. The effect of YAP activation on intestinal stem cell dynamics and differentiation thus remains controversial [36–40,45,46]. Discrepancies may be explained by the context-dependent role of YAP in homeostasis and regeneration, when cells can de-differentiate to restore tissue integrity.

Tissue curvature also influences patterning of cell types. Organoids growing on curved surfaces spontaneously position the crypt and villus cells in concave and convex regions, respectively [10,11••,15]. Yet, organoids growing on flat surfaces self-organize to develop crypt-like and villus-like structures that can spontaneously fold [8,9,12,13•,14••]. Thus, although curvature influences the location of crypt and villus compartments, it is dispensable for cell fate determination. Interestingly, compartment-specific forces appear in parallel with cell fate [14••]. How forces determine cell fate and, conversely, how specific cell types are encoded to generate a characteristic set of forces, needs further investigation.

Proliferation

Mitotic events in the intestinal epithelium occur at the apical surface, a common feature of pseudostratified tissues [47]. During apical division at the intestinal stem cell niche, some sister cells remain neighbours, while other sisters separate and reintegrate into the tissue apart from each other (Figure 1), promoting the exit from the stem cell niche into the transit amplifying zone and their subsequent differentiation [48]. It was suggested that sister stem cell separation is more efficient in cases where a stem cell divides on top of the stiffer [49] and more substrate-adherent [50] Paneth cell. Interestingly, in less crowded tissue regions of intestinal organoids, intestinal cells can also divide non-apically, suggesting that increased tissue density in the intestinal epithelium promotes apical nuclear movement [48], similar to what has been reported in other systems [51].

Intestinal proliferation is regulated by a complex interplay of niche signals and a plethora of other factors (Figure 1) [52], including mechanical inputs such as stretch or compression. Stretch enhances stem cell proliferation in engrafted human organoids, intestinal cell culture and isolated pig intestines [53–55]. In organoids, however, tissue compression fosters cell proliferation by enhancing Wnt signalling [34]. It is possible that in the lower density regime of organoids, compression aids proliferation by concentrating proliferation-inducing factors, while in the more constrained and packed *in vivo* epithelium, compression slows proliferation and stretch stimulates it, through mechanoresponsive factors such as YAP. The extracellular matrix composition can also modulate proliferation through integrin signalling. In organoids cultured in synthetic 3D networks, collagen IV and Laminin 111 efficiently support cyst expansion, in contrast to other ECM proteins (fibronectin, perlecan or hyaluronic acid) [42].

Migration

The intestinal epithelium self-renews through the constant migration of new cells from the bottom of the crypt to the tip of the villus. Migration was assumed to be driven by pushing compressive forces generated by proliferation at the crypt. This idea was recently ruled out as inhibiting cell proliferation at the crypt does not stop migration at the villus [56••]. Moreover, stress measurements *in vivo* and in organoids revealed that the intestinal epithelium is under tension [14••,56••], not compression.

The first step in the intestinal cell journey is exiting the stem cell niche. *In vivo* lineage tracing experiments show a neutral competition for niche space between stem cells, where cells at the edge of the niche have a higher probability to leave the compartment and differentiate [57]. Recent experiments in organoids combined particle image velocimetry, traction force microscopy and monolayer stress microscopy to systematically study the

mechanics of crypt cell migration, unraveling different migratory strategies within and between compartments [14••]. Average stem cell velocities reveal a persistent radial migration away from the niche. Radial tractions point opposite to cell velocity, suggesting that cells actively crawl away from the niche center. When cells cross the crypt-villus boundary, radial tractions change sign to point in the direction of movement, a traction pattern that resembles cells in a trailing edge that are being pulled by forces generated upper in the villus. This hypothesis needs to be explored *in vivo*, where crypt migratory forces have never been addressed.

The combination of laser ablation, quantitative imaging *in vivo* and theoretical modelling [56••] established that differentiated cells develop a front-rear polarity besides their apicobasal polarity. This allows them to actively migrate by extending front basal actin-rich protrusions resembling cryptic lamellipodia (Figure 1). These protrusions likely exert traction forces to propel the epithelium, generating a gradient of increasing tension from the top to the bottom of the villus. An appealing hypothesis is that this tension drags cells out of the crypt. When cells approach the tip of the villus, they slow down, leading to cell crowding before being extruded.

Open questions include how cells cross the mechanical boundary between the crypt and the villus, how they are internally organized to simultaneously sustain apicobasal and front-rear polarity, and which cues guide the directionality of migration. Appealing candidate mechanisms are chemotactic gradients secreted by epithelial or stromal cells, or gradients in basement membrane ligand concentration (haptotaxis) or stiffness (durotaxis) along the crypt-villus axis.

Extrusion

Upon reaching the tip of the villus, cells are extruded and shed into the luminal space (Figure 1). Although a small number of cells die inside the healthy crypt, villus shedding accounts for the vast majority of intestinal cell death, with about 1400 cells estimated to be lost at each tip daily in mice [58]. A portion of these cells is apoptotic (caspase-3 positive) before extrusion, but most extruded differentiated cells in the healthy tissue are living cells. Once extruded, loss of attachment to the basement membrane triggers cell death by anoikis [59].

During extrusion, the cell disassembles its E-cadherin junctions while gradually redistributing its tight junctions basolaterally. In parallel, neighbouring cells connect to each other by basolateral adhesions to maintain barrier integrity during extrusion [59]. The extrusion mechanism is based on actomyosin contraction; in the zebrafish epidermis and MDCK cells, live cell extrusion is triggered by cell compression resulting from locally increased cell density within the epithelium [60]. At that point, through a cascade involving the stretch-responsive channel Piezo1, the lipid Sphingosine-1-phosphate and Rho, a basolateral actomyosin accumulation in the extruding cell and its neighbours extrudes the cell by squeezing it from the base up. Similar extrusion phenotypes have been observed in the colonic epithelium [60], and increased cell density at the mouse villus tip has been demonstrated in the small intestine [56••]. It is therefore plausible that contraction and cell compression drives cell shedding in the epithelium of the small intestine as well. However, in open-lumen organoids [14••], the villus-like region exhibits low cell density and extruding cells have large spreading areas. Although the apoptotic state of extruding cells in these organoids should be examined, the extrusion of large cells suggests that increased cell density is not necessary for extrusion in the intestinal epithelium.

Independently of cell density, topological defects in cell nematic alignment trigger local compression and cell extrusion in some epithelial tissues [61]. Topological defects might also emerge at the villus tip due to the convergence of cell flows, triggering extrusion. With organoid systems at hand, future studies could measure and perturb cell density, alignment and compression to clarify the molecular mechanisms and forces driving cell extrusion in the intestinal epithelium.

Conclusion

The dazzling dynamic equilibrium of the intestinal epithelium relies on mechanical forces that have remained elusive for a long time. Today, we are beginning to understand how cells coordinate these forces in space and time thanks to technological advances in quantitative *in vivo* and *ex vivo* imaging, organoid culture, force measurements and theoretical modelling (Box 1). The convergence of mechanobiology and intestinal physiology is unveiling how each functional compartment of the intestinal epithelium performs its mechanical homeostatic tasks to sustain tissue folding, identity and self-renewal. Many outstanding questions remain to be further explored, such as the mechanisms of epithelial folding *in vivo* and the contribution of non-epithelial cells in this process, the interplay between signalling and forces leading to cell compartmentalization, the mechanical regulation of stemness and differentiation, or the cues guiding collective cell migration and extrusion. The development and combination of *in vivo* imaging, *ex vivo* culture and *in vitro* tissue engineering now provides a rich toolset to experimentally tackle all these questions (Box 1). These advances made the intestinal epithelium a paradigmatic example of mechanical multitasking, demonstrating the importance of force patterning in tissue function and leading the way towards a quantitative understanding of the role of mechanical forces in tissue homeostasis.

Conflict of interest statement

Nothing declared.

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