

Modeling perspectives on the intestinal crypt, a canonical system for growth, mechanics, and remodeling

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Abstract

The intestinal epithelium is crucial to maintaining a healthy gut. Central to this are the crypts of Lieberkühn, which coordinate the rapid self-renewal of the epithelium to protect the small intestine and colon during digestion. Further, mutations in crypt cells may initiate colorectal cancer. It is, therefore, important to understand how crypts function during homeostasis and disease. Mathematical and computational modeling has contributed to increasing our understanding of crypt dynamics. However, many open questions remain to be addressed, particularly regarding the role of mechanics in intestinal crypt dynamics. In this article, we review the state-of-the-art in crypt modeling and explain why further progress requires the integration of new theory from continuum mechanics with cell-based computational models and experimental data.

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Introduction

The intestinal epithelium is a monolayer that protects the small intestine and colon during digestion and simultaneously facilitates nutrient absorption [44,19]. Its rapid self-renewal is controlled by millions of crypts of Lieberkühn, uniformly-spaced invaginations lining the gut. In the intestines, multipotent stem cells are localized in crypt bases where they coordinate cell proliferation, death, and migration to facilitate the constant turnover of the epithelium every 3–5 days [19], with increased turnover in chronic inflammation [40]. Consequently, the crypt has become a canonical model

for studying stem cell dynamics [5]. In general, proliferative cells are only found in the crypts. Hence, when gut tissue is injured or inflamed, crypts are crucial to restoring barrier function by generating new tissue [27]. Moreover, colonic cancer is known to originate in colonic crypts [5]. For these reasons, understanding how crypt dynamics maintain homeostasis in health, how they are restored following injury, and disrupted in disease is of high clinical importance.

While considerable research attention has been directed toward the crypt, many questions remain unresolved. For example, what processes ensure that, during homeostasis, each crypt maintains a uniform and robust structure, while large numbers of cells proliferate and migrate? Additionally, little is known about how the crypt's specialized cell populations are generated and how its invaginated structure arises during development. Such fundamental questions are intimately related to the concept of tissue growth and its interactions with tissue mechanics.

The crypt's compartmentalized nature renders it ideal for mathematical and computational modeling. Significant efforts have focussed on using cell-based approaches to model the crypt, validate existing hypotheses, and generate new ones [17]. While such theory has yielded insight into the dynamics of healthy and diseased crypts, more can be achieved, particularly regarding the role of mechanics in regulating subcellular signaling pathways and thereby rates of cell proliferation, migration, and death. This is now possible due to the development of morphoelasticity theory, which extends continuum mechanics to account for biological tissue growth [21].

This review provides an introduction to the crypt, and relevant modeling approaches. We describe the crypt structure and the signaling pathways known to contribute to homeostasis. Relevant modeling approaches (discrete and continuum) are then introduced, with their strengths and limitations, and a summary of the advances they have enabled. We close by discussing open questions that will advance the modeling field, and in turn, increase understanding of the role of biomechanics in regulating healthy and diseased intestinal crypts.

Biological background

We now introduce the main components of the crypt. We describe its structure and surrounding environment, including signaling pathways involved in its regulation of health and disease; the reviews by Gehart and Clevers [19] and Spit et al. [44] contain further details.

Each test-tube-shaped crypt is lined with an epithelial sheet (Figure 1). Adjacent colonic crypts are separated by flat tissue regions. In the small intestine, crypts are connected to large protrusions called villi that increase gut surface area to aid nutrient absorption. Each crypt contains a pool of slowly-cycling [45] (or even paused [10]) stem cells in its base. In the small intestine, Paneth cells are also found in the crypt base, where they secrete factors that support the stem cell niche (the stem cell pool and its microenvironment) [19,44]. As stem cells divide, their offspring push upward toward the lumen, in a conveyor belt fashion. Transit-amplifying cells proliferate rapidly, before differentiating into specialized, non-proliferating cells, such as goblet cells [44]. Directly underneath the crypt is an extracellular matrix network called the basement membrane, which aids crypt maintenance and facilitates

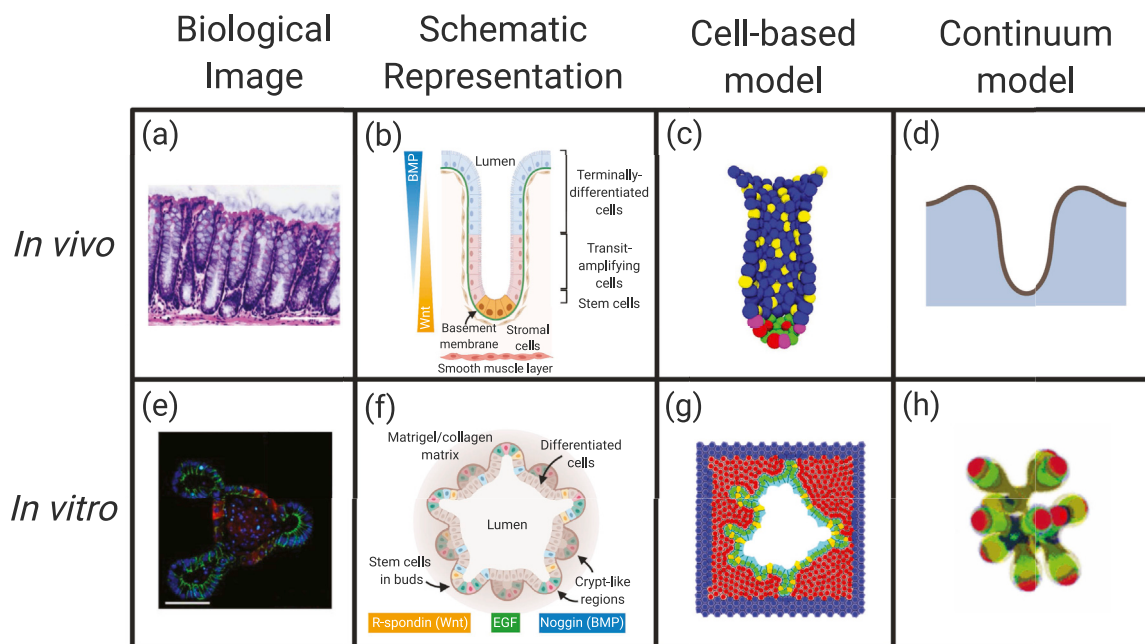
communication with stromal cells [34]. The stroma provides structural stability and additional regulatory signals [29]. It is supported by a layer of smooth muscle cells. A schematic of a crypt is presented in Figure 1(b).

The development of crypt organoids has accelerated understanding of crypt biology. Crypt organoids are *in vitro* cell cultures generated from single stem cells or isolated crypts, which grow into a confluent layer of epithelial tissue, with crypt-like buds surrounding an inner lumen (see Figure 1(f)). Organoids enable detailed investigation of cell–cell signaling, by knocking out genes and/or embedding them in different media. Moreover, as they are *in vitro* cultures, cell behavior can be tracked at greater spatiotemporal resolution using imaging techniques [20,42].

While multiple signaling pathways contribute to crypt homeostasis [19,44], we describe the dominant ones:

1. Wnt signaling is the principal signaling pathway in the crypt, and multiple Wnt proteins regulate cell proliferation, stemness, cell migration, differentiation, and apoptosis [19,44]. A decreasing Wnt

Figure 1



The crypt at varying levels of biological and mathematical abstractions. (a) Histological image of *in vivo* murine colonic crypts; the scale bar represents $100\mu\text{m}$ (adapted with permission from Shoshkes-Carmel et al. [43]). (b) Schematic diagram of a single colonic crypt and essential components. (c) 3D crypt model by Thalheim et al. [48] (adapted with permission). (d) 2D continuum representation of crypt (adapted with permission from Almet et al. [1]). (e) Fluorescence image of *in vitro* murine small intestinal organoid; the scale bar represents $50\mu\text{m}$ (adapted with permission from Gjorevski et al. [20]). (f) Schematic diagram of the organoid structure and relevant components. (g) 2D organoid model used in Langlands et al. [31] (adapted with permission). (h) 3D colon cancer organoid model from Yan et al. [51] (reproduced with permission).

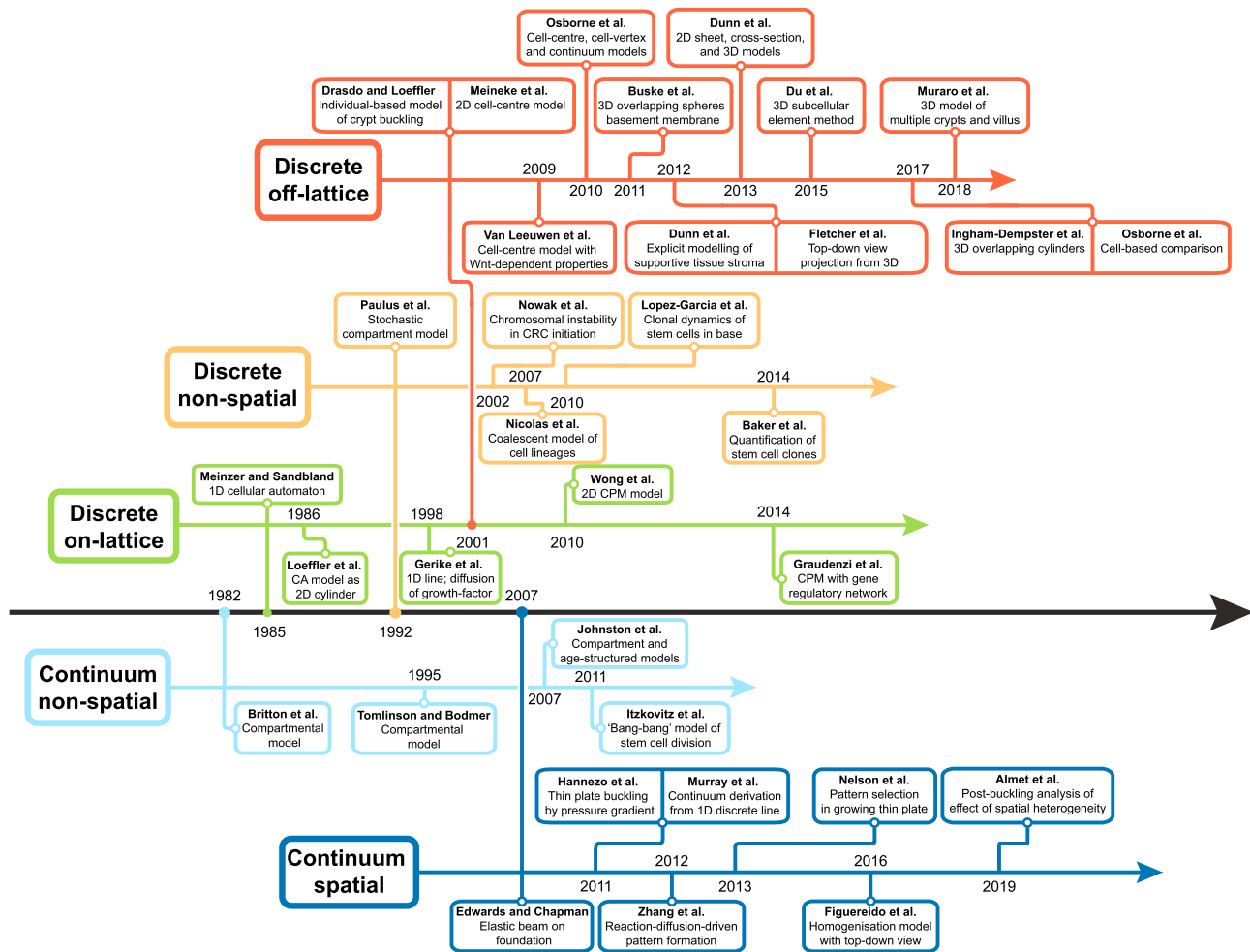
gradient exists along the crypt axis, but it is not fully known how this gradient arises or is maintained. Furthermore, Wnt pathway mutations are among the earliest to occur in colorectal cancer [19].

2. Bone Morphogenetic Protein (BMP) signaling suppresses stem cell multipotency and drives terminal differentiation. An increasing BMP gradient from the crypt base is maintained by the surrounding stroma, and BMP inhibition has been shown to cause excessive crypt formation [44].
3. Notch signaling governs cell fate specification [28]. In the small intestine, Notch-active stem and Paneth cells arise through lateral inhibition caused by cell-cell contact [44].
4. The Hippo pathway regulates Wnt and Notch signaling [4,42]. Inactive during homeostasis, its transducers YAP and TAZ facilitate regeneration

following injury [47], while their loss results in uncontrolled proliferation [23].

The involvement of YAP and TAZ in crypt homeostasis is significant because of their mechanotransductive roles; their regulation of Wnt and Notch is triggered by mechanical cues. Indeed, Gjorevski et al. [20] showed that culturing organoids in soft matrices increased stem cell proliferation, while culturing them in stiffer matrices stimulated stem cell differentiation and organoid formation, through a YAP-dependent pathway. Serra et al. [42] showed that transient activation of YAP/TAZ was required to trigger Paneth cell differentiation, which introduced mechanical heterogeneity in organoids, leading to crypt budding. Despite increasing evidence suggesting a regulatory role for mechanical

Figure 2



A timeline of modeling approaches that have been applied to the crypt. Models have been highlighted for the novelty of their approach and classified according to the discrete or continuum approach that is used, and the spatial effects that are considered.

stimuli, such mechanisms can be difficult to probe experimentally. In such situations, mathematical and computational modeling can provide significant insight.

Mathematical and computational models of the crypt

The approaches reviewed here can be termed “discrete”, where the crypt is viewed as a collection of individual cells (see Figure 1(c and g)) or “continuum”, where it is modeled as a smooth tissue (Figure 1(d and h)). Here, we focus on spatially-resolved models (for a review of models that neglect spatial effects, see Carulli et al. [11]). We discuss their strengths and weaknesses, focusing on recent modeling studies (see Figure 2 for a timeline of crypt modeling approaches).

Cell-based models

Cell-based models represent each cell as one or several points in space with a prescribed cell region [35]. They permit extraction of cell-scale data, such as migration velocities and lineage [48,39], and tissue scale information, such as mitotic distributions [13], for comparison with experimental data [5]. An example of a recent and comprehensive cell-based model of the crypt is that of Thalheim et al. [48]. We use this model as a benchmark for comparison with other models.

Thalheim et al. consider a 3D overlapping spheres model, in which crypt geometry is specified by a surface of revolution (see Figure 1(c)), a common representation among 3D models [13,12,36]. In 2D geometries, cell shape can be modeled via a Voronoi Tessellation, with spatial connectivity defined through a Delaunay Triangulation [31,2,39]; this is avoided in 3D due to computational costs. Adhesion to the crypt surface is enforced by a triangular fiber network, representing the basement membrane and included to ensure shape stability [26,31,2]. Cells interact with the membrane through a weakly-adhesive force. Detachment from the basement membrane results in cell death [13,31,2]. Cell-based models typically include forces that restrict cells to the crypt surface [13,12,36]. Cells at the crypt top are removed from simulations [12,13,39]. In Muraro et al. [36], cells migrate out of the crypt and onto the villus.

Thalheim et al. model cells as elastic spheres, using Langevin dynamics to update cell positions and sizes. In other words, cells were modeled as cylinders [26], while the subcellular element method represented cells as collections of overlapping spheres [12]. Langevin equations incorporate adhesion forces between overlapping cells, deformations induced by cell–cell contact, changes to cell volumes, and interactions with the basement membrane. A popular alternative approach to update cell positions is an over-damped form of Newton’s Second Law [31,2,36,13,39,26]. In Thalheim et al., proliferating cells grow in stochastic increments,

dividing into two cells when their initial volume doubles [12]. If cells are mechanically compressed, proliferation may stop due to contact inhibition [13,39]. Cell proliferative capacity and fate specification are determined by Wnt and Notch levels. Wnt signaling is modeled as a decreasing gradient from the base, a *de facto* approach [31,2,36,13,39,26,12]. Stem cells that leave the base to become transit-amplifying cells, which differentiate as they migrate toward the crypt lumen. Notch activity is determined by Notch levels from the nearest neighbors. Stem cells are assumed to express high levels of Notch. Transit cells differentiate into either Paneth cells (high Wnt, low Notch) that migrate down toward the base, or Goblet cells (low Wnt, low Notch) that migrate up the crypt. Du et al. [12] consider the additional role of BMP signaling in preventing the over-expansion of the stem cell population.

A strength of cell-based models is their ability to integrate processes acting on different spatial and temporal scales within a multiscale framework. For example, logic-based rules at the cell scale may be coupled to subcellular mathematical models for gene regulatory networks [22], which model gene activation as binary states, or ordinary differential equations for biochemical signaling pathways that describe continuous changes [50]. This allows more biological detail to be incorporated and investigation of how perturbations subcellular processes, for example due to genetic mutations, may impact tissue-level behavior.

However, with greater biological detail comes greater model complexity. For cell-based models, there is no standard way to analyze simulation results, which are obtained by averaging multiple realizations. Furthermore, incorporating biological detail increases the number of model parameters. As the dimension of the parameter space increases, it becomes more difficult to identify the mechanism(s) driving observed results and/or to fit the models to experimental data. Individual-based models can be computationally expensive to simulate, particularly when 3D geometries and/or sophisticated implementations, such as the immersed boundary method, which accounts for fluid–structure interactions between cells [16], are used.

Cell-based models also generally assume a fixed geometry. Processes, such as crypt invagination [46] and fission [31] occur in development, cancer, and regeneration, but cannot be modeled properly without accounting for deformability. As forces are specified at the cell scale, it can be difficult to estimate deformations on the scale observed *in vivo* [13,48]. The rigid geometry, thus, limits the scope of what can be investigated. While some cell-based organoid models account for tissue deformation [41,31,2,49], they do not produce deformations on the scale needed to study fission or invagination.

Continuum models

When studying processes characterized by large deformations, such as crypt fission or invagination, an alternative approach is to focus on the macroscale. The crypt tissue may be represented as multiple, interacting diffusible species [15,51], viscous fluids [30], or elastic sheets [25,18]. Morphoelasticity theory, which extends nonlinear elasticity to account for tissue growth [21], is another promising approach to tackle problems that cannot be addressed with cell-based models.

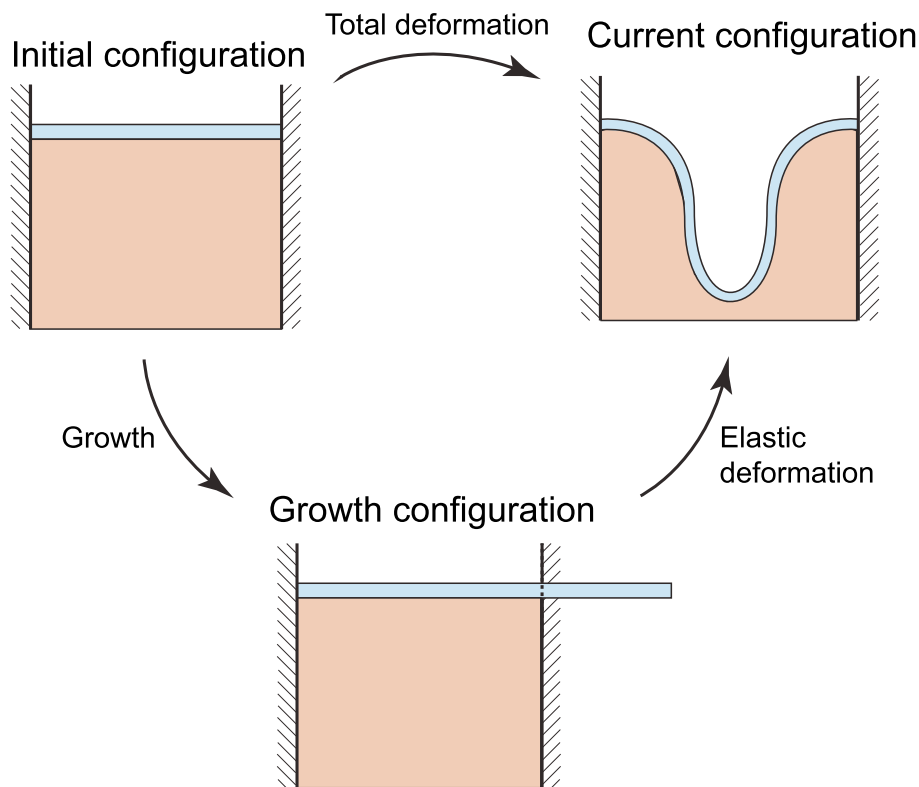
Continuum mechanics models are rooted in more developed branches of mathematics than cell-based models; governing equations come from first principles and deformations arise naturally due to mechanical forces and torques. As growth typically occurs on longer timescales than those associated with elastic/viscoelastic deformations, quasi-static mechanical equilibrium may be assumed. At each time step, the material deformation gradient can be decomposed into distinct growth and elastic deformations. First, growth is defined locally, and different tissue regions may overlap after growth. The elastic deformation map then reassembles the grown material to be geometrically compatible, which may induce residual stresses (see Figure 3).

Morphoelasticity has been used to study many biological processes [3], including wound healing, artery remodeling, and brain cortical folding, the latter sharing many similarities with crypt invagination. Several continuum-mechanics models for crypt and/or villi buckling have been proposed [14,37,8,38], but lack biological specificity. Morphoelasticity has recently been used to study cylindrical buckling along the gastrointestinal tract [7], while Almet et al. [1] considered the effect of material heterogeneity and large deformations on the growth of an idealized crypt model (Figure 1(d)). Despite these advances, the biological realism of continuum crypt models remains limited.

Continuum frameworks, formulated using ordinary and partial differential equations, can be analyzed using a range of analytical tools, including asymptotic methods and linear stability and weakly nonlinear analysis. In this way, it is possible to assess the qualitative and quantitative effects of varying different model parameters and systematically reveal the mechanisms driving observed behaviors.

There are several challenges with tissue-level continuum modeling. By construction, in such models, there is

Figure 3



Potential application of morphoelasticity to the crypt. Here, the crypt is treated as a growing, elastic rod. Crypt evolution is decomposed into an axial growth phase, as a proxy for tissue proliferation, and the subsequent elastic deformations due to residual stresses induced by growth, mechanical effects of the underlying substrate, and boundary conditions.

Table 1

Desirable features of an ideal crypt model, and the current efficacy of continuum and cell-based frameworks to capture them. The two classes of frameworks have complementary strengths and limitations.

Crypt feature	Continuum	Discrete (cell-based)
Deformability	✓✓: Can use continuum mechanics theory	✓: Not well-developed
Mechanical effects	✓✓: Very well-placed	
Clonal cell tracking	✓: Possible for a few models	✓✓: Can easily track cell lineages
Subcellular detail	Has not been considered in detail	✓✓: Very well-suited for this
Stochasticity	×	✓
Cell migration	Possible for certain models	✓✓
Population control	✓: Laws to prescribe local growth	✓✓
Interaction with external environment	✓✓: Can prescribe mechanical forces	✓: Computationally expensive
Cellular heterogeneity	✓: Coarse-grained heterogeneity	✓✓: Individual cell heterogeneity
Cell sorting	×	✓: Possible in some models
Experimental parametrization	Possible but has not been implemented	✓: Possible with enough biological detail

no individual cellular unit, despite the need to incorporate relevant cellular-level detail. This is evident in the crypt, which is spatially and temporally heterogeneous in numerous ways, including growth capacity and stiffness, which depend on cellular processes. Describing such features in a continuous setting is non-trivial, but feasible. For instance, one may need to couple the various processes, spanning subcellular, cellular, and tissue scales. However, multiscale approaches, such as homogenization theory or micro-mechanics, are, in principle, available. Parameterizing such models poses a different challenge. For instance, a key mechanical parameter is the stiffness, e.g. Young's modulus; stiffness is easily obtained with a macroscale material, but less so with a small biological sample. Atomic Force Microscopy has become useful for measuring the mechanical properties of individual cells, including stem and Paneth cell stiffnesses [41]. However, there is a clear need to perform such experiments across the entire crypt, although it is unclear how the stiffness that may be determined from a biological experiment is related to the value in the continuum setting.

Outlook

As crypt modeling has become more mature and experimental data collection methods more sophisticated [24], our understanding of the crypt and its role in gut health has increased. The next challenge is to understand how biochemical and biomechanical processes interact across multiple spatiotemporal scales. A particular challenge relates to crypt fission: during fission, a bifurcation at the crypt base results in the formation of two new crypts. In disease, crypt fission drives the expansion of adenomas, allowing mutated crypts to spread [5,31], but it is also vital for tissue

regeneration after injury [23]. Further complicating the issue, it has been recently suggested that crypts may fuse together [9,6]. The ubiquity of crypt fission, during homeostasis, cancer and regeneration, make understanding how it arises of great clinical significance, while its distinctive mechanical features, particularly the tissue deformations akin to buckling, render it amenable to investigation with mechanistic modeling.

Addressing the above challenges requires further development of continuum and cell-based models, particularly biologically-specialized continuum models of the crypt. We believe that morphoelasticity theory is well-suited to fill this void, but doing so in a way that is consistent with cell activity is a significant challenge. A related challenge is model validation against experimental data. As it is now possible to collect dynamic, cell-scale data [24], this challenge can start to be addressed.

As indicated in Table 1, neither cell-based nor continuum frameworks capture all aspects of an "ideal" crypt model. Instead, we advocate an integrated approach: by linking discrete and continuum models by, say, mapping model parameters, we can harness the complementary advantages of each approach and switch between them, depending on the considered problem. These studies are also needed to establish the robustness of model predictions to changes in the assumptions about microscopic processes.

Another significant challenge concerns parameter estimation. Aside from the sheer number of parameters and uncertainty in their values, equally important for a given model is the number of chosen rules that do not emerge from first principles. In evaluating the results of a model,

the ultimate test is whether it can reproduce data. But even though research is becoming more interdisciplinary [36], many models are not validated against biological data. As experimental data become more detailed and widely available, and as models become more complex and multiscale, in attempting to connect the two, it becomes ever more important to consider how parameter uncertainty affects results. Model inference tools represent a promising way forward [32,33], but significant work remains to be done.

Conflict of interest statement

Nothing declared.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Almet Axel A, Byrne Helen M, Maini Philip K, Moulton Derek E: **•• Post-buckling behaviour of a growing elastic rod.** *J Math Biol* 2019, **78**:777–814.
Analysis of idealized morphoelastic rod model of crypt that extends on previous continuum analyses of the crypt.
 2. Almet Axel A, Hughes Barry D, Landman Kerry A, Näthke Inke S, **•• Osborne James M: A multicellular model of intestinal crypt buckling and fission.** *Bull Math Biol* 2018, **80**:335–359.
A cell-based model with tissue deformability that demonstrates how mechanical stiffness heterogeneity stimulates tissue shape instability.
 3. Ambrosi Davide, Ben Amar Martine, Cyron Christian J, DeSimone Antonio, Goriely Alain, Humphrey Ellen, Jay D, Kuhl: **Growth and remodelling of living tissues: perspectives, challenges and opportunities.** *J R Soc Interface* 2019, **16**: 20190233.
 4. Azzolin Luca, Panciera Tito, Soligo Sandra, Enzo Elena, Biciato Silvio, Dupont Sirio, Bresolin Silvia, Frasson Chiara, Basso Giuseppe, Guzzardo Vincenza, *et al.*: **YAP/TAZ incorporation in the β -catenin destruction complex orchestrates the Wnt response.** *Cell* 2014, **158**:157–170.
 5. Baker Ann Marie, Cereser Biancastella, Melton Samuel, Fletcher Alexander G, Rodriguez-Justo Manuel, Tadrous Paul J, Adam Humphries, George Elia, McDonald Stuart AC, Wright Nicholas A, Simons Benjamin D, Jansen Marnix, Graham Trevor A: **Quantification of crypt and stem cell evolution in the normal and neoplastic human colon.** *Cell Rep* 2014, **8**:940–947.
 6. Baker Ann-Marie, Gabbutt Calum, Williams Marc J, Cereser Biancastella, Noor Jawad, Rodriguez-Justo Manuel, Jansen Marnix, Barnes Chris P, Simons Benjamin D, McDonald Stuart AC, Graham Trevor A, Wright Nicholas A: **Crypt fusion as a homeostatic mechanism in the human colon.** *Gut* 2019.
 7. Balbi Valentina, Kuhl Ellen, Ciarletta Pasquale: **Morphoelastic control of gastro-intestinal organogenesis: theoretical predictions and numerical insights.** *J Mech Phys Solids* 2015, **78**: 493–510.
 8. Ben Amar Martine, Jia Fei: **Anisotropic growth shapes intestinal tissues during embryogenesis.** *Proc Natl Acad Sci* 2013, **110**:10525–10530.
 9. Bruens Lotte, Ellenbroek Saskia IJ, van Rheenen Jacco, Snippert Hugo J: **In vivo imaging reveals existence of crypt fission and fusion in adult mouse intestine.** *Gastroenterology* 2017, **153**:674–677.
 10. Carroll Thomas D, Newton Ian P, Chen Yu, Blow J Julian, **• Näthke Inke: Lgr5⁺ intestinal stem cells reside in an uncensored G¹ phase.** *J Cell Biol* 2018, **217**. jcb.201708023.
Demonstration that intestinal crypt stem cells are paused and not cycling.
 11. Carulli Alexis J, Samuelson Linda C, Schnell Santiago: **Uraveling intestinal stem cell behavior with models of crypt dynamics.** *Integr Biol* 2014, **6**:243–257.
 12. Du Huijing, Nie Qing, Holmes William R: **The interplay between wnt mediated expansion and negative regulation of growth promotes robust intestinal crypt structure and homeostasis.** *PLoS Comput Biol* 2015, **11**:1–23.
 13. Dunn Sara-Jane, Osborne James M, Appleton Paul L, Näthke Inke S: **Combined changes in Wnt signaling response and contact inhibition induce altered proliferation in radiation-treated intestinal crypts.** *Mol Biol Cell* 2016, **27**: 1863–1874.
 14. Edwards Carina M, Jonathan Chapman S: **Biomechanical modelling of colorectal crypt budding and fission.** *Bull Math Biol* 2007, **69**:1927–1942.
 15. Figueiredo Isabel N, Leal Carlos, Romanazzi Giuseppe, Engquist Bjorn: **Homogenization model for aberrant crypt foci.** *SIAM J Appl Math* 2016, **76**:1152–1177.
 16. Fletcher Alexander G, Cooper Fergus, Baker Ruth E: **Mechano-cellular models of epithelial morphogenesis.** *Philos Trans R Soc B Biol Sci* 2017, **372**:20150519.
 17. Fletcher Alexander G, Murray Philip J, Maini Philip K: **Multiscale modelling of intestinal crypt organization and carcinogenesis.** *Math Model Methods Appl Sci* 2015, **25**:2563–2585.
 18. Freddo Andrew M, Shoffner Suzanne K, Yue Shao, Taniguchi Kenichiro, Grosse Ann S, Guysinger Margaux N, Wang Sha, Rudraraju Shiva, Margolis Benjamin, Garikipati Krishna, *et al.*: **Coordination of signaling and tissue mechanics during morphogenesis of murine intestinal villi: a role for mitotic cell rounding.** *Integr Biol* 2016, **8**:918–928.
 19. Gehart Helmuth, Clevers Hans: **Tales from the crypt: new insights into intestinal stem cells.** *Nat Rev Gastroenterol Hepatol* 2018:1.
Introductory review on the intestinal crypt, focussing on the stem cell niche.
 20. Gjorevski Nikolce, Sachs Norman, Manfrin Andrea, Giger Sonja, E Bragina Maiia, Ordóñez-Morán Paloma, Clevers Hans, Lutolf Matthias P: **Designer matrices for intestinal stem cell and organoid culture.** *Nature* 2016, **539**:560.
 21. Goriely Alain: *The mathematics and mechanics of biological growth.* Springer; 2017.
A comprehensive introduction to the mathematical theory of morphoelasticity.
 22. Graudenzi Alex, Caravagna Giulio, De Matteis Giovanni, Antoniotti Marco: **Investigating the relation between stochastic differentiation, homeostasis and clonal expansion in intestinal crypts via multiscale modeling.** *PLoS One* 2014, **9**.
 23. Gregorieff Alex, Liu Yu, Inanlou Mohammad R, Khomchuk Yuliya, Wrana Jeffrey L: **Yap-dependent reprogramming of Lgr5⁺ stem cells drives intestinal regeneration and cancer.** *Nature* 2015, **526**:715.
 24. Guiu Jordi, Hannezo Edouard, Shiro Yui, Demharter Samuel, **• Ulyanchenko Svetlana, Maimets Marti, Jørgensen Anne, Perlman Signe, Lundvall Lene, Mamsen Linn Salto, et al.: Tracing the origin of adult intestinal stem cells.** *Nature* 2019, **570**:107.
Current state-of-the-art for single-cell and cell lineage experimental data.
 25. Hannezo Edouard, Prost Jacques, Joanny Jean-François: **Instabilities of monolayered epithelia: shape and structure of villi and crypts.** *Phys Rev Lett* 2011, **107**:1–5.

26. Tim Ingham-Dempster, Walker Dawn C, Corfe Bernard M: **An agent-based model of anikis in the colon crypt displays novel emergent behaviour consistent with biological observations.** *Royal Society Open Science* 2017, **4**. 160858.
27. Karin Michael, Clevers Hans: **Reparative inflammation takes charge of tissue regeneration.** *Nature* 2016, **529**:307.
28. Kay Sophie K, Harrington Heather A, Shepherd Sarah, Brennan Keith, Dale Trevor, Osborne James M, Gavaghan David J, Byrne Helen M: **The role of the hes1 crosstalk hub in notch-wnt interactions of the intestinal crypt.** *PLoS Comput Biol* 2017, **13**. e1005400.
29. Koch Stefan: **Extrinsic control of wnt signaling in the intestine.** *Differentiation* 2017, **97**.
30. Krndija Denis, El Marjou Fatima, Guirao Boris, Richon Sophie, Leroy Olivier, Bellaiche Yohanns, Hannezo Edouard, Vignjevic Danijela Matic: **Active cell migration is critical for steady-state epithelial turnover in the gut.** *Science* 2019, **365**: 705–710.
31. Langlands Alistair J, Almet Axel A, Appleton Paul L, Newton Ian P, Osborne James M, Näthke Inke S: **Paneth cell-rich regions separated by a cluster of lgr5+ cells initiate crypt fission in the intestinal stem cell niche.** *PLoS Biol* Jun 2016, **14**.
32. Maclaren Oliver J, Parker Aimée, Pin Carmen, Carding Simon R, Alastair J, Watson M, Fletcher Alexander G, Byrne Helen M, Maini Philip K: **A hierarchical bayesian model for understanding the spatiotemporal dynamics of the intestinal epithelium.** *PLoS Comput Biol* 2017, **13**. e1005688.
- Application of Bayesian inference to estimate parameters of an intestinal crypt model.
33. Maclaren Oliver J, Fletcher Alexander G, Byrne Helen M, Maini Philip K: **Models, measurement and inference in epithelial tissue dynamics.** *Math Biosci Eng* 2015, **12**: 1321–1340.
34. Meran Laween, Anna Baulies, Li Vivian SW: **Intestinal stem cell niche: the extracellular matrix and cellular components.** *Stem Cell Int* 2017, **2017**.
35. Metzcar John, Wang Yafei, Heiland Randy, Macklin Paul: **A review of cell-based computational modeling in cancer biology.** *JCO Clin Canc Inf* 2019, **2**.
- A detailed review of cell-based modeling approaches, with the crypt as a model system.
36. Muraro Daniele, Parker Aimee, Vaux Laura, Filippi Sarah, Almet Axel A, Fletcher Alexander G, Watson Alastair JM, Pin Carmen, Maini Philip K, Byrne Helen M: **Chronic TNF α -driven injury delays cell migration to villi in the intestinal epithelium.** *J R Soc Interface* 2018, **15**. 20180037.
37. Nelson Martin R, Howard Daniel, Jensen Oliver E, King John R, Rose Felicity RAJ, Waters Sarah L: **Growth-induced buckling of an epithelial layer.** *Biomech Model Mech* 2011, **10**: 883–900.
38. Nelson Martin R, King John R, Jensen Oliver E: **Buckling of a growing tissue and the emergence of two-dimensional patterns.** *Math Biosci* 2013, **246**:229–241.
39. Osborne James M, Fletcher Alexander G, Pitt-Francis Joe M, Maini Philip K, Gavaghan David J: **Comparing individual-based approaches to modelling the self-organization of multicellular tissues.** *PLoS Comput Biol* 2017, **13**. e1005387.
- Cell-based model comparison with 2D crypt model as case study.
40. Parker Aimée, Vaux Laura, Patterson Angela M, Modasia Amisha, Muraro Daniele, Fletcher Alexander G, Byrne Helen M, Maini Philip K, Watson Alastair JM, Pin Carmen: **Elevated apoptosis impairs epithelial cell turnover and shortens villi in TNF-driven intestinal inflammation.** *Cell Death Dis* 2019, **10**:108.
41. Pin Carmen, Parker Aimee, Patrick Gunning A, Ohta Yuki, Johnson Ian T, Carding Simon R, Sato Toshiro: **An individual based computational model of intestinal crypt fission and its application to predicting unrestrictive growth of the intestinal epithelium.** *Integr Biol* 2015, **7**:213–228.
42. Serra Denise, Mayr Urs, Andrea Boni, Lukonin Ilya, Rempfler Markus, Meylan Ludivine Challet, Stadler Michael B, Strnad Petr, Papasaikas Panagiotis, Vischi Dario, *et al.*: **Self-organization and symmetry breaking in intestinal organoid development.** *Nature* 2019, **1**.
- Links transient YAP activation to stem cell differentiation, which initiates crypt budding within intestinal organoids.
43. Shoshkes-Carmel Michal, Wang Yue J, Wangenstein Kirk J, Tóth Beáta, Kondo Ayano, Massasa Efi E, Itzkovitz Shalev, Kaestner Klaus H: **Subepithelial telocytes are an important source of Wnts that supports intestinal crypts.** *Nature* 2018, **557**:242.
44. Spit Maureen, Koo Bon-Kyoung, Maurice Madelon M: **Tales from the crypt: intestinal niche signals in tissue renewal, plasticity and cancer.** *Open Biology* 2018, **8**. 180120.
- Detailed review of signaling pathways implicated in crypt homeostasis.
45. Sugimoto Shinya, Ohta Yuki, Fujii Masayuki, Matano Mami, Shimokawa Mariko, Nanki Kosaku, Date Shoichi, Nishikori Shingo, Nakazato Yoshihiro, Nakamura Tetsuya, *et al.*: **Reconstruction of the human colon epithelium in vivo.** *Cell Stem Cell* 2018, **22**:171–176.
46. Sumigraey Kaelyn D, Terwilliger Michael, Terry Lechler: **Morphogenesis and compartmentalization of the intestinal crypt.** *Dev Cell* 2018, **45**:183–197.
47. Taniguchi Koji, Wu Li-Wha, Grivennikov Sergei I, Petrus R, De Jong, Lian Ian, Yu Fa-Xing, Wang Kepeng, Ho Samuel B, Boland Brigid S, Chang John T, Sandborn William J, Hardiman Gary, Raz Eyal, Maehara Yoshihiko, Yoshimura Akihiko, Zucman-Rossi Jessica, Guan Kun-Liang, Karin Michael: **A gp130–Src–YAP module links inflammation to epithelial regeneration.** *Nature* 2015, **519**:57.
48. Thalheim Torsten, Peter Buske, Przybilla Jens, Rother Karen, Loeffler Markus, Galle Joerg: **Stem cell competition in the gut: insights from multi-scale computational modelling.** *J R Soc Interface* 2016, **13**. 20160218.
49. Thalheim Torsten, Quaas Marianne, Herberg Maria, Braumann Ulf-Dietrich, Kerner Christiane, Loeffler Markus, Aust Gabriela, Galle Joerg: **Linking stem cell function and growth pattern of intestinal organoids.** *Dev Biol* 2018, **433**: 254–261.
50. Ward Daniel, Fletcher Alexander G, Homer Martin, Marucci Lucia: **Cross-talk between Hippo and Wnt signalling pathways in intestinal crypts: insights from an agent-based model.** *BioRxiv*; 2019.
51. Yan Huaming, Anna Konstorum, Lowengrub John S: **Three-dimensional spatiotemporal modeling of colon cancer organoids reveals that multimodal control of stem cell self-renewal is a critical determinant of size and shape in early stages of tumor growth.** *Bull Math Biol* 2018, **80**:1404–1433.
- Continuum multispecies model of colon cancer organoid.