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Gut homeostasis at a glance

Jieun Choo*,‡, Neda Glisovic*,‡ and Danijela Matic Vignjevic

ABSTRACT

The intestine, a rapidly self-renewing organ, is part of the gastrointestinal system. Its major roles are to absorb food-derived nutrients and water, process waste and act as a barrier against potentially harmful substances. Here, we will give a brief overview of the primary functions of the intestine, its structure and the luminal gradients along its length. We will discuss the dynamics of the intestinal epithelium, its turnover, and the maintenance of homeostasis. Finally, we will focus on the characteristics and functions of intestinal mesenchymal and immune cells. In this Cell Science at a Glance article and the accompanying poster, we aim to present the most recent information about gut cell biology and physiology, providing a resource for further exploration.

KEY WORDS: Gut homeostasis, Intestine structure, Stromal cells, Immune cells

Institut Curie, PSL Research University, CNRS UMR 144, F-75005 Paris, France.
*These authors contributed equally to this work

‡Authors for correspondence (neda.glisovic@curie.fr; jieun.choo@curie.fr)

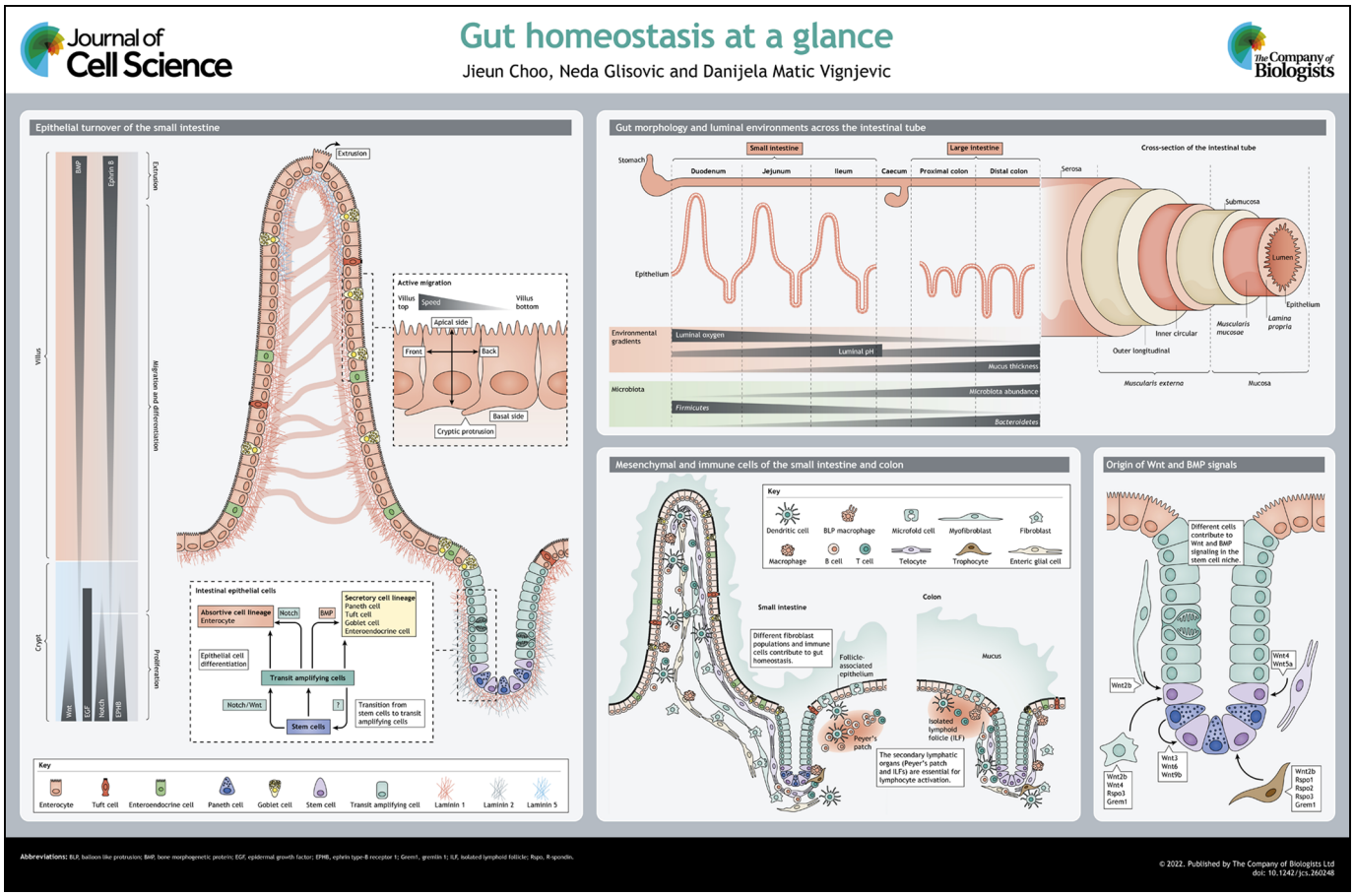
J.C., 0000-0002-1921-9307; N.G., 0000-0002-4714-8884; D.M.V., 0000-0003-4250-703X

Introduction

The intestine is part of the digestive system. It is a tubular organ that digests food, absorbs food-derived nutrients, transports water and electrolytes, and excretes waste metabolites (Kararli, 1995). It harbors the largest population of microorganisms in the body, called the gut microbiota (Thursby and Juge, 2017). In addition, this organ is closely linked to the host immune system given that it is constantly exposed to potentially harmful substances and immunomodulatory agents originating from what we ingest and the resident microbiota (Mowat and Agace, 2014). In this Cell Science at a Glance article, we will briefly describe the function, mucosal structure, and luminal environments in each segment of the intestine. In addition, we will highlight the cell populations constituting the intestinal tissue (epithelial, mesenchymal, and immune cells) based on recent published findings in the field. We will conclude our review by discussing open questions and future perspectives.

Function of intestinal segments

Ingested food and water pass from the mouth through the esophagus and stay in the stomach for a short time. There, carbohydrate digestion, initiated by amylase in saliva, continues while triglyceride and protein degradation begins. Moreover, acidic



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fluid is mixed with the food, which forms a semi-solid, viscus content known as chyme. The formed chyme is then moved into the small intestine. The small intestine is divided into three sections: the duodenum, the jejunum and the ileum (Mescher, 2013).

The duodenum receives digestive enzymes secreted by the pancreas and liver, and further breaks down the content released from the stomach. The semi-solid material then enters the jejunum, where chemical digestion continues. In those two gut segments, most nutrients, vitamins, ions and water are absorbed, whereas others are excreted. The chyme then travels to the ileum, where nutrients, such as vitamin C, B12, zinc and bile salts, are absorbed. The next stop for chyme is the colon (also called the large intestine). Although the primary function of the proximal colon is to partially digest and then absorb the remaining nutrients, water and electrolytes delivered by the ileum, the primary function of the distal colon is to transfer concentrated, solidified feces to the rectum (Rhoades and Bell, 2013; Sensoy, 2021).

Structure and luminal environments along the intestine

As discussed above, the intestine is a multi-functional organ. Each segment of the intestine has a distinct physiological role, specific mucosal structure and unique luminal environment (see poster). Besides those differences, distinct gut segments have general histological features in common: mucosa composed of epithelium, *lamina propria* and *muscularis mucosae*, then submucosa, *muscularis externa* (inner and outer layer), and, finally, serosa (see poster).

The inner lining of the small intestine consists of the villi, the finger-like protrusions that extend into the gut lumen, and the crypts, the invaginations into the mucosal *lamina propria* (Clevers, 2013). It has been reported that the villus height changes along the small intestine in the rat and pigs, referred to as the villus size gradient (Altmann and Leblond, 1970; Clarke, 1970; Lu et al., 2014). Indeed, according to our observations in adult mice (3 to 6 months old), the villus height gradually decreases from duodenum to ileum (∼500 µm in duodenum, ∼320 µm in jejunum and ∼250 µm ileum; our unpublished observations). Along the small intestine, the difference in crypt depth (deeper in ileum compared to duodenum and jejunum) is not as significant as the difference in villus height. In contrast to the small intestine, the colon comprises only crypts, whose depth also varies in different segments (∼60 µm in proximal colon and 130 µm in distal colon), as was also observed in a previously published study (Neumann et al., 2014).

Given that most of the absorption takes place in the duodenum and jejunum, the length of the villi correlates with the function of these segments. Villi increase the absorptive area of the gut, so the longer they are, the larger the surface of absorption is. As the chyme moves forward, from the duodenum to the ileum, the amount of nutrients to be absorbed is lower, therefore the length of villi decreases. In recent years, there have been numerous reports revealing the single-cell profiles of intestinal epithelial cells. For instance, a spatial zonation of enterocytes with differing functions along the villi axis has been uncovered by using single-cell and transcriptomic analysis coupled with a microdissection technique (Moor et al., 2018). Therefore, further analysis of enterocytes and cell-type mapping along villi and crypts might help us to better understand the described structural differences. Do different segments of the gut contain different cell types with distinct functions? What is their density? How are they distributed along the crypt–villus axis?

In contrast, in the colon, the depth of the crypts increases as we move from the proximal to distal colon. A study performed in rats suggests that the surface epithelium facing the lumen (between crypts) has very low permeability to hydrophilic substances, whereas the crypt epithelium contains pores (tight junctions) with significantly larger radii, particularly in the proximal colon (Fihn and Jodal, 2001). Therefore, the difference in crypt depth might be the result of a compensation mechanism – smaller crypts and larger pores in the proximal colon and larger crypts and smaller pores in the distal colon. In addition, the human distal colon has been shown to have a reserve capacity meaning that it can accommodate intracolonic fluid that was not absorbed in the proximal colon (Hammer et al., 1997). Thus, it is possible that additional mucosal absorption can take place in the distal colon. As a result, longer crypts in the distal colon might perform this function. Finally, it is possible that crypts protect stem cells from potentially toxic microbiota metabolites. Therefore, as the microbiota abundance (increased total number as well as the number of different species of microorganisms) is the highest in the distal colon (see poster and Box 1), it is possible that longer crypts protect stem cells more efficiently.

Besides the structure of the intestinal mucosa, the luminal environments, such as the thickness of the mucus layer, pH, oxygen and composition of the microbiota also change along the intestine (see poster). On the poster, we present the different luminal gradients found in the tissues of mice or rats (Chikina and Matic Vignjevic, 2021; Lkhagva et al., 2021). The colon has a thicker mucus layer (Ermund et al., 2013), less oxygen (Friedman et al., 2018) and more abundant microbiota (Lkhagva et al., 2021) compared to the small intestine. Similarly, pH levels are raised, starting from the duodenum to ileum, dropping in the caecum and increasing towards the distal colon (Lkhagva et al., 2021).

Although it is not entirely understood how the variations in the structure and luminal environments affect region-specific activities of the gut in homeostasis, together these environmental factors shape the microbiota and immune system in each segment (see

Box 1. Gut microbiota

The mammalian gastrointestinal tract is home to trillions of microorganisms, including bacteria, viruses, fungi and protozoa, known as the gut microbiota (Beresford-Jones et al., 2022). A growing body of literature acknowledges the effect of gut microbiota in the maintenance of gut homeostasis and emphasizes inter-individual differences in gut microbiota composition. Nevertheless, despite being a continuous space, the intestinal lumen also exhibits fluctuation in both taxa identity and abundance due to the local microenvironments present along its length.

Metagenome sequencing of the luminal content of mice gut has unveiled the existence of 13 phyla of microorganisms and the location-specific diversity of gut microbiome in the intestine. The abundance of the two most numerous groups of microbes was reversed from the stomach to the colon. The abundance of *Firmicutes* gradually decreased from the stomach to the colon, while the abundance of *Bacteroidetes* gradually increased (Lkhagva et al., 2021).

In the small intestine, where sugar and protein metabolism is favored and the concentration of oxygen is high, the microbiota is composed of facultative anaerobes, such as *Proteobacteria* and *Lactobacillales*. In contrast, in the colon, where fermentation of complex polysaccharides takes place and the luminal environment is hypoxic, increased species diversity and dominance of the (obligate) anaerobes, such as saccharolytic *Bacteroidales* and *Clostridiales* orders, is observed. Within the colon, microbiota also differ from the middle of the lumen to the mucosa. For instance, closer to the mucosa, where the oxygen concentration is higher, aerotolerant taxa such as *Actinobacteria*, are present (Tropini et al., 2017).

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Box 2. Immune system of the gut

The composition and function of the gut immune system change in response to differences in the physiology and morphology of the gut along its length. In contrast to the small intestine, where the immune system is primarily involved in preserving the sterility and barrier properties of an epithelium involved in digestion and nutrient absorption, the immune system of the colon aims to inhibit any inflammatory reactions towards commensal microbiota. Therefore, the small intestine immune system is focused on defense against extracellular infections, which includes the production of IL-17, IL-22 and antimicrobial peptides. The colonic immune system protects commensal microbes by comprising a large number of IgA-producing plasma cells, IL-10-producing macrophages and FoxP3^{+} Treg cells, together with the thick mucus layer that is produced by goblet cells (Mazzini et al., 2014; Mowat and Agace, 2014) (see poster).

In terms of function, primary lymphatic organs of the immune system (thymus and bone marrow) are involved in T and B lymphocyte development and selection, whereas secondary lymphatic organs (spleen and lymph nodes, etc.) provide a microenvironment that is essential for lymphocyte activation and differentiation, namely initiation and coordination of adaptive immune response. The secondary lymphatic organs of the intestine are the gut-associated lymphoid tissues (GALT) and the draining lymph nodes, which are spread across the intestinal tube. The GALT encompasses lymphoid aggregates in mucosa and submucosa, placed just below the epithelium, named the follicle-associated epithelium. The follicle-associated epithelium contains microfold cells (M cells), which are specialized in the uptake of antigens and their transport to the underlying DC-rich subepithelial dome regions. In the small intestine, GALT is composed mainly of Peyer's patches. Peyer's patches comprise numerous B cell lymphoid follicles surrounded by smaller T cell areas. In contrast to the lymph nodes, Peyer's patches are not encapsulated and contain germinal centers, suggesting their continuous immune stimulation. Isolated lymphoid follicles (ILFs) play the same role in the colon, as well as being a major source of IgA-producing plasma cells (Buettner and Lochner, 2016; Mowat, 2003).

Boxes 1 and 2). The changes in luminal environments in diseases, such as inflammatory bowel disease and colorectal cancer, are not further discussed here, and we refer the reader to a recent review on the topic (Chikina and Matic Vignjevic, 2021).

Intestinal epithelial cells

The intestinal epithelium consists of a single layer of cells. In mice, turnover of epithelial cells occurs every 3 to 5 days in the small intestine and every 5 to 7 days in the colon (Barker, 2014). This turnover must be tightly regulated to maintain tissue integrity and function. In the small intestine, the continuous renewal of the epithelium is fueled by Lgr5^{+} intestinal stem cells (ISCs) (Barker et al., 2007). ISCs give rise to highly proliferative transit-amplifying (TA) cells that differentiate into enterocytes, goblet cells, Paneth cells, enteroendocrine cells and Tuft cells (Barker, 2014; Vermeulen and Snippert, 2014). These differentiated cells, except Paneth cells, progressively migrate up to the villus tip where they are extruded into the lumen (see poster). In contrast to other intestinal cells, Paneth cells are long-lived and are renewed only every 3 to 6 weeks. They reside close to the ISCs and have a role in establishing the intestinal stem cell niche by producing important signaling molecules, such as Wnt ligands, epidermal growth factor (EGF) and Notch ligands. The signaling pathways between ISC and epithelial cell lineages within the crypt are depicted in the poster. Although Notch and Wnt are important for the ISC to TA transition, Notch and bone morphogenetic proteins (BMPs) are involved in intestinal epithelial cell differentiation (Bonis et al., 2021; Sato et al., 2011; Scoville et al., 2008). The colon also contains enterocytes, goblet cells, enteroendocrine cells and Tuft cells, but few or no Paneth cells (Barker, 2014; Parikh et al., 2019). Therefore, in the colon, some stem cell niche factors are provided by the fibroblasts and myofibroblasts instead (Rees et al., 2020).

The basal surface of the epithelium is underlined by the basement membrane, a thin and dense sheet-like structure on which cells adhere and migrate. The basement membrane is composed of a network of collagen IV and laminin. Surprisingly, its composition is not uniform; although laminin-1 is present all along the crypt–villus axis, laminin-2 is only restricted to crypts and laminin-5 to the top of villi (Glentis et al., 2014; Kedinger et al., 1998; Leivo et al., 1996; Orian-Rousseau et al., 1996) (see poster). The role of this patterning in the basement membrane remains unknown. It was thought that cell migration on the basement membrane is a passive process driven by mitotic pressure generated in the crypts (Cheng and Leblond, 1974; Parker et al., 2017). However, it has been recently shown that mitotic pressure is restricted to the crypts and that active migration of differentiated cells is required to reach the villus tip (Krndija et al., 2019). Although cells migrate collectively, maintaining their apicobasal polarity, they also display a second polarity axis (front–back), characterized by a basal actin-rich protrusion oriented in the direction of migration (Krndija et al., 2019) (see poster). What the cue for this directional migration is, and how cells establish and maintain this double polarity remains to be understood.

Intestinal mesenchymal cells

The subepithelial region of the intestinal mucosa is populated by mesenchymal cells, such as fibroblasts, myofibroblasts and pericytes, which are referred as non-epithelial, non-hematopoietic and non-endothelial cells (Pinchuk et al., 2010; Powell et al., 2011). The fine structure of mesenchymal cells, their surface and/or intracellular markers, roles and rough localizations have been thoroughly described (Mifflin et al., 2011; Pinchuk et al., 2010; Powell et al., 1999, 2011; Roulis and Flavell, 2016). Furthermore, recent studies using single-cell RNA sequencing technology and high-resolution microscopy have revealed the diversity of mesenchymal cell populations, including telocytes, myofibroblasts, fibroblasts, trophocytes and enteric glial cells, and further described their distinct functions and localizations (Baghdadi et al., 2022; Brügger et al., 2020; Kim et al., 2020; Kinchen et al., 2018; McCarthy et al., 2020b; Roulis et al., 2020).

For instance, telocytes, also known as subepithelial α-SMA^{low} (alpha smooth muscle actin low) myofibroblasts, are located just below the epithelial cells. All other resident mesenchymal cells, including α-SMA^{high} myofibroblasts (presented as 'myofibroblasts' in the poster), PDGFRA^{low} fibroblasts (presented as 'fibroblasts' in the poster) and trophocytes, are based in *lamina propria*, encompassing the mucosal vascular elements. Their specific localization and divergent molecular signatures, both in small intestinal villi and peri-cryptal space, have been extensively reviewed recently (McCarthy et al., 2020a). Despite the fact that the colon has not yet been thoroughly studied, it has been suggested that its mesenchymal architecture is also fundamentally the same as that of the small intestine. In addition, GFAP-expressing enteric glial cells have been reported as one of the mesenchymal cell populations that surround the ISC-containing crypts, both in the small intestine and colon (Baghdadi et al., 2022).

In terms of their function, intestinal mesenchymal cells have been recognized as being essential in providing the structural support and the instructive signals that keep the epithelium healthy. They produce the signaling molecules and extracellular matrix

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components, which together constitute the stem cell niche. The establishment of the stem cell niche by both epithelial cells and mesenchymal cells, and the regulation of key signaling pathways maintaining intestinal homeostasis, such as Wnt signaling, BMP signaling, Notch signaling, Hedgehog signaling, EGF signaling and Eph–ephrin signaling, have been extensively reviewed elsewhere (Baulies et al., 2020; Brizzi et al., 2012; Loe et al., 2021; Meran et al., 2017; Spit et al., 2018; Wang et al., 2018; Zhu et al., 2021).

Here, we will discuss in more detail the Wnt and BMP pathways, which are essential for the maintenance of the intestinal stem cell niche. It has been shown that mesenchymal cells express a variety of signaling molecules that regulate Wnt and BMP pathways, including Wnt, Rspo, Dkk, Grem and BMP proteins (Qi et al., 2017; Sato et al., 2011; Takahashi and Shiraishi, 2020). Interestingly, the secretion of the specific molecules is not restricted to only one mesenchymal cell population. In fact, some molecules are produced by different mesenchymal cell types (McCarthy et al., 2020a; Zhu et al., 2021). Furthermore, both Paneth cells in the intestinal epithelium and mesenchymal cells are considered two independent sources providing stem cell niche factors (Zhu et al., 2021). It has been shown that blocking Wnt secretion only in the epithelial compartment has no significant effect on stem cell niche, whereas, in contrast, blocking Wnt secretion both in epithelial and mesenchymal compartments causes loss of ISCs (Farin et al., 2012; Kabiri et al., 2014; San Roman et al., 2014; Valenta et al., 2016). In fact, both Paneth cells and mesenchymal cells produce several Wnt ligands – Paneth cells produce Wnt3, Wnt6 and Wnt9b, whereas mesenchymal cells express Wnt2b, Wnt4, Wnt5a and Wnt5b (Farin et al., 2012; Gregorieff et al., 2005) (see poster).

Eph–ephrin signaling also plays important role in specific positioning and differentiation of intestinal epithelial cells. Eph receptors and ephrins have similar expression patterns in the mucosal epithelium of the small and large intestines. Although expression of EphB decreases in crypt–villus axis (towards the epithelial lumen), the expression of ephrin B increases. These two molecules reverse gradients of expression play an important role in the maintenance and segregation of stem cell and differentiated cell compartments (see poster; Papadakos et al., 2022; Perez White and Getsios, 2014).

All these studies point to a great variety of different cell types with similar physiological functions, suggesting the existence of numerous compensatory mechanisms in gut homeostasis. But, what is the reason for this? Why is the stem cell niche maintenance divided between mesenchymal and epithelial cell types? Why is it important that the gut encompasses so many cells that can compensate for the functions of one another? Therefore, despite the vast knowledge we have gained about mesenchymal and epithelial cells, we still need to explore how their complexity and heterogeneity relate to the maintenance of gut homeostasis.

Intestinal immune cells

As mentioned above, the intestine is continually exposed to food antigens and potentially harmful agents from what we ingest (Mowat and Agace, 2014). In addition, it acts as a major reservoir of commensal microbiota (see Box 1), which produce foreign antigens that are not encoded by host genes (Swiatczak and Cohen, 2015). Thus, to maintain homeostasis, one of the important tasks of the intestine is to distinguish what is dangerous and what is safe for the host. Accordingly, the intestinal immune system recognizes and eliminates exogenous harmful agents or pathogens but is tolerant to harmless food antigens and to the commensal microbiota, facilitating a healthy symbiotic relationship with the host (Mowat and Agace, 2014; Swiatczak and Cohen, 2015). Of note, abnormal, excessive activation of the mucosal immune system in response to dietary antigens and/or commensal microbiota can lead to immune-mediated intestinal disorders, such as celiac and inflammatory bowel disease (Pascual et al., 2014).

In the lamina propria and the submucosa, distinct cells belonging to the innate and adaptive immune systems are found (Mowat and Agace, 2014). Innate immune cells, including macrophages, dendritic cells (DCs), invariant lymphocytes (ILCs), eosinophils and mast cells, work together to maintain intestinal homeostasis at a steady-state, and they can also regulate adaptive immune responses (Kayama and Takeda, 2016; Mowat and Agace, 2014) (see poster). Macrophages have multiple roles in intestinal homeostasis. For example, they ingest and degrade microorganisms and dead tissue cells, producing mediators that drive epithelial cell renewal (Mowat and Agace, 2014). In addition, a specific population of macrophages in the distal colon has been recently discovered that instruct epithelial cells to stop absorbing fluids when loaded with fungal toxins. This is required to maintain the survival of epithelial cells and, consequently, the integrity of the epithelial barrier (Chikina et al., 2020). Although the underlying mechanism remains to be dissected, this feature could explain how colonic epithelial cells absorb fluids, which is their major physiological function, while avoiding poisoning by the numerous microbiota-derived toxic products present in the local environment.

DCs provide a critical link between the innate and adaptive immune systems by presenting antigenic peptides to naïve T cells (Persson et al., 2013). They are required for sampling of the gut luminal content and mediate tolerance to food antigens and gut microbiota (Stagg, 2018). Once activated, they migrate to mesenteric lymph nodes and gut-associated lymphoid tissue (GALT; e.g. Peyer's patches; see Box 2), where they suppress immune responses through the induction of regulatory T cells. DCs are also essential to promote B cell differentiation into IgA^+ plasma cells (Hooper and Macpherson, 2010; Tezuka and Ohteki, 2019).

Conclusion

In conclusion, the intestinal mucosa, together with the gut immune system and microbiome, represents a functional and complex system involved in the maintaining organism homeostasis. Even though the gut has been intensively investigated, many questions remain to be answered. For example, what is the functional relevance of the different villi height along different regions of the gut? What is the precise function of distinct mesenchymal cell types? This could be addressed by experiments using the numerous recently available Gut-on-Chip models, which allow addressing the function of individual mesenchymal types in the maintenance of epithelial homeostasis (Verhulsel et al., 2021; Nikolaev et al., 2020). It also remains to be determined whether the distribution and function of mesenchymal and immune cells differ between the colon and small intestine. How do epithelial cells, mesenchymal and immune cells, together with the microbiota, cooperate to maintain homeostasis? Recent developments in single-cell RNA sequencing combined with spatial transcriptomics could shed light on whether and how the spatial distribution of different cell types plays a role in gut homeostasis. What is the consequence of perturbation of this cooperation? What is the contribution of the external factors, such as extracellular matrix, chyme, mechanical forces and luminal chemical gradients, to homeostasis and disease? High-end microfluidics devices could be used to address those questions and thus to help provide a better understanding of the gut homeostasis at the tissue, cellular and molecular level.

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Competing interests
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Cell science at a glance
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