Role of Wnt signaling in the maintenance and regeneration of the intestinal epithelium

Maxime Gasnier[a, †], Hui Yi Grace Lim[a, †], Nick Barker[a,b,c, *]
[a] A*STAR Institute of Molecular and Cell Biology, Singapore, Singapore
[b] Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[c] Cancer Research Institute, Kanazawa University, Kanazawa, Japan
*Corresponding author: e-mail address: nicholas_barker@imcb.a-star.edu.sg

Abstract

The intestinal epithelium plays a key role in digestion and protection against external pathogens. This tissue presents a high cellular turnover with the epithelium being completely renewed every 5 days, driven by intestinal stem cells (ISCs) residing in the crypt bases. To sustain this dynamic renewal of the intestinal epithelium, the maintenance, proliferation, and differentiation of ISCs must be precisely controlled. One of the central pathways supporting ISC maintenance and dynamics is the Wnt pathway. In this chapter, we examine the role of Wnt signaling in intestinal epithelial homeostasis and tissue regeneration, including mechanisms regulating ISC identity and fine-tuning of Wnt pathway activation. We extensively discuss the contribution of the stem cell niche in maintaining Wnt signaling in the intestinal crypts that support ISC functions. The integration of these findings highlights the complex interplay of multiple niche signals and cellular components sustaining ISC behavior and maintenance, which together supports the immense plasticity of the intestinal epithelium.

1. Introduction

The small intestine makes up a major part of the gastrointestinal tract that functions to complete digestion, absorb nutrients, and form a protective barrier against external agents. It is composed of the duode-

[†] These authors contributed equally.

Current Topics in Developmental Biology
ISBN 0070-2153
https://doi.org/10.1016/bs.ctdb.2023.01.001

num, jejunum, and ileum, three segments that are each approximately one-third of the entire length of the small intestine, which connects to the colon, or large intestine, whose main role is water reabsorption. Within this tissue, the intestinal epithelial layer harbors a dedicated stem cell compartment supported by neighboring epithelial and stromal cells that together make up the stem cell niche, allowing the tissue-resident stem cells to self-renew and differentiate to make up the secretory and absorptive cell lineages represented within the intestinal epithelium. Under homeostatic conditions, the intestinal epithelium undergoes complete renewal within 5 days (Tan & Barker, 2014).

How the intestinal epithelium regulates this homeostatic balance is determined by niche factors and signaling pathways that work together to exert precise control over stem cell maintenance, proliferation, and differentiation. As evident in disease states, any imbalance in this process can lead to hyperproliferation and cancerous growth. In this chapter, we discuss the role of one of the central pathways regulating the ISC compartment—Wnt signaling—and its contribution to the maintenance and regeneration of the intestinal epithelium.

2. Overview of the Wnt pathway

The Wnt pathway plays central roles in stem cell maintenance, proliferation, and differentiation in many tissues. Broadly, there are three major pathways associated with Wnt: the canonical Wnt/β-catenin pathway and the two non-canonical β-catenin-independent pathways—Wnt/planar cell polarity (PCP) and Wnt/calcium (Ca^{2+}). In this chapter, we will focus on the roles of the well-characterized canonical Wnt/β-catenin pathway in the intestine (Fig. 1).

The Wnt ligand family comprises 19 members shared by mice and humans. During its synthesis, Wnt is post-translationally palmitoylated by the palmitoyl transferase Porcupine (Porcn) in the endoplasmic reticulum (ER). This process facilitates the binding of Wntless (Wls) to the lipidated Wnt to transport it from the ER to the Golgi apparatus for its extracellular secretion (Najdi et al., 2012). Palmitoylation of the Wnt ligand is also required for proper binding of secreted Wnts to Frizzled (Fzd) receptors of the receiving cell (Janda, Waghray, Levin, Thomas, & Garcia, 2012). Due to its hydrophobic nature, Wnt exerts a short-range action from the membrane in an autocrine or paracrine fashion.

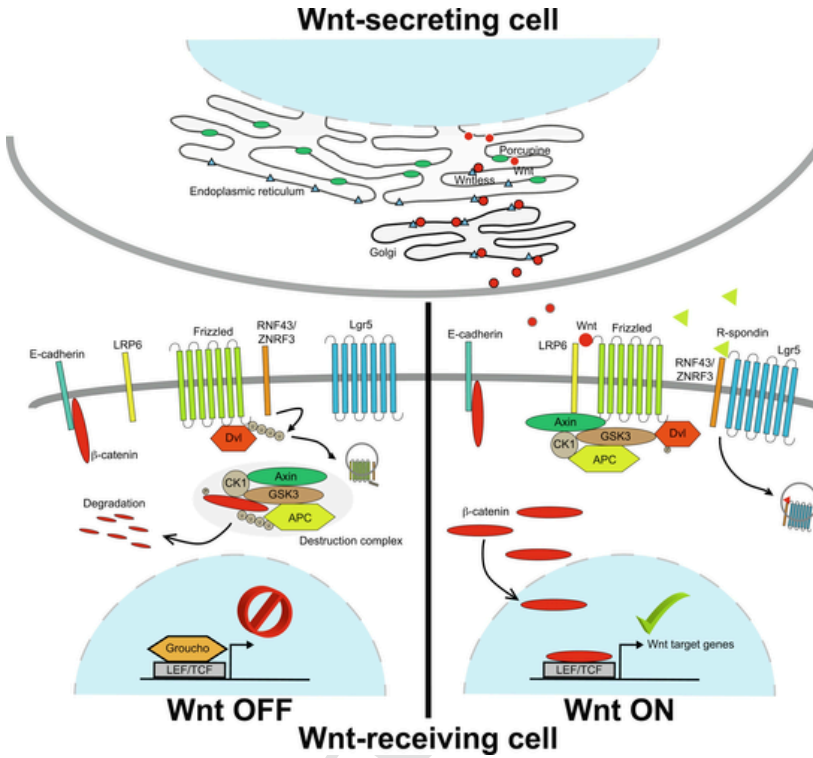

Fig. 1 Wnt/β-catenin pathway in the absence and presence of Wnt and R-spondin ligand binding. In the "Wnt off" state, RNF43/ZNRF3 ubiquitinates the Frizzled receptor resulting in its degradation. The Wnt pathway effector β-catenin in the cytoplasm is also marked for degradation by the destruction complex, blocking the activation of Wnt target genes. Conversely, in the "Wnt on" state, Wnt is post-translationally modified by Porcupine and transported to the membrane for secretion by Wntless within Wnt-secreting cells. Upon binding to Frizzled receptors on Wnt-receiving cells, Axin is sequestered at the membrane, leading to the degradation of the destruction complex. This enables β-catenin to translocate into the nucleus to interact with the LEF/TCF complex to promote transcription of Wnt target genes.

During the activation of the canonical Wnt/β-catenin pathway, Wnt binds to the seven-pass transmembrane receptor Fzd (10 members in mouse and human) present on the surface of Wnt-responsive cells, which also interacts with the single-pass low-density lipoprotein receptor protein 5 or 6 (Lrp5/6). Upon Wnt ligand binding, Axin2, a component of the β-catenin destruction complex that also includes Adenomatous Polyposis Coli (APC), Glycogen Synthase Kinase 3 (GSK3), and casein ki-

nase 1α (CK1α), becomes sequestered to the cell membrane. This initiates the disassembly of the destruction complex, allowing for the translocation and accumulation of β-catenin in the nucleus. There, β-catenin binds to the T-cell factor/Lymphoid enhancing factor (TCF/LEF) transcription factors and activates the transcription of Wnt target genes. In the absence of Wnt, cytoplasmic β-catenin is actively degraded by the destruction complex and TCF/LEF transcription factors bind Groucho co-repressor proteins to actively silence Wnt target genes (Clevers & Nusse, 2012).

One target gene of the Wnt/β-catenin pathway is the Leucine-rich repeat containing G protein-coupled receptor 5 (Lgr5), a seven-pass transmembrane protein that binds the Wnt agonist R-spondin family of ligands (Rspo1/2/3/4) (Barker et al., 2007; Carmon, Gong, Lin, Thomas, & Liu, 2011; de Lau et al., 2011; Glinka et al., 2011). Rspo binding to Lgr4/5/6 receptors inhibits the activity of the E3 ubiquitin ligases RNF43/ZRNF3 that normally ubiquitinate Fzd receptors for subsequent degradation, thereby promoting Fzd receptor accumulation at the cell membrane and potentiating Wnt/β-catenin signaling (Hao et al., 2012; Koo et al., 2012). While Lgr5 is a Wnt target gene that can promote pathway activation, other Wnt target genes, including Rnf43/Zrnf3 and Axin2, are involved in a negative feedback loop, ensuring that Wnt activation is tightly controlled by multiple inputs.

3. Organization of the intestinal epithelium

The intestinal tissue is composed of four layers: the serosa, the outermost layer containing mesothelial cells; the muscularis propria, composed of smooth muscle cells responsible for the peristaltic movement of the small intestine; the submucosa connecting the muscularis propria and mucosa that is made up of fibroblasts, immune cells, blood vessels and nerves; and the innermost mucosa layer formed by the lamina propria, comprising connective tissue, capillaries, and lymphatics, as well as the absorptive epithelium. This epithelium is organized into repeating crypt-villi structures in the small intestine: the proliferative region is located within invaginations referred to as crypts of Lieberkühn, whereas the differentiated epithelium forms protrusions extending from the crypts known as villi, which maximize the absorptive surface area available (Fig. 2). The colon is organized in a similar fashion, with sev-

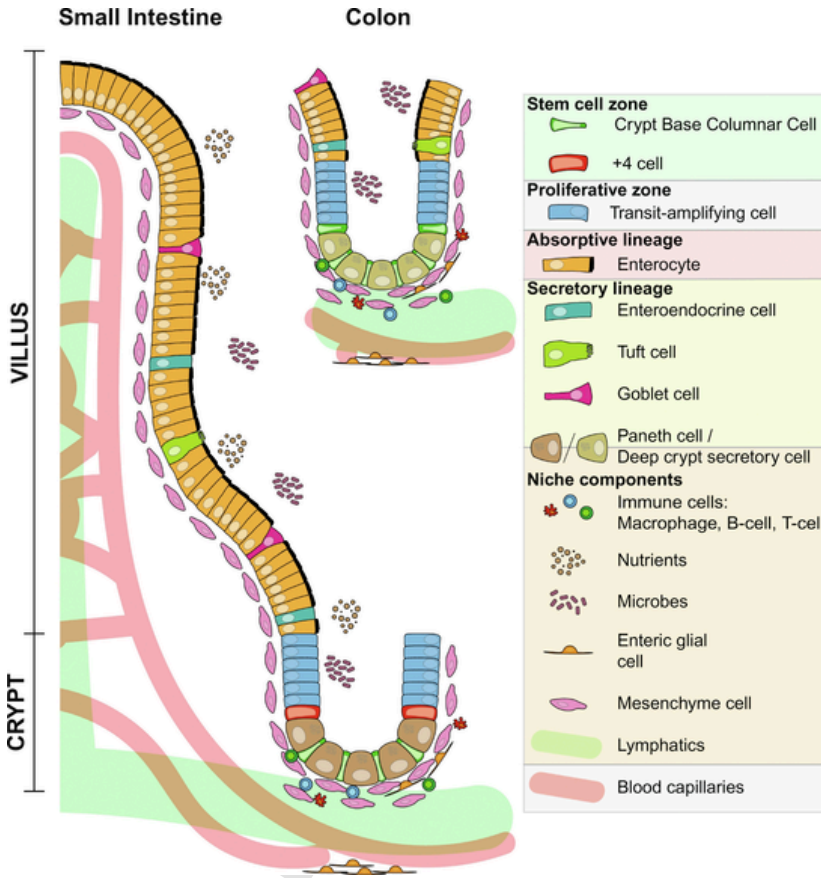

Fig. 2 Organization of the intestinal epithelium and surrounding niche components in the small intestine and colon. The small intestinal epithelium is organized in a crypt-villus structure with the stem cell compartment located at the base of the crypts. There, the Lgr5+ ISCs are intermingled with Paneth cells. Higher up the crypts are the +4 stem cells and transit amplifying (TA) cells. Within the villi, differentiated secretory and absorptive cell lineages reside. Surrounding the small intestinal epithelium is a wide diversity of cell types and niche factors that contribute to intestinal homeostasis and ISC maintenance, including the mesenchyme, immune cells, neural cells, gut flora, ECM proteins and nutritional components. In contrast, the colonic epithelium lacks villi, but the cell lineages and niche components are organized in a largely similar manner. An exception is the absence of Paneth cells, which are replaced by deep crypt secretory cells also interspersed between the Lgr5+ colonic stem cells.

eral key differences. The colonic epithelium lacks villi, presenting instead a flat surface epithelium harboring crypt-like invaginations with the ISC compartment located at their base.

Intestinal crypts contain approximately 20 Lgr5-expressing ISCs, also known as crypt-base-columnar (CBC) cells due to their distinct location and cellular morphology. These ISCs are interspersed with secretory Paneth cells at the crypt bases. Paneth cells are absent in the colon, and ISCs are instead intermingled with deep crypt secretory cells (Sasaki et al., 2016). Using lineage tracing experiments, Lgr5+ ISCs in both the small intestine and colon were found to be capable of generating all the differentiated cell types that populate the intestinal epithelium (Barker et al., 2007). Located directly above the ISCs at position +4 is another population of cells postulated to be capable of giving rise to all intestinal cell lineages. These cells, termed +4 cells, are thought to be a slow-cycling, reserve stem cell compartment independent from the Lgr5 crypt-base stem cells, labeled by Bmi1, Lrig1, Hopx and Tert, among other proposed markers (Breault et al., 2008; Montgomery et al., 2011; Powell et al., 2012; Sangiorgi & Capecchi, 2008; Takeda et al., 2011; Tian et al., 2011; Wong et al., 2012). Nevertheless, it was found that expression of +4 markers overlaps with Lgr5 within intestinal crypts, and that Lgr5+ ISCs and +4 cells have the ability to interconvert (Haber et al., 2017; Itzkovitz et al., 2011; Munoz et al., 2012; Wong et al., 2012; Yan et al., 2017), complicating the functional validation of stem potential for these +4 cells.

As crypt-base ISCs divide, they compete for space with neighboring cells, pushing these cells outwards and away from the crypt base (Ritsma et al., 2014). Cells that exit the stem cell niche then initiate a differentiation process to become progenitor cells in the transit-amplifying zone, where they perform several rounds of cell division as they migrate from the crypt to the villus compartment. In the villus, these cells undergo their final differentiation toward the secretory lineage (enteroendocrine cells, goblet cells, and tuft cells) or absorptive lineage (enterocytes and microfold cells). An exception is the Paneth cell that migrates downwards to the crypt base where it eventually resides (Batlle et al., 2002). Interestingly, it has recently been shown that cells continue to migrate even after inhibition of mitosis, suggesting that mitotic pressure from the dividing ISCs is not the only factor contributing to the migration process (Krndija et al., 2019). Using biophysical modeling coupled with three-dimensional tissue imaging, the intestinal cells were

found to migrate collectively with an increasing velocity as they approach the villus tip. Migrating cells formed actin-related protein 2 and 3 (Arp2/3)-dependent protrusions oriented in the direction of migration. Blocking Arp2/3 activity was sufficient to inhibit migration, though the effect was more pronounced at the villus tips than within the crypts. These data support a model in which migration of cells in the crypt is driven passively by mitotic pressure, which switches to an active migration at the villus level (Krndija et al., 2019). Finally, upon reaching the villus tips, these differentiated cells initiate a cell death program also known as anoikis. This involves detachment of the dying cells from the basal membrane accompanied by remodeling of tight junctions between the dying cell and its neighbors to maintain the epithelial barrier, leading to the extrusion of the dying cell from the epithelium into the lumen (Williams et al., 2015).

3.1 The organoid model

To study ISC functions *in vitro*, Sato et al. developed a system whereby ISCs can be isolated and maintained in long-term culture within an extracellular matrix gel that supports three-dimensional growth. Using a minimal medium supplemented with the mitogen protein EGF, the Wnt pathway enhancer Rspo and the BMP pathway inhibitor Noggin, ISCs proliferate and form organoids that are composed of all the cell lineages and recapitulate native crypt-villus morphologies. Moreover, these organoids can be maintained long-term over multiple passages. Importantly, these culture conditions highlight the minimal niche pathways necessary for ISC homeostasis: Wnt, EGF and BMP (Sato et al., 2009).

The organoid model also provides an invaluable tool to accurately study *in vitro* intestinal homeostasis, facilitating direct visualization along with genetic and chemical manipulations to trigger damage or cancer transformation (Lim, Kostic, & Barker, 2022). Since its introduction in 2009, this system has been further developed with the establishment of human organoid culture (Sato et al., 2011), the growth of intestinal organoids from human pluripotent stem cells (Spence et al., 2011) and the development of techniques including genome editing (Cong et al., 2013; Mali et al., 2013) and co-culture methods (Lindemans et al., 2015) to improve its versatility. In particular, the organoid co-culture system has been extensively used to highlight the niche functions of several cell types. This will be discussed later in this chapter.

4. Wnt pathway in intestinal homeostasis and regeneration

4.1 Modulation of Wnt signaling during homeostasis

Within the crypt bases of the intestine, the canonical Wnt/β-catenin pathway is robustly activated, with a decreasing gradient toward the crypt-villus junction (Farin et al., 2016). Indeed, the expression of multiple Wnt target genes, including *Lgr5, Olfactomedin 4* (*Olfm4*) and *Achaete-Scute Family BHLH Transcription Factor 2* (*Ascl2*), has been identified within the stem cells located at the base of the intestinal crypts (Barker et al., 2007; van der Flier et al., 2009; van der Flier, Haegebarth, Stange, van de Wetering, & Clevers, 2009). Wnt/β-catenin signaling is essential in driving intestinal homeostasis, as inhibiting this pathway using knockout mice for the main effector protein Tcf4 or ectopic expression of the Wnt/β-catenin inhibitor Dickkopf-1 (Dkk1) led to the depletion of ISCs and secretory cell lineages (Korinek et al., 1998; Kuhnert et al., 2004; Pinto, Gregorieff, Begthel, & Clevers, 2003). In contrast, forced expression of a modified β-catenin construct or deletion of the negative regulator Apc to hyperactivate the Wnt/β-catenin pathway promoted intestinal epithelial proliferation and blocked enterocyte differentiation (Sansom et al., 2004; Wong, Rubinfeld, & Gordon, 1998). At the same time, Wnt signaling is also required for Paneth cell differentiation and localization. Genetic deletion of *Tcf4* to block endogenous Wnt/β-catenin signaling leads to a reduction in Paneth cell numbers (van Es et al., 2012). Loss of the Wnt receptor, *Fzd5*, which is specifically expressed by Paneth cells, blocks Paneth cell differentiation and results in their mislocalization along the crypt-villus axis (van Es et al., 2005), an effect also seen upon deletion of the Wnt target gene EphB3 (Batlle et al., 2002). Thus, Wnt signaling controls both ISC maintenance and differentiation of secretory cell lineages.

Maintaining the appropriate level of Wnt activation is crucial for the regulation of intestinal homeostasis. Deletion of B-cell lymphoma 9 and 9l (*Bcl9/9l*), a known transcriptional co-activator of β-catenin (Kramps et al., 2002), induces a decrease in nuclear β-catenin and an enrichment of membrane-localized β-catenin within cells in intestinal crypts. This suppression of Wnt activity has no effect on the expression of most Wnt

target genes, with the exception of *Lgr5*, which is also downregulated (Gay et al., 2019). These results support the fact that Lgr5-expressing cells are the most dependent on the highest level of Wnt signaling (Huels et al., 2018; Yan et al., 2017).

Interestingly, binding of transcriptional co-activators to different regions of the β-catenin protein can differentially regulate Wnt/β-catenin pathway activation and downstream transcriptional outputs. While the co-activators Bcl9 and Bcl9l are known to bind the N-terminal part of β-catenin, other factors such as the CREB-binding protein (CBP), Brahma-related gene 1 (BRG-1), and p300 interact with its C-terminus (Barker et al., 2001; Soderholm & Cantu, 2021). To resolve these interactions, mutant knock-in mice for different forms of β-catenin were generated, harboring mutations impairing the recruitment of either N-terminal (NTF) or C-terminal (CTF) transcriptional co-activators (Borrelli et al., 2021). Strikingly, loss of CTF interaction leads to complete loss of ISCs and proliferative arrest of the transit-amplifying compartment, a phenotype reminiscent of complete loss of the whole β-catenin gene. Conversely, blocking NTF interaction results in a transient expansion of the ISC compartment that is followed by the subsequent loss of stem and proliferation markers. These NTF interaction mutants also display impaired differentiation, with the presence of cells harboring gene signatures of both Paneth and goblet lineages. Together, these findings indicate that CTF co-activator binding to β-catenin acts as an on/off switch for Wnt/β-catenin pathway activation, while NFT interaction is required for the long-term maintenance of the stem cell state (Borrelli et al., 2021).

4.2 Determination of the stem cell state

To sustain epithelial cell turnover in the intestine under homeostatic conditions, crypt-base ISCs continuously divide, with an average of one cell division per day (Barker et al., 2007). Two models of ISC division have been proposed: a hierarchical model based on asymmetric cell division, and a stochastic symmetric division model with random division outcomes (Fig. 3). In the first model, stem cell components are asymmetrically distributed during cytokinesis, resulting in an asymmetric fate specification between the two daughter cells that gives rise to one stem cell and one transit-amplifying cell (Snippert et al., 2010). The second model proposes instead that ISC divisions can lead, by random

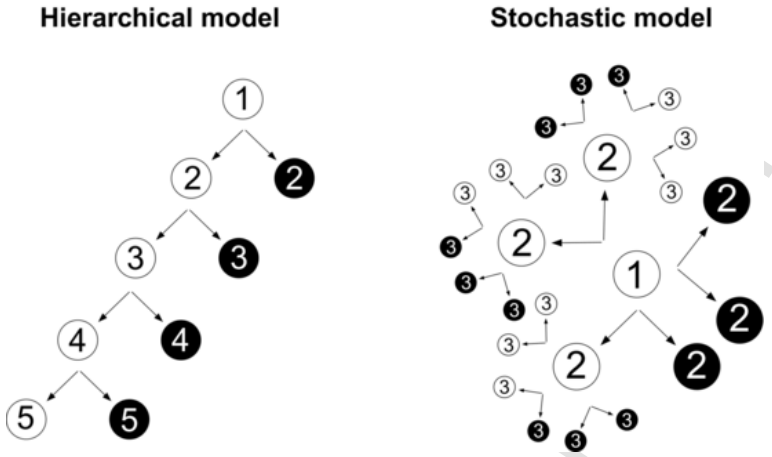

Fig. 3 Two models of ISC division to explain the homeostatic renewal of the intestinal epithelium. In the stochastic model, ISCs divide in a random manner to give rise to two ISCs, one ISC and one TA cell, or two TA cells. The hierarchical model proposes instead that ISCs divide asymmetrically, producing one stem and one differentiated daughter cell each time.

chance, to three possible outcomes: the generation of two stem cells, one stem cell and one transit-amplifying cell, or two transit-amplifying cells. To distinguish between the two proposed models, lineage tracing experiments using a confetti marker activated in ISCs were performed to facilitate the visualization of independently labeled clonal populations arising from each activated stem cell. Within one to 6 months, intestinal crypts were completely labeled by a single fluorescent marker indicative of monoclonality, supporting the symmetric division model in which clones arising from a single ISC eventually populate the entire crypt (Lopez-Garcia, Klein, Simons, & Winton, 2010; Snippert et al., 2010).

A question raised by the data so far is if the stem cell property is intrinsic to the cell or if its localization, within a dedicated niche, determines its stemness. Stem cells residing in the upper parts of the niche, named border cells, can be passively displaced into the transit amplifying domain by dividing neighboring cells, suggesting that stem cell identity can be uncoupled from cell division. As such, stem cells located at the crypt base, named central cells, display a survival advantage over the border cells. On the other hand, targeted ablation of Lgr5-expressing cells can induce Lgr5-negative cells to colonize the crypt and re-express

Lgr5 to act as functional stem cells (Ritsma et al., 2014). This supports the idea that stem cell potential is not cell-intrinsic but can be induced by localized microenvironmental cues within the stem cell niche that drive the acquisition of stem cell properties.

To further dissect the mechanisms behind stem cell proliferation during intestinal homeostasis, a mathematical model was recently proposed in which ISC division sets up a conveyor belt mechanism that drives daughter cells to exit the niche while random cell rearrangements allow cells to re-enter this niche (Corominas-Murtra et al., 2020). This model predicts that the number of functional stem cells is dependent on the distance from the base of the crypt. More recently, this mathematical prediction has been validated and further investigated by Azkanaz et al. Here, the authors show that while the distribution and number of Lgr5-expressing cells are similar between crypts in the small intestine and colon, the number of effective stem cells in the small intestine is significantly higher compared to the colon. Moreover, cells located further from the crypt base can still act as effective stem cells by re-entering the stem cell zone using an active anterograde movement dependent on Wnt signaling, which can be visualized using intravital microscopy. Inhibition of Wnt signaling *in vivo* decreases the number of effective stem cells and in turn accelerates the rate of monoclonal conversion of crypts (Azkanaz et al., 2022). In support of the role of Wnt signaling in this process, Lgr5-expressing cells migrate further *in vitro* when co-cultured with Wnt-secreting Paneth cells, an effect that is abolished upon treatment with a Porcupine inhibitor that blocks Wnt secretion (Azkanaz et al., 2022). Indeed, while the small intestine harbors a Paneth cell population that is a major source of epithelial Wnt ligands, there is no known source of Wnts in the colonic epithelium, which does not contain Paneth cells. Instead, stromal cells make up the primary source of Wnt ligands in the colon (Das et al., 2022). Thus, it is plausible that the level of stromal Wnt signaling is insufficient to drive retrograde movement in the colon but nonetheless sufficient to maintain homeostasis in both the small intestine and colon.

In summary, the current models suggest that ISC identity is linked primarily to their localization to the stem cell niche in crypt bases rather than an intrinsic property of these cells. Nevertheless, it is worth noting that in a cancer context, the majority of colorectal cancer metastases are seeded by Lgr5-negative cells, suggesting that these cells could have an

intrinsic capacity that confers them with cancer stem cell potential in a niche-independent manner (Fumagalli et al., 2020).

4.3 Reconstituting the stem cell pool after injury

During irradiation-induced injury, Lgr5+ CBCs are frequently depleted from the crypts, in part due to their high Wnt activity rendering them more sensitive to DNA damage (Tao et al., 2015). To compensate for loss of Lgr5+ CBCs, slow cycling +4 cells become highly proliferative to repopulate the neighboring crypts and villi before converting into Lgr5+ ISCs (Tian et al., 2011; Yan et al., 2012). These +4 cells are radiation resistant and insensitive to the Wnt pathway (Yan et al., 2012), which is negatively regulated by the transcription factor Sox9 (Bastide et al., 2007; Roche et al., 2015). This exit of quiescent state is under the control of Musashi (Msi) RNA binding protein as its gene ablation renders the epithelium unable to regenerate after irradiation (Yousefi et al., 2016).

Differentiated cells also have the capacity to dedifferentiate to replenish the ISC compartment. Several cell types from the secretory and absorptive lineages have been shown to have the potential to revert to a stem cell state during tissue repair, including the Dll1+ secretory precursor, the Alpi+ enterocyte precursor, the Krt19+ progenitor, enteroendocrine progenitor and Paneth cells (Jadhav et al., 2017; Schmitt et al., 2018; Tetteh et al., 2016; van Es et al., 2012; Yan, Gevaert, et al., 2017; Yu et al., 2018).

Moreover, following ISC ablation, most of the regenerative capacity arises from dedifferentiated cells from both secretory and absorptive progenitors, with little to no contribution from the +4 stem cells (Murata et al., 2020). This dedifferentiation process is driven by the Wnt target gene *Ascl2*, whose expression appears much earlier than *Lgr5*. In Ascl2-null mice, this dedifferentiation process is blocked, resulting in the loss of small intestinal tissue integrity following irradiation (Murata et al., 2020). These results challenge the long-standing notion that Bmi1-expressing +4 cells compensate for the loss of Lgr5+ ISCs, rendering Lgr5+ ISCs dispensable for the maintenance of intestinal homeostasis (Tian et al., 2011). Indeed, by using a more efficient Lgr5-2A-DTR diphtheria toxin ablation mouse model to achieve constant ablation of Lgr5+ cells that blocked their regeneration, severe disruptions to intestinal epithelial integrity were observed, highlighting the importance of this

cell population (Tan et al., 2021). Thus, the intestinal epithelium is a highly plastic tissue with remarkable regenerative potential, supported by the Lgr5+ ISCs.

5. Regulation of Wnt signaling by the intestinal niche

5.1 Paneth cells

Paneth cells are terminally differentiated cells that migrate downwards to reside within small intestinal crypt bases, where they intermingle with Lgr5+ ISCs. The Wnt pathway regulates differentiation along the Paneth cell lineage: Wnt/β-catenin signaling activates Atoh1, a transcription factor promoting the secretory lineage identity (Yang, Bermingham, Finegold, & Zoghbi, 2001). Following lineage specification, Paneth cells also require Wnt signaling *via* the Wnt receptor Fzd5 to complete maturation (van Es et al., 2005), indicating that high Wnt activity within intestinal crypts functions, somewhat paradoxically, to maintain both the undifferentiated stem cell state and to complete the differentiation of Paneth cells. Paneth cells have diverse functions, including the release of antimicrobial peptides such as lysozymes and defensins that influence the intestinal microbial composition and immune response (Cray, Sheahan, & Dekaney, 2021). At the same time, Paneth cells are also critical players in the regulation of intestinal homeostasis, serving as the primary epithelial source of niche factors like Wnt ligands that support the ISC niche. The Wnt pathway is thus intimately linked to both the formation and function of Paneth cells in maintaining intestinal homeostasis.

ISCs isolated in culture require exogenous supplementation of the niche factors EGF, Rspo, and Noggin in order to generate intestinal organoids containing stem and differentiated cell lineages that can be passaged long term, serving as a near-physiological *ex vivo* model reflecting the niche pathways that support intestinal homeostasis within native crypts (Sato et al., 2009). In addition to these pathways, Notch signaling has also been identified as a major regulator of ISC activity *in vivo* (Fre et al., 2005), mediated by Notch1 and Notch2 receptors present on Lgr5+ ISCs (Pellegrinet et al., 2011; VanDussen et al., 2012). In support of Paneth cells as an epithelial niche supplying these signaling factors required for stem cell maintenance, Paneth cells were found to

express high levels of the secreted factors EGF and Wnt3, as well as the surface-bound Notch ligand Dll4 (Gregorieff et al., 2005; Sato et al., 2011). Moreover, Paneth cells and ISCs maintain extensive cell-cell contact that is required for Notch receptor-ligand interactions to occur, a feature that is recapitulated both in the crypt-like invaginations present in murine intestinal organoids *in vitro* as well as within intestinal crypts *in vivo* (Sato, van Es, et al., 2011; Snippert et al., 2010). To directly test the role of Paneth cells in ISC maintenance, cell doublets containing a Lgr5+ ISC and a Paneth cell were seeded in culture and the efficiency of organoid formation was evaluated as a readout of stem cell activity. Compared to doublets containing purely Lgr5+ stem cells, these stem-Paneth cell doublets demonstrated a marked increase in organoid outgrowth capacity (Snippert et al., 2010). Importantly, this effect of Paneth cell incorporation can be substituted with the addition of exogenous Wnt3a in culture, suggesting that Wnt secretion from Paneth cells is a major player in promoting stem cell activity within the epithelial organoid system (Sato, van Es, et al., 2011).

In contrast, the indispensability of Paneth cells toward ISC maintenance *in vivo* has been more heavily disputed, in part due to the use of varying Paneth cell ablation models and the contribution of other non-epithelial cell populations that compensate for the loss of niche signaling factors normally secreted by Paneth cells. Indeed, by targeting the secretory lineage transcription factor Atoh1, some studies were able to robustly eliminate the Paneth cell population but found no major alterations in intestinal architecture and stem cell dynamics (Durand et al., 2012; Kim, Escudero, & Shivdasani, 2012). In this model, the Lgr5+ crypt-base stem cells were maintained and expressed high β-catenin levels indicative of active Wnt signaling, even in the absence of epithelial Wnt ligands (Durand et al., 2012). This may point to a contribution of Wnt ligands derived from non-epithelial sources in supporting stem cell maintenance. However, Atoh1 also functions downstream of Notch signaling in ISCs that regulate their differentiation into secretory goblet cells (Kazanjian, Noah, Brown, Burkart, & Shroyer, 2010; van Es, de Geest, van de Born, Clevers, & Hassan, 2010), potentially rendering Notch ligand-presenting Paneth cells dispensable for ISC functions in this Atoh1-null context. Thus, the Atoh1-deficient model remains inadequate in distinguishing the Wnt- and Notch-driven functions of Paneth cells in supporting the maintenance of the intestinal epithelium. In contrast to the Atoh1-null model, other *in vivo* models of Paneth cell

ablation, though incomplete, showed marked reductions in the Lgr5+ stem cell population following Paneth cell loss (Sato, van Es, et al., 2011). These include models expressing diphtheria toxin within Cryptdin2+ Paneth cells (Garabedian, Roberts, McNevin, & Gordon, 1997), knockout of Gfi1 (Bjerknes & Cheng, 2010), and conditional deletion of epithelial Sox9 under a Villin-Cre driver (Bastide et al., 2007). Together, these studies demonstrate a central role of Paneth cells in supporting stem cell functions *in vivo*, likely by maintaining Notch pathway activation through direct Notch ligand binding that cannot be readily compensated by other non-epithelial cells in the surrounding stem cell niche.

An alternative explanation for the differing observations seen across the Paneth cell ablation models is the immense plasticity of the intestinal epithelium in rapidly responding to Paneth cell loss. Using a new pLys^{DTR} mouse line enabling specific ablation of lysozyme-expressing intestinal Paneth cells, the complete loss of Paneth cells can be achieved *in vivo* (van Es et al., 2019). In this model, Lgr5+ crypt-base stem cells were not perturbed. Instead, enteroendocrine and tuft cell populations were found to replace Paneth cells, turning on Notch ligand Dll1 expression and localizing to the crypt base interspersed between Lgr5+ stem cells, precisely restoring Paneth cell functions (van Es et al., 2019). Thus, Notch activity can be sustained even in the absence of Paneth cells to support ISC maintenance by the rapid conversion of other differentiated lineages toward the Paneth cell lineage.

To further dissect the contribution of Paneth cells to intestinal homeostasis *in vivo*, epithelial-specific depletion of Wnt3, a Wnt ligand specifically expressed within Paneth cells (Gregorieff et al., 2005), was found to have no observable effect on Wnt activity or ISC maintenance (Farin, Van Es, & Clevers, 2012), suggesting that other non-epithelial Wnt ligands could be compensating for its functions. In line with this, loss of Atoh1 to deplete Paneth cells or deletion of Wnt3 in intestinal epithelial organoids severely compromised organoid growth, an effect that could be reversed by exogenous Wnt3a supplementation or co-culture of these organoids with a feeder intestinal mesenchymal cell layer (Farin et al., 2012; Kabiri et al., 2014). In the absence of Wnt3, mesenchymal-derived Wnt2b was found to be a major substitute that could restore *ex vivo* epithelial organoid growth (Farin et al., 2012). Together, these findings suggest that Wnt3 derived from Paneth cells is a major requirement for epithelial intestinal organoid growth in culture, but there

exists some redundancy in the form of other Wnt pathway factors secreted from mesenchymal cells *in vivo* that support the ISC niche.

Interestingly, apart from Wnt secretion, Paneth cells from aged mice also secrete Notum, a Wnt deacetylase that inhibits the binding of Wnt to its receptor Fzd. Thus, Notum secretion by Paneth cells can suppress Wnt signaling, impairing self-renewal and regeneration during the aging process (Pentinmikko et al., 2019). In contrast, chemical inhibition or genetic knockout of Notum was able to enhance the regenerative capacity of these aged stem cells, an effect also observed in organoid cultures with Wnt supplementation (Pentinmikko et al., 2019). In line with these findings, a more recent study found that Apc-mutant cells secrete Notum that inhibits the proliferation of neighboring Wnt-sensitive ISCs (Flanagan et al., 2021). These Apc-mutant cells are themselves insensitive to Notum, giving them a clonal advantage over the wild-type cells (Flanagan et al., 2021; Huels et al., 2018). In contrast, when Notum activity is genetically or pharmacologically inhibited, the competitiveness of wild-type stem cells is restored, abrogating the capacity of Apc-mutant cells to expand and form adenomas (Flanagan et al., 2021). Thus, in addition to the well-characterized Wnt ligand family, Notum is another Wnt pathway member that can regulate intestinal epithelial dynamics in different aging and cancer contexts. In particular, Paneth cells that secrete both Wnt and Notum can play opposing roles in Wnt pathway regulation.

5.2 Deep crypt secretory cells

Within colonic crypts, which do not contain Paneth cells, secretory cells interspersed with Lgr5+ stem cells have instead been identified. Like Paneth cells of the small intestine, these colonic secretory cells produce niche factors supporting stem cell maintenance, including the Notch ligands Dll1 and Dll4, and EGF (Rothenberg et al., 2012). One major distinction, however, is the absence of canonical Wnt ligand expression by these colonic deep crypt secretory cells, although both deep crypt secretory cells and colonic stem cells express high levels of the Wnt receptor Fzd5 (Sasaki et al., 2016). Thus, Wnt regulation of the colonic stem cell niche may arise from alternative, non-epithelial sources (Farin et al., 2012; Gregorieff et al., 2005). Moreover, whether these colonic deep crypt secretory cells regulate stem cell homeostasis *via* non-

canonical Wnt pathways or the secretion of other canonical Wnt pathway members such as R-spondins remains an open question.

Deep crypt secretory cells in the colon were first identified by expression of the receptor tyrosine kinase cKit, which marked a subset of Muc2+ goblet cells within the colonic epithelium residing in crypt bases adjacent to Lgr5+ stem cells (Rothenberg et al., 2012). While single Lgr5+ colonic stem cells were able to generate organoids *in vitro*, the addition of isolated colonic epithelial cKit+ cells to Lgr5+ cells greatly improved organoid formation efficiency (Rothenberg et al., 2012), mirroring the effect of Paneth cell addition to Lgr5+ ISC cultures (Sato, van Es, et al., 2011). Thus, cKit+ colonic cells support the maintenance of Lgr5+ stem cells, either through direct physical interactions or secreted niche factors. Interestingly, Lgr5+ colonic stem cells express Stem cell factor/Kit-ligand (Rothenberg et al., 2012), the ligand for the cKit receptor present on deep crypt secretory cells, suggesting that there could be reciprocal interactions between both cellular compartments.

In addition to marking the deep crypt secretory cells, cKit also labels Paneth cells in the small intestine, a small proportion of Lgr5+ colonic stem cells, and a subset of stromal cells, making it hard to distinguish the contribution of these different cell subpopulations. To more conclusively demonstrate the functions of the niche cells interspersed between Lgr5+ colonic stem cells, a subsequent study identified Reg4 as a specific marker of these epithelial cells that make up the colonic stem cell niche (Sasaki et al., 2016). Gene signatures from Reg4+ colonic cells closely resembled those from Paneth cells in the small intestine, reflecting their common functionalities as niche cells supporting the ISC compartment. Like cKit+ colonic cells, co-culture of Reg4+ colonic cells with Lgr5+ colonic stem cells *in vitro* enhanced organoid outgrowth (Sasaki et al., 2016). More strikingly, targeted ablation of Reg4+ colonic cells *in vivo* resulted in the loss of Lgr5+ stem cells accompanied by an expansion of the proliferative cell compartment, indicative of the role of Reg4+ cells in promoting stemness while repressing differentiation (Sasaki et al., 2016).

Together, these findings support a central role for deep crypt secretory cells in colonic stem cell maintenance. Although Wnt involvement in this process appears to be limited given the minimal expression of Wnt ligands by deep crypt secretory cells, Wnt signaling remains active in Lgr5+ colonic stem cells, suggesting the presence of alternative Wnt sources within the colonic stem cell niche. Moreover, Wnt activity in

combination with Notch inhibition remains important in regulating differentiation toward the deep crypt secretory lineage (Sasaki et al., 2016).

5.3 Stromal cells

The stromal cell compartment is composed of a highly heterogenous group of cells including subepithelial myofibroblasts, fibroblasts, pericytes, and smooth muscle cells located within the lamina propria surrounding the basal side of the intestinal epithelial layer (Mifflin, Pinchuk, Saada, & Powell, 2011). Due to their common mesenchymal origin, many of these cells co-express markers such as α-Smooth muscle actin (SMA), making it challenging to isolate and study the functions of specific stromal cell subpopulations. Nevertheless, recent studies combining targeted genetic ablation approaches and single cell RNAseq have made inroads into dissecting the identity of these stromal cell populations and their contribution as a Wnt-secreting niche.

While epithelial Paneth cells are the main producers of Wnt3a, Wnt6 and Wnt9 in the small intestine, stromal cells secrete primarily Wnt2b, Wnt4 and Wnt5a, with the same ligands also observed in the colon (Gregorieff et al., 2005). Within the heterogenous stromal compartment, Wnt ligands also display specific expression patterns, with Wnt4 evenly distributed throughout the villus, Wnt5a enriched in villus tips, and Wnt2b specifically localized to intestinal crypts (Gregorieff et al., 2005). Collectively, these ligands participate in both canonical Wnt/β-catenin and non-canonical Wnt pathways. Precisely how region-specific combinations of secreted Wnt ligands interact with their adjacent epithelial stem or differentiated cells to activate downstream signaling pathways remains an active area of research. One possibility as seen in other systems is for non-canonical Wnts to antagonize canonical Wnt signaling in order to regulate its level of activation (Fan et al., 2017; Nemeth, Topol, Anderson, Yang, & Bodine, 2007).

This multitude of Wnt ligands present within the intestinal epithelium and mesenchyme also suggests some level of redundancy that ensures the robust maintenance of intestinal homeostasis. In line with this, single deletions of Wnt2b and Wnt5a—the primary Wnt ligands produced by the stromal compartment—both failed to elicit major phenotypic alterations in the intestine (Bakker et al., 2012; van Amerongen & Berns, 2006), although Wnt5a deletion under an injury context impaired the

ability of the intestine to fully regenerate (Miyoshi, Ajima, Luo, Yamaguchi, & Stappenbeck, 2012). To overcome the potential compensatory effects of multiple Wnt ligands expressed within the intestinal tissue, subsequent studies assessed the effect of depleting Porcupine (Porcn) or Wntless (Wls), both of which are normally required for the secretion of all Wnt ligands. Indeed, in support of a major contribution of the stromal cell compartment as a Wnt-secreting source, epithelial-specific deletion of Porcn using the Villin-Cre driver did not perturb regular intestinal homeostasis or levels of Wnt activity within the intestinal crypt (Kabiri et al., 2014; San Roman, Jayewickreme, Murtaugh, & Shivdasani, 2014). Similarly, loss of Wls within both the colonic and small intestinal epithelium had no significant effect on the tissue (Degirmenci, Valenta, Dimitrieva, Hausmann, & Basler, 2018), suggesting that other non-epithelial sources are sufficient or become activated to overcome the loss of epithelial-derived Wnt. In contrast, global deletion of Porcn that impact both the epithelial and stromal cell compartments severely disrupted intestinal homeostasis (Valenta et al., 2016), an effect that was replicated by *in vivo* pharmacological inhibition of Porcn (Kabiri et al., 2014). *In vitro*, Porcn-depleted epithelial-derived intestinal organoids cannot be maintained, but this effect can be reversed by co-culturing them with a feeder layer of intestinal stromal cells even in the absence of exogenous Wnt supplementation (Kabiri et al., 2014), Collectively, these findings demonstrate that Wnt ligands supplied by the stromal cell compartment are important for intestinal homeostasis and can compensate for loss of epithelial sources of Wnts.

To further dissect the stromal cell populations that support the ISC niche, various marker combinations have been proposed to identify subsets of this heterogenous cell compartment. One such stromal marker is Pdgf receptor alpha (Pdgfrα), which broadly labels subepithelial myofibroblasts and telocytes. Unsurprisingly, blocking Wnt ligand secretion by Porcn deletion within this large stromal population of Pdgfrα-expressing cells disrupted homeostatic levels of Wnt/β-catenin signaling, formation of intestinal crypts, and epithelial proliferation (Greicius et al., 2018). Using single-cell RNAseq, three Pdgfrα+ subpopulations were subsequently identified: a Pdgfrα-high telocyte population enriched within the villus mesenchyme, and two Pdgfrα-low mesenchymal populations located deeper in the vascular plexus surrounding the intestinal crypts away from the epithelium (McCarthy et al., 2020). Both Wnt ligands and Wnt potentiators are highly expressed in these Pdgfrα+ cells, with

Pdgfrα-high telocytes expressing primarily the non-canonical Wnt ligands Wnt4, Wnt5a and Wnt5b, and Pdgfrα-low cells supplying the canonical Wnt2b ligand and Rspo1-3 (McCarthy et al., 2020). Together, the distinct tissue localization and pattern of Wnt expression exhibited by these Pdgfrα-high and -low cells suggest that they could be playing unique roles in intestinal homeostasis regulation. Strikingly, comparable Pdgfrα-high crypt-top and Pdgfrα-low crypt-bottom fibroblasts have also been identified within the colonic epithelium (Brugger, Valenta, Fazilaty, Hausmann, & Basler, 2020).

Pdgfrα-high cells overlap with a Foxl1-labeled stromal compartment, which was also found to mark telocytes harboring long cellular extensions that could facilitate signal transduction to the epithelium (Aoki et al., 2016; Shoshkes-Carmel et al., 2018). Foxl1+ telocytes similarly express Wnt ligands Wnt2b and Wnt5a, along with Rspo3 (Aoki et al., 2016). Although Foxl1+ cells are situated throughout the mesenchyme, ligand expression is spatially restricted, with Wnt2b ligands primarily detected in Foxl1+ cells at the base of intestinal crypts and Wnt5a around the crypt-villus junction (Shoshkes-Carmel et al., 2018). This may reflect further cell heterogeneity within the Foxl1+ population, or a compartmentalization of the tissue informed by reciprocal interactions between the epithelium and the stroma. Importantly, ablation of Foxl1+ telocytes resulted in decreased Wnt activity accompanied by impaired proliferation and loss of crypt-villus morphology (Aoki et al., 2016), an effect that could be replicated by conditional loss of Porcn to block Wnt secretion by Foxl1+ cells (Shoshkes-Carmel et al., 2018). In the colon, a similar population marked by Tagln or Acta2 displaying a high degree of overlap with Foxl1+ telocytes was found to be a major source of Wnt4 and Wnt5a (Das et al., 2022). Blocking Wnt secretion in Tagln+ or Acta2+ cells severely impaired colonic epithelial regeneration, with a drastically exacerbated phenotype arising upon simultaneous depletion of epithelial Wnt secretion (Das et al., 2022), thus implicating this population as a critical Wnt source.

Another subpopulation of Pdgfrα-high/ Foxl1+ telocytes is the Ng2+ pericyte-like stromal cells, found primarily in the ileum of the small intestine (Kim et al., 2020). Ng2+ cells secrete Wnt2b and Wnt4 ligands, which can be blocked by Wls deletion resulting in reduced numbers of Lgr5-expressing stem cells (Kim et al., 2020). Nevertheless, no major defects in epithelial integrity were observed, indicating alternative niche sources compensating for loss of Ng2+ cells (Kim et al., 2020).

This contrasts with the more severe intestinal phenotype arising from Porcn depletion in Foxl1+ telocytes, suggesting that other Ng2- populations may form the critical Wnt-secreting niche necessary for maintaining intestinal homeostasis.

In contrast to Pdgfrα-high/ Foxl1+ telocytes, the Pdgfrα-low mesenchymal population represents a more heterogenous group that can be subdivided into CD81+ trophocytes and CD81- mesenchymal cells (McCarthy et al., 2020). While isolated intestinal crypts normally require Rspo and Noggin supplementation to form organoids, co-culture of these crypts with Pdgfrα-low cells was sufficient to support organoid outgrowth even in the absence of exogenous Rspo and Noggin, in line with the strong Rspo expression detected in these Pdgfrα-low cells (McCarthy et al., 2020). Nevertheless, only Pdgfrα-low CD81+ cells, not the corresponding CD81− population, were able to support long-term organoid growth, a reflection of organoid self-renewal capacity (McCarthy et al., 2020). *In vivo*, targeted ablation of this cell population using an alternative Grem1 driver induced marked loss of ISCs and eventual collapse of intestinal crypt-villus architecture (McCarthy et al., 2020). However, depletion of Rspo3 in Grem1+ cells alone was not sufficient to induce changes in intestinal morphology as lymphatic endothelial cells serve as a secondary alternative source of Rspo3 ligands; only the simultaneous loss of Rspo3 in both cellular compartments could result in a massive reduction in intestinal crypt depth and villus lengths (Goto et al., 2022). Given that loss of Grem1+ cells alone was able to induce such a deleterious phenotype, it is plausible that CD81+/Grem1+ mesenchymal cells are a major source of Rspo3 along with other yet unidentified niche factors that support the maintenance of the intestinal epithelium.

Pdgfrα-low cells are also strongly enriched for another mesenchymal cell marker, CD34, encompassing the pericryptal CD34+ Gp38+ cells that reside in close proximity to crypt-base stem cells in both the small intestine and colon (Stzepourginski et al., 2017). Much like the Pdgfrα-low CD81+ stromal population, pericryptal CD34+ cells also express Wnt2b, Rspo1 and Grem1 at high levels, and can support intestinal organoid outgrowth *in vitro* without exogenous Rspo1 supplementation (Stzepourginski et al., 2017), suggesting that both sets of markers could be labelling similar, overlapping cell populations. Within the colon, CD90 marks another CD34+ subpopulation that is expressed by a small population of colonic crypt fibroblasts (Karpus et al., 2019). In line

with their proposed role as a Wnt-secreting niche, co-culture of CD90+ colonic fibroblasts with organoids derived from both small intestinal and colonic crypts enhanced organoid growth and budding phenotypes, likely a result of Wnt2b secretion (Karpus et al., 2019). Similar findings were made with a Map3k2-regulated intestinal stromal cell (MRISC) population marked by CD81 and CD34, which was found to upregulate Rspo1 secretion in response to DSS-induced injury of the colonic epithelium (Jacob et al., 2022).

Finally, Gli1 is another stromal cell marker known to label a subset of Pdgfrα+ cells but appears equally represented in Pdgfrα-high and Pdgfrα-low subpopulations (McCarthy, 2020). These Gli1+ mesenchymal cells populate crypt bases of both the small intestine and colon (Degirmenci et al., 2018), where they express the canonical ligand Wnt2b (Valenta et al., 2016). In the small intestine, conditional loss of Wls in Gli1+ cells had no observable effect on tissue homeostasis, but Wls depletion performed in both epithelium and Gli1+ mesenchyme was able to induce complete degeneration of the epithelium, suggesting that Gli1+ cells could serve as a Wnt source substituting for Wnt ligands secreted by Paneth cells in the epithelium upon Paneth cell loss (Degirmenci et al., 2018). Conversely, deleting Wls in Gli1+ cells alone in the colon was already sufficient to elicit major tissue damage together with loss of stem cells and Wnt activation, which could reflect the absence of epithelial Wnt sources in this tissue. In support of these *in vivo* outcomes, Wls knockout intestinal organoids can grow *in vitro* in combination with Gli1+ mesenchymal cells, without the addition of exogenous Wnts. Like the Pdgfrα+ population, Gli1+ mesenchymal cells represent another highly heterogenous cell population, overlapping with CD34+, Foxl1+ and Myh11+ clusters, among others. Among the clusters identified from Gli1+ cells, two of them, which were also marked by CD34, strongly expressed Wnt2b, Wnt4 and Rspo3 (Degirmenci et al., 2018). These cells likely represent the Pdgfrα-low CD34+ pericryptal population.

In summary, the stromal compartment is a highly heterogenous cell population expressing a broad range of Wnt ligands that allows for compensation by alternative or reserve Wnt sources to ensure robustness of the system. In the presence of Wnt-producing Paneth cells in the epithelium, Wnt secretion by some stromal populations is dispensable. Yet, blocking Wnt secretion in other stromal populations can elicit major intestinal tissue collapse, potentially an indication of their varied roles in driving non-canonical Wnt or other niche signaling pathways. Indeed,

canonical and non-canonical Wnt ligands are presented in a unique spatial distribution along the crypt-villus axis, reflecting differences in stromal cell localization but also crosstalk between epithelial and stromal cells in setting up niche gradients within the tissue. While many marker combinations have been proposed to mark different but overlapping subsets of stromal cells, single-cell and spatial transcriptomics methods are beginning to resolve some of these more complex relationships. Ultimately, these studies will build a comprehensive picture of interconnected and reciprocal epithelial-stromal interactions regulated by a network of niche signaling pathways.

5.4 Immune and lymphatic cells

The intestinal tissue also contains a large diversity of immune cell populations, including B and T lymphocytes, macrophages, and dendritic cells. In addition to the epithelial and mesenchymal cell populations, immune cells can also serve as a source of Wnt ligands supporting intestinal epithelial functions. Their role becomes dominant as a consequence of tissue injury, during which time immune cells are recruited and activated to support host defense as well as healing and repair of the tissue.

One major class of immune cells in the intestine is the macrophages that reside in the lamina propria just beneath the epithelium and throughout the external muscle and serosa layers (Cao, Mertens, Sivanathan, Cai, & Xiao, 2022). These macrophages can be broadly classified into M1 macrophages, which secrete pro-inflammatory factors to protect the host from external agents, and M2 macrophages, which are anti-inflammatory and instead regulate tissue repair (Murray & Wynn, 2011). Yet, *in vivo*, these macrophages are challenging to clearly define as they are known to interconvert between the M1 and M2 subtypes depending on the injury context, representing a highly heterogenous population. Importantly, damaged mucosal tissues from patients with intestinal bowel disease (IBD) and ulcerative colitis showed prominent signs of macrophage infiltration accompanied by Wnt ligand expression (Cosin-Roger et al., 2013; Ortiz-Masia et al., 2014). In IBD samples, CD68+ macrophages were found to secrete Wnt1 that activated Wnt/β-catenin signaling within the epithelium in a hypoxia-inducible factor 1a (HIF-1a)-dependent manner (Ortiz-Masia et al., 2014), in line with the exposure of macrophages to localized hypoxic conditions during tissue

injury. CD68, however, marks all macrophages without distinction of the M1 and M2 functional subtypes. To dissect the contribution of M1 and M2 subtypes, macrophages isolated from patients were directed *in vitro* toward the M1 or M2 phenotype, revealing M2 macrophages as the primary source of Wnt ligands and epithelial Wnt activation (Cosin-Roger et al., 2013). Compared to M1 macrophages, the M2 subtype expressed elevated levels of Wnt1 and Wnt3a ligands, promoting β-catenin accumulation and expression of Wnt target genes when grown with Caco-2 intestinal epithelial cells in a co-culture system (Cosin-Roger et al., 2013). Separately, macrophages could also regulate β-catenin signaling *via* an alternative IL-10-dependent mechanism (Quiros et al., 2017). In this model, CD11c+ cells, which include macrophages but also some dendritic cell populations, were found to be the primary source of IL-10 during intestinal mucosal wound healing, as loss of IL-10 in this cell population affected the regeneration process (Quiros et al., 2017). By activating CREB signaling to promote Wnt1-inducible signaling protein 1 (WISP1) secretion, IL-10 expressed by CD11c+ macrophages was able to promote β-catenin signaling and epithelial repair (Quiros et al., 2017). This IL-10-driven mechanism could represent an alternative injury-driven pathway also regulated by Wnt1 from infiltrating intestinal macrophages, or may reflect the contribution from a different cell population marked by CD11c that is responsible for tissue regeneration.

In murine models of intestinal damage, Wnt ligand secretion by macrophages has also been implicated in the regenerative process. This was found to be driven in a STAT6-dependent manner by M2 macrophages upon treatment of mice with 2,4,6-trinitrobenzenesulfonic acid (TNBS) to induce colonic mucosal inflammation and injury (Cosin-Roger et al., 2016). STAT6 is a driver of the M2 phenotype, and STAT6 knockout mice fail to support M2 macrophage induction following TNBS-driven damage (Cosin-Roger et al., 2016). In the regenerating crypts, the ligands Wnt2b, Wnt7b and Wnt10a are upregulated along with activation of Wnt/β-catenin signaling, an effect that was reversed in STAT6 knockout mice (Cosin-Roger et al., 2016), highlighting the contribution of STAT6-dependent M2 macrophages to this process. Moreover, specifically blocking secretion of Wnt ligands by Porcn depletion in Csf1r+ cells, which encompasses both M1 and M2 macrophage subtypes, resulted in loss of Lgr5+ ISCs and attenuated crypt regeneration following injury (Saha et al., 2016). Interestingly, this effect of Porcn depletion can be rescued by exogenous Wnt ligands,

macrophage-derived conditioned medium, or purified extracellular vesicles from this medium, suggesting that Wnt ligands are delivered to the epithelium *via* extracellular vesicles secreted by Csf1r+ macrophages (Saha et al., 2016).

Conversely, during homeostasis, deleting Porcn in Csf1r+ macrophages did not induce significant morphological changes (Saha et al., 2016). Thus, Wnt secretion from this macrophage population appears to be dispensable for intestinal homeostasis, although it remains to be seen if simultaneous deletion of Porcn within the epithelial or mesenchymal compartments could reveal reserve niche functions. Depletion of the macrophage population itself *via* inhibition of CSF1R signaling, however, resulted in loss of Lgr5+ ISCs and impaired the homeostatic balance in the intestine, an effect that could be attributed in part to depletion of Wnt4 and Rspo1 normally secreted by crypt-base macrophages (Sehgal et al., 2018). Given that blocking Wnt secretion in macrophages was not enough to disrupt intestinal homeostasis, macrophages likely regulate homeostatic pathways *via* alternative mechanisms independent of Wnt activity.

Lymphocytes, including T-cells, B-cells, and natural killer cells, comprise another class of immune cells within the intestine that perform a diverse range of functions, including antibody generation and elimination of infected host cells. A large proportion of these lymphocytes are intraepithelial lymphocytes located in close proximity to the intestinal epithelium, while others are located within the lamina propria. How these lymphocyte populations potentially communicate with the intestinal epithelium to maintain proper intestinal functions remain poorly understood. More recently, the use of lymphocyte-depleted mouse models has shed light on the functions of these immune cell populations, revealing a contact-dependent role of integrin αEβ7+ T-cells in regulating Wnt and Notch signaling within crypt-base ISCs (Chen et al., 2021). In this study, knockout mouse models for either integrin β7 or Rag1 were used to deplete T and B lymphocytes. Loss of lymphocytes resulted in downregulation of Wnt signaling accompanied by altered differentiation along the secretory and absorptive cell lineages, although ISC numbers remained unchanged (Chen et al., 2021). Strikingly, changes in lineage differentiation can similarly be achieved when intestinal organoids are directly co-cultured with T cells, but not when T cells are cultured in a separate layer without direct contact with the epithelial cells, highlighting the importance of epithelial-lymphocyte interactions (Chen et al.,

2021). This interaction was found to involve integrin αEβ7 present on T-cells and E-cadherin on the surface of epithelial cells, which induces E-cadherin internalization and promotes Wnt signaling in intestinal crypts (Chen et al., 2021). Whether other lymphocyte populations that are not in direct contact with the epithelium also regulate Wnt signaling *via* alternative mechanisms is still not well understood.

Finally, part of the immune system is the lymphatic network that functions to transport immune cells, fluids, and nutrients from the intestinal mucosa. In the small intestine, lymphatic lacteals located in the central part of each villus are surrounded by a lymphatic capillary network, whereas the colon, which lacks villi, does not have lacteals (Goto et al., 2022; Niec et al., 2022). Strikingly, there is a second lymphatic network concentrated at the base of both small intestinal and colonic crypts, in close proximity and occasionally in direct contact with the epithelium (Goto et al., 2022; Niec et al., 2022). While targeted ablation of the lymphatic cells within native intestinal tissues induced depletion of crypt-base ISCs, co-culture of intestinal crypts with lymphatic endothelial cells significantly enhanced organoid outgrowth, pointing to a role of the lymphatic system in stem cell maintenance (Niec et al., 2022). Importantly, this effect can be mimicked using conditioned medium from lymphatic endothelial cells. Analyses of secreted components that were enriched in crypt-base lymphatic cells identified, among other ligands, Rspo3 and Wnt2, two major niche factors known to promote Wnt activation in the ISC compartment (Niec et al., 2022). Indeed, the predominant populations expressing Rspo3 in the intestine were previously identified as Lyve1+ lymphatic endothelial cells, along with an adjacent group of Grem1+ fibroblasts (Goto et al., 2022; Ogasawara et al., 2018). Therefore, niche factors originating from the lymphatic system promote Wnt activation in the ISC niche.

5.5 Nervous system

The gastrointestinal tract is innervated by neural connections from both the central nervous system (CNS) and enteric nervous system (ENS). While the signals from the CNS derive from the brain and spinal cord, the ENS is intrinsic to the gastrointestinal tissue, with enteric neurons residing either within the smooth muscle layers of the muscularis propria (myenteric) or in the submucosa (submucosal) (Furness, 2012; Ten Hove, Seppen, & de Jonge, 2021). In the small intestine, enteric neu-

rons are supported by glial cells found within the myenteric and submucosal plexuses, as well as directly beneath the mucosal lining adjacent to intestinal villi and crypts (Yu & Li, 2014). Both neurons and glia interact with the intestinal epithelium by secreting signaling factors, or by forming direct physical connections with epithelial cells such as enteroendocrine cells (Bohorquez et al., 2015). Importantly, ablation of the ENS resulted in severe loss of intestinal mucosal integrity and onset of inflammation (Bush et al., 1998), supporting a major contribution of the ENS toward intestinal homeostasis.

To study how the ENS regulates intestinal epithelial functions, *in vitro* co-culture systems have been established to replicate the neural-epithelial interactions that exist within the intestinal crypt. For instance, by combining pluripotent stem cell-derived human intestinal organoids with neural crest cells and inducing their differentiation *in vitro*, human intestinal organoids integrated with functional enteric neurons and glial cells could be generated (Workman et al., 2017). Alternatively, to assess the effect of secreted factors from neural cultures on epithelial growth, a transwell format was used to grow intestinal cell monolayers suspended over an underlying culture of enteric neurons and glia (Puzan, Hosic, Ghio, & Koppes, 2018). In this co-culture system, ENS cultures elicited an altered ISC differentiation program biased toward ChgA-expressing enteroendocrine cells, and increased production of the cytokine IL-10 (Puzan et al., 2018).

More recently, targeted *in vivo* cell ablation approaches were used to dissect the contribution of glial fibrillary acidic protein (GFAP)- and proteolipid protein 1 (PLP1)-positive enteric glial cell populations to the homeostatic maintenance of the intestinal epithelium. PLP1+ cell ablation did not perturb intestinal homeostasis but elimination of GFAP+ enteric glial cells resulted in a corresponding loss of Lgr5+ ISCs, although intestinal epithelial functions could eventually be restored by other compensatory enteric glial populations (Baghdadi et al., 2022). In line with this, combined ablation of both GFAP+ and PLP1+ enteric glial populations resulted in severe disruptions to intestinal morphology and loss of intestinal barrier function. This effect on intestinal homeostasis and stem cell activity is likely due to the loss of Wnt ligands normally expressed within the GFAP+ mucosal glial cells that surround the ISC niche, attenuating Wnt activity (Baghdadi et al., 2022). While this was the first study to demonstrate enteric glial cells as a niche source of Wnts, this finding corroborates earlier work in *C. elegans* identifying the

secretion of Wnt/EGL-20 from neurons that bind to Frizzled receptors on intestinal cells to activate genes involved in mitochondrial unfolded protein response (Zhang et al., 2018). Indeed, enteric neurons have been shown in various models to supply niche factors regulating the ISC niche, including TGF-β, which blocks ISC proliferation (Miyoshi et al., 2012), and 15dPGJ2, which supports ISC differentiation (Bach-Ngohou et al., 2010). Importantly, this neural-epithelial interaction is bidirectional, as intestinal epithelial cells are known to secrete glucagon-like peptide 2 (GLP-2) that binds to receptors expressed specifically on enteric neurons, inducing neuronal signal transmission (Bjerknes & Cheng, 2001). Future studies on this diversity of neurons innervating the ISC niche would yield crucial insights into the additional niche factors and signaling cascades they activate in maintaining intestinal homeostasis.

5.6 Extracellular matrix

In addition to the cellular components of the ISC niche, intestinal crypts are also surrounded by an extracellular matrix (ECM) composed of fibrous proteins, glycoproteins, and glycosaminoglycans that support both structural and biochemical interactions with the intestinal epithelium (Meran, Baulies, & Li, 2017). The fibrous proteins, including collagen, primarily function as structural scaffolds for the surrounding tissue, whereas glycoproteins like fibronectin and laminin are thought to facilitate cell-ECM communication by interacting with cell surface adhesion molecules (Pompili, Latella, Gaudio, Sferra, & Vetuschi, 2021). Glycosaminoglycans, on the other hand, confer hydration pressure to tissues and can also regulate growth factor accessibility to the stem cell niche (Pompili et al., 2021). Some ECM constituents distribute heterogeneously, with laminin-α2 enriched around the intestinal crypt and laminin-α3 restricted to the villus, while collagen and the glycosaminoglycan-containing heparan sulfate proteoglycan (HSPG) are found all along the intestinal crypt-villus axis (Beaulieu, 1992; Oshiro et al., 2001; Teller & Beaulieu, 2001). These region-specific ECM proteins may be important in shaping the signaling pathways and cellular environment supporting the ISC niche. In support of the biological importance of the ECM, the major intestinal ECM proteins collagen IV, laminin, fibronectin, and hyaluronic acid were also found to be requisite

factors supporting intestinal organoid growth in a minimal organoid culture system (Gjorevski et al., 2016).

Studies on the roles of laminin in the small intestine have shed light on how they regulate Wnt pathway activity to support intestinal homeostasis. Laminins can bind to specific membrane-bound integrin receptors, resulting in the activation of intracellular cytoskeletal changes and signaling cascades, or non-integrin receptors such as dystroglycans. In particular, the α5-type laminin, which is localized to both the transit-amplifying and villus regions of the small intestine, has been implicated in intestinal smooth muscle development (Bolcato-Bellemin et al., 2003) and epithelial differentiation (Lepage, Seltana, Thibault, Tremblay, & Beaulieu, 2018). In one mouse model, loss of laminin-α5 expression within the intestinal mucosa resulted in the conversion of the mucosal architecture to one resembling the colon, along with the deposition of colonic laminin proteins (Mahoney, Stappenbeck, & Miner, 2008). By profiling laminin-α5 knockout intestinal tissue, several Wnt targets including Pitx2 and Myod1 and the Wnt receptor Fzd2 were found to be upregulated, indicative of a repressive effect of laminin expression on Wnt activity (Ritie et al., 2012). However, the mechanism by which laminin regulates Wnt remains unclear. Moreover, how the other laminin isoforms within the ISC niche regulate Wnt activity and stem cell maintenance is still not well understood, although laminin-α1 was shown to be a key niche component supporting intestinal organoid formation *in vitro*, suggesting that it may play a role in regulating stem cell activity (Gjorevski et al., 2016).

Another major ECM family that has been implicated in Wnt pathway regulation are the heparan sulfate proteoglycans (HSPGs). HSPGs contain a core protein covalently associated with one or more heparan sulfate side chains and are found on the cell membrane and within the ECM. Importantly, HSPGs have the ability to bind growth factors and signaling molecules, allowing them to regulate the movement of these secreted factors and to set up morphogen gradients critical for tissue homeostasis and growth (Sarrazin, Lamanna, & Esko, 2011). Of note, HSPGs have been shown to regulate the Wnt pathway at multiple levels, including directly binding to Wnt ligands to stabilize and concentrate them at the cell surface (Ai et al., 2003; Fuerer, Habib, & Nusse, 2010), and binding to other Wnt pathway members to facilitate Wnt ligand-receptor interactions (Freeman et al., 2008). In line with this, inhibition of HSPG synthesis in the *Drosophila* embryo was found to perturb Wing-

less (Wg) gradient establishment and Wg target gene expression in wing imaginal discs (Bornemann, Duncan, Staatz, Selleck, & Warrior, 2004). A similar finding was made in mouse intestinal epithelial cells lacking heparan sulfate, which displayed impaired Wnt/β-catenin signaling and repression of downstream Wnt target genes, including Lgr5 (Yamamoto et al., 2013). This effect could be attributed to the lowered stability and binding affinity of Wnt3a ligands to intestinal epithelial cells in the absence of heparan sulfate (Yamamoto et al., 2013). In other contexts, binding of HSPG to Wnt ligands could have the converse effect of downregulating Wnt signaling. Glypican-6 (GPC6), another class of HSPGs, was found to inhibit Wnt activity by binding Wnt5a and blocking its release from intestinal mesenchymal cells (Shi, Kaneiwa, Cydzik, Gariepy, & Filmus, 2020). Together, the interactions between Wnt pathway members and the HSPG family of proteins can regulate the accessibility of signaling molecules within the ISC niche needed for the homeostatic maintenance of the intestinal epithelium.

5.7 Flora

The gut microbiota represents another important regulator of Wnt activity that supports intestinal homeostasis. This microbial community includes bacteria, fungi and viruses that reside within the luminal space and outer mucus layer, rarely in contact with the intestinal epithelium under physiological conditions (Paone & Cani, 2020). This is regulated in part by antimicrobial factors secreted by Paneth cells (Bevins & Salzman, 2011). Interestingly, colonic crypts, which do not contain Paneth cells, display a vastly broader microbiota diversity, and support a resident crypt-specific core microbiota (Pedron et al., 2012).

Studies using germ-free animals have offered key insights into the role of the gut microbiome. Relative to standard specific pathogen-free (SPF) mice, germ-free mice display reduced Wnt/β-catenin activity along with decreased levels of the non-canonical Wnt ligands Wnt5a and Wnt11 within the colon, a phenotype that could be partially reversed with conventionalization of germ-free mice using commensal bacterial strains (Neumann et al., 2014). A similar study using a germ-free zebrafish model found that the resident microbiota was necessary for β-catenin stability and canonical Wnt pathway activation within intestinal epithelial cells (Cheesman, Neal, Mittge, Seredick, & Guillemin, 2011).

To probe the interactions between intestinal epithelial cells and specific microbial populations, *in vitro* intestinal cell line and organoid models incorporating microbial colonization have also been established. Colonization of intestinal epithelial cell lines by a non-pathogenic *Salmonella* strain was sufficient to abrogate proteasomal degradation of β-catenin, promoting its nuclear translocation (Sun, Hobert, Rao, Neish, & Madara, 2004). Similar findings were made using an intestinal organoid co-culture system incorporating *Lactobacillus reuteri*, a common probiotic bacterium, resulting in the upregulation of R-spo1-3 expression, increased nuclear accumulation of β-catenin, and expansion of the Lgr5+ stem cell population (Wu et al., 2020), although how Wnt is activated by bacterial co-culture remains unclear.

One possible mechanism is *via* lactate secreted by lactic-acid-producing bacterial strains that promote Wnt/β-catenin pathway activation and Lgr5+ ISC proliferation in a Gpr81-dependent manner (Lee et al., 2018). Gpr81 is a G-protein-coupled receptor that binds lactate and is present on Paneth and intestinal stromal cells, inducing the upregulation of Wnt3a ligand expression in these niche cells (Lee et al., 2018). Thus, some microbial populations interact with components of the stem cell niche to promote factors inducing Wnt pathway activation in intestinal crypts. It remains unclear whether other microbes can act as a direct source of Wnt ligands or secrete regulators directly targeting Wnt activity within intestinal epithelial cells. Moreover, given that gut microbiota composition varies significantly along the proximal-distal axis of the small intestine and colon (Vuik et al., 2019), there is a need for more accurate models capturing these spatial differences that can be used to decipher the functions of microbial communities in various intestinal epithelial populations.

5.8 Nutrition

As a primary regulator of nutrient absorption, the intestine is well-positioned to sense and respond to changes in nutritional levels within the diet. Indeed, changes in the nutritional balance can have far-reaching implications on human health and disease. To study the effect of nutrition on intestinal functions, mouse models of obesity/high-fat diets, fasting and calorie restriction are commonly used. In the high-fat diet (HFD) model, mice display rapid weight gain accompanied by distinct changes in intestinal morphology and cell lineage allocation. Both short-term

(8–20 weeks) (Mah, Van Landeghem, Gavin, Magness, & Lund, 2014; Mao et al., 2013; Xie et al., 2020) and long-term chronic (9–14 months) (Beyaz et al., 2016) administration of HFD in mice promoted the expansion of the ISC population and concurrent reduction in Paneth cell numbers. In these models, stem cell functions were also enhanced, with HFD mice-derived crypts displaying greater organoid forming potential than those originating from mice on a regular balanced diet (Beyaz et al., 2016; Xie et al., 2020). When single Lgr5+ ISCs were isolated from HFD mice, these cells displayed robust organoid formation capacity even in the absence of Paneth cells, suggesting that components of the HFD can regulate intrinsic ISC factors independent of the Paneth cell niche (Beyaz et al., 2016). Indeed, addition of the fatty acid components palmitic acid or oleic acid to intestinal organoid cultures was sufficient to replicate the phenotypes seen in the HFD model, *via* a Pparδ-driven mechanism that can activate downstream targets of β-catenin including the Notch ligands Jag1 and Jag2 (Beyaz et al., 2016). The induction of Notch ligands within these intestinal cells may be a response to the loss of Paneth cells in a HFD context, maintaining Notch signaling to neighboring Lgr5+ ISCs needed to sustain stem cell functions. At the same time, extrinsic signals such as insulin and insulin-like growth factors (IGFs) upregulated in plasma of HFD mice have also been implicated in β-catenin activation and ISC proliferation (Mah et al., 2014). In stark contrast to the HFD model, an alternative high-fat/high-sugar diet (HFHSD) model found that while ISC proliferation remained elevated, there was an overall reduction in Lgr5+ ISCs as a result of increased differentiation toward enterocyte lineages (Aliluev et al., 2021). This intestinal hyperproliferation phenotype exhibited in HFHSD mice was linked instead to Pparγ and Insr-Igf1r-Akt signaling (Aliluev et al., 2021). Thus, changes in dietary composition can induce vastly opposing effects on stem cell behavior within the intestine *via* the activation of Wnt-dependent and Wnt-independent pathways.

On the other end of the nutritional spectrum, calorie restriction has been linked to longevity and improved health in humans (Alonso & Yilmaz, 2018). Yet, the relationship between calorie restriction and stem cell functions has remained unclear. Similar to the HFD model, ISC populations can also become activated under calorie-restricted conditions. In one model, a 24-h fasting regimen was found to induce a fatty acid oxidation program within ISCs, driving their activation (Mihaylova et al., 2018). The fatty acid oxidation program is mediated by PPAR re-

ceptor activity in ISCs (Mana et al., 2021), resulting in the generation of free fatty acids and acetyl-CoA, which serve as energy sources. Indeed, the ketone body beta-hydroxybutyrate, one of the terminal products in the fatty acid oxidation process, was found in Lgr5+ ISCs to regulate Notch signaling that promote stem cell renewal during homeostasis (Cheng et al., 2019).

At the same time, calorie restriction can also regulate ISC activity indirectly by promoting the niche function of Paneth cells. When grown in combination with isolated ISCs, Paneth cells derived from calorie-restricted mice had a greater capacity to promote organoid outgrowth (Yilmaz et al., 2012). In these calorie-restricted Paneth cells, mTORC1 signaling is downregulated, along with increased secretion of cyclic ADP ribose produced by the ectoenzyme Bst1 (Yilmaz et al., 2012). In Lgr5+ ISCs, mTORC1 signaling is instead elevated in response to calorie restriction, with Paneth cell-derived cyclic ADP ribose promoting SIRT1 activity in these cells to promote ISC renewal (Igarashi & Guarente, 2016). Interestingly, while the Wnt/β-catenin pathway upregulated in ISCs can activate mTOR signaling *via* GSK3 inhibition (Inoki et al., 2006), it is also antagonized by mTORC1 signaling in a cell-autonomous manner (Zeng et al., 2018), possibly representing a negative feedback loop that fine tunes the level of Wnt activation within the intestinal crypts. Thus, multiple direct and indirect mechanisms, including the intersection of the Wnt pathway with other metabolic and signaling pathways, work together to regulate Lgr5+ ISC activity during calorie restriction.

Finally, apart from these diet-induced phenotypes, other studies have focused on delineating the roles of individual nutritional components on ISC functions, including their involvement in Wnt pathway activation. One type of free fatty acid, arachidonic acid (AA), was found to induce β-catenin nuclear accumulation and Wnt pathway activation upon administration to intestinal organoid cultures (Wang et al., 2020). *In vivo*, Wnt signaling was unaffected when mice were treated with AA, though under an irradiation-induced regenerative context, AA-treated mice displayed significantly more regenerating crypts with cells positive for Axin2-LacZ, a readout for Wnt activity (Wang et al., 2020). Apart from fatty acids, the amino acid L-arginine has also been found to promote secretion of Wnt3a by Paneth cells (Hou et al., 2020) and Wnt2b by crypt base fibroblasts marked by CD90 (Hou et al., 2020), both niche factors that support intestinal homeostasis. Therefore, nutritional con-

stituents of the diet can individually influence Wnt signaling and intestinal functions. Future work analyzing the combinatorial effects of these nutritional elements on both ISCs and their supporting niche could yield more relevant insights.

6. Discussion

In this chapter, we have presented the characteristic crypt-villus morphology of the intestinal epithelium, where the stem cell compartment is maintained in a confined structure at the base of the crypts. ISCs continually give rise to precursors capable of differentiating to become cells from all intestinal epithelial lineages. The maintenance of stemness, proliferation, and differentiation of ISCs need to be tightly regulated to ensure the homeostatic balance of the tissue. This is in part controlled by the canonical Wnt/β-catenin pathway, which is essential in sustaining proper intestinal epithelial homeostasis and regeneration of the tissue following damage. Modulation of the Wnt response is critical in ensuring this balance in ISC dynamics. One key example that we have highlighted in this chapter is the Wnt pathway effector β-catenin, which can interact with distinct groups of transcriptional co-factors that recognize either its N- or C-terminal domain to support different modes of Wnt activation. It would be important to evaluate how these co-factors are differentially expressed in intestinal crypts and their role in Wnt signaling modulation during homeostasis and regeneration.

The presence of different protein signatures within intestinal epithelial cells also raises the question of how stemness is established and maintained. Two non-exclusive models have been proposed: stemness that is shaped by the microenvironment in the stem cell niche, or by intrinsic factors within individual stem cells. ISCs indeed harbor intrinsically distinct genetic signatures and expression of unique markers that confer stem cell properties, such as in the case of Lgr5-negative colorectal cancer cells with an intrinsic cancer stem cell potential capable of seeding metastases away from the primary niche. On the other hand, during tissue injury upon ablation of Lgr5+ ISCs, the crypt is capable of regenerating *via* the dedifferentiation of cells originating from different epithelial lineages, suggesting that differentiated cells have the potential to revert into a stem-like state. Moreover, cells further away from the crypt base can move in a retrograde fashion during homeostasis to re-

enter the stem cell zone and act as functional stem cells. These findings support the immense plasticity of the intestinal epithelium in response to different homeostatic and injury stimuli, whereby stem cell potential is not limited to the crypt-base ISCs but can also be shaped by extrinsic niche factors that guide the reversion of more differentiated cells back to a stem-like state.

The stem cell niche supporting the maintenance and function of these crypt-base ISCs is composed of a wide diversity of cell types and secreted factors, including Paneth cells, stroma, immune cells, neuronal cells, gut microbiota, and nutritional components. Many niche cells secrete Wnt and Rspo ligands promoting Wnt/β-catenin activation in the intestinal crypts, often in a redundant manner that can be compensated by other niche components. For instance, blocking Wnt secretion from Paneth cells, the epithelial niche cells in the small intestine, did not impair ISC homeostasis. An alternative source of Wnt and Rspo ligands is the surrounding stromal population, but in the event of damage, other facultative populations such as immune cells and neurons or external agents like microbes and nutrients can also function as a supporting niche. Indeed, these niche components do not function in isolation; how the diversity of cell types supporting the stem cell niche interact together under different contexts to regulate ISCs remains a major direction for future work. Newer, more complex intestinal organoid models incorporating multiple epithelial, stromal, immune, neural, and microbial elements could pave the way for studying interactions among these niche factors and their relative contributions toward Wnt signaling in the native ISC niche. Doing so, we can also better understand how the ISC population integrates the signals coming from these multiple niche sources and the reciprocal signaling from ISCs back to niche cells that could modulate the extent of pathway activation.

To further dissect the cellular and transcriptional complexity of the native ISC niche, spatial transcriptomics is an emerging technology that enables the generation of spatially resolved RNA-seq data that can be directly mapped back to a precise location within a given tissue section. Moreover, by using intact tissue sections to extract transcriptomic data, the cell-cell interactions surrounding the ISC niche can be retained in a native state. This allows gene expression to studied at the tissue level in different physiological or pathological settings. Spatial transcriptomics has already been successfully implemented to characterize the small intestinal stem cell niche (Niec et al., 2022). With continuous improve-

ments being made to attain higher spatial resolution, more precise characterizations of the niche interactions can soon be acquired. In the long term, combining spatial transcriptomics with single cell RNA-seq and live cell imaging would provide both spatial and temporal resolution to decipher the complex interactions occurring between the ISC and its niche.

Along with more complex spatial sequencing methods should come a greater appreciation for the regional differences across the small intestine and colon. The small intestine can be subdivided anatomically into the duodenum, jejunum, and ileum, whereas the colon has at least two distinct proximal and distal regions. These anatomical differences can be translated into varying functions and marker expression. For instance, the +4 marker Bmi1 labels cells at the +4 position in the duodenum but is rarely found in the ileum (Sangiorgi & Capecchi, 2008). Another example is the colonic epithelium, where the numbers of Lgr5+ ISCs are similar throughout the tissue but regional differences in the numbers of deep crypt secretory (DCS) cells exist – the cecum and distal colon contain an average of 13 DCS cells, while the proximal colon has only an average of 2 DCS cells (Sasaki et al., 2016). Whether region-specific levels of Wnt/β-catenin signaling explain these morphological features and how they could translate into functional differences to suit the unique roles of each tissue or tissue region will be an important area of future research. Moreover, analysis of specific tissue regions will allow us to glean more relevant insights into intestinal homeostasis and regeneration, both *in vivo* and also with the development of more accurate *in vitro* models.

In all, by combining greater spatial resolution with more complex models capable of recapitulating the bidirectional interactions and signaling crosstalk taking place within the ISC niche, we can start to tease apart the complex mechanisms at play that ensure the precise control of Wnt/β-catenin signaling needed to support homeostasis and regeneration of the small intestine and colon.

References

Ai, X., Do, A.T., Lozynska, O., Kusche-Gullberg, M., Lindahl, U., & Emerson, C.P., Jr. (2003). QSulf1 remodels the 6-O sulfation states of cell surface heparan sulfate proteoglycans to promote Wnt signaling. The Journal of Cell Biology, 162(2), 341–351. doi:10.1083/jcb.200212083.

Aliluev, A., Tritschler, S., Sterr, M., Oppenlander, L., Hinterdobler, J., & Greisle, T., et al. (2021). Diet-induced alteration of intestinal stem cell function underlies obesity and prediabetes in mice. Nature Metabolism, 3(9), 1202–1216. doi:10.1038/s42255-021-

00458-9.

Alonso, S., & Yilmaz, O.H. (2018). Nutritional Regulation of Intestinal Stem Cells. Annual Review of Nutrition, 38, 273–301. doi:10.1146/annurev-nutr-082117-051644.

Aoki, R., Shoshkes-Carmel, M., Gao, N., Shin, S., May, C.L., & Golson, M.L., et al. (2016). Foxl1-expressing mesenchymal cells constitute the intestinal stem cell niche. Cellular and Molecular Gastroenterology and Hepatology, 2(2), 175–188. doi:10.1016/j.jcmgh.2015.12.004.

Azkanaz, M., Corominas-Murtra, B., Ellenbroek, S.I.J., Bruens, L., Webb, A.T., & Laskaris, D., et al. (2022). Retrograde movements determine effective stem cell numbers in the intestine. Nature, 607(7919), 548–554. doi:10.1038/s41586-022-04962-0.

Bach-Ngohou, K., Mahe, M.M., Aubert, P., Abdo, H., Boni, S., & Bourreille, A., et al. (2010). Enteric glia modulate epithelial cell proliferation and differentiation through 15-deoxy-12,14-prostaglandin J2. The Journal of Physiology, 588(Pt 14), 2533–2544. doi:10.1113/jphysiol.2010.188409.

Baghdadi, M.B., Ayyaz, A., Coquenlorge, S., Chu, B., Kumar, S., & Streutker, C., et al. (2022). Enteric glial cell heterogeneity regulates intestinal stem cell niches. Cell Stem Cell, 29(1), 86–100 e106. doi:10.1016/j.stem.2021.10.004.

Bakker, E.R., Raghoebir, L., Franken, P.F., Helvensteijn, W., van Gurp, L., & Meijlink, F., et al. (2012). Induced Wnt5a expression perturbs embryonic outgrowth and intestinal elongation, but is well-tolerated in adult mice. Developmental Biology, 369(1), 91–100. doi:10.1016/j.ydbio.2012.06.007.

Barker, N., Hurlstone, A., Musisi, H., Miles, A., Bienz, M., & Clevers, H. (2001). The chromatin remodelling factor Brg-1 interacts with beta-catenin to promote target gene activation. The EMBO Journal, 20(17), 4935–4943. doi:10.1093/emboj/20.17.4935.

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., & Cozijnsen, M., et al. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature, 449(7165), 1003–1007. doi:10.1038/nature06196.

Bastide, P., Darido, C., Pannequin, J., Kist, R., Robine, S., & Marty-Double, C., et al. (2007). Sox9 regulates cell proliferation and is required for Paneth cell differentiation in the intestinal epithelium. The Journal of Cell Biology, 178(4), 635–648. doi:10.1083/jcb.200704152.

Batlle, E., Henderson, J.T., Beghtel, H., van den Born, M.M., Sancho, E., & Huls, G., et al. (2002). Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell, 111(2), 251–263. doi:10.1016/s0092-8674(02)01015-2.

Beaulieu, J.F. (1992). Differential expression of the VLA family of integrins along the crypt-villus axis in the human small intestine. Journal of Cell Science, 102(Pt 3), 427–436. doi:10.1242/jcs.102.3.427.

Bevins, C.L., & Salzman, N.H. (2011). Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nature Reviews. Microbiology, 9(5), 356–368. doi:10.1038/nrmicro2546.

Beyaz, S., Mana, M.D., Roper, J., Kedrin, D., Saadatpour, A., & Hong, S.J., et al. (2016). High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. Nature, 531(7592), 53–58. doi:10.1038/nature17173.

Bjerknes, M., & Cheng, H. (2001). Modulation of specific intestinal epithelial progenitors by enteric neurons. Proceedings of the National Academy of Sciences of the United States of America, 98(22), 12497–12502. doi:10.1073/pnas.211278098.

Bjerknes, M., & Cheng, H. (2010). Cell Lineage metastability in Gfi1-deficient mouse intestinal epithelium. Developmental Biology, 345(1), 49–63. doi:10.1016/j.ydbio.2010.06.021.

Bohorquez, D.V., Shahid, R.A., Erdmann, A., Kreger, A.M., Wang, Y., & Calakos, N., et al. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. The Journal of Clinical Investigation, 125(2), 782–786. doi:10.1172/JCI78361.

Bolcato-Bellemin, A.L., Lefebvre, O., Arnold, C., Sorokin, L., Miner, J.H., & Kedinger, M., et al. (2003). Laminin alpha5 chain is required for intestinal smooth muscle development. Developmental Biology, 260(2), 376–390. doi:10.1016/s0012-1606(03)00254-9.

Bornemann, D.J., Duncan, J.E., Staatz, W., Selleck, S., & Warrior, R. (2004). Abrogation of heparan sulfate synthesis in Drosophila disrupts the Wingless, Hedgehog and Decapentaplegic signaling pathways. Development, 131(9), 1927–1938. doi:10.1242/dev.01061.

Borrelli, C., Valenta, T., Handler, K., Velez, K., Gurtner, A., & Moro, G., et al. (2021). Differential regulation of beta-catenin-mediated transcription via N- and C-terminal co-factors governs identity of murine intestinal epithelial stem cells. Nature Communications, 12(1), 1368. doi:10.1038/s41467-021-21591-9.

Breault, D.T., Min, I.M., Carlone, D.L., Farilla, L.G., Ambruzs, D.M., & Henderson, D.E., et al. (2008). Generation of mTert-GFP mice as a model to identify and study tissue progenitor cells. Proceedings of the National Academy of Sciences of the United States of America, 105(30), 10420–10425. doi:10.1073/pnas.0804800105.

Brugger, M.D., Valenta, T., Fazilaty, H., Hausmann, G., & Basler, K. (2020). Distinct populations of crypt-associated fibroblasts act as signaling hubs to control colon homeostasis. PLoS Biology, 18(12), e3001032. doi:10.1371/journal.pbio.3001032.

Bush, T.G., Savidge, T.C., Freeman, T.C., Cox, H.J., Campbell, E.A., & Mucke, L., et al. (1998). Fulminant jejuno-ileitis following ablation of enteric glia in adult transgenic mice. Cell, 93(2), 189–201. doi:10.1016/s0092-8674(00)81571-8.

Cao, Q., Mertens, R.T., Sivanathan, K.N., Cai, X., & Xiao, P. (2022). Macrophage orchestration of epithelial and stromal cell homeostasis in the intestine. Journal of Leukocyte Biology, 112(2), 313–331. doi:10.1002/JLB.3RU0322-176R.

Carmon, K.S., Gong, X., Lin, Q., Thomas, A., & Liu, Q. (2011). R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. Proceedings of the National Academy of Sciences of the United States of America, 108(28), 11452–11457. doi:10.1073/pnas.1106083108.

Cheesman, S.E., Neal, J.T., Mittge, E., Seredick, B.M., & Guillemin, K. (2011). Epithelial cell proliferation in the developing zebrafish intestine is regulated by the Wnt pathway and microbial signaling via Myd88. Proceedings of the National Academy of Sciences of the United States of America, 108(Suppl 1), 4570–4577. doi:10.1073/pnas.1000072107.

Chen, S., Zheng, Y., Ran, X., Du, H., Feng, H., & Yang, L., et al. (2021). Integrin alphaEbeta7(+) T cells direct intestinal stem cell fate decisions via adhesion signaling. Cell Research, 31(12), 1291–1307. doi:10.1038/s41422-021-00561-2.

Cheng, C.W., Biton, M., Haber, A.L., Gunduz, N., Eng, G., & Gaynor, L.T., et al. (2019). Ketone Body Signaling Mediates Intestinal Stem Cell Homeostasis and Adaptation to Diet. Cell, 178(5), 1115–1131 e1115. doi:10.1016/j.cell.2019.07.048.

Clevers, H., & Nusse, R. (2012). Wnt/beta-catenin signaling and disease. Cell, 149(6), 1192–1205. doi:10.1016/j.cell.2012.05.012.

Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., & Habib, N., et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science, 339(6121), 819–823. doi:10.1126/science.1231143.

Corominas-Murtra, B., Scheele, C., Kishi, K., Ellenbroek, S.I.J., Simons, B.D., & van Rheenen, J., et al. (2020). Stem cell lineage survival as a noisy competition for niche access. Proceedings of the National Academy of Sciences of the United States of America, 117(29), 16969–16975. doi:10.1073/pnas.1921205117.

Cosin-Roger, J., Ortiz-Masia, D., Calatayud, S., Hernandez, C., Alvarez, A., & Hinojosa, J., et al. (2013). M2 macrophages activate WNT signaling pathway in epithelial cells: relevance in ulcerative colitis. PLoS One, 8(10), e78128. doi:10.1371/journal.pone.0078128.

Cosin-Roger, J., Ortiz-Masia, D., Calatayud, S., Hernandez, C., Esplugues, J.V., & Barrachina, M.D. (2016). The activation of Wnt signaling by a STAT6-dependent macrophage phenotype promotes mucosal repair in murine IBD. Mucosal Immunology, 9(4), 986–998. doi:10.1038/mi.2015.123.

Cray, P., Sheahan, B.J., & Dekaney, C.M. (2021). Secretory Sorcery: Paneth Cell Control of Intestinal Repair and Homeostasis. Cellular and Molecular Gastroenterology and Hepatology, 12(4), 1239–1250. doi:10.1016/j.jcmgh.2021.06.006.

Das, S., Feng, Q., Balasubramanian, I., Lin, X., Liu, H., & Pellon-Cardenas, O., et al. (2022). Colonic healing requires Wnt produced by epithelium as well as Tagln+ and Acta2+ stromal cells. Development, 149(1). doi:10.1242/dev.199587.

de Lau, W., Barker, N., Low, T.Y., Koo, B.K., Li, V.S., & Teunissen, H., et al. (2011). Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature, 476(7360), 293–297. doi:10.1038/nature10337.

Degirmenci, B., Valenta, T., Dimitrieva, S., Hausmann, G., & Basler, K. (2018). GLI1-expressing mesenchymal cells form the essential Wnt-secreting niche for colon stem cells. Nature, 558(7710), 449–453. doi:10.1038/s41586-018-0190-3.

Durand, A., Donahue, B., Peignon, G., Letourneur, F., Cagnard, N., & Slomianny, C., et al. (2012). Functional intestinal stem cells after Paneth cell ablation induced by the loss of transcription factor Math1 (Atoh1). Proceedings of the National Academy of Sciences of the United States of America, 109(23), 8965–8970. doi:10.1073/pnas.1201652109.

Fan, J., Wei, Q., Liao, J., Zou, Y., Song, D., & Xiong, D., et al. (2017). Noncanonical Wnt signaling plays an important role in modulating canonical Wnt-regulated stemness, proliferation and terminal differentiation of hepatic progenitors. Oncotarget, 8(16), 27105–27119. doi:10.18632/oncotarget.15637.

Farin, H.F., Jordens, I., Mosa, M.H., Basak, O., Korving, J., & Tauriello, D.V., et al. (2016). Visualization of a short-range Wnt gradient in the intestinal stem-cell niche. Nature, 530(7590), 340–343. doi:10.1038/nature16937.

Farin, H.F., Van Es, J.H., & Clevers, H. (2012). Redundant sources of Wnt regulate intestinal stem cells and promote formation of Paneth cells. Gastroenterology, 143(6), 1518–1529 e1517. doi:10.1053/j.gastro.2012.08.031.

Flanagan, D.J., Pentinmikko, N., Luopajarvi, K., Willis, N.J., Gilroy, K., & Raven, A.P., et al. (2021). NOTUM from Apc-mutant cells biases clonal competition to initiate cancer. Nature, 594(7863), 430–435. doi:10.1038/s41586-021-03525-z.

Fre, S., Huyghe, M., Mourikis, P., Robine, S., Louvard, D., & Artavanis-Tsakonas, S. (2005). Notch signals control the fate of immature progenitor cells in the intestine. Nature, 435(7044), 964–968. doi:10.1038/nature03589.

Freeman, S.D., Moore, W.M., Guiral, E.C., Holme, A.D., Turnbull, J.E., & Pownall, M.E. (2008). Extracellular regulation of developmental cell signaling by XtSulf1. Developmental Biology, 320(2), 436–445. doi:10.1016/j.ydbio.2008.05.554.

Fuerer, C., Habib, S.J., & Nusse, R. (2010). A study on the interactions between heparan sulfate proteoglycans and Wnt proteins. Developmental Dynamics, 239(1), 184–190. doi:10.1002/dvdy.22067.

Fumagalli, A., Oost, K.C., Kester, L., Morgner, J., Bornes, L., & Bruens, L., et al. (2020). Plasticity of Lgr5-Negative Cancer Cells Drives Metastasis in Colorectal Cancer. Cell Stem Cell, 26(4), 569–578 e567. doi:10.1016/j.stem.2020.02.008.

Furness, J.B. (2012). The enteric nervous system and neurogastroenterology. Nature Reviews. Gastroenterology & Hepatology, 9(5), 286–294. doi:10.1038/nrgastro.2012.32.

Garabedian, E.M., Roberts, L.J., McNevin, M.S., & Gordon, J.I. (1997). Examining the role of Paneth cells in the small intestine by lineage ablation in transgenic mice. The Journal of Biological Chemistry, 272(38), 23729–23740. doi:10.1074/jbc.272.38.23729.

Gay, D.M., Ridgway, R.A., Muller, M., Hodder, M.C., Hedley, A., & Clark, W., et al. (2019). Loss of BCL9/9l suppresses Wnt driven tumourigenesis in models that recapitulate human cancer. Nature Communications, 10(1), 723. doi:10.1038/s41467-019-08586-3.

Gjorevski, N., Sachs, N., Manfrin, A., Giger, S., Bragina, M.E., & Ordonez-Moran, P., et al. (2016). Designer matrices for intestinal stem cell and organoid culture. Nature, 539(7630), 560–564. doi:10.1038/nature20168.

Glinka, A., Dolde, C., Kirsch, N., Huang, Y.L., Kazanskaya, O., & Ingelfinger, D., et al. (2011). LGR4 and LGR5 are R-spondin receptors mediating Wnt/beta-catenin and Wnt/PCP signalling. EMBO Reports, 12(10), 1055–1061. doi:10.1038/embor.2011.175.

Goto, N., Goto, S., Imada, S., Hosseini, S., Deshpande, V., & Yilmaz, O.H. (2022). Lymphatics and fibroblasts support intestinal stem cells in homeostasis and injury. Cell Stem Cell, 29(8), 1246–1261 e1246. doi:10.1016/j.stem.2022.06.013.

Gregorieff, A., Pinto, D., Begthel, H., Destree, O., Kielman, M., & Clevers, H. (2005). Expression pattern of Wnt signaling components in the adult intestine. Gastroenterology, 129(2), 626–638. doi:10.1016/j.gastro.2005.06.007.

Greicius, G., Kabiri, Z., Sigmundsson, K., Liang, C., Bunte, R., & Singh, M.K., et al. (2018). PDGFRalpha(+) pericryptal stromal cells are the critical source of Wnts and RSPO3 for murine intestinal stem cells in vivo. Proceedings of the National Academy of Sciences of the United States of America, 115(14), E3173–E3181. doi:10.1073/pnas.1713510115.

Haber, A.L., Biton, M., Rogel, N., Herbst, R.H., Shekhar, K., & Smillie, C., et al. (2017). A single-cell survey of the small intestinal epithelium. Nature, 551(7680), 333–339. doi:10.1038/nature24489.

Hao, H.X., Xie, Y., Zhang, Y., Charlat, O., Oster, E., & Avello, M., et al. (2012). ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. Nature, 485(7397), 195–200. doi:10.1038/nature11019.

Hou, Q., Dong, Y., Huang, J., Liao, C., Lei, J., & Wang, Y., et al. (2020). Exogenous L-arginine increases intestinal stem cell function through CD90+ stromal cells producing mTORC1-induced Wnt2b. Communications Biology, 3(1), 611. doi:10.1038/s42003-020-01347-9.

Hou, Q., Dong, Y., Yu, Q., Wang, B., Le, S., & Guo, Y., et al. (2020). Regulation of the Paneth cell niche by exogenous L-arginine couples the intestinal stem cell function. The FASEB Journal, 34(8), 10299–10315. doi:10.1096/fj.201902573RR.

Huels, D.J., Bruens, L., Hodder, M.C., Cammareri, P., Campbell, A.D., & Ridgway, R.A., et al. (2018). Wnt ligands influence tumour initiation by controlling the number of intestinal stem cells. Nature Communications, 9(1), 1132. doi:10.1038/s41467-018-03426-2.

Igarashi, M., & Guarente, L. (2016). mTORC1 and SIRT1 Cooperate to Foster Expansion of Gut Adult Stem Cells during Calorie Restriction. Cell, 166(2), 436–450. doi:10.1016/j.cell.2016.05.044.

Inoki, K., Ouyang, H., Zhu, T., Lindvall, C., Wang, Y., & Zhang, X., et al. (2006). TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell, 126(5), 955–968. doi:10.1016/j.cell.2006.06.055.

Itzkovitz, S., Lyubimova, A., Blat, I.C., Maynard, M., van Es, J., & Lees, J., et al. (2011). Single-molecule transcript counting of stem-cell markers in the mouse intestine. Nature Cell Biology, 14(1), 106–114. doi:10.1038/ncb2384.

Jacob, J.M., Di Carlo, S.E., Stzepourginski, I., Lepelletier, A., Ndiaye, P.D., & Varet, H., et al. (2022). PDGFRalpha-induced stromal maturation is required to restrain postnatal intestinal epithelial stemness and promote defense mechanisms. Cell Stem Cell, 29(5), 856–868 e855. doi:10.1016/j.stem.2022.04.005.

Jadhav, U., Saxena, M., O'Neill, N.K., Saadatpour, A., Yuan, G.C., & Herbert, Z., et al. (2017). Dynamic Reorganization of Chromatin Accessibility Signatures during Dedifferentiation of Secretory Precursors into Lgr5+ Intestinal Stem Cells. Cell Stem Cell, 21(1), 65–77 e65. doi:10.1016/j.stem.2017.05.001.

Janda, C.Y., Waghray, D., Levin, A.M., Thomas, C., & Garcia, K.C. (2012). Structural basis of Wnt recognition by Frizzled. Science, 337(6090), 59–64. doi:10.1126/science.1222879.

Kabiri, Z., Greicius, G., Madan, B., Biechele, S., Zhong, Z., & Zaribafzadeh, H., et al. (2014). Stroma provides an intestinal stem cell niche in the absence of epithelial Wnts. Development, 141(11), 2206–2215. doi:10.1242/dev.104976.

Karpus, O.N., Westendorp, B.F., Vermeulen, J.L.M., Meisner, S., Koster, J., & Muncan, V., et al. (2019). Colonic CD90+ Crypt Fibroblasts Secrete Semaphorins to Support Epithelial Growth. Cell Reports, 26(13), 3698–3708 e3695. doi:10.1016/j.celrep.2019.02.101.

Kazanjian, A., Noah, T., Brown, D., Burkart, J., & Shroyer, N.F. (2010). Atonal homolog 1 is required for growth and differentiation effects of notch/gamma-secretase inhibitors on normal and cancerous intestinal epithelial cells. Gastroenterology, 139(3). doi:10.1053/j.gastro.2010.05.081. 918-928, 928 e911-916.

Kim, J.E., Fei, L., Yin, W.C., Coquenlorge, S., Rao-Bhatia, A., & Zhang, X., et al. (2020). Single cell and genetic analyses reveal conserved populations and signaling mechanisms of gastrointestinal stromal niches. Nature Communications, 11(1), 334. doi:10.1038/s41467-019-14058-5.

Kim, T.H., Escudero, S., & Shivdasani, R.A. (2012). Intact function of Lgr5 receptor-expressing intestinal stem cells in the absence of Paneth cells. Proceedings of the National Academy of Sciences of the United States of America, 109(10), 3932–3937. doi:10.1073/pnas.1113890109.

Koo, B.K., Spit, M., Jordens, I., Low, T.Y., Stange, D.E., & van de Wetering, M., et al. (2012). Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of

Wnt receptors. Nature, 488(7413), 665–669. doi:10.1038/nature11308.

Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., & Peters, P.J., et al. (1998). Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nature Genetics, 19(4), 379–383. doi:10.1038/1270.

Kramps, T., Peter, O., Brunner, E., Nellen, D., Froesch, B., & Chatterjee, S., et al. (2002). Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear beta-catenin-TCF complex. Cell, 109(1), 47–60. doi:10.1016/s0092-8674(02)00679-7.

Krndija, D., El Marjou, F., Guirao, B., Richon, S., Leroy, O., & Bellaiche, Y., et al. (2019). Active cell migration is critical for steady-state epithelial turnover in the gut. Science, 365(6454), 705–710. doi:10.1126/science.aau3429.

Kuhnert, F., Davis, C.R., Wang, H.T., Chu, P., Lee, M., & Yuan, J., et al. (2004). Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. Proceedings of the National Academy of Sciences of the United States of America, 101(1), 266–271. doi:10.1073/pnas.2536800100.

Lee, Y.S., Kim, T.Y., Kim, Y., Lee, S.H., Kim, S., & Kang, S.W., et al. (2018). Microbiota-Derived Lactate Accelerates Intestinal Stem-Cell-Mediated Epithelial Development. Cell Host & Microbe, 24(6), 833–846 e836. doi:10.1016/j.chom.2018.11.002.

Lepage, M., Seltana, A., Thibault, M.P., Tremblay, E., & Beaulieu, J.F. (2018). Knockdown of laminin alpha5 stimulates intestinal cell differentiation. Biochemical and Biophysical Research Communications, 495(1), 1510–1515. doi:10.1016/j.bbrc.2017.11.181.

Lim, H.Y.G., Kostic, L., & Barker, N. (2022). Chapter Two - Organoid systems for recapitulating the intestinal stem cell niche and modeling disease in vitro. In Nilsson, S. (Ed.), Advances in Stem Cells and their Niches (6, pp. 57–96). Elsevier.

Lindemans, C.A., Calafiore, M., Mertelsmann, A.M., O'Connor, M.H., Dudakov, J.A., & Jenq, R.R., et al. (2015). Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. Nature, 528(7583), 560–564. doi:10.1038/nature16460.

Lopez-Garcia, C., Klein, A.M., Simons, B.D., & Winton, D.J. (2010). Intestinal stem cell replacement follows a pattern of neutral drift. Science, 330(6005), 822–825. doi:10.1126/science.1196236.

Mah, A.T., Van Landeghem, L., Gavin, H.E., Magness, S.T., & Lund, P.K. (2014). Impact of diet-induced obesity on intestinal stem cells: hyperproliferation but impaired intrinsic function that requires insulin/IGF1. Endocrinology, 155(9), 3302–3314. doi:10.1210/en.2014-1112.

Mahoney, Z.X., Stappenbeck, T.S., & Miner, J.H. (2008). Laminin alpha 5 influences the architecture of the mouse small intestine mucosa. Journal of Cell Science, 121(Pt 15), 2493–2502. doi:10.1242/jcs.025528.

Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., & DiCarlo, J.E., et al. (2013). RNA-guided human genome engineering via Cas9. Science, 339(6121), 823–826. doi:10.1126/science.1232033.

Mana, M.D., Hussey, A.M., Tzouanas, C.N., Imada, S., Barrera Millan, Y., & Bahceci, D., et al. (2021). High-fat diet-activated fatty acid oxidation mediates intestinal stemness and tumorigenicity. Cell Reports, 35(10), 109212. doi:10.1016/j.celrep.2021.109212.

Mao, J., Hu, X., Xiao, Y., Yang, C., Ding, Y., & Hou, N., et al. (2013). Overnutrition

stimulates intestinal epithelium proliferation through beta-catenin signaling in obese mice. Diabetes, 62(11), 3736–3746. doi:10.2337/db13-0035.

McCarthy, N., Manieri, E., Storm, E.E., Saadatpour, A., Luoma, A.M., & Kapoor, V.N., et al. (2020). Distinct Mesenchymal Cell Populations Generate the Essential Intestinal BMP Signaling Gradient. Cell Stem Cell, 26(3), 391–402 e395. doi:10.1016/j.stem.2020.01.008.

Meran, L., Baulies, A., & Li, V.S.W. (2017). Intestinal Stem Cell Niche: The Extracellular Matrix and Cellular Components. Stem Cells International, 2017, 7970385. doi:10.1155/2017/7970385.

Mifflin, R.C., Pinchuk, I.V., Saada, J.I., & Powell, D.W. (2011). Intestinal myofibroblasts: targets for stem cell therapy. American Journal of Physiology. Gastrointestinal and Liver Physiology, 300(5), G684–G696. doi:10.1152/ajpgi.00474.2010.

Mihaylova, M.M., Cheng, C.W., Cao, A.Q., Tripathi, S., Mana, M.D., & Bauer-Rowe, K.E., et al. (2018). Fasting Activates Fatty Acid Oxidation to Enhance Intestinal Stem Cell Function during Homeostasis and Aging. Cell Stem Cell, 22(5), 769–778 e764. doi:10.1016/j.stem.2018.04.001.

Miyoshi, H., Ajima, R., Luo, C.T., Yamaguchi, T.P., & Stappenbeck, T.S. (2012). Wnt5a potentiates TGF-beta signaling to promote colonic crypt regeneration after tissue injury. Science, 338(6103), 108–113. doi:10.1126/science.1223821.

Montgomery, R.K., Carlone, D.L., Richmond, C.A., Farilla, L., Kranendonk, M.E., & Henderson, D.E., et al. (2011). Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. Proceedings of the National Academy of Sciences of the United States of America, 108(1), 179–184. doi:10.1073/pnas.1013004108.

Munoz, J., Stange, D.E., Schepers, A.G., van de Wetering, M., Koo, B.K., & Itzkovitz, S., et al. (2012). The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent "+4" cell markers. The EMBO Journal, 31(14), 3079–3091. doi:10.1038/emboj.2012.166.

Murata, K., Jadhav, U., Madha, S., van Es, J., Dean, J., & Cavazza, A., et al. (2020). Ascl2-Dependent Cell Dedifferentiation Drives Regeneration of Ablated Intestinal Stem Cells. Cell Stem Cell, 26(3), 377–390 e376. doi:10.1016/j.stem.2019.12.011.

Murray, P.J., & Wynn, T.A. (2011). Protective and pathogenic functions of macrophage subsets. Nature Reviews. Immunology, 11(11), 723–737. doi:10.1038/nri3073.

Najdi, R., Proffitt, K., Sprowl, S., Kaur, S., Yu, J., & Covey, T.M., et al. (2012). A uniform human Wnt expression library reveals a shared secretory pathway and unique signaling activities. Differentiation, 84(2), 203–213. doi:10.1016/j.diff.2012.06.004.

Nemeth, M.J., Topol, L., Anderson, S.M., Yang, Y., & Bodine, D.M. (2007). Wnt5a inhibits canonical Wnt signaling in hematopoietic stem cells and enhances repopulation. Proceedings of the National Academy of Sciences of the United States of America, 104(39), 15436–15441. doi:10.1073/pnas.0704747104.

Neumann, P.A., Koch, S., Hilgarth, R.S., Perez-Chanona, E., Denning, P., & Jobin, C., et al. (2014). Gut commensal bacteria and regional Wnt gene expression in the proximal versus distal colon. The American Journal of Pathology, 184(3), 592–599. doi:10.1016/j.ajpath.2013.11.029.

Niec, R.E., Chu, T., Schernthanner, M., Gur-Cohen, S., Hidalgo, L., & Pasolli, H.A., et al. (2022). Lymphatics act as a signaling hub to regulate intestinal stem cell activity. Cell Stem Cell. doi:10.1016/j.stem.2022.05.007.

Ogasawara, R., Hashimoto, D., Kimura, S., Hayase, E., Ara, T., & Takahashi, S., et al. (2018). Intestinal Lymphatic Endothelial Cells Produce R-Spondin3. Scientific Reports, 8(1), 10719. doi:10.1038/s41598-018-29100-7.

Ortiz-Masia, D., Cosin-Roger, J., Calatayud, S., Hernandez, C., Alos, R., & Hinojosa, J., et al. (2014). Hypoxic macrophages impair autophagy in epithelial cells through Wnt1: relevance in IBD. Mucosal Immunology, 7(4), 929–938. doi:10.1038/mi.2013.108.

Oshiro, M., Ono, K., Suzuki, Y., Ota, H., Katsuyama, T., & Mori, N. (2001). Immunohistochemical localization of heparan sulfate proteoglycan in human gastrointestinal tract. Histochemistry and Cell Biology, 115(5), 373–380. doi:10.1007/s004180100271.

Paone, P., & Cani, P.D. (2020). Mucus barrier, mucins and gut microbiota: the expected slimy partners? Gut, 69(12), 2232–2243. doi:10.1136/gutjnl-2020-322260.

Pedron, T., Mulet, C., Dauga, C., Frangeul, L., Chervaux, C., & Grompone, G., et al. (2012). A crypt-specific core microbiota resides in the mouse colon. mBio, 3(3). doi:10.1128/mBio.00116-12.

Pellegrinet, L., Rodilla, V., Liu, Z., Chen, S., Koch, U., & Espinosa, L., et al. (2011). Dll1- and dll4-mediated notch signaling are required for homeostasis of intestinal stem cells. Gastroenterology, 140(4). doi:10.1053/j.gastro.2011.01.005. 1230-1240 e1231-1237.

Pentinmikko, N., Iqbal, S., Mana, M., Andersson, S., Cognetta, A.B., 3rd, & Suciu, R.M., et al. (2019). Notum produced by Paneth cells attenuates regeneration of aged intestinal epithelium. Nature, 571(7765), 398–402. doi:10.1038/s41586-019-1383-0.

Pinto, D., Gregorieff, A., Begthel, H., & Clevers, H. (2003). Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes & Development, 17(14), 1709–1713. doi:10.1101/gad.267103.

Pompili, S., Latella, G., Gaudio, E., Sferra, R., & Vetuschi, A. (2021). The Charming World of the Extracellular Matrix: A Dynamic and Protective Network of the Intestinal Wall. Frontiers in Medicine, 8, 610189. doi:10.3389/fmed.2021.610189.

Powell, A.E., Wang, Y., Li, Y., Poulin, E.J., Means, A.L., & Washington, M.K., et al. (2012). The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. Cell, 149(1), 146–158. doi:10.1016/j.cell.2012.02.042.

Puzan, M., Hosic, S., Ghio, C., & Koppes, A. (2018). Enteric Nervous System Regulation of Intestinal Stem Cell Differentiation and Epithelial Monolayer Function. Scientific Reports, 8(1), 6313. doi:10.1038/s41598-018-24768-3.

Quiros, M., Nishio, H., Neumann, P.A., Siuda, D., Brazil, J.C., & Azcutia, V., et al. (2017). Macrophage-derived IL-10 mediates mucosal repair by epithelial WISP-1 signaling. The Journal of Clinical Investigation, 127(9), 3510–3520. doi:10.1172/JCI90229.

Ritie, L., Spenle, C., Lacroute, J., Bolcato-Bellemin, A.L., Lefebvre, O., & Bole-Feysot, C., et al. (2012). Abnormal Wnt and PI3Kinase signaling in the malformed intestine of lama5 deficient mice. PLoS One, 7(5), e37710. doi:10.1371/journal.pone.0037710.

Ritsma, L., Ellenbroek, S.I.J., Zomer, A., Snippert, H.J., de Sauvage, F.J., & Simons, B.D., et al. (2014). Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging. Nature, 507(7492), 362–365. doi:10.1038/nature12972.

Roche, K.C., Gracz, A.D., Liu, X.F., Newton, V., Akiyama, H., & Magness, S.T. (2015). SOX9 maintains reserve stem cells and preserves radioresistance in mouse small

intestine. Gastroenterology, 149(6), 1553–1563 e1510. doi:10.1053/j.gastro.2015.07.004.

Rothenberg, M.E., Nusse, Y., Kalisky, T., Lee, J.J., Dalerba, P., & Scheeren, F., et al. (2012). Identification of a cKit(+) colonic crypt base secretory cell that supports Lgr5(+) stem cells in mice. Gastroenterology, 142(5), 1195–1205 e1196. doi:10.1053/j.gastro.2012.02.006.

Saha, S., Aranda, E., Hayakawa, Y., Bhanja, P., Atay, S., & Brodin, N.P., et al. (2016). Macrophage-derived extracellular vesicle-packaged WNTs rescue intestinal stem cells and enhance survival after radiation injury. Nature Communications, 7, 13096. doi:10.1038/ncomms13096.

San Roman, A.K., Jayewickreme, C.D., Murtaugh, L.C., & Shivdasani, R.A. (2014). Wnt secretion from epithelial cells and subepithelial myofibroblasts is not required in the mouse intestinal stem cell niche in vivo. Stem Cell Reports, 2(2), 127–134. doi:10.1016/j.stemcr.2013.12.012.

Sangiorgi, E., & Capecchi, M.R. (2008). Bmi1 is expressed in vivo in intestinal stem cells. Nature Genetics, 40(7), 915–920. doi:10.1038/ng.165.

Sansom, O.J., Reed, K.R., Hayes, A.J., Ireland, H., Brinkmann, H., & Newton, I.P., et al. (2004). Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. Genes & Development, 18(12), 1385–1390. doi:10.1101/gad.287404.

Sarrazin, S., Lamanna, W.C., & Esko, J.D. (2011). Heparan sulfate proteoglycans. Cold Spring Harbor Perspectives in Biology, 3(7). doi:10.1101/cshperspect.a004952.

Sasaki, N., Sachs, N., Wiebrands, K., Ellenbroek, S.I., Fumagalli, A., & Lyubimova, A., et al. (2016). Reg4+ deep crypt secretory cells function as epithelial niche for Lgr5+ stem cells in colon. Proceedings of the National Academy of Sciences of the United States of America, 113(37), E5399–E5407. doi:10.1073/pnas.1607327113.

Sato, T., Stange, D.E., Ferrante, M., Vries, R.G., Van Es, J.H., & Van den Brink, S., et al. (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology, 141(5), 1762–1772. doi:10.1053/j.gastro.2011.07.050.

Sato, T., van Es, J.H., Snippert, H.J., Stange, D.E., Vries, R.G., & van den Born, M., et al. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature, 469(7330), 415–418. doi:10.1038/nature09637.

Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., & Stange, D.E., et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature, 459(7244), 262–265. doi:10.1038/nature07935.

Schmitt, M., Schewe, M., Sacchetti, A., Feijtel, D., van de Geer, W.S., & Teeuwssen, M., et al. (2018). Paneth Cells Respond to Inflammation and Contribute to Tissue Regeneration by Acquiring Stem-like Features through SCF/c-Kit Signaling. Cell Reports, 24(9), 2312–2328 e2317. doi:10.1016/j.celrep.2018.07.085.

Sehgal, A., Donaldson, D.S., Pridans, C., Sauter, K.A., Hume, D.A., & Mabbott, N.A. (2018). The role of CSF1R-dependent macrophages in control of the intestinal stem-cell niche. Nature Communications, 9(1), 1272. doi:10.1038/s41467-018-03638-6.

Shi, W., Kaneiwa, T., Cydzik, M., Gariepy, J., & Filmus, J. (2020). Glypican-6 stimulates intestinal elongation by simultaneously regulating Hedgehog and non-canonical Wnt signaling. Matrix Biology, 88, 19–32. doi:10.1016/j.matbio.2019.11.002.

Shoshkes-Carmel, M., Wang, Y.J., Wangensteen, K.J., Toth, B., Kondo, A., & Massasa, E.E., et al. (2018). Subepithelial telocytes are an important source of Wnts that supports intestinal crypts. Nature, 557(7704), 242–246. doi:10.1038/s41586-018-

0084-4.

Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., & Kroon-Veenboer, C., et al. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell, 143(1), 134–144. doi:10.1016/j.cell.2010.09.016.

Soderholm, S., & Cantu, C. (2021). The WNT/beta-catenin dependent transcription: A tissue-specific business. WIREs Mechanisms of Disease, 13(3), e1511. doi:10.1002/wsbm.1511.

Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., & Tolle, K., et al. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature, 470(7332), 105–109. doi:10.1038/nature09691.

Stzepourginski, I., Nigro, G., Jacob, J.M., Dulauroy, S., Sansonetti, P.J., & Eberl, G., et al. (2017). CD34+ mesenchymal cells are a major component of the intestinal stem cells niche at homeostasis and after injury. Proceedings of the National Academy of Sciences of the United States of America, 114(4), E506–E513. doi:10.1073/pnas.1620059114.

Sun, J., Hobert, M.E., Rao, A.S., Neish, A.S., & Madara, J.L. (2004). Bacterial activation of beta-catenin signaling in human epithelia. American Journal of Physiology. Gastrointestinal and Liver Physiology, 287(1), G220–G227. doi:10.1152/ajpgi.00498.2003.

Takeda, N., Jain, R., LeBoeuf, M.R., Wang, Q., Lu, M.M., & Epstein, J.A. (2011). Interconversion between intestinal stem cell populations in distinct niches. Science, 334(6061), 1420–1424. doi:10.1126/science.1213214.

Tan, D.W., & Barker, N. (2014). Intestinal stem cells and their defining niche. Current Topics in Developmental Biology, 107, 77–107. doi:10.1016/B978-0-12-416022-4.00003-2.

Tan, S.H., Phuah, P., Tan, L.T., Yada, S., Goh, J., & Tomaz, L.B., et al. (2021). A constant pool of Lgr5(+) intestinal stem cells is required for intestinal homeostasis. Cell Reports, 34(4), 108633. doi:10.1016/j.celrep.2020.108633.

Tao, S., Tang, D., Morita, Y., Sperka, T., Omrani, O., & Lechel, A., et al. (2015). Wnt activity and basal niche position sensitize intestinal stem and progenitor cells to DNA damage. The EMBO Journal, 34(5), 624–640. doi:10.15252/embj.201490700.

Teller, I.C., & Beaulieu, J.F. (2001). Interactions between laminin and epithelial cells in intestinal health and disease. Expert Reviews in Molecular Medicine, 3(24), 1–18. doi:10.1017/S1462399401003623.

Ten Hove, A.S., Seppen, J., & de Jonge, W.J. (2021). Neuronal innervation of the intestinal crypt. American Journal of Physiology. Gastrointestinal and Liver Physiology, 320(2), G193–G205. doi:10.1152/ajpgi.00239.2020.

Tetteh, P.W., Basak, O., Farin, H.F., Wiebrands, K., Kretzschmar, K., & Begthel, H., et al. (2016). Replacement of Lost Lgr5-Positive Stem Cells through Plasticity of Their Enterocyte-Lineage Daughters. Cell Stem Cell, 18(2), 203–213. doi:10.1016/j.stem.2016.01.001.

Tian, H., Biehs, B., Warming, S., Leong, K.G., Rangell, L., & Klein, O.D., et al. (2011). A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature, 478(7368), 255–259. doi:10.1038/nature10408.

Valenta, T., Degirmenci, B., Moor, A.E., Herr, P., Zimmerli, D., & Moor, M.B., et al. (2016). Wnt Ligands Secreted by Subepithelial Mesenchymal Cells Are Essential for the Survival of Intestinal Stem Cells and Gut Homeostasis. Cell Reports, 15(5),

911–918. doi:10.1016/j.celrep.2016.03.088.

van Amerongen, R., & Berns, A. (2006). Knockout mouse models to study Wnt signal transduction. Trends in Genetics, 22(12), 678–689. doi:10.1016/j.tig.2006.10.001.

van der Flier, L.G., Haegebarth, A., Stange, D.E., van de Wetering, M., & Clevers, H. (2009). OLFM4 is a robust marker for stem cells in human intestine and marks a subset of colorectal cancer cells. Gastroenterology, 137(1), 15–17. doi:10.1053/j.gastro.2009.05.035.

van der Flier, L.G., van Gijn, M.E., Hatzis, P., Kujala, P., Haegebarth, A., & Stange, D.E., et al. (2009). Transcription factor achaete scute-like 2 controls intestinal stem cell fate. Cell, 136(5), 903–912. doi:10.1016/j.cell.2009.01.031.

van Es, J.H., de Geest, N., van de Born, M., Clevers, H., & Hassan, B.A. (2010). Intestinal stem cells lacking the Math1 tumour suppressor are refractory to Notch inhibitors. Nature Communications, 1, 18. doi:10.1038/ncomms1017.

van Es, J.H., Haegebarth, A., Kujala, P., Itzkovitz, S., Koo, B.K., & Boj, S.F., et al. (2012). A critical role for the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal. Molecular and Cellular Biology, 32(10), 1918–1927. doi:10.1128/MCB.06288-11.

van Es, J.H., Jay, P., Gregorieff, A., van Gijn, M.E., Jonkheer, S., & Hatzis, P., et al. (2005). Wnt signalling induces maturation of Paneth cells in intestinal crypts. Nature Cell Biology, 7(4), 381–386. doi:10.1038/ncb1240.

van Es, J.H., Sato, T., van de Wetering, M., Lyubimova, A., Yee Nee, A.N., & Gregorieff, A., et al. (2012). Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. Nature Cell Biology, 14(10), 1099–1104. doi:10.1038/ncb2581.

van Es, J.H., Wiebrands, K., Lopez-Iglesias, C., van de Wetering, M., Zeinstra, L., & van den Born, M., et al. (2019). Enteroendocrine and tuft cells support Lgr5 stem cells on Paneth cell depletion. Proceedings of the National Academy of Sciences of the United States of America. doi:10.1073/pnas.1801888117.

VanDussen, K.L., Carulli, A.J., Keeley, T.M., Patel, S.R., Puthoff, B.J., & Magness, S.T., et al. (2012). Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. Development, 139(3), 488–497. doi:10.1242/dev.070763.

Vuik, F., Dicksved, J., Lam, S.Y., Fuhler, G.M., van der Laan, L., & van de Winkel, A., et al. (2019). Composition of the mucosa-associated microbiota along the entire gastrointestinal tract of human individuals. United European Gastroenterology Journal, 7(7), 897–907. doi:10.1177/2050640619852255.

Wang, Q., Lin, Y., Sheng, X., Xu, J., Hou, X., & Li, Y., et al. (2020). Arachidonic Acid Promotes Intestinal Regeneration by Activating WNT Signaling. Stem Cell Reports, 15(2), 374–388. doi:10.1016/j.stemcr.2020.06.009.

Williams, J.M., Duckworth, C.A., Burkitt, M.D., Watson, A.J., Campbell, B.J., & Pritchard, D.M. (2015). Epithelial cell shedding and barrier function: a matter of life and death at the small intestinal villus tip. Veterinary Pathology, 52(3), 445–455. doi:10.1177/0300985814559404.

Wong, M.H., Rubinfeld, B., & Gordon, J.I. (1998). Effects of forced expression of an NH2-terminal truncated beta-Catenin on mouse intestinal epithelial homeostasis. The Journal of Cell Biology, 141(3), 765–777. doi:10.1083/jcb.141.3.765.

Wong, V.W., Stange, D.E., Page, M.E., Buczacki, S., Wabik, A., & Itami, S., et al. (2012). Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. Nature Cell Biology, 14(4), 401–408. doi:10.1038/ncb2464.

Workman, M.J., Mahe, M.M., Trisno, S., Poling, H.M., Watson, C.L., & Sundaram, N., et al. (2017). Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. Nature Medicine, 23(1), 49–59. doi:10.1038/nm.4233.

Wu, H., Xie, S., Miao, J., Li, Y., Wang, Z., & Wang, M., et al. (2020). Lactobacillus reuteri maintains intestinal epithelial regeneration and repairs damaged intestinal mucosa. Gut Microbes, 11(4), 997–1014. doi:10.1080/19490976.2020.1734423.

Xie, Y., Ding, F., Di, W., Lv, Y., Xia, F., & Sheng, Y., et al. (2020). Impact of a highfat diet on intestinal stem cells and epithelial barrier function in middleaged female mice. Molecular Medicine Reports, 21(3), 1133–1144. doi:10.3892/mmr.2020.10932.

Yamamoto, S., Nakase, H., Matsuura, M., Honzawa, Y., Matsumura, K., & Uza, N., et al. (2013). Heparan sulfate on intestinal epithelial cells plays a critical role in intestinal crypt homeostasis via Wnt/beta-catenin signaling. American Journal of Physiology. Gastrointestinal and Liver Physiology, 305(3), G241–G249. doi:10.1152/ajpgi.00480.2012.

Yan, K.S., Chia, L.A., Li, X., Ootani, A., Su, J., & Lee, J.Y., et al. (2012). The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. Proceedings of the National Academy of Sciences of the United States of America, 109(2), 466–471. doi:10.1073/pnas.1118857109.

Yan, K.S., Gevaert, O., Zheng, G.X.Y., Anchang, B., Probert, C.S., & Larkin, K.A., et al. (2017). Intestinal Enteroendocrine Lineage Cells Possess Homeostatic and Injury-Inducible Stem Cell Activity. Cell Stem Cell, 21(1), 78–90 e76. doi:10.1016/j.stem.2017.06.014.

Yan, K.S., Janda, C.Y., Chang, J., Zheng, G.X.Y., Larkin, K.A., & Luca, V.C., et al. (2017). Non-equivalence of Wnt and R-spondin ligands during Lgr5(+) intestinal stem-cell self-renewal. Nature, 545(7653), 238–242. doi:10.1038/nature22313.

Yang, Q., Bermingham, N.A., Finegold, M.J., & Zoghbi, H.Y. (2001). Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. Science, 294(5549), 2155–2158. doi:10.1126/science.1065718.

Yilmaz, O.H., Katajisto, P., Lamming, D.W., Gultekin, Y., Bauer-Rowe, K.E., & Sengupta, S., et al. (2012). mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. Nature, 486(7404), 490–495. doi:10.1038/nature11163.

Yousefi, M., Li, N., Nakauka-Ddamba, A., Wang, S., Davidow, K., & Schoenberger, J., et al. (2016). Msi RNA-binding proteins control reserve intestinal stem cell quiescence. The Journal of Cell Biology, 215(3), 401–413. doi:10.1083/jcb.201604119.

Yu, S., Tong, K., Zhao, Y., Balasubramanian, I., Yap, G.S., & Ferraris, R.P., et al. (2018). Paneth Cell Multipotency Induced by Notch Activation following Injury. Cell Stem Cell, 23(1), 46–59 e45. doi:10.1016/j.stem.2018.05.002.

Yu, Y.B., & Li, Y.Q. (2014). Enteric glial cells and their role in the intestinal epithelial barrier. World Journal of Gastroenterology, 20(32), 11273–11280. doi:10.3748/wjg.v20.i32.11273.

Zeng, H., Lu, B., Zamponi, R., Yang, Z., Wetzel, K., & Loureiro, J., et al. (2018). mTORC1 signaling suppresses Wnt/beta-catenin signaling through DVL-dependent regulation of Wnt receptor FZD level. Proceedings of the National Academy of Sciences of the United States of America, 115(44), E10362–E10369. doi:10.1073/pnas.1808575115.

Zhang, Q., Wu, X., Chen, P., Liu, L., Xin, N., & Tian, Y., et al. (2018). The Mitochondrial Unfolded Protein Response Is Mediated Cell-Non-autonomously by Retromer-

Dependent Wnt Signaling. Cell, 174(4), 870–883 e817. doi:10.1016/j.cell.2018.06.029.