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REVIEW

Mammalian Intestinal Development and Differentiation—The State of the Art

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SUMMARY

The past 15 years have seen tremendous progress in our understanding of mammalian intestinal development and differentiation, driven by technological advances in mouse genetics, organoid biology, and single-cell approaches. This review synthesizes the most salient findings into a consensus view of how multiple signaling systems and transcriptional regulators work in concert to build this complex organ system during fetal life and maintain it in adulthood in the face of massive regenerative demands caused by the sometimes hostile internal environment of the gut.

The development of the mammalian intestine, from its earliest origins as a morphologically uniform sheet of endoderm cells during gastrulation into the complex organ system that is essential for the life of the organism, is a truly fascinating process. During midgestation development, reciprocal interactions between endoderm-derived epithelium and mesoderm-derived mesenchyme enable villification, or the conversion of a radially symmetric pseudostratified epithelium into the functional subdivision of crypts and villi. Once a mature crypt–villus axis is established, proliferation and differentiation of new epithelial cells continue throughout life. Spatially localized signals including the wingless and Int-1, fibroblast growth factor, and Hippo systems, among others, ensure that new cells are being born continuously in the crypt. As cells exit the crypt compartment, a gradient of bone morphogenetic protein signaling limits proliferation to allow for the specification of multiple mature cell types. The first major differentiation decision is dependent on Notch signaling, which specifies epithelial cells into absorptive and secretory lineages. The secretory lineage is subdivided further into Paneth, goblet, tuft, and enteroendocrine cells via a complex network of transcription factors. Although some of the signaling molecules are produced by epithelial cells, critical components are derived from specialized crypt-adjacent mesenchymal cells termed *telocytes*, which are marked by Forkhead box l1, GLI Family Zinc Finger 1, and platelet-derived growth factor receptor α. The crucial nature of these processes is evidenced by the multitude of intestinal disorders such as colorectal cancer, short-bowel syndrome, and inflammatory bowel disease, which all reflect perturbations of the development and/or differentiation of the intestine. *(Cell Mol Gastroenterol Hepatol 2023;16:809–821; https://doi.org/10.1016/j.jcmgh.2023.07.011)*

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The mammalian intestine is a complex and highly regulated organ responsible for several essential functions including nutrient digestion and absorption, immunologic protection from pathogens, and endocrine regulation. Throughout development and in adult homeostasis, intestinal stem and progenitor cells must adopt distinct fates to produce and maintain a functional intestinal epithelium, which contains multiple distinct differentiated cell types. Importantly, failure of stem or progenitor cells to differentiate correctly can lead to pathologic disorders such as colorectal cancer.[1] Understanding the signaling, transcription factor, and chromatin basis of intestinal cell fate decisions therefore is critical to advancing our understanding of gastrointestinal health and disease. Most studies on mammalian intestinal development have been performed in mice because it is a genetically tractable model. Therefore, in this article, stages of development refer to those of the mouse unless noted otherwise. In addition to rodent models, both mouse and human organoid systems have provided major contributions to our understanding of intestinal biology,[2] and select findings discovered using this system also are discussed later. Because of space limitations, the development of the enteric nervous and mucosal immune systems is not covered here.

Intestinal Development

During development, the intestinal epithelium is transformed by a series of morphologic events from a pseudostratified fetal gut tube into a mature columnar epithelium organized into repeating sets of epithelial invaginations

Abbreviations used in this paper: ATOH1, atonal bHLH transcription factor 1; BMP, bone morphogenetic protein; E, embryonic day; EEC, enteroendocrine cell; FGF, fibroblast growth factor; HNF, hepatocyte nuclear factor; IL, interleukin; Lgr5, leucine-rich repeat containing G-protein–coupled receptor 5; PDGFRa, platelet-derived growth factor receptor α; RANKL, receptor activator of nuclear factor kappa B; RSPO, R-spondin; Wnt, wingless and Int-1.

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called *crypts of Lieberkühn* and fingerlike projections termed *villi*.[3] After specification of the 3 primary embryonic germ layers, cells of the endodermal lineage are arranged as a flat sheet, with pockets that form at the anterior (anterior intestinal portal) and posterior (caudal intestinal portal) ends of the embryo.[4] As the anterior and posterior intestinal portals elongate and migrate away from each other, the lateral endoderm folds ventrally along the longitudinal axis, joining to produce an enclosed gut tube.[3,5] By embryonic day 9.5 (E9.5) (gestational week 8 in human beings), the endoderm has finished folding and forms a closed pseudostratified epithelial tube ensheathed by the mesoderm-derived mesenchyme. By this stage of development, the gut tube already has been regionally specified into the anterior-most foregut, the midgut, and the posterior-most hindgut.[6] Importantly, the foregut will give rise to the epithelia of the esophagus, stomach, and duodenum, as well as the gut-associated liver and pancreas, while the midgut and hindgut will develop into the small intestine and large intestine, respectively.[7]

From E9.5 through E14.5 (gestational weeks 4–9 in human beings), the pseudostratified intestinal epithelium and mesenchyme rapidly proliferate, resulting in gut tube elongation and a significant increase in cell number, luminal area, and girth.[8,9] Beginning at E14.5 (gestational week 9 in human beings), the pseudostratified epithelium undergoes extensive remodeling to give rise to villus structures and produce a columnar epithelium[8,10] (Figure 1). In a proximal-to-distal wave, clusters of postmitotic, platelet-derived growth factor receptor α (PDGFRa)-expressing mesenchymal cells coalesce under the sites of future epithelial villi.[11,12] Although the mechanism by which villi emerge remains uncertain, it is believed that PDGFRa^{+} mesenchymal clusters signal to the overlying epithelium to inhibit proliferation in the nascent villi and trigger epithelial folding.[12] Furthermore, epithelial-derived Hedgehog signaling is required for this process because blocking Hedgehog signaling perturbs mesenchymal cell clustering as well as villus formation.[13] Indeed, expression of the Hedgehog-responsive transcription factor *Foxl1*, previously termed *forkhead homolog 6*, is required in the underlying mesenchyme for normal villus morphogenesis, as mice deficient in *Foxl1* show delayed villus formation and fewer villi that are shorter in length at birth.[14,15] Furthermore, studies using the chicken model have suggested that compressive stresses generated by differentiating smooth muscle layers of the gut induce buckling and folding of the epithelium, thereby producing nascent villi.[16] However, a recent publication by Rao-Bhatia et al[17] using multiple mutant mouse strains suggested that endoderm-derived Hedgehog signaling induces activation of planar cell polarity genes in a GLI-dependent fashion, and that the planar cell polarity genes *Fat4* and *Dcsh1* are critical for villus formation. Thus, it is possible that the mechanisms of villification differ between mammals and birds.

By the end of villus morphogenesis at E16.5, epithelial cell proliferation has become restricted to intervillus domains connecting nascent villi, and cytodifferentiation gives rise to the functional cell types of the small intestine, including absorptive enterocytes as well as secretory goblet cells and enteroendocrine cells.[3,18] In human intestinal development, proliferating cells are initially present randomly in the pseudostratified epithelium but are confined to crypt and intervillus regions by week 12 of gestation.[19] Although in human beings morphogenesis and maturation of the intestine are complete by birth, in the mouse these processes continue postnatally, with emergence of intestinal crypts and differentiation of secretory Paneth cells in the small intestine occuring by postnatal day 14.[20–23] During the third postnatal week, crypts expand rapidly through a process called *crypt fission*, and villi lengthen as the rate of cell production exceeds the rate of apoptosis at the villus tip.[24] However, by postnatal day 28, an equilibrium is reached, and the mature epithelium is established.[20]

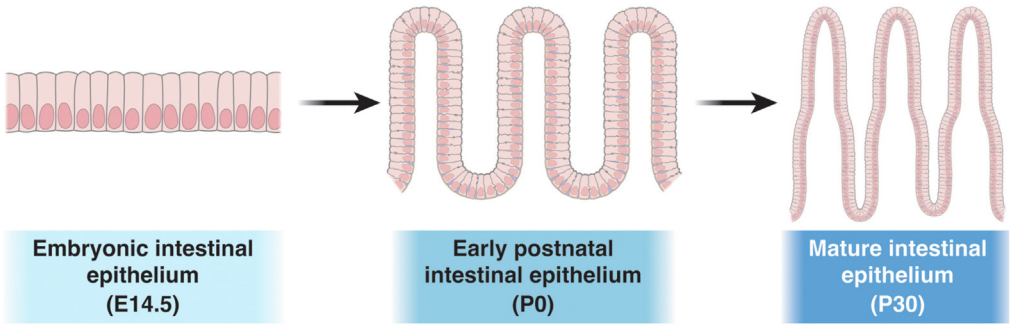

Figure 1. The epithelial transition of the developing small intestine. Around embryonic day 14.5 in the mouse, the pseudostratified, nearly flat epithelium undergoes a dramatic expansion and transition to eventually produce the columnar epithelium of the adult gut tube. In a proximal-to-distal wave, clusters of postmitotic mesenchymal cells coalesce under the sites of future epithelial villi. These expand into the gut lumen to form nascent villi, and proliferating cells coalesce into the intervillus regions. Whereas in human beings, intestinal development is complete at birth, in mice, crypt and villus formation is completed only by the fourth week of life. Of note, during human gestation, the primitive gut tube closes by week 4, the first enteroendocrine cells appear by week 8, mucosal remodeling for villification occurs in an anterior to posterior fashion beginning at week 9, Paneth cells appear at the base of developing crypts by week 11, intestinal morphogenesis is considered complete by week 13, absorptive enterocytes resemble those of the adult intestine by week 22, and mature gut motility is achieved by week 36.

The Adult Intestine

Intestinal Architecture and Cell Types

The adult intestine must maintain a robust barrier to the outside world, while simultaneously completing its critical digestive, absorptive, and immunologic functions. Given constant exposure to environmental insults, the intestinal epithelium has adopted a robust regenerative capacity and completely self-renews every 3–5 days.[8,25,26] This high regenerative output is maintained by a population of rapidly dividing (approximately once per day) multipotent *Lgr5*-expressing intestinal stem cells, which reside in an intestinal stem cell niche at the crypt base.[27] The discovery of leucine-rich repeat containing G-protein–coupled receptor 5 (Lgr5) as a marker was driven by prior knowledge that Wingless and Int-1 (Wnt) signaling is the key mitogen responsible for intestinal epithelial renewal. By screening for Wnt-responsive genes (ie, genes that were turned off via activation of a dominant-negative T-cell factor 4 mutant transcription factor) and selecting those that were confined to the crypt base and expressed in crypt base columnar cells, Barker et al[27] identified *Lgr5* as a Wnt pathway target gene. Using genetic lineage tracing with the causes recombination - locus of crossing over P1 (Cre-loxP) system, the investigators could show that $Lgr5^+$ cells can be traced to all differentiated cell types of the intestinal epithelium from duodenum to colon.[27] Importantly, the Clevers laboratory showed shortly thereafter that sorted $Lgr5^+$ cells have the ability to form self-renewing organoids under specific culture conditions containing critical growth factors provided by niche cells in vivo, establishing the proliferative potential of $Lgr5^+$ cells in an in vitro system.[28] Of note, genetic ablation of $Lgr5^+$ cells does not lead to cessation of intestinal proliferation and death of the organism; rather, reserve stem cell populations[29] and plasticity of committed progenitor cells[30–32] allow for the replacement of the proliferative compartment.

Another unresolved question is whether these rapidly cycling $Lgr5^+$ cells represent the most upstream, self-renewing stem cell type, or whether they themselves are derived from another, even more stem-like cell population. Regardless of this issue, $Lgr5^+$ cells produce transit-amplifying absorptive and secretory progenitor cells, which are pushed out of the crypt base and undergo additional rounds of cell division as they migrate up the crypt wall. Progenitor cells differentiate progressively, giving rise to the mature postmitotic cell types of the epithelium as they transit from the crypt into the villus unit. The differentiated cells continue their migration in ordered cohorts up to the villus tip, where they are shed into the lumen in a process called *anoikis*.[33,34] The secretory Paneth cell of the small intestine is the only differentiated cell type to escape this upward trajectory and instead resides in the crypt.[23] An overview of the architecture of the adult small intestinal epithelium and stem cell niche is shown in Figure 2, and Table 1 summarizes the key features and marker genes of the major cell types considered, as discussed later.

Several differentiated cell types of the absorptive and secretory lineages populate the intestinal epithelium.

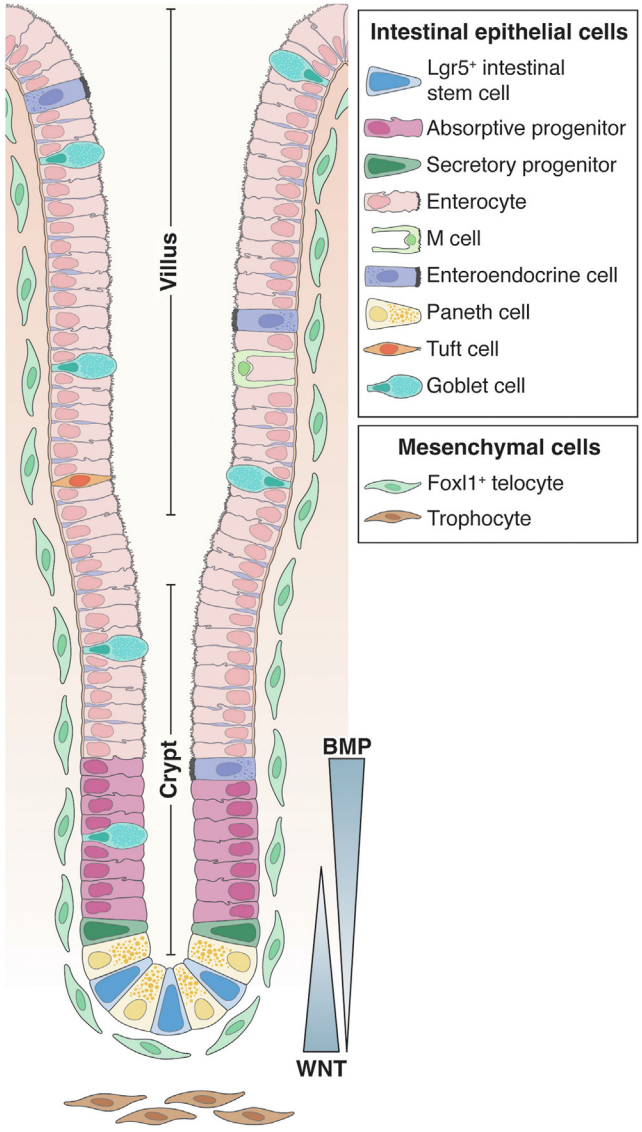

Figure 2. Cell types of the small intestine. In the small intestine, the intestinal epithelium is organized into repeating units called crypts and villi. *Lgr5*-expressing intestinal stem cells (ISCs) residing at the crypt base divide and differentiate to give rise to absorptive and secretory progenitor cells, which mature into the differentiated cell types of the epithelium. $Lgr5^+$ ISCs are supported by the intestinal stem cell niche, which produces opposing gradients of Wnt and BMP signaling that balance ISC self-renewal and differentiation. Populations of mesenchymal cells, including $Foxl1^+$ telocytes and trophocytes, have been ascribed niche signaling function. Figure created with BioRender.com.

Absorptive enterocytes, comprising 80% of the intestinal epithelium, fulfill the primary nutrient absorptive functions of the intestine and are responsible for ion, water, sugar, peptide, and lipid uptake.[35] Interestingly, enterocytes show a zonated gene expression pattern along the villus axis, suggesting that they traverse a series of functional states as they migrate to the villus tip.[36] For example, based on expression profiling, enterocytes in the midvillus region

Table 1. Major Cell Types of the Intestinal Epithelium and the Intestinal Stem Cell Niche

Cell type	Function	Markers
Lgr5 stem cell	Rapid cycling, replenishes epithelium	LGR5, OLFM4
Absorptive progenitor	Transit-amplifying cell	HES1
Secretory progenitor	Transit-amplifying cell	ATOH1
Enterocyte	Digestion/absorption	IAP, iFABP, LDH, SI
M cell	Antigen sampling	GP2
Enteroendocrine cells	Multiple subtypes, hormone production	CHGA
Paneth cell	Innate immunity	LYZ
Tuft cell	Defense against parasites	DCLK1
Goblet cell	Mucous secretion	MUC2
Telocyte	Stem cell niche	FOXL1
Trophocyte	Stem cell niche	PDGFRa

ATOH1, atonal bHLH (basic helix loop helix) transcription factor 1; CHGA, chromogranin A; DCLK1, doublecortin-like kinase 1; FOXL1, forkhead box L1; GP2, glycoprotein 2; HES1, hes family bHLH transcription factor 1; IAP, intestinal alkaline phosphatase; iFABP, intestinal fatty acid binding protein; LDH, lactose dehydrogenase; LYZ, lysozyme; MUC2, mucin 2; OLFM4, olfactomedin 4; SI, sucrase isomaltase.

likely specialize in amino acid and carbohydrate absorption, whereas enterocytes at the villus tip likely function in chylomicron secretion. In addition, microfold cells (M cells) represent a specialized member of the absorptive lineage that resides in the follicle-associated epithelium overlaying mucosal lymphoid tissues.[35,37] Here, M cells sample antigens from the lumen, transfer luminal particles to underlying lymphoid cells, and contribute to mucosal immunity.[38]

Four cell types comprise the mature secretory lineage of the small intestinal epithelium: the goblet cell, tuft cell, enteroendocrine cell, and Paneth cell. Goblet cells secrete mucins to create a dense mucus network that separates the epithelium from the luminal content and microbiota.[39] At the same time, the mucus layer also can act as a rich nutrient source for commensal bacterial species.[40,41] Comprising less then 0.4% of the epithelium, tuft cells act as chemosensors in the gut and use taste receptors to monitor the intestinal lumen.[42,43] Tuft cells are identifiable by their expression of doublecortin-like kinase 1 and function in the host defense against parasitic infection through their secretion of interleukin (IL)25, a key cytokine for parasite clearance.[44–47]

The enteroendocrine cell (EEC) lineage represents a heterogenous population of hormone-producing cells that function in metabolic regulation, appetite control, intestinal motility, and mucosal immunity.[48] Subsets of EECs initially were defined based on their secretion of individual hormones, such as L cells producing glucagon-like peptide 1, I cells producing cholecystokinin, N cells producing neurotensin, D cells producing somatostatin, and S cells producing secretin.[49] However, more recently, it was suggested that EEC subsets can express more than one hormone and show hormonal plasticity during the maturation process.[50,51] Indeed, L cells were shown to acquire transcriptional I cell or N cell identity as they matured.[52]

Finally, the Paneth cell represents a unique cell population that resides at the crypt base of the small intestine and has a lifespan of approximately 60 days, contrasting sharply with short-lived differentiated villus cells.[53] In addition to expressing key signaling molecules, Paneth cells express several antimicrobial peptides including alpha-defensins, lysozyme, angiogenin-4, secretory phospholipase A2, and Regenerating islet-derived protein III-gamma.[54,55] Antimicrobial peptides localize to secretory granules, which coalesce at the apical region of Paneth cells and are released into the crypt lumen when exposed to bacteria, bacterial antigens, or pharmacologic stimulants.[54,56–58] Antimicrobial activity initiated by Paneth cells provides important protection against enteric infection and shapes the commensal microbiota, thereby maintaining intestinal health. Indeed, mice null for *Mmp7*, which encodes a proteolytic enzyme required for α-defensin maturation, show increased susceptibility to infection with *Salmonella typhimurium* and altered microbiota that are marked by increased *Firmicute* species and decreased *Bacteroidetes* species.[59,60] Furthermore, subsets of patients with inflammatory bowel disease present with compromised expression of α-defensins and display abnormal Paneth cell granules, suggesting a role for Paneth cell dysfunction in inflammatory bowel disease pathogenesis.[61–63]

Of note, Paneth cells are absent from the mammalian colon. However, a population of mucus-secreting, nongoblet cells was identified deep in the crypts of the rat colon in 1983.[64] These colonic "crypt base secretory cells" were shown by Rothenberg et al[65] to have properties reminiscent of small intestinal Paneth cells. Thus, these c-kit–positive cells express the Notch ligands Dll1 and Dll4, as well as epidermal growth factor, just like Paneth cells. Importantly, when co-cultured with $Lgr5^{+}$ rapidly cycling cells, these crypt base secretory cells increased colonoid formation in vitro.[65] Recently, it was discovered that the differentiation of this unique cell type is regulated by Sprouty2, a negative regulator of multiple receptor tyrosine kinases, including the epidermal growth factor receptor and IL13

signaling derived from adjacent innate lymphoid cells.[66,67] Another marker of crypt base secretory cells identified by Rothenberg et al[65] is regenerating islet-derived family member 4. Sasaki et al[68] subsequently used a gene replacement strategy to place the human diphtheria toxin receptor under the control of the *Reg4* promoter to allow for inducible cell ablation to investigate the contribution of this cell type in vivo. Administration of diphtheria toxin led to complete loss of regenerating islet-derived family member 4–positive cells, and also goblet cells. Remarkably, however, epithelial proliferation as well as crypt depth were not impaired, even though $Lgr5^{+}$ cells lost their confinement to the crypt base. Thus, crypt base secretory cells in the colon are transcriptionally similar to small intestinal Paneth cells, can support organoid formation in culture, but are dispensable for epithelial renewal in vivo.

Intestinal Stem Cell Differentiation

To produce the functional cell types of the intestinal epithelium, distinct cell fates must be specified accurately during intestinal stem cell differentiation, which occurs continuously throughout life to enable the rapid turnover of the intestinal epithelium. Most notably, the Notch signaling pathway plays a central role in determining the absorptive and secretory fate in differentiating intestinal stem cells. Notch signaling regulates expression of *Atoh1*, a basic helix-loop-helix (bHLH) transcription factor that specifies the secretory fate. Indeed, mice deficient in *Atoh1* are depleted of goblet cells, enteroendocrine cells, and Paneth cells, whereas enterocytes are not affected.[69,70] Secretory progenitors express the membrane-bound Notch ligands delta-like proteins 1 and 4 (DLL1 and DLL4) and signal to their neighboring cells through the NOTCH1 receptor.[71,72] Activation of Notch signaling in the receiving cell results in transcriptional activation of the Notch target gene, *Hes1*, which transcriptionally represses *Atoh1*, thereby locking the signal receiving cell out of the secretory lineage.[35,73] Thus, cells with active Notch signaling will become absorptive progenitors and differentiate to produce enterocytes. Conversely, secretory progenitors will continue to express *Atoh1*, which acts in a positive feedback loop to reinforce *Dll1* and *Dll4* expression while also regulating downstream transcription factors necessary for secretory lineage differentiation.[74,75]

In secretory progenitor cells, ATOH1 binds and transcriptionally regulates several transcriptional factors that define secretory sublineages, including Neuorgenin 3 (*Neurog3*), growth factor independent 1 (*Gfi1*), *SAM pointed domain-containing Ets transcription factor (Spdef)*, and *SRY-related HMG-box 9 (Sox9)*.[74,75] First, NEUROG3 is required for enteroendocrine cell differentiation, as intestinal removal of *Neurog3* depletes all EEC subtypes without affecting nonendocrine epithelial cell types.[76] Mechanistically, *Neurog3* is repressed transcriptionally by GFI1, a zinc-finger transcription factor expressed in goblet cells and Paneth cells.[77] Mice deficient in *Gfi1* show an increased number of enteroendocrine cells, with an associated reduction in the number of goblet and Paneth cells.[78] Thus, GFI1 likely selects the goblet and Paneth cell fate in secretory progenitors by repressing *Neurog3*, thereby preventing enteroendocrine cell differentiation. The enteroendocrine cell lineage is subdivided further through the action of multiple transcription factors, including the FoxA proteins for the L and D cell lineages as well as NEUROD1 for the S and I lineages.[79,80]

Maturation and terminal differentiation of both the goblet cell and Paneth cell lineages requires the Ets-domain transcription factor, SPDEF. Indeed, ablation of *Spdef* results in an accumulation of immature secretory progenitor cells at the expense of mature goblet and Paneth cells.[81] Accordingly, SPDEF overexpression promotes differentiation toward the goblet cell lineage.[82] Furthermore, Paneth cell differentiation requires the Wnt signaling target, SOX9, and removal of *Sox9* from the intestinal epithelium results in a complete absence of Paneth cells.[83,84]

Surprisingly, tuft cell differentiation can occur independently of ATOH1, raising questions as to the specification process for this secretory cell type.[85,86] Recent studies have identified 2 transcription factors, POU domain, class 2, transcription factor 3 (POU2F3) and SRY-related HMG-box 4 (SOX4), that are required for tuft cell differentiation. Indeed, removal of *Pou2f3* from the intestinal epithelium completely depletes the tuft cell population, whereas loss of *Sox4* results in a significant reduction in their number.[44,86] Interestingly, tuft cell and goblet cell numbers are increased in response to helminth infection via IL4 and IL13 signaling, showing the dynamic regulation of these cell populations in response to environmental stimuli.[44]

As described, differentiation toward the secretory lineages depends largely on ATOH1 activity in the absence of Notch signaling. Conversely, in absorptive progenitors, the Notch target gene atonal bHLH transcription factor 1 represses all prosecretory factors, thereby promoting enterocyte differentiation.[73] Several additional transcriptional factors, including those belonging to the caudal type homeobox (CDX), GATA binding protein, and hepatocyte nuclear factor (HNF) families, are involved in enterocyte differentiation. For example, HNF4A and HNF4G promote enterocyte differentiation because their simultaneous ablation depletes enterocyte numbers and perturbs enterocyte gene activation.[87,88] Furthermore, HNF4G has been shown to cooperate with SMAD family member 4 (SMAD4), a downstream transcription factor of the BMP signaling pathway, in a feed-forward loop to promote enterocyte differentiation.[87] CDX2, a master regulator of intestinal identity, also promotes enterocyte differentiation because removal of *Cdx2* from the adult intestine impairs enterocyte maturation and compromises expression of genes involved in enterocyte functioning.[89–91] Most recently, the myeloid transformation gene 16 transcriptional co-repressor was found to promote enterocyte differentiation, possibly by binding and repressing ATOH1 target genes in enterocyte progenitor cells.[92]

Finally, differentiation of M cells occurs through a receptor activator of nuclear factor kappa B ligand (RANKL)-dependent signaling mechanism. Stromal cells underlying the follicle-associated epithelium secrete RANKL, which

then binds to the RANKL receptor (RANK) expressed on intestinal epithelial cells and triggers M-cell differentiation.[93,94] RANKL is sufficient to induce M-cell differentiation because RANKL treatment of intestinal organoids increases M-cell numbers.[95] Downstream of RANKL signaling, activation of the transcription factor SPI1-related protein B (SPIB) promotes M-cell differentiation, and removal of *Spib* results in reduced M-cell numbers and defective luminal antigen uptake.[94,95]

In sum, after Notch-regulated specification of the absorptive and secretory lineages, a complex transcription factor network orchestrates intestinal stem cell differentiation to produce the functionally mature cell types of the intestinal epithelium.

The Intestinal Stem Cell Niche

Signaling in the Niche

To preserve intestinal homeostasis, the production of functionally mature cell types must be balanced by intestinal stem cell self-renewal. To maintain their self-renewal and epithelial function, intestinal stem and progenitor cells rely on signals supplied by the surrounding intestinal stem cell niche. The canonical Wnt signaling pathway, mediated by the effector protein β-catenin, represents the principal force governing intestinal homeostasis and intestinal stem cell proliferation.[96] Binding of Wnt ligands to the frizzled and low-density lipoprotein receptor 5/6 co-receptors initiates a cascade of cytosolic signaling events that inhibits proteasomal degradation of β-catenin.[97–101] Stabilized β-catenin translocates to the nucleus and associates with the lymphoid enhancer-binding factor/T-cell factor transcription factors, leading to the transcriptional activation of downstream Wnt target genes.[102–104] Importantly, Wnt signaling is potentiated by binding of R-spondin (RSPO)1–4 agonists to leucine-rich G-protein–coupled receptors, which stabilizes the frizzled and low-density lipoprotein receptor-related proteins at the plasma membrane, thereby amplifying the Wnt signal.[105–109]

In addition to the Wnt signaling pathway, BMP signaling is a key regulator of intestinal crypt cell proliferation and differentiation. After the formation of homodimers or heterodimers, BMP ligands bind to their type II receptors (BMPR2), which results in the phosphorylation and activation of type I receptors (BMPR1A and BMPR1B) and the initiation of an intracellular signaling cascade mediated by the SMAD transcription factors.[110] Expression of BMP inhibitors, such as Noggin and Gremlin1, provides additional regulation of the BMP signaling cascade.[111,112]

Numerous studies have defined the coordinated activities of the Wnt and BMP signaling pathways as part of the intestinal stem cell niche. First, canonical Wnt signaling is required for intestinal stem cell maintenance and epithelial cell proliferation because overexpression of the Wnt inhibitor Dickkopf-1 represses Wnt target genes and induces rapid cessation of crypt cell proliferation.[113,114] Similarly, systemic deletion of *Tcf4*, a transcriptional activator of Wnt target genes, leads to a complete loss of proliferative compartments in the embryonic and neonatal intestinal epithelium, while removal of *Tcf4* from the adult intestinal epithelium results in a rapid loss of *Lgr5*$^+$ intestinal stem cells.[115,116] Conversely, excessive Wnt signaling pathway activation results in robust intestinal growth and frequently is observed in colorectal cancer.[117,118] Finally, potentiation of Wnt signaling via RSPO agonists is required for intestinal homeostasis because simultaneous removal of the *Lgr4* and *Lgr5* receptors abrogates crypt cell proliferation and induces intestinal stem cell attrition.[107] Thus, Wnt signaling is required for the development and maintenance of intestinal stem cells and plays a pivotal role in maintaining intestinal homeostasis.

Conversely, BMP signaling counteracts the proliferative signals of the intestinal stem cell niche, functioning to restrict proliferation, inhibit intestinal stem cell renewal, and promote terminal differentiation. Indeed, inhibiting BMP signaling by Noggin overexpression or *Bmpr1a* deletion results in crypt hyperplasia, development of ectopic crypts, and disrupted villus morphogenesis.[119–121] Furthermore, BMP signaling directly suppresses expression of *Lgr5*$^+$ intestinal stem cell signature genes and restricts stem cell expansion during intestinal homeostasis.[122] Accordingly, long-term growth of intestinal crypts into budding intestinal organoids requires BMP inhibition through the addition of recombinant Noggin.[28] Finally, removal of *Bmpr1a* from the intestinal epithelium impairs terminal differentiation of secretory lineage cells and perturbs expression of zonated enterocyte and goblet cell gene signatures,[123,124] showing that BMP signaling not only restricts intestinal stem cell self-renewal and epithelial proliferation, but also regulates differentiated cell states in the intestinal epithelium.

Given the contrasting outputs of the Wnt and BMP signaling pathways, whereby Wnt signaling promotes and BMP suppresses intestinal stem cell self-renewal, a model of the intestinal stem cell niche emerges in which opposing gradients of Wnt and BMP signaling activity guide proliferative, immature cells to a postmitotic, terminally differentiated state (Figure 2). At the crypt base, enrichment of Wnt ligands, RSPO agonists, and BMP inhibitors produces a pro-proliferative, high Wnt signaling environment that is conducive to intestinal stem cell self-renewal.[35] Further up the crypt, enrichment of BMP ligands, coupled with attenuated Wnt signaling, produces a high BMP signaling environment that promotes cellular maturation and terminal differentiation.[125] Thus, through coordinated Wnt and BMP signaling activities, the intestinal stem cell niche supports intestinal stem cell self-renewal and maintains crypt homeostasis.

Signaling input to intestinal proliferation and differentiation is of course not limited to the Wnt and BMP pathways, and even the rate of glycolytic flux can impact stem cell function.[126] Among other key systems is the highly complex Hippo pathway, recently reviewed in detail by Hong et al.[127] Core components of the Hippo pathway include a cascade of protein kinases that ultimately control the cytoplasmic vs nuclear localization of the transcriptional co-activators Yes-associated protein (YAP) and Transcriptional coactivator with PDZ-binding motif (TAZ), which when localized to the

nucleus complex with TEA domain family member (TEAD) proteins to activate genes involved in cell survival and growth, including survivin (baculoviral inhibitor of apoptosis repeat-containing 5 [BRIC5]) and cellular myelocytomatosis (c-MYC).[127] Even though studies using overexpression vs gene ablation mouse models have led to sometimes conflicting results, a picture has emerged in which under homeostatic conditions the pathway is active to limit YAP/TAZ activity. Thus, when mammalian sterile 20-like kinase 1 and 2 (Mst1 and Mst2), the serine/threonine protein kinases that normally limit YAP activity, are deleted simultaneously in the mouse intestinal epithelium, villus structure becomes perturbed, the proportion of undifferentiated cells increases, and mutant mice become susceptible to colonic dysplasia and adenoma formation.[128] In addition, Hippo pathway activation appears to play an important role in epithelial injury because mice lacking intestinal YAP show reduced crypt proliferation in the early phases of recovery from radiation injury, which was a transient effect attributed to YAP inhibition of Wnt signaling at the beginning of regeneration.[129]

Another critical signaling system active in intestinal development and homeostasis is the highly complex fibroblast growth factor (FGF) pathway, which counts 22 ligands and 4 receptors in both mice and human beings.[130] FGF signaling impacts all stages of gut life, from embryonic development to injury response. For instance, mesenchymal FGF10 is critical to organ development, as evidenced by duodenal and colonic atresia in Fgf10 null mice.[131] Curiously, although FGF10 is expressed at high levels in the duodenum and colon in mouse embryos, in human beings it is found primarily in the ileum.[132] FGF4 and FGF9 also play important roles in gut development.[130] In the adult intestine, stromal overexpression of FGF7, also known as keratinocyte growth factor, can increase villus height and crypt depth, possibly mediated by the epithelially expressed FGF receptor 3 (FGFR3).[133] A critical role for FGFR3 in intestinal crypt signaling is supported by the fact that *Fgfr3* null mice display only half the number of intestinal crypts, the likely result of fewer stem cells.[134]

Cellular Components of the Niche

Until recently, the cellular source of the critical Wnt and BMP signals in the intestinal niche remained unclear. Initial studies suggested that epithelial Paneth cells may constitute the intestinal stem cell niche because they express the canonical Wnt ligand *Wnt3* and reside juxtaposed to intestinal stem cells in a position well suited for short-range signaling.[55] Furthermore, growth of intestinal stem cells into budding organoids is improved significantly when cocultured with Paneth cells, indicating that Paneth cells may retain intrinsic niche functionality.[55,135] Finally, partial ablation of Paneth cells in vivo reduces intestinal stem cell numbers, suggesting a role for this cell population as part of the intestinal stem cell niche, although these findings remain controversial.[55]

However, more recent studies have raised doubt as to whether Paneth cell–derived niche factors are necessary for intestinal homeostasis. Importantly, mouse models of complete and permanent Paneth cell ablation showed that this epithelial cell type is dispensable for intestinal stem cell maintenance and crypt cell proliferation.[23,136,137] Furthermore, epithelial-specific ablation of *Wnt3* or *Porcupine*, an O-acyltransferase required for the functional maturation of all Wnts, has little effect on intestinal stem cells, suggesting the existence of compensatory nonepithelial Wnt sources.[135,138–140] Indeed, whole-body removal of Wnt signaling results in crypt failure that can be rescued with exogenous Wnt2b, a canonical Wnt ligand expressed specifically in the mesenchyme.[135,141,142] Finally, co-culture of growth-arrested *Wnt3*-deficient crypts with a mesenchymal cell feeder layer restores robust organoid formation, showing that mesenchymal cell populations can support self-renewal of the intestinal epithelium.[135] Together, these findings indicate that nonepithelial sources of critical niche factors can maintain intestinal stem cell self-renewal and intestinal homeostasis.

Addressing this possibility, our laboratory recently identified a population of *Foxl1*-expressing subepithelial mesenchymal cells as a key cellular component of the intestinal stem cell niche. Foxl1^{+} mesenchymal cells reside just beneath the epithelial layer and are identifiable by their long cytoplasmic processes that extend for more than 100 μm[143,144] (Figure 3). Termed *telocytes* because of their large cell bodies and flat morphology, this unusual cell type was

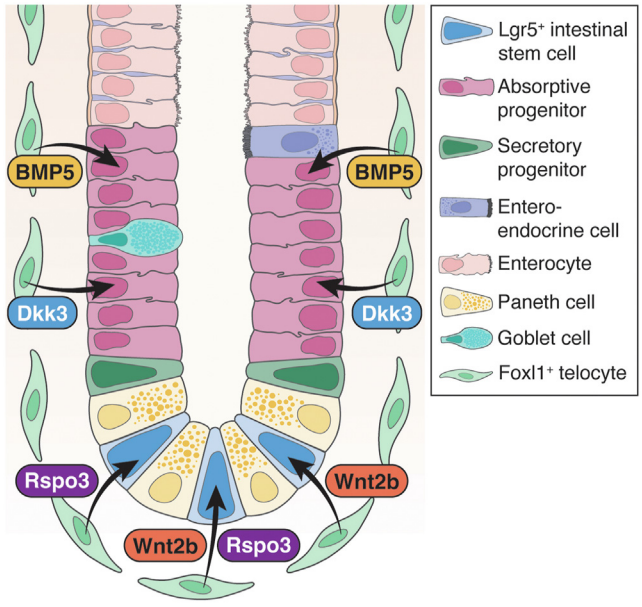

Figure 3. Foxl1+ telocytes express intestinal stem cell niche signaling factors in a region-specific manner. Foxl1+ telocytes compartmentalize expression of signaling molecules, as telocytes residing at the crypt base express Wnt ligands and agonists that contribute to a high Wnt signaling environment (Wnt2b, Rspo3). Foxl1+ telocytes further up the crypt express Wnt antagonists and BMP ligands that contribute to a high BMP signaling environment (BMP5, dickkopf WNT signaling pathway inhibitor 3 [Dkk3]). Intestinal epithelial cells are as described in the legend of Figure 2. Figure created with BioRender.com.

characterized morphologically by Popescu et al.[145] Foxl1$^+$ telocytes hug the epithelium from crypt base to villus tip and form a plexus that runs from the stomach to the colon.[144–146] Well positioned to provide the epithelium with critical niche signals, Foxl1$^+$ cells express numerous signaling molecules including *Wnt2b*, *Rspo3*, *Grem1*, and several BMP ligands.[125,143,144,147] Foxl1$^+$ telocytes compartmentalize expression of these signaling molecules because single-molecule RNA fluorescence in situ hybridization showed that *Wnt2b* and *Rspo3* transcripts are enriched in Foxl1$^+$ cells located at the crypt base, consistent with the high Wnt signaling environment required near intestinal stem cells (Figure 3). Conversely, Foxl1$^+$ cells located at the crypt–villus junction are enriched for the Wnt antagonist *Dkk3* and the BMP ligand *Bmp5*, which likely function to inhibit proliferation and promote differentiation[144] (Figure 3). Importantly, ablation of Foxl1$^+$ cells from the intestine results in intestinal stem cell loss, shortened villi, and crypt collapse, indicating that this cell population contributes significantly to intestinal homeostasis.[143] Furthermore, removal of all Wnt secretion from Foxl1$^+$ telocytes leads to intestinal stem cell attrition and abrogated stem and transit-amplifying cell proliferation.[144] Together, these studies show that Foxl1$^+$ telocytes are the critical source of Wnt signals to the intestinal epithelium and are a critical component of the intestinal stem cell niche.

In addition to Foxl1$^+$ telocytes, several additional and likely overlapping nonepithelial cell populations have been reported to function as part of the intestinal stem cell niche. Mesenchymal cell populations marked by the expression of *Gli1*,[148] *Pdgfra*,[125,149] *Cd34*,[150] and *Cspg4*[151] all have been ascribed niche function, while, most recently, lymphatic endothelial cells marked by lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) were found to contribute to intestinal stem cell maintenance.[152] However, the extent to which these mesenchymal cell populations overlap remains unclear. Indeed, by single-cell RNA sequencing, *Gli1* and *Cspg4* are broadly expressed among resident mesenchymal cell populations, and a subset of colonic GLI1$^+$ cells was found to express *Foxl1* transcripts.[125,148] By immunostaining, Foxl1$^+$ telocytes overlap with PDGFRa$^+$ cells and, by RNA sequencing, Foxl1$^+$ telocyte cells express high levels of *Pdgfra*, *Cd34*, and *Cspg4*.[144,151] Accordingly, a recent study identified a subset of Foxl1$^+$ telocytes based on their increased expression of PDGFRa.[125] They found that PDGFRa^{High} telocytes concentrate largely at the crypt–villus junction and primarily express BMP ligands, whereas a subset of mesenchymal cells the authors termed "trophocytes"[125] express low PDGFRa levels, are enriched under the crypt base, and express high levels of the BMP inhibitor Gremlin1 (Figure 2). Together, these investigators posited that PDGFRa^{High} Foxl1$^+$ telocytes create a high BMP signaling environment at the tops of crypts, whereas PDGFRa^{Low} trophocyte cells create a low BMP signaling environment at the crypt base. However, it is important to note that our lineage labeling studies found that mesenchymal cells located at the crypt base also express *Foxl1* and have telocyte morphology and produce Wnt pathway ligands.[144] Thus, it is unclear whether trophocytes truly represent a separate cell type or are simply a subset of telocytes with lower Foxl1 expression.

In sum, significant overlap in molecular marker expression confounds studies seeking to identify a single distinct mesenchymal cell population that constitutes the intestinal stem cell niche. Nonetheless, ample evidence supports the notion that the intestinal mesenchyme acts as a critical signaling hub to support intestinal stem cells, and that Foxl1$^+$ telocytes play a major role in maintaining intestinal homeostasis.

Conclusions

Through a combination of advanced histologic and immunostaining analysis, genetic lineage tracing, the use of advanced genetic models, and the deployment of organoid culture systems, the past 20 years has seen a dramatic improvement in our understanding of intestinal development and homeostatic differentiation. A complex picture has evolved in which cascades of transcriptional regulators impact the epigenetic state and thus transcriptional programs that govern these processes and integrate the reciprocal crosstalk of signaling molecules between the endoderm-derived epithelium and mesoderm-derived underlying mesenchyme. These insights already have enabled advanced disease modeling and form the basis of understanding of pathogenic processes in the gut. Importantly, recent advances in the analysis of human tissues including singe-cell approaches[147] have documented that the cell types and regulatory processes identified in mice also are operative in the human intestine. It indeed is likely that the advanced methodologies available to researchers today, from advanced mouse genetic models that can target any gene in vivo, whether in the stroma or epithelium and complex organoid models that even allow for the inclusion of immune cells to model inflammatory bowel disease,[153] to the powerful epigenomic methodologies that enable analysis of transcriptomes and epigenomes at the single-cell level (for a recent example see Burclaff et al[154]) will enable researchers worldwide to completely elucidate mammalian intestinal development in the near future. Going forward, it will be of particular interest to exploit the new spatial transcriptomic methodologies, which examine transcript levels of thousands of genes while retaining spatial resolution of complex small and large intestine architecture,[155] not just during development but also in human diseases such as Crohn's disease, ulcerative colitis, and cancer. These new techniques promise to enable a detailed understanding of the various immune cell types in the submucosa of inflammatory bowel disease and the interaction of cancer-associated fibroblasts and the immune system in colorectal cancer. Furthermore, it will be fascinating to use nanometer-scale analysis of the chromatin structure such as "optical reconstruction of chromatin architecture"[156] in both intestinal epithelial and stromal cells to determine how genetic risk variants, often very distant to the target gene, are impacting their effector transcripts. In sum, the future is bright for the analysis of mammalian development and differentiation, and its pathogenic perturbations in disease.

References

1. Hammoud SS, Cairns BR, Jones DA. Epigenetic regulation of colon cancer and intestinal stem cells. Curr Opin Cell Biol 2013;25:177–183.
2. Pimenta J, Ribeiro R, Almeida R, et al. Organ-on-Chip approaches for intestinal 3D in vitro modeling. Cell Mol Gastroenterol Hepatol 2022;13:351–367.
3. Chin AM, Hill DR, Aurora M, Spence JR. Morphogenesis and maturation of the embryonic and postnatal intestine. Semin Cell Dev Biol 2017;66:81–93.
4. Spence JR, Lauf R, Shroyer NF. Vertebrate intestinal endoderm development. Dev Dyn 2011;240:501–520.
5. Lawson KA, Meneses JJ, Pedersen RA. Cell fate and cell lineage in the endoderm of the presomite mouse embryo, studied with an intracellular tracer. Dev Biol 1986; 115:325–339.
6. Zorn AM, Wells JM. Vertebrate endoderm development and organ formation. Annu Rev Cell Dev Biol 2009; 25:221–251.
7. Guiu J, Jensen KB. From definitive endoderm to gut-a process of growth and maturation. Stem Cells Dev 2015;24:1972–1983.
8. Wells JM, Spence JR. How to make an intestine. Development 2014;141:752–760.
9. Cervantes S, Yamaguchi TP, Hebrok M. Wnt5a is essential for intestinal elongation in mice. Dev Biol 2009; 326:285–294.
10. Trier JS, Moxey PC. Morphogenesis of the small intestine during fetal development. Ciba Found Symp 1979; 70:3–29.
11. Walton KD, Kolterud Å, Czerwinski MJ, et al. Hedgehog-responsive mesenchymal clusters direct patterning and emergence of intestinal villi. Proc Natl Acad Sci U S A 2012;109:15817–15822.
12. Karlsson L, Lindahl P, Heath JK, Betsholtz C. Abnormal gastrointestinal development in PDGF-A and PDGFR-(alpha) deficient mice implicates a novel mesenchymal structure with putative instructive properties in villus morphogenesis. Development 2000;127: 3457–3466.
13. Mao J, Kim B-M, Rajurkar M, et al. Hedgehog signaling controls mesenchymal growth in the developing mammalian digestive tract. Development 2010; 137:1721–1729.
14. Madison BB, McKenna LB, Dolson D, et al. FoxF1 and FoxL1 link hedgehog signaling and the control of epithelial proliferation in the developing stomach and intestine. J Biol Chem 2009;284:5936–5944.
15. Kaestner KH, Silberg DG, Traber PG, Schütz G. The mesenchymal winged helix transcription factor Fkh6 is required for the control of gastrointestinal proliferation and differentiation. Genes Dev 1997;11:1583–1595.
16. Shyer AE, Tallinen T, Nerurkar NL, et al. Villification: how the gut gets its villi. Science 2013;342:212–218.
17. Rao-Bhatia A, Zhu M, Yin WC, et al. Hedgehog-activated Fat4 and PCP pathways mediate mesenchymal cell clustering and villus formation in gut development. Dev Cell 2020;52:647–658 e6.
18. Walker EM, Thompson CA, Battle MA. GATA4 and GATA6 regulate intestinal epithelial cytodifferentiation during development. Dev Biol 2014;392:283–294.
19. Arsenault P, Menard D. Cell proliferation in developing human jejunum. Biol Neonate 1987;51:297–304.
20. Calvert R, Pothier P. Migration of fetal intestinal intervillous cells in neonatal mice. Anat Rec 1990; 227:199–206.
21. Sumigray KD, Terwilliger M, Lechler T. Morphogenesis and compartmentalization of the intestinal crypt. Dev Cell 2018;45:183–197.e5.
22. Bry L, Falk P, Huttner K, et al. Paneth cell differentiation in the developing intestine of normal and transgenic mice. Proc Natl Acad Sci U S A 1994;91:10335–10339.
23. Kim T-H, Escudero S, Shivdasani RA. Intact function of Lgr5 receptor-expressing intestinal stem cells in the absence of Paneth cells. Proc Natl Acad Sci U S A 2012; 109:3932–3937.
24. Gordon JI, Hermiston ML. Differentiation and self-renewal in the mouse gastrointestinal epithelium. Curr Opin Cell Biol 1994;6:795–803.
25. Creamer B, Shorter RG, Bamforth J. The turnover and shedding of epithelial cells. I. The turnover in the gastrointestinal tract. Gut 1961;2:110–118.
26. Darwich AS, Aslam U, Ashcroft DM, Rostami-Hodjegan A. Meta-analysis of the turnover of intestinal epithelia in preclinical animal species and humans. Drug Metab Disp 2014;42:2016–2022.
27. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007;449:1003–1007.
28. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262–265.
29. Tian H, Biehs B, Warming S, et al. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature 2012;478:255–259.
30. Tetteh PW, Basak O, Farin HF, et al. Replacement of lost Lgr5-positive stem cells through plasticity of their enterocyte-lineage daughters. Cell Stem Cell 2016; 18:203–213.
31. Yu S, Tong K, Zhao Y, et al. Paneth cell multipotency induced by notch activation following injury. Cell Stem Cell 2018;23:46–59 e5.
32. Meyer AR, Brown ME, McGrath PS, Dempsey PJ. Injury-induced cellular plasticity drives intestinal regeneration. Cell Mol Gastroenterol Hepatol 2022;13:843–856.
33. Potten CS. Epithelial cell growth and differentiation. II. Intestinal apoptosis. Am J Physiol 1997; 273:G253–G257.
34. Grossmann J, Walther K, Artinger M, et al. Apoptotic signaling during initiation of detachment-induced apoptosis ("anoikis") of primary human intestinal epithelial cells. Cell Growth Differ 2001;12:147–155.
35. Gehart H, Clevers H. Tales from the crypt: new insights into intestinal stem cells. Nat Rev Gastroenterol Hepatol 2019;16:19–34.
36. Moor AE, Harnik Y, Ben-Moshe S, et al. Spatial reconstruction of single enterocytes uncovers broad zonation

along the intestinal villus axis. Cell 2018;175:1156–1167.e15.
37. Neutra MR, Mantis NJ, Kraehenbuhl JP. Collaboration of epithelial cells with organized mucosal lymphoid tissues. Nat Immunol 2001;2:1004–1009.
38. Dillon A, Lo DD. M cells: intelligent engineering of mucosal immune surveillance. Front Immunol 2019;10:1499.
39. Birchenough GM, Johansson ME, Gustafsson JK, et al. New developments in goblet cell mucus secretion and function. Mucosal Immunol 2015;8:712–719.
40. Martens EC, Chiang HC, Gordon JI. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 2008;4:447–457.
41. Schroeder BO. Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. Gastroenterol Rep (Oxf) 2019;7:3–12.
42. Gerbe F, van Es JH, Makrini L, et al. Distinct ATOH1 and Neurog3 requirements define tuft cells as a new secretory cell type in the intestinal epithelium. J Cell Biol 2011;192:767–780.
43. Hendel SK, Kellermann L, Hausmann A, et al. Tuft cells and their role in intestinal diseases. Front Immunol 2022;13:822867.
44. Gerbe F, Sidot E, Smyth DJ, et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature 2016;529:226–230.
45. Von Moltke J, Ji M, Liang H-E, Locksley RM. Tuft-cell-derived IL-25 regulates an intestinal ILC2–epithelial response circuit. Nature 2016;529:221–225.
46. Gerbe F, Brulin B, Makrini L, et al. DCAMKL-1 expression identifies tuft cells rather than stem cells in the adult mouse intestinal epithelium. Gastroenterology 2009;137:2179–2180.
47. Howitt MR, Lavoie S, Michaud M, et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science 2016;351:1329–1333.
48. Worthington JJ, Reimann F, Gribble FM. Enteroendocrine cells-sensory sentinels of the intestinal environment and orchestrators of mucosal immunity. Mucosal Immunol 2018;11:3–20.
49. Guo X, Lv J, Xi R. The specification and function of enteroendocrine cells in Drosophila and mammals: a comparative review. FEBS J 2022;289:4773–4796.
50. Habib AM, Richards P, Cairns LS, et al. Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. Endocrinology 2012;153:3054–3065.
51. Haber AL, Biton M, Rogel N, et al. A single-cell survey of the small intestinal epithelium. Nature 2017;551:333–339.
52. Gehart H, van Es JH, Hamer K, et al. Identification of enteroendocrine regulators by real-time single-cell differentiation mapping. Cell 2019;176:1158–1173 e16.
53. Ireland H, Houghton C, Howard L, Winton DJ. Cellular inheritance of a Cre-activated reporter gene to determine Paneth cell longevity in the murine small intestine. Dev Dyn 2005;233:1332–1336.
54. Holly MK, Smith JG. Paneth cells during viral infection and pathogenesis. Viruses 2018;10:225.
55. Sato T, van Es JH, Snippert HJ, et al. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 2011;469:415–418.
56. Deckx RJ, Vantrappen GR, Parein MM. Localization of lysozyme activity in a Paneth cell granule fraction. Biochim Biophys Acta 1967;139:204–207.
57. Clevers HC, Bevins CL. Paneth cells: maestros of the small intestinal crypts. Annu Rev Physiol 2013;75:289–311.
58. Ayabe T, Satchell DP, Wilson CL, et al. Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. Nat Immunol 2000;1:113–118.
59. Wilson CL, Ouellette AJ, Satchell DP, et al. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science 1999;286:113–117.
60. Salzman NH, Hung K, Haribhai D, et al. Enteric defensins are essential regulators of intestinal microbial ecology. Nat Immunol 2010;11:76–82.
61. Wehkamp J, Harder J, Weichenthal M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. Gut 2004;53:1658–1664.
62. Wehkamp J, Salzman NH, Porter E, et al. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci U S A 2005;102:18129–18134.
63. Cadwell K, Liu JY, Brown SL, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature 2008;456:259–263.
64. Altmann GG. Morphological observations on mucus-secreting nongoblet cells in the deep crypts of the rat ascending colon. Am J Anat 1983;167:95–117.
65. Rothenberg ME, Nusse Y, Kalisky T, et al. Identification of a cKit(+) colonic crypt base secretory cell that supports Lgr5(+) stem cells in mice. Gastroenterology 2012;142:1195–1205 e6.
66. McCarthy H, Keely S. Re-evaluating the role of deep crypt secretory cells in intestinal homeostasis. Cell Mol Gastroenterol Hepatol 2023;15:1020–1021.
67. Schumacher MA, Liu CY, Katada K, et al. Deep crypt secretory cell differentiation in the colonic epithelium is regulated by sprouty2 and interleukin 13. Cell Mol Gastroenterol Hepatol 2023;15:971–984.
68. Sasaki N, Sachs N, Wiebrands K, et al. Reg4+ deep crypt secretory cells function as epithelial niche for Lgr5+ stem cells in colon. Proc Natl Acad Sci U S A 2016;113:E5399–E5407.
69. Yang Q, Bermingham NA, Finegold MJ, Zoghbi HY. Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. Science 2001;294:2155–2158.
70. Shroyer NF, Helmrath MA, Wang VYC, et al. Intestine-specific ablation of mouse atonal homolog 1 (Math1) reveals a role in cellular homeostasis. Gastroenterology 2007;132:2478–2488.
71. Van Es JH, Sato T, Van De Wetering M, et al. Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. Nat Cell Biol 2012;14:1099–1104.

72. Pellegrinet L, Rodilla V, Liu Z, et al. Dll1- and Dll4-mediated Notch signaling are required for homeostasis of intestinal stem cells. Gastroenterology 2011; 140:1230–1240.e7.
73. Beumer J, Clevers H. Cell fate specification and differentiation in the adult mammalian intestine. Nat Rev Mol Cell Biol 2021;22:39–53.
74. Kim TH, Li F, Ferreiro-Neira I, et al. Broadly permissive intestinal chromatin underlies lateral inhibition and cell plasticity. Nature 2014;506:511–515.
75. Lo Y-H, Chung E, Li Z, et al. Transcriptional regulation by ATOH1 and its target SPDEF in the intestine. Cell Mol Gastroenterol Hepatol 2017;3:51–71.
76. Jenny M, Uhl C, Roche C, et al. Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. EMBO J 2002; 21:6338–6347.
77. Bjerknes M, Cheng H. Cell lineage metastability in Gfi1-deficient mouse intestinal epithelium. Dev Biol 2010; 345:49–63.
78. Shroyer NF, Wallis D, Venken KJT, et al. Gfi1 functions downstream of Math1 to control intestinal secretory cell subtype allocation and differentiation. Genes Dev 2005; 19:2412–2417.
79. Ye DZ, Kaestner KH. Foxa1 and Foxa2 control the differentiation of goblet and enteroendocrine L- and D-cells in mice. Gastroenterology 2009;137:2052–2062.
80. May CL, Kaestner KH. Gut endocrine cell development. Mol Cell Endocrinol 2010;323:70–75.
81. Gregorieff A, Stange DE, Kujala P, et al. The ets-domain transcription factor Spdef promotes maturation of goblet and Paneth cells in the intestinal epithelium. Gastroenterology 2009;137:1333–1345, e1-3.
82. Noah TK, Kazanjian A, Whitsett J, Shroyer NF. SAM pointed domain ETS factor (SPDEF) regulates terminal differentiation and maturation of intestinal goblet cells. Exp Cell Res 2010;316:452–465.
83. Mori–Akiyama Y, Van Den Born M, Van Es JH, et al. SOX9 is required for the differentiation of Paneth cells in the intestinal epithelium. Gastroenterology 2007; 133:539–546.
84. Bastide P, Darido C, Pannequin J, et al. Sox9 regulates cell proliferation and is required for Paneth cell differentiation in the intestinal epithelium. J Cell Biol 2007; 178:635–648.
85. Herring CA, Banerjee A, McKinley ET, et al. Unsupervised trajectory analysis of single-cell RNA-seq and imaging data reveals alternative tuft cell origins in the gut. Cell Syst 2018;6:37–51.e9.
86. Gracz AD, Samsa LA, Fordham MJ, et al. Sox4 promotes Atoh1-independent intestinal secretory differentiation toward tuft and enteroendocrine fates. Gastroenterology 2018;155:1508–1523.e10.
87. Chen L, Toke NH, Luo S, et al. A reinforcing HNF4-SMAD4 feed-forward module stabilizes enterocyte identity. Nat Genet 2019;51:777–785.
88. Chen L, Toke NH, Luo S, et al. HNF4 factors control chromatin accessibility and are redundantly required for maturation of the fetal intestine. Development 2019; 146:dev179432.
89. Verzi MP, Shin H, He HH, et al. Differentiation-specific histone modifications reveal dynamic chromatin interactions and partners for the intestinal transcription factor CDX2. Dev Cell 2010;19:713–726.
90. Fang R, Santiago NA, Olds LC, Sibley E. The homeodomain protein Cdx2 regulates lactase gene promoter activity during enterocyte differentiation. Gastroenterology 2000;118:115–127.
91. Gao N, White P, Kaestner KH. Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2. Dev Cell 2009;16:588–599.
92. Baulies A, Angelis N, Foglizzo V, et al. The transcription co-repressors MTG8 and MTG16 regulate exit of intestinal stem cells from their niche and differentiation into enterocyte vs secretory lineages. Gastroenterology 2020;159:1328–1341.e3.
93. Knoop KA, Kumar N, Butler BR, et al. RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. J Immunol 2009;183:5738–5747.
94. Kanaya T, Hase K, Takahashi D, et al. The Ets transcription factor Spi-B is essential for the differentiation of intestinal microfold cells. Nat Immunol 2012;13:729–736.
95. De Lau W, Kujala P, Schneeberger K, et al. Peyer's patch M cells derived from Lgr5+ stem cells require SpiB and are induced by RankL in cultured "miniguts". Mol Cell Biol 2012;32:3639–3647.
96. Krausova M, Korinek V. Wnt signaling in adult intestinal stem cells and cancer. Cell Signal 2014;26:570–579.
97. Pinson KI, Brennan J, Monkley S, et al. An LDL-receptor-related protein mediates Wnt signalling in mice. Nature 2000;407:535–538.
98. Tamai K, Semenov M, Kato Y, et al. LDL-receptor-related proteins in Wnt signal transduction. Nature 2000; 407:530–535.
99. Yang-Snyder J, Miller JR, Brown JD, et al. A frizzled homolog functions in a vertebrate Wnt signaling pathway. Curr Biol 1996;6:1302–1306.
100. Bhanot P, Brink M, Samos CH, et al. A new member of the frizzled family from Drosophila functions as a Wingless receptor. Nature 1996;382:225–230.
101. Liu C, Li Y, Semenov M, et al. Control of β-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 2002;108:837–847.
102. Huber O, Korn R, McLaughlin J, et al. Nuclear localization of β-catenin by interaction with transcription factor LEF-1. Mech Dev 1996;59:3–10.
103. Behrens J, Von Kries JP, Kühl M, et al. Functional interaction of β-catenin with the transcription factor LEF-1. Nature 1996;382:638–642.
104. Van De Wetering M, Cavallo R, Dooijes D, et al. Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell 1997; 88:789–799.
105. Koo B-K, Spit M, Jordens I, et al. Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. Nature 2012;488:665–669.
106. Hao H-X, Xie Y, Zhang Y, et al. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. Nature 2012;485:195–200.

107. De Lau W, Barker N, Low TY, et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature 2011;476:293–297.
108. Carmon KS, Gong X, Lin Q, et al. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/β-catenin signaling. Proc Natl Acad Sci U S A 2011;108:11452–11457.
109. Jin Y-R, Yoon JK. The R-spondin family of proteins: Emerging regulators of WNT signaling. Int J Biochem Cell Biol 2012;44:2278–2287.
110. Gomez-Puerto MC, Iyengar PV, García De Vinuesa A, et al. Bone morphogenetic protein receptor signal transduction in human disease. J Pathol 2019;247:9–20.
111. Hsu DR, Economides AN, Wang X, et al. The Xenopus dorsalizing factor gremlin identifies a novel family of secreted proteins that antagonize BMP activities. Mol Cell 1998;1:673–683.
112. Smith WC, Harland RM. Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in Xenopus embryos. Cell 1992;70:829–840.
113. Kuhnert F, Davis CR, Wang H-T, et al. Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. Proc Natl Acad Sci 2004;101:266–271.
114. Pinto D, Gregorieff A, Begthel H, Clevers H. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev 2003;17:1709–1713.
115. Korinek V, Barker N, Moerer P, et al. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 1998;19:379–383.
116. Van Es JH, Haegebarth A, Kujala P, et al. A critical role for the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal. Mol Cell Biol 2012;32:1918–1927.
117. Morin PJ, Sparks AB, Korinek V, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997;275:1787–1790.
118. Barker N, Ridgway RA, Van Es JH, et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 2009; 457:608–611.
119. Haramis AP, Begthel H, van den Born M, et al. De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. Science 2004;303:1684–1686.
120. Batts LE, Polk DB, Dubois RN, Kulessa H. Bmp signaling is required for intestinal growth and morphogenesis. Dev Dyn 2006;235:1563–1570.
121. He XC, Zhang J, Tong W-G, et al. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt–β-catenin signaling. Nat Genet 2004;36:1117–1121.
122. Qi Z, Li Y, Zhao B, et al. BMP restricts stemness of intestinal Lgr5+ stem cells by directly suppressing their signature genes. Nat Commun 2017;8:13824.
123. Auclair BA, Benoit YD, Rivard N, et al. Bone morphogenetic protein signaling is essential for terminal differentiation of the intestinal secretory cell lineage. Gastroenterology 2007;133:887–896.
124. Beumer J, Puschhof J, Yengej FY, et al. BMP gradient along the intestinal villus axis controls zonated enterocyte and goblet cell states. Cell Rep 2022;38:110438.
125. McCarthy N, Manieri E, Storm EE, et al. Distinct mesenchymal cell populations generate the essential intestinal BMP signaling gradient. Cell Stem Cell 2020; 26:391–402 e5.
126. Li C, Zhou Y, Wei R, et al. Glycolytic regulation of intestinal stem cell self-renewal and differentiation. Cell Mol Gastroenterol Hepatol 2023;15:931–947.
127. Hong AW, Meng Z, Guan KL. The Hippo pathway in intestinal regeneration and disease. Nat Rev Gastroenterol Hepatol 2016;13:324–337.
128. Zhou D, Zhang Y, Wu H, et al. Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance. Proc Natl Acad Sci U S A 2011;108:E1312–E1320.
129. Gregorieff A, Liu Y, Inanlou MR, et al. Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. Nature 2015;526:715–718.
130. Danopoulos S, Schlieve CR, Grikscheit TC, Al Alam D. Fibroblast growth factors in the gastrointestinal tract: twists and turns. Dev Dyn 2017;246:344–352.
131. Kanard RC, Fairbanks TJ, De Langhe SP, et al. Fibroblast growth factor-10 serves a regulatory role in duodenal development. J Pediatr Surg 2005;40:313–316.
132. Al Alam D, Danopoulos S, Schall K, et al. Fibroblast growth factor 10 alters the balance between goblet and Paneth cells in the adult mouse small intestine. Am J Physiol Gastrointest Liver Physiol 2015;308:G678–G690.
133. Cai Y, Wang W, Liang H, et al. Keratinocyte growth factor improves epithelial structure and function in a mouse model of intestinal ischemia/reperfusion. PLoS One 2012;7:e44772.
134. Vidrich A, Buzan JM, Brodrick B, et al. Fibroblast growth factor receptor-3 regulates Paneth cell lineage allocation and accrual of epithelial stem cells during murine intestinal development. Am J Physiol Gastrointest Liver Physiol 2009;297:G168–G178.
135. Farin HF, Van Es JH, Clevers H. Redundant sources of Wnt regulate intestinal stem cells and promote formation of Paneth cells. Gastroenterology 2012;143:1518–1529.e7.
136. Durand A, Donahue B, Peignon G, et al. Functional intestinal stem cells after Paneth cell ablation induced by the loss of transcription factor Math1 (Atoh1). Proc Natl Acad Sci U S A 2012;109:8965–8970.
137. Garabedian EM, Roberts LJJ, McNevin MS, Gordon JI. Examining the role of Paneth cells in the small intestine by lineage ablation in transgenic mice. J Biol Chem 1997;272:23729–23740.
138. San Roman AK, Jayewickreme CD, Murtaugh LC, Shivdasani RA. Wnt secretion from epithelial cells and subepithelial myofibroblasts is not required in the mouse intestinal stem cell niche in vivo. Stem Cell Rep 2014; 2:127–134.
139. Najdi R, Proffitt K, Sprowl S, et al. A uniform human Wnt expression library reveals a shared secretory pathway and unique signaling activities. Differentiation 2012; 84:203–213.
140. Proffitt KD, Virshup DM. Precise regulation of porcupine activity is required for physiological Wnt signaling. J Biol Chem 2012;287:34167–34178.
141. Banziger C, Soldini D, Schutt C, et al. Wntless, a conserved membrane protein dedicated to the secretion

of Wnt proteins from signaling cells. Cell 2006; 125:509–522.
142. Valenta T, Degirmenci B, Moor AE, et al. Wnt ligands secreted by subepithelial mesenchymal cells are essential for the survival of intestinal stem cells and gut homeostasis. Cell Rep 2016;15:911–918.
143. Aoki R, Shoshkes-Carmel M, Gao N, et al. Foxl1-expressing mesenchymal cells constitute the intestinal stem cell niche. Cell Mol Gastroenterol Hepatol 2016; 2:175–188.
144. Shoshkes-Carmel M, Wang YJ, Wangensteen KJ, et al. Subepithelial telocytes are an important source of Wnts that supports intestinal crypts. Nature 2018; 557:242–246.
145. Cretoiu C, Cretoiu SM, Simionescu AA, Popescu LM. Telocytes, a distinct type of cell among the stromal cells present in the lamina propria of jejunum. Histol Histopathol 2012;27:1067–1078.
146. Carmona IC, Bartolomé MJL, Escribano CJ. Identification of telocytes in the lamina propria of rat duodenum: transmission electron microscopy. J Cell Mol Med 2011; 15:26–30.
147. Kinchen J, Chen HH, Parikh K, et al. Structural remodeling of the human colonic mesenchyme in inflammatory bowel disease. Cell 2018;175:372–386.e17.
148. Degirmenci B, Valenta T, Dimitrieva S, et al. GLI1-expressing mesenchymal cells form the essential Wnt-secreting niche for colon stem cells. Nature 2018; 558:449–453.
149. Greicius G, Kabiri Z, Sigmundsson K, et al. PDGFRα+ pericryptal stromal cells are the critical source of Wnts and RSPO3 for murine intestinal stem cells in vivo. Proc Natl Acad Sci U S A 2018;115:E3173–E3181.
150. Stzepourginski I, Nigro G, Jacob J-M, et al. CD34+ mesenchymal cells are a major component of the intestinal stem cells niche at homeostasis and after injury. Proc Natl Acad Sci U S A 2017; 114:E506–E513.
151. Kim J-E, Fei L, Yin W-C, et al. Single cell and genetic analyses reveal conserved populations and signaling mechanisms of gastrointestinal stromal niches. Nat Commun 2020;11:334.
152. Niec RE, Chu T, Schernthanner M, et al. Lymphatics act as a signaling hub to regulate intestinal stem cell activity. Cell Stem Cell 2022;29:1067–1082 e18.
153. Tsuruta S, Kawasaki T, Machida M, et al. Development of human gut organoids with resident tissue macrophages as a model of intestinal immune responses. Cell Mol Gastroenterol Hepatol 2022;14:726–729 e5.
154. Burclaff J, Bliton RJ, Breau KA, et al. A proximal-to-distal survey of healthy adult human small intestine and colon epithelium by single-cell transcriptomics. Cell Mol Gastroenterol Hepatol 2022;13:1554–1589.
155. He S, Bhatt R, Brown C, et al. High-plex imaging of RNA and proteins at subcellular resolution in fixed tissue by spatial molecular imaging. Nat Biotechnol 2022; 40:1794–1806.
156. Mateo LJ, Murphy SE, Hafner A, et al. Visualizing DNA folding and RNA in embryos at single-cell resolution. Nature 2019;568:49–54.

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