cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

Injury-Induced Cellular Plasticity Drives Intestinal Regeneration

Anne R. Meyer, Monica E. Brown, Patrick S. McGrath, and Peter J. Dempsey

Section of Developmental Biology, Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado

SUMMARY

Intestinal regeneration after damage occurs through the activation of several signaling pathways to promote cellular plasticity and dedifferentiation of remaining cells to a stem cell state. This review highlights many of the key cell types and signaling networks involved in the intestinal regenerative response.

The epithelial lining of the intestine, particularly the stem cell compartment, is affected by harsh conditions in the luminal environment and also is susceptible to genotoxic agents such as radiation and chemotherapy. Therefore, the ability for intestinal epithelial cells to revert to a stem cell state is an important physiological damage response to regenerate the intestinal epithelium at sites of mucosal injury. Many signaling networks involved in maintaining the stem cell niche are activated as part of the damage response to promote cellular plasticity and regeneration. The relative contribution of each cell type and signaling pathway is a critical area of ongoing research, likely dependent on the nature of injury as well as the regional specification within the intestine. Here, we review the current understanding of the multicellular cooperation to restore the intestinal epithelium after damage. *(Cell Mol Gastroenterol Hepatol 2022;13:843–856; https://doi.org/10.1016/j.jcmgh.2021.12.005)*

Keywords: Intestinal Homeostasis; Stem Cell Niche; Cellular Plasticity; Intestinal Regeneration.

The epithelial lining of the intestine is composed of a single layer of cells that serve many functions, including digestion, nutrient absorption, barrier function, and immunity. In the small intestine, the epithelium is organized into repetitive crypt–villus units that have slightly different architecture and cellular composition along the proximal–distal axis reflecting unique functions of each region (ie, duodenum, jejunum, and ileum). By contrast, the colon has a much simpler organization containing only crypt units that directly merge into surface epithelium. Remarkably, the entire intestinal epithelial lining is replaced approximately every 3–5 days, and this constant renewal is required to maintain intestinal homeostasis and tissue integrity.[1] Multipotent leucine-rich repeat-containing G-protein–coupled receptor-5–positive (LGR5$^+$) intestinal stem cells (ISCs) located in the crypt base are responsible for the constant renewal and rapid replenishment of all epithelial cell types lining the crypt–villus axis.[2] The intestine must maintain a high rate of renewal under normal homeostasis to withstand a harsh luminal environment. The spatial distribution of ISCs at the base of crypt invaginations is important because it offers some isolation and protection from luminal stressors. However, in the event of damage to the stem cell compartment, the intestinal epithelium has a remarkable ability to regenerate after injury. In this review, we highlight some of the key findings related to the signaling pathways and mechanisms involved in intestinal homeostasis, cellular plasticity, and regeneration after injury.

Cellular Architecture in the Intestine

LGR5$^+$ ISCs give rise to a bipotent progenitor cell population known as transit-amplifying (TA) cells. TA cells rapidly proliferate and eventually undergo lineage commitment by differentiating into either absorptive or secretory progenitors. Ultimately, absorptive progenitors go through a limited number of divisions and upon exiting the crypt differentiate into postmitotic absorptive enterocytes or into rare microfold cells that are associated with lymphoid structures and involved in immune surveillance.[3] In contrast, secretory progenitors differentiate directly into several postmitotic secretory cell types including hormone-secreting enteroendocrine cells, mucus-secreting goblet cells, and antimicrobial-secreting Paneth cells.[4] Chemosensory tuft cells also have been proposed to be a secretory cell type, although recent studies have raised questions about the cellular origin of distinct tuft cell populations within the gastrointestinal tract.[5] Constant proliferation and differentiation in the crypt drives migration of differentiated cells toward the tip of the villus where they eventually undergo anoikis and are shed into the lumen.[6] Paneth cells are the exception and are retained at the crypt base where they are intercalated between LGR5$^+$ ISCs and have an extended half-life of approximately 30 days.

Abbreviations used in this paper: **ASCL2, Achaete-scute complex homolog 2; ATOH, atonal bHLH transcription factor 1; BMI1, B cell-specific Moloney murine leukemia virus integration site 1; BMP, bone morphogenetic protein; CLU, clusterin; DCLK1, Doublecortin-like kinase 1; DLL, delta like canonical Notch ligand; DSS, dextran sodium sulfate; EGF, epidermal growth factor; FOXL1, forkhead box L1; IL, interleukin; ILC, innate lymphoid cell; ISC, intestinal stem cells; KRAS, kirsten rat sarcoma viral oncogene homolog; LGR5$^+$, leucine-rich repeat-containing G-protein–coupled receptor-5–positive; LRC, label-retaining cell; PDGFRα, platelet derived growth factor receptor alpha; RTK, receptor tyrosine kinase; SCF, stem cell factor; TA, transit-amplifying; WNT, wingless-related integration site.**

Most current article

2352-345X
https://doi.org/10.1016/j.jcmgh.2021.12.005

Cell Fate Specification

LGR5$^+$ ISCs historically have been defined through the use of *LacZ* or *eGFP-IRES-CreERT2* knock-in reporter alleles.[2] However, there is increasing evidence that not all LGR5$^+$ stem cells are the same.[7] Data suggest that LGR5^{high} cells that are located deeper in the crypt base intercalated between mature Paneth cells are more likely to be retained and to serve as bona fide ISCs that contribute to ISC renewal and crypt homeostasis.[8] ISC retention is achieved through symmetrical cell division of LGR5^{high} cells in a process called *neutral drift*.[9,10] LGR5$^+$ ISCs are actively dividing and have a cell-cycle time of approximately 1 day. A complex signaling system tightly regulates LGR5$^+$ stem cell renewal as well as cell fate decisions in the intestine. For example, Notch signaling initially is required for the maintenance and survival of LGR5$^+$ ISCs, but also triggers bipotent progenitors to repress the secretory lineage transcription factor, atonal bHLH transcription factor 1 (ATOH1), and drive absorptive enterocyte differentiation.[11,12] On the other hand, Paneth cells are dependent on both Notch signaling for their cell fate specification and wingless-related integration site (WNT) signaling for their functional differentiation.[13–15] Establishment of these signaling gradients are critical for lineage commitment and terminal differentiation of each of the mature cell types in the intestine.

Intestinal Stem Cell Niche

The microenvironment that precisely regulates the maintenance, self-renewal, proliferation, and differentiation of stem cells is referred to as a *stem cell niche*. In the small intestine the stem cell niche is critically dependent on the crypt architecture, with LGR5$^+$ ISCs positioned between Paneth cells at the crypt base and an intricate network of support cells including stromal cells, immune cells, neural cells, and endothelial cells embedded in the extracellular matrix of the lamina propria surrounding the crypt. The physical orientation of the intestinal epithelium and crypt architecture within this microenvironment allows the establishment of spatially restricted growth factor gradients for WNT, epidermal growth factor (EGF), NOTCH, bone morphogenetic protein (BMP), and other signaling molecules that are essential for maintaining a precise balance between stemness and differentiation. Both intrinsic signals derived from intestinal epithelial cells found within the crypt compartment and extrinsic short-range signals generated by surrounding stromal cell populations contribute to the stem cell niche. Additional signals derived from other cell types found within the lamina propria and submucosa (eg, macrophages, dendritic cells, lymphocytes, enteric neurons, glial cells, endothelial cells, pericytes, lacteal cells, myocytes, and so forth) also contribute to maintaining crypt homeostasis. Furthermore, the intestinal stem cell niche continuously monitors and responds to the luminal environment for changes in nutrients, metabolites, and signals from the microbiome to maintain integrity and function (Figure 1).

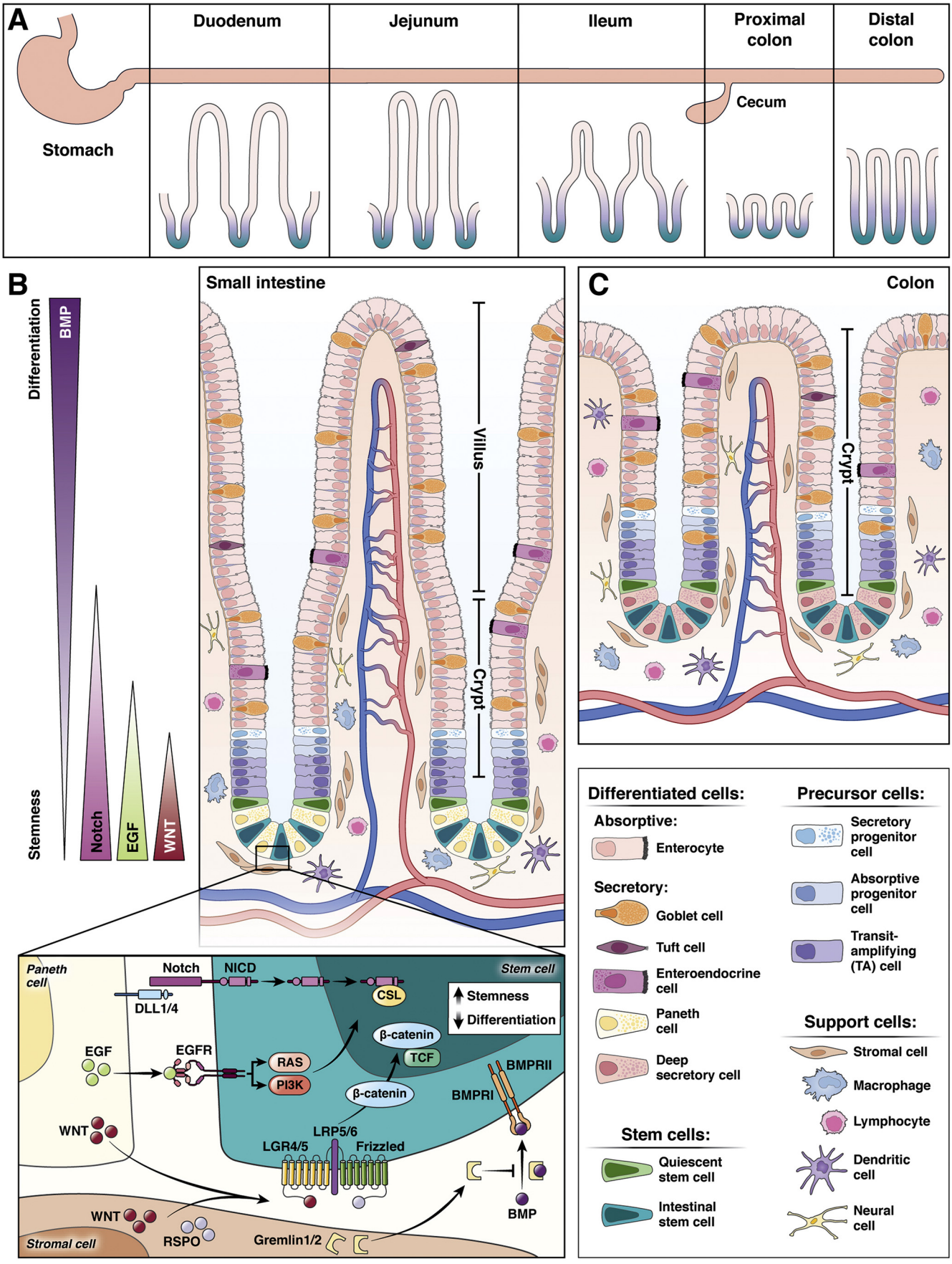
Epithelial Compartment

In the small intestine, Paneth cells are in direct physical contact with LGR5$^+$ ISCs and provide both cell-to-cell contact and secreted signals to support the stem cell niche.[16] For example, Paneth cells are essential for initiating Notch signaling, which is critical for LGR5$^+$ ISC maintenance and survival. Paneth cells (signal-sending cells) directly communicate with stem cells through the expression of membrane-bound delta like canonical Notch ligands DLL-1 and DLL-4, which bind to NOTCH1 and NOTCH2 receptors expressed on the surface of LGR5$^+$ ISCs (signal-receiving cells). Notch ligand binding to the receptor triggers cleavage and release of the intracellular signaling fragment of the receptor (Notch intracellular domain), which is translocated to the nucleus and activates transcription of Notch target genes in ISCs. Because Notch ligand presentation requires cell-to-cell contact, Paneth cells can produce a highly localized Notch gradient at the crypt base. Still, normal crypt homeostasis can be maintained after Paneth cell ablation, at least in the short term, by other secretory cell types including enteroendocrine and tuft cells that gain DLL-1 expression and migrate down to occupy the space left by Paneth cells at the crypt base.[17] Such cellular redundancy highlights the importance of maintaining functional Notch signaling within the intestinal stem cell niche. In the colon, regenerating family member 4 (REG4)-expressing deep crypt secretory cells have been proposed to function as the colon equivalent to Paneth cells and maintain the colon stem cell niche.[18]

In addition to Notch signaling, Paneth cells produce short-range secreted ligands including WNT ligands such as WNT3, WNT6, and WNT9b, and EGF family ligands such as EGF and transforming growth factor α. In vitro studies with murine small intestine organoid cultures supplemented with stem cell niche factors (EGF, Noggin, and R-spondin) indicate that WNT production from Paneth cells is sufficient to support organoid stem cell maintenance and proliferation. However, epithelial-specific WNT ligand production is not required or sufficient to maintain crypt homeostasis in vivo, suggesting redundancy of WNT signaling from the stromal compartment to maintain the intestinal stem cell niche. Similarly, EGF receptor (EGFR) signaling is critical for organoid growth in vitro, but not for normal intestinal homeostasis in vivo, suggesting signaling redundancy with other erb-b2 receptor tyrosine kinase (ERBB) receptors or receptor tyrosine kinases (RTKs). For example, Joosten et al. showed that MET proto-oncogene receptor tyrosine kinase (MET) signaling can compensate for a loss of EGFR signaling in normal and colorectal cancer stem cells.[19,20] Furthermore, the stromal compartment provides several ligands that also can activate ERBB receptors and other RTKs. Similar to Notch and WNT signaling, this redundancy highlights the importance of RTK signaling in supporting the intestinal stem cell niche. In addition to secreting niche factors, Paneth cells also provide antimicrobial peptides to ensure a sterile crypt environment and important metabolic niche signals to support ISCs.[21]

Stromal Compartment

Multiple stromal cell types are embedded in the lamina propria and contribute to a network that establishes a gradient of signaling molecules and supports the stem cell


A
Duodenum
Jejunum
Ileum
Proximal colon
Distal colon
Stomach
Cecum
B
Small intestine
Differentiation
BMP
Stemness
Notch
EGF
WNT
Villus
Crypt
C
Colon
Crypt
Paneth cell
Notch
NICD
Stem cell
DLL1/4
CSL
Stemness
Differentiation
EGF
EGFR
RAS
PI3K
β-catenin
TCF
β-catenin
BMPRII
BMPRI
WNT
LRP5/6
LGR4/5
Frizzled
BMP
WNT
RSPO
Gremlin1/2
Stromal cell
Differentiated cells:
Absorptive:
Enterocyte
Secretory:
Goblet cell
Tuft cell
Enteroendocrine cell
Paneth cell
Deep secretory cell
Stem cells:
Quiescent stem cell
Intestinal stem cell
Precursor cells:
Secretory progenitor cell
Absorptive progenitor cell
Transit-amplifying (TA) cell
Support cells:
Stromal cell
Macrophage
Lymphocyte
Dendritic cell
Neural cell

niche. Many of these supporting cell types have long projections that assist in cell-to-cell communication with the epithelial compartment. Recent studies have implicated overlapping populations of stromal cells that are defined by different cell surface markers and transcription factors including platelet derived growth factor receptor alpha (PDGFRα), CD34, glioma-associated oncogene homolog 1 (GLI1), and forkhead box L1 (FOXL1), which produce diverse agonistic and antagonistic ligands that regulate WNT/R-spondin and BMP pathways.[22–26] Despite marked mesenchymal expression of WNT2B, WNT4, and WNT5A in stromal cells surrounding intestinal crypts, studies blocking WNT secretion in either epithelial cells or myosin heavy chain 11 (MYH11)-positive myofibroblasts had no effect on ISC renewal.[27–32] However, ablation of FOXL1$^+$ telocytes (a distinct subset of mesenchymal cells) or blockade of WNT secretion from FOXL1$^+$ cells results in the rapid loss of ISCs and crypt failure, indicating that FOXL1$^+$ cells are an essential source of WNT ligands for ISC renewal. Interestingly, gene expression studies have shown that telocytes located in different regions of the crypt–villus axis have distinct functions. Telocytes located near the crypt base express WNT2B and R-spondin 3 (RSPO3), consistent with the high WNT activity that is required to maintain the intestinal stem cell niche. On the other hand, telocytes located at the tips of villi express WNT antagonists and noncanonical WNT5A, likely to inhibit proliferation and promote differentiation, cellular polarity, and villus formation.[33] Similarly, recent analysis of PDGFRα expression has identified 3 distinct stromal populations that establish a BMP signaling gradient in the intestine. Two of the PDGFRα^+ populations show low expression of PDGFRα and can be distinguished through the presence of the cell surface marker CD81 and the BMP inhibitor Gremlin1.[34] PDGFRα^{lo}CD81$^+$ cells (also known as trophocytes) that express high levels of Gremlin1 are located primarily surrounding the crypt base and block BMP signaling in the ISC compartment. PDGFRα^{lo} stromal cells that are negative for CD81 and Gremlin1 are situated above and around crypts. Contrastingly, PDGFRα^{hi} telocytes are abundant at the crypt–villus shoulder and express BMP ligands that drive differentiation in epithelial cells exiting the crypt. The stromal compartment also produces other signaling molecules including other EGF-like ligands, insulin-like growth factor 1 and 2 (IGF1/2), and metabolites such as prostaglandin E2 (PGE_2) that act directly on the epithelial compartment.[35–40]

Other supporting cells also can contribute to the intestinal stem cell niche. For example, Zhu et al[41] provided evidence that interleukin (IL)13 produced by group 2 innate lymphoid cells (ILCs) promotes the maintenance and self-renewal of LGR5$^+$ ISCs. IL10 secreted by regulatory T cells also help maintain the LGR5$^+$ ISC niche.[42] Likewise, Kosinski et al[43] showed that myofibroblasts and smooth muscle cells are a source of BMP antagonists (Gremlin1, Gremlin2, and Chordin-like 1) that maintain the stem cell niche at the crypt base.[43] Furthermore, each of the lineages that contribute to the intestinal stem cell niche are continuously responding to environmental cues and likely are activated in response to acute or chronic intestinal injury to aid in intestinal regeneration. The crosstalk between these cell populations and their relative contributions to the intestinal stem cell niche and regeneration after injury is an important area of ongoing research.

Intestinal Injury and Repair

The epithelium that lines the intestine faces harsh conditions from the luminal environment and is susceptible to a number of damaging events including infection with pathogenic organisms, acute or chronic inflammatory disease, as well as genotoxic stress associated with chemotherapy/radiation treatment. Therefore, numerous model systems have been developed to study intestinal injury and repair. Mice are the most frequently used animal model for intestinal studies because their intestinal development is similar to human beings, and they share many of the same genes and immune responses while remaining small and easy to genetically manipulate. The use of transgenic mouse lines has been imperative for advances in our understanding of cellular plasticity and regenerative responses in the intestine. For instance, knocking-in the *DTR* gene into the *Lgr5* locus allows toxin-induced LGR5$^+$ cell ablation and provides a way to conditionally study the response to specific stem cell loss in the intestine.[44] Other common injury models used to investigate the regenerative response in the intestine include treatment with high-dose radiation or doxorubicin (a chemotherapy drug), which results in the rapid loss of LGR5$^+$ ISCs as well as proliferating progenitor cells. Although each of these models affect the stem cell compartment, specific LGR5$^+$ ISC ablation allows proliferating progenitor cells to contribute to regeneration, whereas radiation or chemotherapy destroy most proliferating cells (including progenitors) and limits the pool of cells that contribute to regeneration to postmitotic cells. It also is important to note that there are regional differences in the response to epithelial damage along the proximal–distal axis. For example, it is well documented that the colon is more radioresistant than the small intestine.[45,46] This highlights the

Figure 1. (***See previous page***). **Diagram of the intestinal structure and the stem cell niche.** The intestinal epithelium is a monolayer of cells with a distinct composition along the proximal–distal axis. In both the small intestine and colon, the stem cell compartment is located at the base of the crypt. Stem cells continuously generate transit-amplifying and progenitor populations that further differentiate into 5 main functional cell types: enterocytes, goblet cells, Paneth cells, enteroendocrine cells, and tuft cells. Signaling molecules produced by cells within the epithelial compartment as well as a network of supporting cells establish a gradient to balance between stemness and differentiation in the crypt. WNT, EGF, and Notch signaling are restricted to the base of the crypt and work to maintain intestinal stem cells. BMP antagonists such as Gremlin1/2 also are restricted to the crypt base to establish a BMP signaling gradient from the crypt–villus shoulder to the villus tip and promote differentiation in cells exiting the crypt. BMPRI, bone morphogenetic protein receptor type 1B; BMPRII, bone morphogenetic protein receptor type 2; CSL, CBF-1/Supressor of Hairless/Lag-1; EGFR, epidermal growth factor receptor; PI3K, phosphatidylinositol 3-kinase; RAS, rat sarcoma viral oncogene; RSPO, R-spondin; TCF, transcription factor 4.

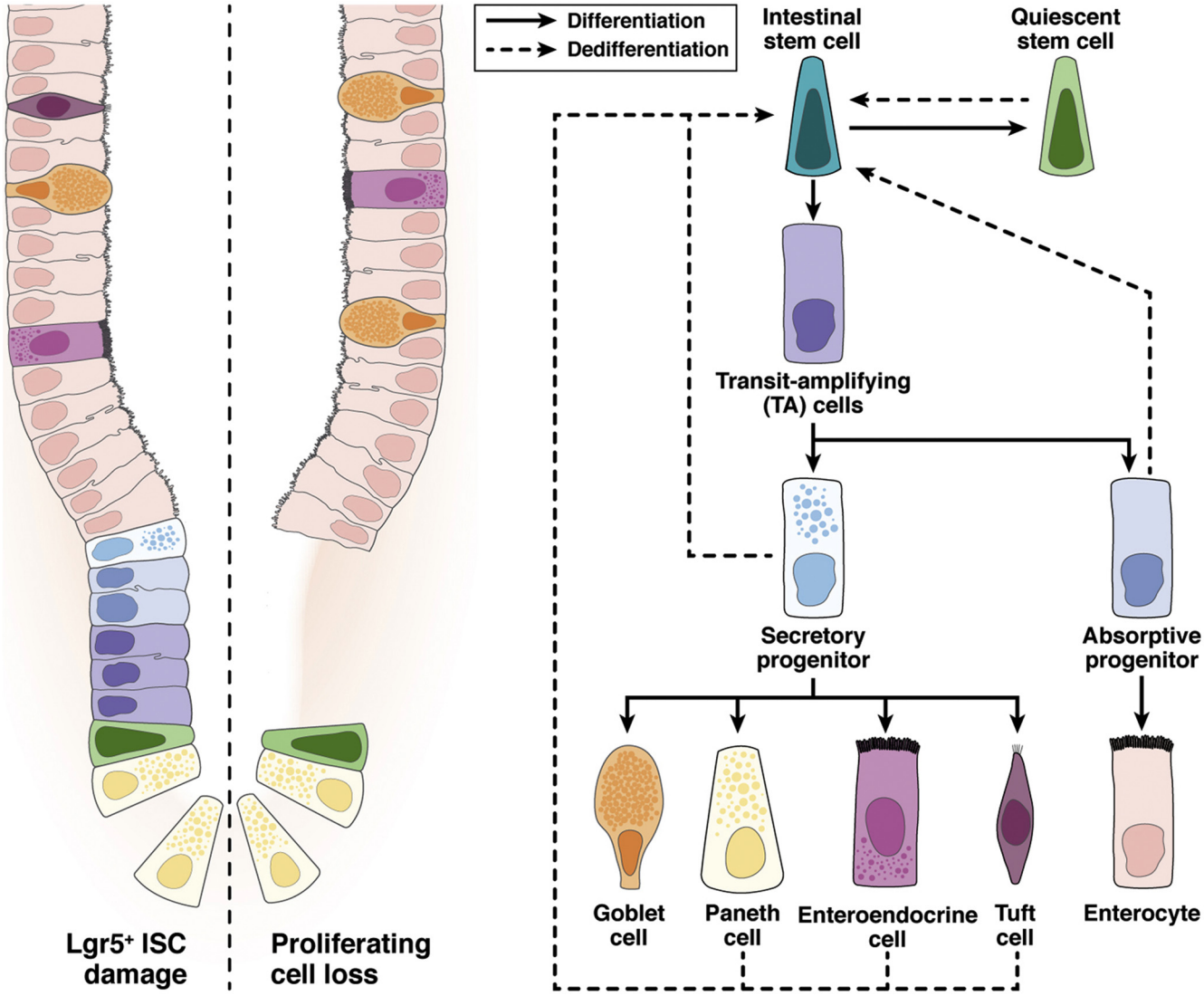


Figure 2. Diagram of cells that show cellular plasticity and dedifferentiate after damage. In an injury-dependent manner, there is hierarchical dedifferentiation and mobilization of epithelial cells that contribute to regeneration. Cells from both the secretory and absorptive lineage branches are capable of dedifferentiating to LGR5^{+} intestinal stem cells to repopulate the crypt after damage.

need for further investigations that take both the damaging event and the regional specification into consideration.

Cellular Plasticity

The intestinal epithelium shows remarkable flexibility upon damage. Acute injury that results in ISC loss activates a regenerative response to restore the stem cell compartment. Several groups have tried to identify the cellular source of intestinal regeneration after damage using genetic labeling and lineage tracing of distinct cell types. These approaches fail to assess the level of contribution from each population in overall epithelial regeneration and cannot exclude the possibility that other cellular sources exist. It appears that a variety of cell types are involved synchronously in damage-induced epithelial regeneration and several different cell types can acquire stem cell characteristics to repair the intestinal epithelium after stress, damage, infection, or disease (Figure 2). To clarify the degree each cell population contributes to overall epithelial regeneration, it is necessary to combine genetic lineage tracing, depletion studies, single-cell gene expression profiling, chromatin structure analysis, and organoid-formation assays.

Quiescent Stem Cells

LGR5^{+} cells are considered active multipotent intestinal stem cells because they rapidly divide and give rise to TA cells and all differentiated intestinal cell types. There is also a second population of ISCs, considered reserve ISCs, that are quiescent and resistant to stress but are mobilized upon injury to repopulate the crypt. Reserve intestinal stem cells generally reside in the +4 to +6 cell position from the crypt base. Several markers have been proposed for the reserve

stem cell population including B cell-specific Moloney murine leukemia virus integration site 1 (BMI1), leucine rich repeats and immunoglobulin like domains (LRIG1) HOP homeobox (HOPX), and mouse telomerase reverse transcriptase (MTERT). Lineage-tracing studies using *Bmi1*, *Lrig1*, *Hopx*, or *mTert* drivers show that reserve stem cell populations proliferate in response to irradiation damage.[44,47–49] Reserve stem cells appear to share some properties with label-retaining cells (LRCs) originally described by Potten et al,[50] but represent a molecularly and functionally distinct population.[51] LRCs consist of both Paneth cells and non-Paneth cells located in the +4 position of the crypt or above. Importantly, Buczacki et al[52] showed that LRCs are heterogeneous and non-Paneth LRCs are a secretory progenitor that also have reserve stem cell activity. It has been proposed that reserve stem cells are required to protect the niche from stress, and to generate LGR5$^+$ stem cells and/or TA cells in response to damage. Therefore, maintenance of the quiescent stem cell population is considered a critical element of radioresistance. However, there is overlapping gene expression between many of the active and reserve stem markers, highlighting both the plasticity and inter-relation of cells within the stem cell zone, but also raising the question of whether these cells represent discrete stem cell populations.

Progenitor Cells

Several lineage-committed progenitor cells, including ATOH1$^+$ or DLL-1$^+$ secretory progenitors or intestinal alkaline phosphatase (ALPI)-positive enterocyte progenitors, also can act as facultative stem cell populations capable of repopulating the crypt after injury.[53,54] For example, lineage tracing studies have indicated that all differentiated cell types can be derived from ALPI$^+$ enterocyte progenitors upon LGR5$^+$ ablation.[55] Similarly, DLL-1^{high} secretory progenitors retain cellular plasticity and can revert to a stem cell state after radiation damage.[56] It appears that more committed precursor populations also can contribute to regeneration, such as CD69$^+$CD274$^+$ goblet cell precursors.[57] However, it is important to remember that proliferating progenitor cell populations are more susceptible to radiation or chemotherapy treatment and therefore only are available to contribute to regeneration if they survive the damaging event.

Paneth Cells

Under homeostatic conditions, Paneth cells are postmitotic and support the stem cell niche and host defense. However, recent studies have provided evidence that mature Paneth cells and/or Paneth cell progenitors also can contribute to epithelial regeneration after damage to the intestine. For instance, Yu et al[58] showed that a subset of lysozyme 1$^+$ Paneth cells proliferate and produce differentiated cell types after radiation damage. Likewise, acute injury with doxorubicin treatment triggers defensin α4$^+$ Paneth cells to dedifferentiate into multipotent stem cells in a Notch-dependent manner.[59] On the other hand, Hayakawa et al[60] found that basic helx-loop-helix family member a15 (BHLHA15)-positive Paneth cells and short-lived secretory precursors showed no plasticity after LGR5$^+$ ISC ablation or radiation damage, but did contribute to regeneration after doxorubicin-induced injury. These results suggest that Paneth cell and/or Paneth cell progenitors represent at least one differentiated lineage that is capable of repopulating the crypt after at least some types of damage. However, the minimal regenerative contribution from defensin α6$^+$ Paneth cells after doxorubicin treatment highlights the need for a detailed analysis of Cre-expressing cells in the crypt compartment at a single-cell level.[61]

Enteroendocrine Cells

In contrast to the reserve ISC studies, Jadhav et al[57] suggested that BMI1$^+$ cells are not a dedicated population of quiescent stem cell, but instead are mature enteroendocrine cells that express *ChgA* and *Neurod1*. Whether a reserve ISC or enteroendocrine cell, it appears that BMI1$^+$ cells do contribute to intestinal regeneration after injury. In fact, Yan et al[62] showed that BMI1$^+$ cells are enriched for other enteroendocrine markers, including *Prox1*. Lineage tracing using a *Prox1* driver showed that mature enteroendocrine cells can acquire stemness during homeostasis and injury-induced regeneration.

Tuft Cells

Doublecortin-like kinase 1 (DCLK1) also previously was reported as a marker of quiescent stem cells, but it is highly expressed in chemosensory tuft cells residing in the stomach and intestine. Westphalen et al[63] described a rare subpopulation of long-lived DCLK1$^+$ cells that acquired stem cell properties under homeostatic conditions and played a critical role in regeneration. Ablation of DCLK1$^+$ cells before irradiation or dextran sodium sulfate (DSS) injury in mice diminished epithelial repair and caused crypt dropout. Not only did tuft cells show cellular plasticity during homeostasis and regeneration, but both tuft cells and enteroendocrine cells showed cellular plasticity and up-regulated Notch signals to support the stem cell niche upon Paneth cell depletion.[17]

For many of the cell types involved in the regenerative response, reversion to a stem cell state analogous to LGR5$^+$ ISCs is required. Indeed, Metcalfe et al[64] showed that recovery after radiation-induced damage is acutely perturbed in the presence of sustained depletion of LGR5$^+$ ISCs with diphtheria toxin. In this case, regenerating cells must transition to a LGR5$^+$ ISC state before they contribute to repair of the intestinal epithelium. Other studies have suggested that a subpopulation of slow-cycling mex-3 RNA binding family member A (MEX3A) or Keratin 15–expressing LGR5+ ISCs are less radiosensitive, allowing them to contribute to radiation-induced regeneration. On the other hand, it has been suggested that early progeny of LGR5$^+$ and Olfactomedin 4 (OLFM4)-positive cells are available to support regeneration after chemotherapy damage owing to a reduced DNA damage response and lack of apoptosis induction.[61] Sheng et al[65] described a heterogenous population of Musashi RNA Binding Protein 1 (MSI1)-positive cells

that also showed DNA damage resistance. Interestingly, rapid-cycling MSI1$^+$ cells can contribute to postradiation regeneration in the absence of LGR5 expression, questioning the need to transition to an LGR5-expressing cell population before initiating a regenerative response. Thus, the efficiency and hierarchical contribution of different intestinal cell types likely is dependent on the type and extent of injury and the intestinal region involved. Regardless, it appears that the simultaneous activation of multiple cell lineages to acquire stemness and proliferate is required to combat and overcome many different types of intestinal injury.

Regulation of Plasticity

Many of the signaling pathways that support the intestinal stem cell niche also are up-regulated to promote postinjury cellular plasticity and regeneration, including both the WNT and Notch signaling networks.

Achaete-Scute Complex Homolog 2 Protein

WNT signaling is one of the most important regulators of adult intestinal stem cells. Under homeostasis, *Ascl2* is a WNT target gene that is restricted to LGR5$^+$ ISCs. Achaete-scute complex homolog 2 (ASCL2) is a basic helix-loop-helix transcription factor that works together with β-catenin/transcription factor 4 (TCF4) to activate transcription of stem cell genes and coordinate stemness in the intestinal epithelium in a WNT-dependent manner.[66] Murata et al[67] showed increased expression of *Ascl2* in regenerating cells and showed that ASCL2 is essential for crypt cell dedifferentiation after ISC injury. Therefore, it is likely that the transcription factor ASCL2 can regulate gene expression in both adult ISCs and in the course of cellular plasticity after injury. Murata et al[67] suggested that *Il11rα1* may be a functional target gene of ASCL2, however, further studies are needed to fully understand the mechanistic role of ASCL2 in intestinal regeneration.

Hippo Pathway: Yes-Associated Protein 1/Tafazzin Signaling

The Hippo signaling pathway plays a significant role in intestinal development, regeneration, and cancer.[68] At the center of this pathway is a kinase cascade that starts with activation of macrophage stimulating 1 (MST) and ends with phosphorylation of transcriptional regulators yes-associated protein (YAP) and tafazzin (TAZ), resulting in their cytoplasmic sequestration and/or degradation. Hippo activity is regulated by several input signals including immune modulators, mechanotransduction, prostaglandins, and WNT signaling. Interestingly, regenerating crypts have increased YAP levels and YAP inactivation severely impairs intestinal regeneration. It appears that, at least in part, the Hippo pathway functions to counterbalance WNT signaling because YAP/TAZ inhibit WNT target genes.[69,70] In addition, YAP accumulation in the nuclei of regenerating crypt cells activates the transcription of a number of genes that help with wound healing including *Areg*, *Ereg*, *IL33*, C*tgf*, and *Ccn1*. Hyperactivation of YAP, on the other hand, is associated with cancer development.[71] Thus, the Hippo pathway must be tightly regulated to promote tissue regeneration, but also block tumorigenesis.

Stem Cell Factor/c-Kit Receptor Signaling

Stem cell factor (SCF) is a ligand for the receptor tyrosine kinase c-Kit. SCF-mediated activation of c-Kit results in autophosphorylation and initiation of downstream signaling. It has been reported that SCF/c-Kit signaling plays a role in cell survival, proliferation, and migration. Although certain signaling events may be present in all regenerating cells, other pathways may be restricted to specific cell subsets or types of injury. For example, levels of SCF expression are increased with inflammation, as seen with inflammatory bowel disease patients or in DSS mouse models, while the c-Kit receptor is expressed by antimicrobial-secreting Paneth cells. Schmitt et al[72] showed that the SCF/c-Kit signaling axis contributes to Paneth cell plasticity after inflammation-driven ISC injury. It appears that Paneth cells acquire stem-like features downstream of inflammatory activation of c-Kit through glycogen synthase kinase 3 beta (GSK3β) inhibition and WNT activation. Several other tyrosine kinases also appear to play a role in intestinal regeneration, including SRC proto-oncogene non-receptor tyrosine kinase (SRC) and MET.[19,73]

Notch Signaling Pathway

Although Notch signaling remains a critical component of the intestinal stem cell niche, it appears that up-regulation of Notch signaling also is associated with aspects of cellular plasticity and regeneration. Yu et al[58] showed that Paneth cells from irradiated mice that were undergoing dedifferentiation gained ectopic expression of *Notch1* and activated the Notch signaling pathway. In addition, forced expression of the Notch intracellular domain in Paneth cells promoted dedifferentiation in the absence of injury. Furthermore, Paneth cell dedifferentiation after doxorubicin-induced injury was inhibited after loss of Notch signaling in Paneth cell progenitors.[59] Taken together, these results suggest that Paneth cell plasticity after intestinal injury is regulated by Notch activation.

Fetal-Like Transcriptional Program

Although the role of WNT, YAP/TAZ, RTKs, and Notch signaling in regeneration is well established, there are other signaling networks that coordinate cellular plasticity in response to damage to the intestinal epithelium. For instance, several groups have reported a fetal-like transcriptional program, distinguished by the expression of *Sca-1*, in regenerative lineages.[74–76] In this case, intestinal epithelial cells repurpose aspects of fetal development to promote repair. Interestingly, single-cell transcriptomics have identified a rare population of quiescent facultative stem cells termed *revival stem cells*, which are marked by clusterin (CLU) expression. CLU$^+$ revival stem cells express stem cell antigen-1 (SCA-1 or Ly6a) after injury, suggesting potential overlap with a fetal gene signature involved in regeneration. CLU$^+$ cells do not contribute to homeostasis

but are mobilized in a YAP-dependent manner to produce LGR5$^+$ ISCs after various mechanisms of intestinal injury including irradiation, LGR5$^+$ cell ablation, and DSS-induced colitis.[77]

Other Nonepithelial Signals

In addition to the intrinsic signaling networks that regulate cellular plasticity, stromal and immune cell populations accumulate at sites of damage in the intestine and secrete factors that promote the regenerative process. Jarde et al[36] showed that neuregulin 1, an EGF family ligand, is up-regulated by stromal cells, macrophages, and Paneth cells to drive stemness and proliferation upon radiation or chemotherapy agent–induced injury. Neuregulin activates the mitogen-activated protein kinase and phosphatidylinositol 3-kinase/AKT signaling pathways, and loss of *Neuregulin 1* severely compromises the regenerative capacity of the intestinal epithelium. Similarly, Zhang et al[37] showed that a Cdc42–mitogen-activated protein kinase program is required for regeneration, and elevating a splice variant of Cdc42 (V2) enhances tissue repair. Another example of regulation by the microenvironment is CD34$^+$ stromal cells that up-regulate *Gremlin1* and *R-spondin1* in response to DSS-induced colitis in mice to inhibit BMP and amplify WNT signaling in nearby epithelial cells.[26] Likewise, Degirmenci et al[23] showed that a subset of GLI1$^+$ mesenchymal cells also contribute to post-DSS treatment recovery by overexpressing RSPO3 to boost WNT signaling. Complementary changes were observed in immune cells, for example, macrophage-derived WNT ligands packaged in extracellular vesicles are critical for recovery after radiation-induced injury.[78] Group 3 ILC-derived IL22 also promoted repair of the intestinal epithelium after graft-versus-host disease or chemotherapy-induced damage.[79,80] Alternatively, Romera-Hernandez et al[81] reported that group 3 ILC-driven tissue regeneration is independent of IL22 and relies on amplification of the Hippo/YAP signaling pathways. Altogether, it is likely that multicellular cooperation and a sophisticated network of connected signaling pathways is required to restore intestinal homeostasis after injury.

Epigenomic State and Chromatin Remodeling

Although there is direct evidence that upon damage crypt regeneration is achieved through the mobilization and dedifferentiation of a diverse population of facultative stem cells, the precise role that epigenetic mechanisms play in regulating cell fate decisions during homeostasis and restoring ISC function after injury still is poorly understood. Early studies on chromatin status indicated that adult ISCs and differentiated cells generally possess similar DNA methylation as well as enhancer and open chromatin profiles.[82–84] Not only does the broadly permissive epigenomic state between ISCs and committed lineages provide a platform for Notch/ATOH1-dependent lateral inhibition and lineage specification, but the lack of chromatin barrier provides a simple explanation for achieving cellular plasticity during regeneration. Therefore, it is likely that transcription factor occupancy plays a major role in the outcome for both cell fate decisions and dedifferentiation, and activation of distinct signaling pathways to induce appropriate transcription factor expression is required. Recent studies have arrived at a conflicting conclusion that suggests a more dynamic chromatin regulation during cell fate specification into differentiated intestinal cell types.[57,85–88] In the case of injury-induced dedifferentiation of committed progenitor populations such as preterminal enteroendocrine cells and maturing goblet cell progenitors, histone methylation remodeling is required to overcome the chromatin barrier and allow dedifferentiation. Although investigations into epigenetic factors that regulate intestinal differentiation are in their infancy, data suggest that different signaling pathways and transcription factors are used based on the region of the intestine and how the epithelium is injured (eg, whole-body irradiation, ISC ablation, chronic inflammation, and so forth). Remodeling a chromatin barrier likely is dependent on the extent of injury, for example, LGR5$^+$ cell ablation leaves proliferating progenitors available for dedifferentiation, whereas dedifferentiation after radiation-induced injury is dependent on the level of radiation and radiosensitivity in different regions of the intestine. Thus, the signaling pathways involved in driving dedifferentiation in the context of permissive chromatin likely are different from those required to initiate chromatic remodeling.

Implications for Tumor Initiation

LGR5$^+$ stem cells and committed progenitor populations share a similar epigenomic profile, which provides a reasonable mechanism for crypt progenitor dedifferentiation and repopulation of the stem cell compartment after stress or damage. An important area of ongoing research is whether similar mechanisms of cellular plasticity are present during tumor initiation. Two key models for the cellular origin of intestinal tumorigenesis have been proposed: stem vs non–stem cell origin. The bottom up model claims that early adenoma formation originates from stem cells at the bottom of the crypt, while the top down model asserts that tumor initiation occurs at the top of the crypt independent of crypt stem cells.[89,90] To test both models, WNT-activating mutations or perturbations in other signaling pathways (such as kirsten rat sarcoma viral oncogene homolog (KRAS) and nuclear factor-κB) are introduced to specific cell types throughout the crypt–villus axis. For example, loss of adenomatous polyposis coli (*APC)* in LGR5$^+$ stem cells triggers adenoma formation, supporting the bottom up model. Likewise, WNT-activating mutations in specific +4 reserve stem cell populations (eg, prominin 1 (PROM1)-positive, or LRIG1$^+$) also can initiate tumor formation.[49,91] However, because of the overlapping gene expression of +4 reserve stem cell markers in LGR5$^+$ stem cells, it is uncertain whether adenoma formation in +4 stem cells represents a distinct transforming event that occurs separate of the LGR5$^+$ ISCs. Dissimilar to stem cell populations, Barker et al[92] showed that *APC* inactivation in short-lived TA cells using an *AhCre* driver produced foci with limited

expansion that failed to progress to adenomas. In the early stages of tumorigenesis, dysplastic cells often were observed in the upper crypt region with morphologically normal cells at the base in support of the top down model. Furthermore, Metcalfe et al[64] showed that adenoma initiation occurred in LGR5$^-$ cell populations when diphtheria toxin receptor–mediated LGR5$^+$ ISC ablation was combined with *APC* loss. Although it is clear that tumor initiation can originate outside the LGR5$^+$ stem cells, a non–stem cell origin has required constitutive WNT activation in combination with tissue damage, compound mutations, or alterations in the microenvironment to drive tumorigenesis. For instance, WNT-dependent tumor initiation in radioresistant keratin 19$^+$ cells only occurred after radiation-induced injury and loss of LGR5$^+$ ISCs.[93] Likewise, constitutive WNT activation in X-box binding protein 1 (XBP1)-positive villus cells and Paneth cells failed to produce adenomas until WNT signaling was coupled with activation of oncogenic KRAS or loss of NFKB inhibitor alpha (IκBα).[94] In addition, *APC*-deficient Paneth cells are insensitive to WNT activation, but upon Notch activation and dedifferentiation into stem cell/progenitors they become WNT responsive and readily are transformed. *APC*-deficient DCLK1$^+$ tuft cells also readily can form tumors in the presence of chronic inflammation in the colon.[63] Unlike other intestinal cell types, *APC* or *APC/KRAS* mutations in ALPI$^+$ enterocyte progenitors fail to form tumors. It has been proposed that mutated enterocyte progenitors cannot form adenomas because these cells rapidly migrate toward the villus tips where they are shed into the lumen, not allowing enough time for tumor initiation.[55] Although cellular plasticity is implicated in tumor initiation, cancer cells also harness plasticity to evade and become resistant to therapy.[95] Understanding the role of cellular plasticity and the underlying signaling networks that drive cancer development in the intestine are important to identify preventative approaches, diagnostic tools, and therapeutic strategies.

Concluding Remarks and Future Perspectives

In this review, we have highlighted some of the key regulatory signals and pathways that are required for homeostasis, cellular plasticity, and postinjury regeneration of the intestinal epithelium. Under homeostasis, the intestinal stem cell niche is composed of a multicellular and highly interactive signaling network that continually monitors its surroundings to achieve balance between stemness and differentiation. The epithelium that lines the intestine is highly susceptible to damage from the constant exposure to harsh luminal insults and pathogenic states including infections and inflammation. However, the intestine has a remarkable ability to repair itself. Dedifferentiation of intestinal cells into multipotent stem cells, a process cumulatively termed *intestinal cell plasticity*, provides a mechanism to maintain barrier integrity and homeostatic stability in the face of persistent injury. Still, one of the major outstanding questions is as follows: how is disruption or loss of the LGR5$^+$ stem cell compartment sensed? It is probable that different types of injury produce distinct signals and cellular responses, but this remains an active area of ongoing research. Nevertheless, it appears that many intestinal cell types can undergo dedifferentiation to multipotent ISCs and there is likely a hierarchical mobilization of different cell populations in an injury-dependent manner. For example, the regenerative response after radiation-induced genotoxic injury, which kills LGR5$^+$ ISCs and most proliferating cell progeny, is reliant on different radioresistant cell populations including more quiescent cells such as secretory cell progenitors or distinct proliferating progenitors that display reduced DNA damage responses. How these different populations contribute to regeneration may depend on the extent of injury and regional differences in sensitivity to injury. For example, the colon is more radioresistant than the small intestine, likely owing to differences in the composition and regulation of the stem cell niche in each region. It also remains to be seen whether the facultative stem cell populations mobilized by genotoxic insults are the same as those involved in regenerative responses to more pathophysiological stressors such as infection or inflammation. If progenitor cells or other committed precursor lineages withstand the injury, their ability to dedifferentiate and contribute to regeneration also likely is dependent on having the appropriate signaling networks necessary to overcome any chromatin barrier. The chromatin barrier to dedifferentiate is lower in early progenitor cell populations than more mature cell types.

Most of the work in the field of intestinal cellular plasticity and regeneration has combined mouse intestinal injury models with Cre-dependent reporter systems that often lack cell-type specificity and sufficient spatial and temporal control. In the future, it is important to implement more unbiased approaches with single-cell resolution to examine the hierarchy and relative contribution of distinct cell types and signaling networks to overall repair. This will be particularly important for addressing whether similar facultative stem cell populations and mechanisms of regeneration occur in the human intestine. To date, studies of cellular plasticity in the human intestine have been limited. There are likely significant differences between mouse and human intestine stem cell niches. For instance, in human beings, crypt–villus structures are formed during fetal development, unlike mice, in which the crypt–villus axis is established postnatally. In addition, the kinetics of stem cell proliferation and neutral drift are slower in human beings, indicating that the dynamics of regeneration in human beings also may be different. Indeed, organoid cultures suggest the human intestinal stem cell niche is far more complex and requires more stem cell signals than mice. Although it remains unclear if current human intestinal organoids are an appropriate in vitro model to study such regenerative events, the development of more complex multicellular in vitro systems that recapitulate the intestinal stem cell niche is an important avenue for further study.

Recent advances in intestinal regeneration have suggested that cellular plasticity and dedifferentiation are a common feature of injury-induced regenerative events and

in many cases involve up-regulation of intestinal stem cell niche signaling pathways. Understanding the hierarchical nature of these regenerative events may provide new insights and approaches to improved intestinal regeneration and repair during chronic injury such as in inflammatory bowel disease and new therapeutic avenues to address tumor resistance that focus on impacting multiple aspects of cancer stem cells and the tumor microenvironment.

References

1. Barker N. Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nat Rev Mol Cell Biol 2014;15:19–33.
2. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007;449:1003–1007.
3. Nicoletti C. Unsolved mysteries of intestinal M cells. Gut 2000;47:735–739.
4. Bottcher A, Buttner M, Tritschler S, Sterr M, Aliluev A, Oppenlander L, Burtscher I, Sass S, Irmler M, Beckers J, Ziegenhain C, Enard W, Schamberger AC, Verhamme FM, Eickelberg O, Theis FJ, Lickert H. Non-canonical Wnt/PCP signalling regulates intestinal stem cell lineage priming towards enteroendocrine and Paneth cell fates. Nat Cell Biol 2021;23:23–31.
5. Herring CA, Banerjee A, McKinley ET, Simmons AJ, Ping J, Roland JT, Franklin JL, Liu Q, Gerdes MJ, Coffey RJ, Lau KS. 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.
6. Patankar JV, Becker C. Cell death in the gut epithelium and implications for chronic inflammation. Nat Rev Gastroenterol Hepatol 2020;17:543–556.
7. Barriga FM, Montagni E, Mana M, Mendez-Lago M, Hernando-Momblona X, Sevillano M, Guillaumet-Adkins A, Rodriguez-Esteban G, Buczacki SJA, Gut M, Heyn H, Winton DJ, Yilmaz OH, Attolini CS, Gut I, Batlle E. Mex3a marks a slowly dividing subpopulation of Lgr5+ intestinal stem cells. Cell Stem Cell 2017;20:801–816 e7.
8. Basak O, van de Born M, Korving J, Beumer J, van der Elst S, van Es JH, Clevers H. Mapping early fate determination in Lgr5+ crypt stem cells using a novel Ki67-RFP allele. EMBO J 2014;33:2057–2068.
9. Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD, Clevers H. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell 2010;143:134–144.
10. Lopez-Garcia C, Klein AM, Simons BD, Winton DJ. Intestinal stem cell replacement follows a pattern of neutral drift. Science 2010;330:822–825.
11. Fre S, Huyghe M, Mourikis P, Robine S, Louvard D, Artavanis-Tsakonas S. Notch signals control the fate of immature progenitor cells in the intestine. Nature 2005;435:964–968.
12. Kim TH, Shivdasani RA. Genetic evidence that intestinal Notch functions vary regionally and operate through a common mechanism of Math1 repression. J Biol Chem 2011;286:11427–11433.
13. Tian H, Biehs B, Chiu C, Siebel CW, Wu Y, Costa M, de Sauvage FJ, Klein OD. Opposing activities of Notch and Wnt signaling regulate intestinal stem cells and gut homeostasis. Cell Rep 2015;11:33–42.
14. van Es JH, van Gijn ME, Riccio O, van den Born M, Vooijs M, Begthel H, Cozijnsen M, Robine S, Winton DJ, Radtke F, Clevers H. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. Nature 2005;435:959–963.
15. Andreu P, Peignon G, Slomianny C, Taketo MM, Colnot S, Robine S, Lamarque D, Laurent-Puig P, Perret C, Romagnolo B. A genetic study of the role of the Wnt/beta-catenin signalling in Paneth cell differentiation. Dev Biol 2008;324:288–296.
16. Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 2011;469:415–418.
17. van Es JH, Wiebrands K, Lopez-Iglesias C, van de Wetering M, Zeinstra L, van den Born M, Korving J, Sasaki N, Peters PJ, van Oudenaarden A, Clevers H. Enteroendocrine and tuft cells support Lgr5 stem cells on Paneth cell depletion. Proc Natl Acad Sci U S A 2019;116:26599–26605.
18. Sasaki N, Sachs N, Wiebrands K, Ellenbroek SI, Fumagalli A, Lyubimova A, Begthel H, van den Born M, van Es JH, Karthaus WR, Li VS, Lopez-Iglesias C, Peters PJ, van Rheenen J, van Oudenaarden A, Clevers H. 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.
19. Joosten SPJ, Zeilstra J, van Andel H, Mijnals RC, Zaunbrecher J, Duivenvoorden AAM, van de Wetering M, Clevers H, Spaargaren M, Pals ST. MET signaling mediates intestinal crypt-villus development, regeneration, and adenoma formation and is promoted by stem cell CD44 isoforms. Gastroenterology 2017;153:1040–1053 e4.
20. Joosten SPJ, Mizutani T, Spaargaren M, Clevers H, Pals ST. MET signaling overcomes epidermal growth factor receptor inhibition in normal and colorectal cancer stem cells causing drug resistance. Gastroenterology 2019;157:1153–1155 e1.
21. Rodriguez-Colman MJ, Schewe M, Meerlo M, Stigter E, Gerrits J, Pras-Raves M, Sacchetti A, Hornsveld M, Oost KC, Snippert HJ, Verhoeven-Duif N, Fodde R, Burgering BM. Interplay between metabolic identities in the intestinal crypt supports stem cell function. Nature 2017;543:424–427.
22. Aoki R, Shoshkes-Carmel M, Gao N, Shin S, May CL, Golson ML, Zahm AM, Ray M, Wiser CL, Wright CV, Kaestner KH. Foxl1-expressing mesenchymal cells constitute the intestinal stem cell niche. Cell Mol Gastroenterol Hepatol 2016;2:175–188.
23. Degirmenci B, Valenta T, Dimitrieva S, Hausmann G, Basler K. GLI1-expressing mesenchymal cells form the

essential Wnt-secreting niche for colon stem cells. Nature 2018;558:449–453.
24. Greicius G, Kabiri Z, Sigmundsson K, Liang C, Bunte R, Singh MK, Virshup DM. PDGFRalpha(+) 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.
25. Shoshkes-Carmel M, Wang YJ, Wangensteen KJ, Toth B, Kondo A, Massasa EE, Itzkovitz S, Kaestner KH. Subepithelial telocytes are an important source of Wnts that supports intestinal crypts. Nature 2018; 557:242–246.
26. Stzepourginski I, Nigro G, Jacob JM, Dulauroy S, Sansonetti PJ, Eberl G, Peduto L. 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.
27. Klostermeier UC, Barann M, Wittig M, Hasler R, Franke A, Gavrilova O, Kreck B, Sina C, Schilhabel MB, Schreiber S, Rosenstiel P. A tissue-specific landscape of sense/antisense transcription in the mouse intestine. BMC Genomics 2011;12:305.
28. 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.
29. Durand A, Donahue B, Peignon G, Letourneur F, Cagnard N, Slomianny C, Perret C, Shroyer NF, Romagnolo B. 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.
30. Kim TH, 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.
31. Kabiri Z, Greicius G, Madan B, Biechele S, Zhong Z, Zaribafzadeh H, Edison, Aliyev J, Wu Y, Bunte R, Williams BO, Rossant J, Virshup DM. Stroma provides an intestinal stem cell niche in the absence of epithelial Wnts. Development 2014;141:2206–2215.
32. 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.
33. Gregorieff A, Pinto D, Begthel H, Destree O, Kielman M, Clevers H. Expression pattern of Wnt signaling components in the adult intestine. Gastroenterology 2005; 129:626–638.
34. McCarthy N, Manieri E, Storm EE, Saadatpour A, Luoma AM, Kapoor VN, Madha S, Gaynor LT, Cox C, Keerthivasan S, Wucherpfennig K, Yuan GC, de Sauvage FJ, Turley SJ, Shivdasani RA. Distinct mesenchymal cell populations generate the essential intestinal BMP signaling gradient. Cell Stem Cell 2020;26:391–402 e5.
35. Lee D, Pearsall RS, Das S, Dey SK, Godfrey VL, Threadgill DW. Epiregulin is not essential for development of intestinal tumors but is required for protection from intestinal damage. Mol Cell Biol 2004; 24:8907–8916.
36. Jarde T, Chan WH, Rossello FJ, Kaur Kahlon T, Theocharous M, Kurian Arackal T, Flores T, Giraud M, Richards E, Chan E, Kerr G, Engel RM, Prasko M, Donoghue JF, Abe SI, Phesse TJ, Nefzger CM, McMurrick PJ, Powell DR, Daly RJ, Polo JM, Abud HE. Mesenchymal niche-derived neuregulin-1 drives intestinal stem cell proliferation and regeneration of damaged epithelium. Cell Stem Cell 2020;27:646–662 e7.
37. Zhang X, Bandyopadhyay S, Araujo LP, Tong K, Flores J, Laubitz D, Zhao Y, Yap G, Wang J, Zou Q, Ferraris R, Zhang L, Hu W, Bonder EM, Kiela PR, Coffey R, Verzi MP, Ivanov II, Gao N. Elevating EGFR-MAPK program by a nonconventional Cdc42 enhances intestinal epithelial survival and regeneration. JCI Insight 2020; 5:e135923.
38. Ziegler AN, Feng Q, Chidambaram S, Testai JM, Kumari E, Rothbard DE, Constancia M, Sandovici I, Cominski T, Pang K, Gao N, Wood TL, Levison SW. Insulin-like growth factor II: an essential adult stem cell niche constituent in brain and intestine. Stem Cell Rep 2019;12:816–830.
39. Zhou W, Rowitz BM, Dailey MJ. Insulin/IGF-1 enhances intestinal epithelial crypt proliferation through PI3K/Akt, and not ERK signaling in obese humans. Exp Biol Med (Maywood) 2018;243:911–916.
40. Miyoshi H, VanDussen KL, Malvin NP, Ryu SH, Wang Y, Sonnek NM, Lai CW, Stappenbeck TS. Prostaglandin E2 promotes intestinal repair through an adaptive cellular response of the epithelium. EMBO J 2017; 36:5–24.
41. Zhu P, Zhu X, Wu J, He L, Lu T, Wang Y, Liu B, Ye B, Sun L, Fan D, Wang J, Yang L, Qin X, Du Y, Li C, He L, Ren W, Wu X, Tian Y, Fan Z. IL-13 secreted by ILC2s promotes the self-renewal of intestinal stem cells through circular RNA circPan3. Nat Immunol 2019; 20:183–194.
42. Biton M, Haber AL, Rogel N, Burgin G, Beyaz S, Schnell A, Ashenberg O, Su CW, Smillie C, Shekhar K, Chen Z, Wu C, Ordovas-Montanes J, Alvarez D, Herbst RH, Zhang M, Tirosh I, Dionne D, Nguyen LT, Xifaras ME, Shalek AK, von Andrian UH, Graham DB, Rozenblatt-Rosen O, Shi HN, Kuchroo V, Yilmaz OH, Regev A, Xavier RJ. T helper cell cytokines modulate intestinal stem cell renewal and differentiation. Cell 2018; 175:1307–1320 e22.
43. Kosinski C, Li VSW, Chan ASY, et al. Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors. Proc Natl Acad Sci 2007;104:15418–15423.
44. Tian H, Biehs B, Warming S, Leong KG, Rangell L, Klein OD, de Sauvage FJ. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature 2011;478:255–259.
45. Hua G, Wang C, Pan Y, Zeng Z, Lee SG, Martin ML, Haimovitz-Friedman A, Fuks Z, Paty PB, Kolesnick R. Distinct levels of radioresistance in Lgr5(+) colonic epithelial stem cells versus Lgr5(+) small intestinal stem cells. Cancer Res 2017;77:2124–2133.
46. Hua G, Thin TH, Feldman R, Haimovitz-Friedman A, Clevers H, Fuks Z, Kolesnick R. Crypt base columnar

stem cells in small intestines of mice are radioresistant. Gastroenterology 2012;143:1266–1276.
47. Takeda N, Jain R, LeBoeuf MR, Wang Q, Lu MM, Epstein JA. Interconversion between intestinal stem cell populations in distinct niches. Science 2011; 334:1420–1424.
48. Montgomery RK, Carlone DL, Richmond CA, Farilla L, Kranendonk ME, Henderson DE, Baffour-Awuah NY, Ambruzs DM, Fogli LK, Algra S, Breault DT. Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. Proc Natl Acad Sci U S A 2011;108:179–184.
49. Powell AE, Wang Y, Li Y, Poulin EJ, Means AL, Washington MK, Higginbotham JN, Juchheim A, Prasad N, Levy SE, Guo Y, Shyr Y, Aronow BJ, Haigis KM, Franklin JL, Coffey RJ. The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. Cell 2012; 149:146–158.
50. Potten CS, Owen G, Booth D. Intestinal stem cells protect their genome by selective segregation of template DNA strands. J Cell Sci 2002;115:2381–2388.
51. Li N, Nakauka-Ddamba A, Tobias J, Jensen ST, Lengner CJ. Mouse label-retaining cells are molecularly and functionally distinct from reserve intestinal stem cells. Gastroenterology 2016;151:298–310 e7.
52. Buczacki SJ, Zecchini HI, Nicholson AM, Russell R, Vermeulen L, Kemp R, Winton DJ. Intestinal label-retaining cells are secretory precursors expressing Lgr5. Nature 2013;495:65–69.
53. Tomic G, Morrissey E, Kozar S, Ben-Moshe S, Hoyle A, Azzarelli R, Kemp R, Chilamakuri CSR, Itzkovitz S, Philpott A, Winton DJ. Phospho-regulation of ATOH1 is required for plasticity of secretory progenitors and tissue regeneration. Cell Stem Cell 2018;23:436–443 e7.
54. Ishibashi F, Shimizu H, Nakata T, Fujii S, Suzuki K, Kawamoto A, Anzai S, Kuno R, Nagata S, Ito G, Murano T, Mizutani T, Oshima S, Tsuchiya K, Nakamura T, Watanabe M, Okamoto R. Contribution of ATOH1(+) cells to the homeostasis, repair, and tumorigenesis of the colonic epithelium. Stem Cell Rep 2018; 10:27–42.
55. Tetteh PW, Basak O, Farin HF, Wiebrands K, Kretzschmar K, Begthel H, van den Born M, Korving J, de Sauvage F, van Es JH, van Oudenaarden A, Clevers H. Replacement of lost Lgr5-positive stem cells through plasticity of their enterocyte-lineage daughters. Cell Stem Cell 2016;18:203–213.
56. van Es JH, Sato T, van de Wetering M, Lyubimova A, Yee Nee AN, Gregorieff A, Sasaki N, Zeinstra L, van den Born M, Korving J, Martens ACM, Barker N, van Oudenaarden A, Clevers H. Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. Nat Cell Biol 2012;14:1099–1104.
57. Jadhav U, Saxena M, O'Neill NK, Saadatpour A, Yuan GC, Herbert Z, Murata K, Shivdasani RA. Dynamic reorganization of chromatin accessibility signatures during dedifferentiation of secretory precursors into Lgr5+ intestinal stem cells. Cell Stem Cell 2017; 21:65–77 e5.
58. Yu S, Tong K, Zhao Y, Balasubramanian I, Yap GS, Ferraris RP, Bonder EM, Verzi MP, Gao N. Paneth cell multipotency induced by notch activation following injury. Cell Stem Cell 2018;23:46–59 e5.
59. Jones JC, Brindley CD, Elder NH, Myers MG Jr, Rajala MW, Dekaney CM, McNamee EN, Frey MR, Shroyer NF, Dempsey PJ. Cellular plasticity of Defa4(Cre)-expressing Paneth cells in response to notch activation and intestinal injury. Cell Mol Gastroenterol Hepatol 2019;7:533–554.
60. Hayakawa Y, Tsuboi M, Asfaha S, Kinoshita H, Niikura R, Konishi M, Hata M, Oya Y, Kim W, Middelhoff M, Hikiba Y, Higashijima N, Ihara S, Ushiku T, Fukayama M, Tailor Y, Hirata Y, Guha C, Yan KS, Koike K, Wang TC. BHLHA15-positive secretory precursor cells can give rise to tumors in intestine and colon in mice. Gastroenterology 2019;156:1066–1081 e16.
61. Sheahan BJ, Freeman AN, Keeley TM, Samuelson LC, Roper J, Hasapis S, Lee CL, Dekaney CM. Epithelial regeneration after doxorubicin arises primarily from early progeny of active intestinal stem cells. Cell Mol Gastroenterol Hepatol 2021;12:119–140.
62. Yan KS, Gevaert O, Zheng GXY, Anchang B, Probert CS, Larkin KA, Davies PS, Cheng ZF, Kaddis JS, Han A, Roelf K, Calderon RI, Cynn E, Hu X, Mandleywala K, Wilhelmy J, Grimes SM, Corney DC, Boutet SC, Terry JM, Belgrader P, Ziraldo SB, Mikkelsen TS, Wang F, von Furstenberg RJ, Smith NR, Chandrakesan P, May R, Chrissy MAS, Jain R, Cartwright CA, Niland JC, Hong YK, Carrington J, Breault DT, Epstein J, Houchen CW, Lynch JP, Martin MG, Plevritis SK, Curtis C, Ji HP, Li L, Henning SJ, Wong MH, Kuo CJ. Intestinal enteroendocrine lineage cells possess homeostatic and injury-inducible stem cell activity. Cell Stem Cell 2017;21:78–90 e6.
63. Westphalen CB, Asfaha S, Hayakawa Y, Takemoto Y, Lukin DJ, Nuber AH, Brandtner A, Setlik W, Remotti H, Muley A, Chen X, May R, Houchen CW, Fox JG, Gershon MD, Quante M, Wang TC. Long-lived intestinal tuft cells serve as colon cancer-initiating cells. J Clin Invest 2014;124:1283–1295.
64. Metcalfe C, Kljavin NM, Ybarra R, de Sauvage FJ. Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration. Cell Stem Cell 2014;14:149–159.
65. Sheng X, Lin Z, Lv C, Shao C, Bi X, Deng M, Xu J, Guerrero-Juarez CF, Li M, Wu X, Zhao R, Yang X, Li G, Liu X, Wang Q, Nie Q, Cui W, Gao S, Zhang H, Liu Z, Cong Y, Plikus MV, Lengner CJ, Andersen B, Ren F, Yu Z. Cycling stem cells are radioresistant and regenerate the intestine. Cell Rep 2020;32:107952.
66. Schuijers J, Junker JP, Mokry M, Hatzis P, Koo BK, Sasselli V, van der Flier LG, Cuppen E, van Oudenaarden A, Clevers H. Ascl2 acts as an R-spondin/Wnt-responsive switch to control stemness in intestinal crypts. Cell Stem Cell 2015;16:158–170.
67. Murata K, Jadhav U, Madha S, van Es J, Dean J, Cavazza A, Wucherpfennig K, Michor F, Clevers H, Shivdasani RA. Ascl2-dependent cell dedifferentiation drives regeneration of ablated intestinal stem cells. Cell Stem Cell 2020;26:377–390 e6.

68. Gregorieff A, Wrana JL. Hippo signalling in intestinal regeneration and cancer. Curr Opin Cell Biol 2017; 48:17–25.
69. Barry ER, Morikawa T, Butler BL, Shrestha K, de la Rosa R, Yan KS, Fuchs CS, Magness ST, Smits R, Ogino S, Kuo CJ, Camargo FD. Restriction of intestinal stem cell expansion and the regenerative response by YAP. Nature 2013;493:106–110.
70. Guillermin O, Angelis N, Sidor CM, Ridgway R, Baulies A, Kucharska A, Antas P, Rose MR, Cordero J, Sansom O, Li VSW, Thompson BJ. Wnt and Src signals converge on YAP-TEAD to drive intestinal regeneration. EMBO J 2021;40:e105770.
71. Kim HB, Kim M, Park YS, Park I, Kim T, Yang SY, Cho CJ, Hwang D, Jung JH, Markowitz SD, Hwang SW, Yang SK, Lim DS, Myung SJ. Prostaglandin E2 activates YAP and a positive-signaling loop to promote colon regeneration after colitis but also carcinogenesis in mice. Gastroenterology 2017;152:616–630.
72. Schmitt M, Schewe M, Sacchetti A, Feijtel D, van de Geer WS, Teeuwssen M, Sleddens HF, Joosten R, van Royen ME, van de Werken HJG, van Es J, Clevers H, Fodde R. Paneth cells respond to inflammation and contribute to tissue regeneration by acquiring stem-like features through SCF/c-kit signaling. Cell Rep 2018; 24:2312–2328 e7.
73. Cordero JB, Ridgway RA, Valeri N, Nixon C, Frame MC, Muller WJ, Vidal M, Sansom OJ. c-Src drives intestinal regeneration and transformation. EMBO J 2014; 33:1474–1491.
74. Nusse YM, Savage AK, Marangoni P, Rosendahl-Huber AKM, Landman TA, de Sauvage FJ, Locksley RM, Klein OD. Parasitic helminths induce fetal-like reversion in the intestinal stem cell niche. Nature 2018; 559:109–113.
75. Goldsmith JR, Spitofsky N, Zamani A, Hood R, Boggs A, Li X, Li M, Reiner E, Ayyaz A, Etwebi Z, Lu L, Rivera Guzman J, Bou-Dargham MJ, Cathoupolis T, Hakonarson H, Sun H, Wrana JL, Gonzalez MV, Chen YH. TNFAIP8 controls murine intestinal stem cell homeostasis and regeneration by regulating microbiome-induced Akt signaling. Nat Commun 2020; 11:2591.
76. Yui S, Azzolin L, Maimets M, Pedersen MT, Fordham RP, Hansen SL, Larsen HL, Guiu J, Alves MRP, Rundsten CF, Johansen JV, Li Y, Madsen CD, Nakamura T, Watanabe M, Nielsen OH, Schweiger PJ, Piccolo S, Jensen KB. YAP/TAZ-dependent reprogramming of colonic epithelium links ECM remodeling to tissue regeneration. Cell Stem Cell 2018;22:35–49 e7.
77. Ayyaz A, Kumar S, Sangiorgi B, Ghoshal B, Gosio J, Ouladan S, Fink M, Barutcu S, Trcka D, Shen J, Chan K, Wrana JL, Gregorieff A. Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell. Nature 2019;569:121–125.
78. Saha S, Aranda E, Hayakawa Y, Bhanja P, Atay S, Brodin NP, Li J, Asfaha S, Liu L, Tailor Y, Zhang J, Godwin AK, Tome WA, Wang TC, Guha C, Pollard JW. Macrophage-derived extracellular vesicle-packaged WNTs rescue intestinal stem cells and enhance survival after radiation injury. Nat Commun 2016;7:13096.
79. Aparicio-Domingo P, Romera-Hernandez M, Karrich JJ, Cornelissen F, Papazian N, Lindenbergh-Kortleve DJ, Butler JA, Boon L, Coles MC, Samsom JN, Cupedo T. Type 3 innate lymphoid cells maintain intestinal epithelial stem cells after tissue damage. J Exp Med 2015; 212:1783–1791.
80. Hanash AM, Dudakov JA, Hua G, O'Connor MH, Young LF, Singer NV, West ML, Jenq RR, Holland AM, Kappel LW, Ghosh A, Tsai JJ, Rao UK, Yim NL, Smith OM, Velardi E, Hawryluk EB, Murphy GF, Liu C, Fouser LA, Kolesnick R, Blazar BR, van den Brink MR. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. Immunity 2012; 37:339–350.
81. Romera-Hernandez M, Aparicio-Domingo P, Papazian N, Karrich JJ, Cornelissen F, Hoogenboezem RM, Samsom JN, Cupedo T. Yap1-driven intestinal repair is controlled by group 3 innate lymphoid cells. Cell Rep 2020;30:37–45 e3.
82. Kaaij LT, van de Wetering M, Fang F, Decato B, Molaro A, van de Werken HJ, van Es JH, Schuijers J, de Wit E, de Laat W, Hannon GJ, Clevers HC, Smith AD, Ketting RF. DNA methylation dynamics during intestinal stem cell differentiation reveals enhancers driving gene expression in the villus. Genome Biol 2013;14:R50.
83. Sheaffer KL, Kim R, Aoki R, Elliott EN, Schug J, Burger L, Schubeler D, Kaestner KH. DNA methylation is required for the control of stem cell differentiation in the small intestine. Genes Dev 2014;28:652–664.
84. Kim R, Sheaffer KL, Choi I, Won KJ, Kaestner KH. Epigenetic regulation of intestinal stem cells by Tet1-mediated DNA hydroxymethylation. Genes Dev 2016; 30:2433–2442.
85. Raab JR, Tulasi DY, Wager KE, Morowitz JM, Magness ST, Gracz AD. Quantitative classification of chromatin dynamics reveals regulators of intestinal stem cell differentiation. Development 2020;147:dev181966.
86. Lindeboom RG, van Voorthuijsen L, Oost KC, Rodriguez-Colman MJ, Luna-Velez MV, Furlan C, Baraille F, Jansen PW, Ribeiro A, Burgering BM, Snippert HJ, Vermeulen M. Integrative multi-omics analysis of intestinal organoid differentiation. Mol Syst Biol 2018;14:e8227.
87. Kazakevych J, Sayols S, Messner B, Krienke C, Soshnikova N. Dynamic changes in chromatin states during specification and differentiation of adult intestinal stem cells. Nucleic Acids Res 2017;45:5770–5784.
88. Uribe-Lewis S, Carroll T, Menon S, Nicholson A, Manasterski PJ, Winton DJ, Buczacki SJA, Murrell A. 5-hydroxymethylcytosine and gene activity in mouse intestinal differentiation. Sci Rep 2020;10:546.
89. Huels DJ, Sansom OJ. Stem vs non-stem cell origin of colorectal cancer. Br J Cancer 2015;113:1–5.
90. Shih IM, Wang TL, Traverso G, Romans K, Hamilton SR, Ben-Sasson S, Kinzler KW, Vogelstein B. Top-down morphogenesis of colorectal tumors. Proc Natl Acad Sci U S A 2001;98:2640–2645.

91. Zhu L, Gibson P, Currle DS, Tong Y, Richardson RJ, Bayazitov IT, Poppleton H, Zakharenko S, Ellison DW, Gilbertson RJ. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. Nature 2009;457:603–607.
92. Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 2009;457:608–611.
93. Asfaha S, Hayakawa Y, Muley A, Stokes S, Graham TA, Ericksen RE, Westphalen CB, von Burstin J, Mastracci TL, Worthley DL, Guha C, Quante M, Rustgi AK, Wang TC. Krt19(+)/Lgr5(-) cells are radioresistant cancer-initiating stem cells in the colon and intestine. Cell Stem Cell 2015;16:627–638.
94. Schwitalla S, Fingerle AA, Cammareri P, Nebelsiek T, Goktuna SI, Ziegler PK, Canli O, Heijmans J, Huels DJ, Moreaux G, Rupec RA, Gerhard M, Schmid R, Barker N, Clevers H, Lang R, Neumann J, Kirchner T, Taketo MM, van den Brink GR, Sansom OJ, Arkan MC, Greten FR. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. Cell 2013;152:25–38.
95. Shimokawa M, Ohta Y, Nishikori S, Matano M, Takano A, Fujii M, Date S, Sugimoto S, Kanai T, Sato T. Visualization and targeting of LGR5(+) human colon cancer stem cells. Nature 2017;545:187–192.

Received July 9, 2021. Accepted December 7, 2021.

Correspondence
Address correspondence to: Peter J. Dempsey, PhD, Section of Developmental Biology, Department of Pediatrics, University of Colorado School of Medicine, 1775 Aurora Court, Barbara Davis Center, M20–3306, Aurora, Colorado 80045. e-mail: peter.dempsey@cuanschutz.edu; fax: (303) 724-6538.

Conflicts of interest
The authors disclose no conflicts.

Funding
This work was supported by National Institutes of Health grant R01-DK120921 and National Science Foundation grant NSF-2033723. ARM is supported by the Postdoctoral Training Program in Developmental Biology and Regenerative Medicine, Section of Developmental Biology, Dept. of Pediatrics, University of Colorado Anschutz Medical Campus.