© 2016. Published by The Company of Biologists Ltd | Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

REVIEW

Regulation and plasticity of intestinal stem cells during homeostasis and regeneration

Joep Beumer[1,2] and Hans Clevers[1,2,*]

ABSTRACT

The intestinal epithelium is the fastest renewing tissue in mammals and has a large flexibility to adapt to different types of damage. Lgr5$^+$ crypt base columnar (CBC) cells act as stem cells during homeostasis and are essential during regeneration. Upon perturbation, the activity of CBCs is dynamically regulated to maintain homeostasis and multiple dedicated progenitor cell populations can reverse to the stem cell state upon damage, adding another layer of compensatory mechanisms to facilitate regeneration. Here, we review our current understanding of how intestinal stem and progenitor cells contribute to homeostasis and regeneration, and the different signaling pathways that regulate their behavior. Nutritional state and inflammation have been recently identified as upstream regulators of stem cell activity in the mammalian intestine, and we explore how these systemic signals can influence homeostasis and regeneration.

KEY WORDS: Cellular dynamics, Homeostasis, Intestine, Regeneration

Introduction

The intestinal epithelium is organized into modules termed crypt-villus units. Villi are surface-extending protrusions into the intestinal lumen that are covered by differentiated cell types. Intestinal stem cells (called crypt base columnar cells, or CBCs) are easily identifiable by their slender morphology and localization at the crypt base, intermingled with the much larger, granule-containing and post-mitotic Paneth cell (Fig. 1). CBCs divide each day to produce rapidly proliferating daughter cells that move up the wall of the crypt onto the flanks of the villus, sometimes referred to as the intestinal 'conveyor belt'. At the villus tips, mature cells are continuously lost by apoptosis, 4-5 days after their birth. Only the stem cells in the crypt retain long-term self-renewing ability. A second population of non-dividing stem cells has been suggested to exist at the so-called '+4' position, four cell diameters above the base of the crypt and directly adjacent to the CBC/Paneth region. Within the intestine, two major differentiated epithelial lineages are distinguished: (1) the enterocyte or absorptive lineage, responsible for absorbing nutrients, and (2) the secretory lineage (Fig. 2). The latter consists of Paneth cells, which act as niche cells for stem cells and secrete antimicrobial molecules; the mucus-secreting goblet cells; a variety of hormone-producing enteroendocrine cell; and the mechanosensing tuft cells (Clevers, 2013). The intestinal epithelium is exposed to a hostile luminal environment, which may explain its rapid turnover rate of 4-5 days, the fastest among mammalian tissues.

Different extrinsic or intrinsic causes can upset the homeostatic self-renewal, and/or result in overt damage. Under such circumstances, the epithelium displays an impressive regenerative response. For instance, a 12 Gy dose of irradiation causes hematopoietic failure, yet still triggers an effective regenerative response in the intestine, classically involving hyperproliferation of non-differentiated crypt cells, as well as crypt fission – the process by which one crypt produces two crypts – to repopulate the epithelium (Withers and Elkind, 1970). This response is illustrative of the adaptive capacity of the intestine. Flexibility in the regenerative response also occurs upon surgical resection and acute inflammation (Cordero and Sansom, 2012). Regeneration is generally believed to be facilitated by stem cells, and multiple strategies can be conceived that could facilitate such a flexible response to injury (Fig. 3). For example, separate stem cell populations may exist in a 'two-stem cell' model that either act during homeostasis or are activated during damage. The CBC stem cells that fuel intestinal renewal during homeostasis are rapidly dividing. A separate non-dividing, or quiescent, population of 'reserve' stem cells has been proposed to co-exist, i.e. the intestinal '+4' stem cell. Such non-dividing reserve stem cells could be less radiosensitive, and may divide only when the actively dividing stem cells are challenged (Li and Clevers, 2010). A second scenario could be that the activity of a single stem cell population is adaptively controlled upon injury. Indeed, inflammation increases the activity of intestinal stem cells (ISCs) in a cell-autonomous manner (Lindemans et al., 2015). Similarly, calorie restriction can impinge on stem cell activity, for example by augmenting the function of Paneth cells, which indirectly increases the number of CBCs (Richmond et al., 2015; Yilmaz et al., 2012). In a third model, committed progenitor cells could regain stem cell potential by reverting to a stem cell state. Such plasticity could be termed 'reverse' stem cell potential (as opposed to 'reserve' stem cells), and by its very nature would exist in a non-stem cell population (Buczacki et al., 2013; Tetteh et al., 2016; van Es et al., 2012a). The capacity to dedifferentiate has been identified in a variety of multicellular organisms, pointing to a potentially universal mechanism for regeneration (Sánchez Alvarado and Yamanaka, 2014), and has been described for mammalian endodermal tissues such as the lung (Hogan et al., 2014).

In this Review, we discuss the range of cellular responses that enable the intestine to adapt to different perturbations, with a focus on stem and progenitor cell dynamics. We will first give a general overview of the identification of intestinal stem cells. Next, we will review the requirement for different signaling pathways to maintain homeostasis, both under normal physiological conditions as well as in response to injury. We focus on recent advances in the understanding of how dynamics in stem cell activity occur, including how specific stressors such as inflammation or nutritional deprivation might directly impact on stem cell behavior.

[1]Hubrecht Institute for Developmental Biology and Stem Cell Research, 3584 CT, Utrecht, The Netherlands. [2]Cancer Genomics Netherlands, University Medical Center Utrecht, 3584 CX, Utrecht, The Netherlands.

*Author for correspondence (h.clevers@hubrecht.eu)

H.C., 0000-0002-3077-5582

Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

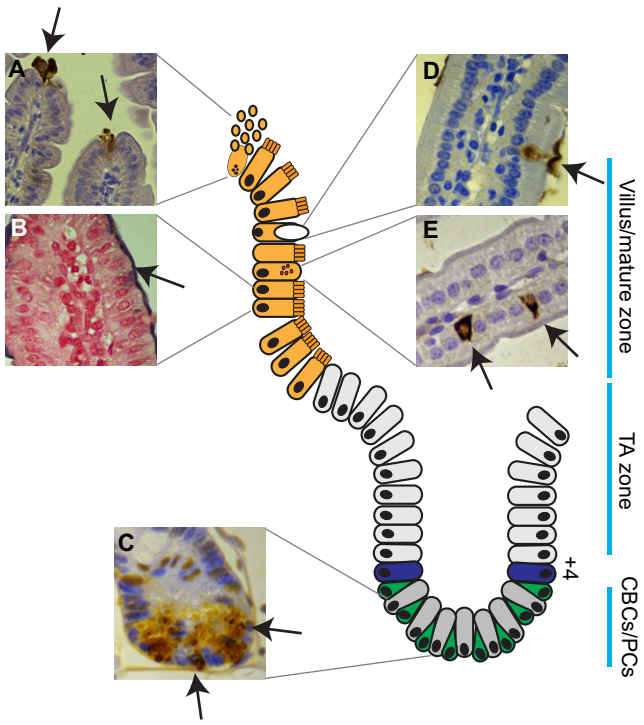

Fig. 1. The intestinal crypt-villus unit in mice. The intestinal epithelium is organized as units of crypt villi. Stem cells and transit-amplifying cells in the crypt proliferate continuously to renew mature cells in the villi (central schematic). At the tip of villi (A), mature cells undergo apoptosis and are shed in the lumen, stained by cleaved caspase 3 (brown, arrows). The brush border on the apical surface of the enterocytes (B) can be visualized by alkaline phosphatase staining (black, arrow). Paneth cells (C) at the bottom of the crypt are stained by lysozyme (brown, arrows). A goblet cell (D) is shown stained by mucin 2 (brown, arrow) and enteroendocrine cells (E) are stained by chromogranin A (brown, arrows). In the schematic, orange indicates differentiated cells in villi, light gray indicates transit-amplifying (TA) progenitor cells, blue indicates the +4 cell position, green indicates CBCs, and dark gray indicates Paneth cells (PCs).

The quest for the intestinal stem cells

Radioactive nucleotide labeling studies have been used to show that intestinal proliferation is restricted to crypts, and that cells after their generation (with the exception of Paneth cells) move upwards towards the villus tips to eventually self-sacrifice (Clevers, 2013). This conveyer-belt model of intestinal renewal and cell migration suggests that the cells that drive homeostatic renewal reside at the crypt bottom. The CBC stem cells were originally identified by Cheng and Leblond and are located at the crypt base, interspersed between Paneth cells (Fig. 1; Cheng and Leblond, 1974). Functional evidence to identify crypt stem cells definitively, as in other epithelial tissues, had to await the identification of specific marker genes and the development of lineage-tracing technology, as the gold standard for assessing stem cell potential (Kretzschmar and Watt, 2012). Leucine-rich repeat containing G-protein coupled receptor 5 (Lgr5), a target gene of the Wnt signaling pathway, was identified as marker of CBCs (Barker et al., 2007). With the generation of appropriate Lgr5 knock-in alleles, murine CBCs were shown to generate all differentiated cell types of the intestine over long time periods (Fig. 2). Surprisingly at the time, they were observed to be rapidly dividing, with an average cell cycle time of 21.5 h (Schepers et al., 2011). Single Lgr5^{+} cells can be isolated from mouse intestine and under defined culture conditions can form mini-guts – miniature intestines with crypt-villus domains that contain all the mature intestinal cell types (Sato et al., 2009). Therefore, CBCs fulfill the defining criteria of stem cells, namely multipotency and the capacity to self-renew.

Stem cells are generally believed to be slowly cycling/quiescent in order to protect genome integrity. The rapid cell division rate of CBCs challenges this belief, building upon the idea that quiescence might not be a defining hallmark of stem cells (Clevers, 2015). However, a second stem cell population has been proposed to exist alongside CBCs. Potten and colleagues identified DNA label-retaining cells at the +4 position, four cells above the base of the crypt (Fig. 1; Potten et al., 1974). Bmi1, Lrig1, Hopx and mTert (Tert) have been described as markers of these cells (Fig. 2; Montgomery et al., 2011; Powell et al., 2012; Sangiorgi and Capecchi, 2008; Takeda et al., 2011). +4 cells are insensitive to injury, and lineage-tracing experiments based on these markers show increased stem cell activity upon damage, suggesting that these cells are reserve stem cells capable of replacing CBCs in the injury setting (Montgomery et al., 2011). The study of +4 cells became complicated when it was found that these marker genes were broadly expressed at the transcript level, including in CBCs (Grün et al., 2015; Li et al., 2014; Muñoz et al., 2012). Lengner and colleagues subsequently showed that the Bmi1-CreER and Hopx-CreER proteins are more specifically localized than the corresponding mRNA transcripts, and that these reporters – based on transcriptional profiling – can identify cells that are largely distinct from those marked by Lgr5 (Li et al., 2014). Moreover, lineage tracing from Hopx-CreER and subsequent single-cell profiling demonstrated that Hopx^{+} and Bmi1^{+} cells can generate

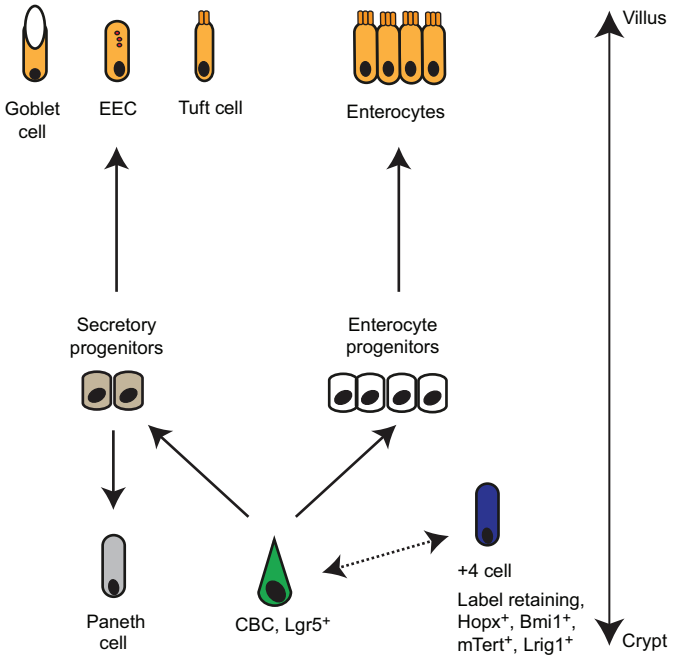

Fig. 2. Stem cells and differentiated progeny in the intestine. Crypt base columnar cells (CBCs, green) are intestinal stem cells that generate all major intestinal lineages, including secretory cells and enterocytes (orange). Paneth cells are the exception of the differentiated lineage, and do not migrate upwards. +4 cells (blue), marked by Hopx, mTert, Bmi1 and Lrig1 or identified as label retaining, have been proposed as a second stem cell population (dashed arrow). The double-headed arrow on the right indicates the relative position of the cells along the crypt-villus axis. EEC, enteroendocrine cell.

Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

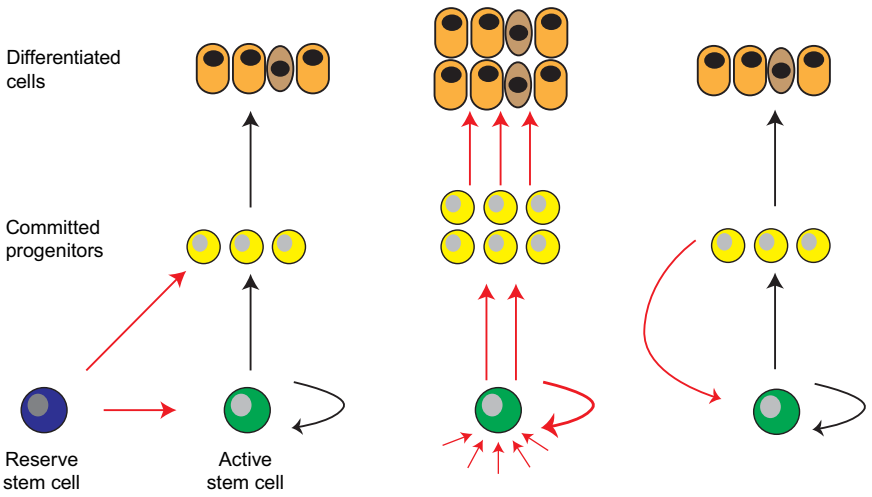

Fig. 3. Models of regeneration in the intestine. A variety of possible scenarios exist for intestinal regeneration. In the first (left panel), actively dividing stem cells (green) are responsible for intestinal renewal during homeostasis, producing committed progenitor cells (yellow), which produce differentiated cells (orange). A co-existing quiescent, reserve population of stem cells (blue) is activated upon injury, and might contribute directly to the generation of progeny or active stem cells (red arrows). In the second scenario (center panel), niche and inflammatory signals (red arrows, bottom) can directly enhance stem cell activity to promote a regenerative response, leading to increased numbers of committed progenitors and differentiated cells (red arrows). In the third scenario (right panel), committed progenitor cells retain stem cell potential and can revert to the stem cell state in response to perturbations (red arrow).

CBCs during homeostasis (Li et al., 2014), suggesting a bona fide separate stem cell population (Fig. 2). However, using the original experimental conditions, our lab has shown that lineage tracing from the Bmi1 locus initiates with equal efficiency along the entire crypt axis, including in CBCs (Muñoz et al., 2012). It is clear that +4 cells as defined by each of these markers require more attention to unequivocally demonstrate their homeostatic stem cell potential, and their relation to CBCs.

Signaling pathways that regulate intestinal stem cells

The activity of CBCs is tightly controlled by signaling molecules that derive from the epithelium and from mesenchymal cells outside the epithelial crypt-villus unit depicted in Fig. 1. Mesenchymal cells are vital components of the intestinal niche, and include fibroblasts, immune cells, enteric neurons and capillaries. These cells have been shown to secrete a wide variety of growth factors and cytokines that, together with epithelial signals, control proliferation and differentiation of CBCs (Powell et al., 2011). Perturbations such as irradiation, toxins, chemotherapy, inflammation and nutritional deprivation can all induce rewiring of signaling pathways to modulate CBC activity and compensate for cellular loss in order to accommodate regeneration. In this section, we summarize and discuss the main signaling pathways that are involved in the regulation of intestinal stem cell activity in homeostasis and upon injury (Table 1).

Wnt

The Wnt pathway is essential for the maintenance of intestinal stem cells. When Wnt ligands bind to the frizzled-LRP5/6 receptor complex, the cytoplasmic APC destruction complex is inhibited, leading to accumulation of the key mediator of Wnt signals: β-catenin. β-Catenin translocates to the nucleus and acts as a transcriptional co-factor for T-cell factor (TCF) transcription factors, inducing transcription of Wnt target genes (Clevers and Nusse, 2012). Inactivating mutations in Tcf7l2 (also known as TCF4), one of the downstream transcription factors of the Wnt pathway, prevent the formation of proliferative crypts in neonatal mice (Korinek et al., 1998). In the adult intestine, ubiquitous deletion of TCF4 using a Villin-CreERT2 transgenic model caused rapid loss of Lgr5^{+} CBCs (van Es et al., 2012b). Similarly, mice with an intestinal overexpression of the secreted Wnt inhibitor Dickkopf 1 (Dkk1) showed loss of crypts and decreased epithelial proliferation (Kuhnert et al., 2004; Pinto et al., 2003). CBC-specific activation of the Wnt pathway by inactivating mutations in APC stabilizes β-catenin and confers stem cells with a competitive advantage over their wild-type counterparts resulting in the rapid formation of adenomas (Barker et al., 2009; Snippert et al., 2014; Vermeulen et al., 2013).

Wnt ligands are redundantly expressed in epithelial Paneth cells and in the mesenchyme surrounding the crypt (Farin et al., 2012; Kabiri et al., 2014). Paneth cell-derived Wnt3 is essential to maintain proper growth of cultured epithelial 3D intestinal organoids, but can be depleted *in vivo* without a phenotype. This is because, in the latter, the mesenchyme acts as alternative source of Wnt, whereas *in vitro* the mesenchyme was not present (Farin et al., 2012). Indeed, *Wnt3* knockout intestinal organoids can be rescued by co-culturing them with mesenchyme (Farin et al., 2012). Removal of Foxl1-expressing peri-cryptal mesenchymal cells *in vivo* causes loss of Wnt activity, and of proliferation, in crypts (Aoki et al., 2016). This recent study identifies the mesenchyme as a crucial component of the CBC niche, though it remains to be determined whether Wnt itself or another signal is non-redundant with the Paneth cell niche function.

Activation of the Wnt pathway is restricted to the lower crypt, and forms a signaling gradient along the crypt axis (Batlle et al., 2002; Muñoz et al., 2012). Different strategies for Wnt-controlled growth and patterning have been proposed, with both long-distance and local signaling activity observed (Mikels and Nusse, 2006). In *Drosophila* larvae, Wnt is expressed by a narrow stripe of cells in the imaginal discs and forms a long-distance gradient into the prospective wing. Tethering of the *Drosophila* Wnt ligand Wingless to the membrane of the producing cell does not, however, disturb normal wing development (Alexandre et al., 2014). Surprisingly, in the mammalian intestinal crypt, Paneth cell-produced Wnt3 does not freely diffuse, but is bound to the neighboring stem cell membranes, as visualized by an HA-tagged Wnt3 allele (Farin et al., 2016). Binding to the membrane occurs directly through frizzled, the cognitive Wnt receptor, and transfer of Wnt3 from Paneth cells to neighboring cells depends on direct cell-to-cell contact. When proliferation is inhibited in intestinal organoids, the Wnt gradient in the crypts collapses, implying that Wnt spreads by proliferative dilution of Wnt-binding membranes (Farin et al., 2016). It is tempting to speculate that the coupling between cell cycle activity and the concentration of a crucial stem cell niche signal is part of a functional feedback loop. When proliferation is overstimulated, rapid dilution of Wnt might lead to loss of CBCs or of their transit-

Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

Table 1. Summary of signaling pathways involved in dynamic control of ISC activity

Signaling pathway	Role in ISC dynamics	Evidence	References
Wnt	Stem cell maintenance	TCF4 inactivation causes loss of stem cells in mice.	(Korinek et al., 1998; van Es et al., 2012b)
		Overexpression of Dkk1 in mice causes loss of crypts in mice.	(Kuhnert et al., 2004; Pinto et al., 2003)
		APC inactivation confers stem cells with a competitive advantage in mice.	(Snippert et al., 2014; Vermeulen et al., 2013)
	Regeneration	FAK deletion prevents regenerative proliferation after intestinal injury in mice.	(Ashton et al., 2010)
		Wnt5a-deficient murine crypts show abnormal regenerative response with prolonged proliferation.	(Miyoshi et al., 2013)
		R-spondin 1 administration improves regeneration in mice.	(Kim et al., 2005; Zhou et al., 2013)
Notch	Stem cell maintenance	Inhibition of Notch through γ-secretase causes stem cell conversion towards secretory lineages.	(Milano et al., 2004; van Es et al., 2010)
		Inactivation of Notch effectors Hes1, Hes3 and Hes5 in the intestine causes increased secretory formation in mice.	(Ueo et al., 2012)
EGF/EphB	Stem cell proliferation	Proliferation is reduced in mouse intestinal crypts lacking EphB2/3.	(Holmberg et al., 2006)
		Loss of Lrig1 causes activation of ErbB signaling and expansion of the intestinal crypt in mice.	(Wong et al., 2012)
	Regeneration	Doxorubicin treatment induces EGF-ligand/BMP inhibitor expression in sub-epithelial tissue in mice, although its relevance remains to be determined.	(Seiler et al., 2015)
BMP	Stem cell differentiation	BMP inhibition by transgenic noggin overexpression, or conditional loss of Bmpr1a, causes formation of ectopic crypts in mice.	(Haramis, 2004; He et al., 2004)
	Regeneration	BMP ligand expression is increased in *Drosophila* gut after injury and limits stem cell expansion.	(Guo et al., 2013)
		Doxorubicin treatment induces EGF-ligand/BMP inhibitor expression in sub-epithelial tissue in mice, although its relevance remains to be determined.	(Seiler et al., 2015)
Hippo	Stem cell proliferation	Yorkie overexpression increases stem cell proliferation in *Drosophila*.	(Karpowicz et al., 2010)
		Knockdown of YAP/TAZ in mouse crypts suppresses proliferation.	(Imajo et al., 2014)
	Regeneration	YAP deletion interferes with regeneration in DSS-induced colitis in mice, shown by reduced proliferation and increased mortality.	(Cai et al., 2010)
		YAP deletion interferes with regeneration after irradiation in mice indicated by reduced proliferation.	(Gregorieff et al., 2015)
		YAP/TAZ deletion impairs organoid formation.	(Azzolin et al., 2014; Gregorieff et al., 2015)

amplifying (TA) cells. This protective feedback could explain why intestinal oncogenesis is virtually always initiated by mutations in the Wnt pathway (Morin et al., 1997). Indeed, recent work indicates that *Braf* mutations cause loss of CBCs, which could be reversed by activating the Wnt pathway by exogenous Wnt ligands *in vitro* or β-catenin mutations *in vivo* (Riemer et al., 2015). Vice versa, a decrease in CBC proliferation in the event of injury or other perturbations could lead to a large CBC-bound pool of Wnt that stimulates proliferation or symmetric stem cell expansion during subsequent regeneration.

Wnt signaling is a crucial mediator of regeneration (Cordero and Sansom, 2012). Deletion of the Wnt target gene Myc impedes regeneration by causing massive crypt loss following 14 Gy irradiation (Ashton et al., 2010). Downstream of Wnt/Myc, focal adhesion kinase (FAK; also known as Ptk2) is upregulated following irradiation (Ashton et al., 2010). FAK is essential for maintaining Wnt-driven proliferation during regeneration, and suppresses apoptosis by activating AKT/mTOR signaling (Ashton et al., 2010). Non-canonical Wnt signaling also seems to partly mediate regenerative responses in the colon (Miyoshi et al., 2013). After mechanical injury, one of the transcripts enriched in wound beds compared with normal epithelium is *Wnt5a*, which encodes a non-canonical Wnt ligand. Wnt5a is expressed by stroma surrounding the site of injury, and lack of Wnt5a causes a failure to develop new crypts at the wounding site. Wnt5a has been shown to limit the proliferation of crypt cells after injury in a TGFβ-dependent manner (Miyoshi et al., 2013); however, it remains unclear whether cell cycle inhibition is a functionally relevant element of the Wnt5a-mediated regenerative response, or whether Wnt5a has additional effects.

Multiple studies have found that enhancing Wnt signaling can stimulate recovery after intestinal damage. Administration of the Wnt agonist R-spondin 1 improves recovery after chemoradiotherapy in mice (Kim et al., 2005). E3 ligases and the Wnt targets RNF43 and ZNRF3 can downregulate frizzled receptors, thereby effectively inhibiting the Wnt pathway (Hao et al., 2012; Koo et al., 2012). In vertebrates, four different R-spondin proteins can short circuit this negative feedback by binding to their receptors, Lgr4, Lgr5 or Lgr6. Together, this ligand-receptor complex associates with and inhibits RNF43 and ZNFR3 (Hao et al., 2012; Koo et al., 2012). The effect of R-spondin 1 on regeneration can be further improved by simultaneous treatment with Slit2 (Zhou et al., 2013). Slit2 is expressed by CBCs, along with its receptor roundabout 1 (Robo1). Knockout of *Robo1* decreases the number of CBCs, suggesting that a Robo1/Slit2 signaling axis is important for CBC maintenance (Zhou et al., 2013). When administered simultaneously, R-spondin 1 and Slit2 expand the number of $Lgr5^+$ cells, and act synergistically to stimulate intestinal recovery, thereby preventing lethality of mice treated with chemoradiotherapy. It remains unclear whether Slit2 is a potentiator of Wnt signaling in a similar manner to R-spondin 1 in this regenerative context. Slit2 is overexpressed in some intestinal cancers, and has been found to downregulate E-cadherin (cadherin 1), thereby possibly releasing β-catenin and activating Wnt

Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

signaling (Zhang et al., 2015). Paradoxically, signaling through Slit2/Robo2 decreases β-catenin in mammary stem cells, limiting stem cell self-renewal (Harburg et al., 2014). Future studies are clearly required to dissect downstream targets of Slit2 activity in intestinal regeneration. Taken together, current evidence implies a large therapeutic opportunity for Wnt signal enhancement in promoting gastrointestinal recovery, for example upon chemotherapy.

Notch

The Notch signaling pathway depends on a cell presenting a Notch ligand to an adjacent cell expressing the Notch receptor. When the Notch receptor binds its ligand, the Notch intracellular domain (NCID) is released by proteolysis. NCID subsequently translocates to the nucleus where it activates target genes through the transcription factor RBP-J. Paneth cells express the Notch ligands Delta-like 1 and 4 (Dll1 and Dll4) and present these ligands to adjacent CBCs (Sato et al., 2011). Notch inhibition causes rapid conversion of all proliferative crypt cells, including the ISCs, into goblet cells (Milano et al., 2004; van Es et al., 2005, 2010). Notch signaling activates expression of Hes family transcription factors, including Hes1, which repress the helix-loop-helix transcription factor Atoh1 (also known as Math1). Similar to Notch inhibition, induction of Atoh1 in turn promotes differentiation towards a secretory fate (Ueo et al., 2012), whereas loss of Atoh1 results in an absence of secretory cells (Shroyer et al., 2007). Therefore, Notch acts as a binary switch through lateral inhibition, promoting CBCs to undergo Atoh1-dependent secretory differentiation when inactivated. Secretory progenitors express Dll1 (van Es et al., 2012a) and suppress a secretory fate in their neighbors, driving the fate of these cells towards the enterocyte lineage.

EGF

Epidermal growth factor (EGF) is a crucial component of the intestinal organoid culture (Sato et al., 2009). The EGF receptor (EGFR) is highly expressed in CBCs, whereas its ligands are expressed by Paneth cells (Sato et al., 2011). The activity of ErbB signaling is controlled by the negative regulator Lrig1, a transmembrane protein that is co-expressed with Lgr5 in CBCs (Wong et al., 2012). Loss of Lrig1 causes enhanced receptor activation and a concomitant rapid expansion of crypts and stem cell numbers. It is currently unknown at which level Lrig1 affects ErbB signaling. These lines of evidence illustrate the importance of this pathway as an inductive signal for stem cell proliferation. Although the role of EGFR/ErbB signaling for ISC proliferation is clearly established, it is not known if it is a prerequisite for CBC identity.

BMP

Bone morphogenetic protein (BMP) signaling acts as inducer of differentiation in the crypt. Mesenchyme surrounding CBCs create a ‘BMP-low’ environment by secreting BMP inhibitors (Kosinski et al., 2007), whereas BMP ligands expressed in villi create a ‘BMP-high’ environment that promotes differentiation. Consistent with this, transgenic expression of the BMP inhibitor noggin leads to excessive crypt formation (Haramis, 2004). Moreover, the BMP inhibitor noggin is an essential ingredient of the organoid culture medium (Sato et al., 2009), as without it stem cells undergo differentiation. Similarly, conditional inactivation of the BMP receptor Bmpr1a in mice elevates Wnt signaling activity and causes a rapid expansion of the stem cell compartment (He et al., 2004). These phenotypes are reminiscent of patients with juvenile polyposis who carry inactivating mutations in the BMP pathway (Howe et al., 2001). In the *Drosophila* midgut, BMP is induced upon injury and acts directly on ISCs to limit their expansion (Guo et al., 2013). In the murine intestine, the BMP inhibitor chordin-like 2 is upregulated together with EGF ligand amphiregulin in sub-epithelial tissues after treatment with the chemotherapeutic doxorubicin (Seiler et al., 2015). This upregulation occurs in concert with an increase in ISC proliferation. The requirement of differential BMP or EGF signaling during regeneration remains to be assessed in future studies.

Hippo

The Hippo signaling pathway is a key player in the regulation of organ size and has been shown to act as an interpreter of mechanical cues (Varelas, 2014). Recent studies indicate a role for Hippo signaling in intestinal homeostasis and regeneration, although its exact function remains controversial (Li and Clevers, 2013). Upon Hippo pathway activation, a kinase cascade consisting of MST1/2 (MST2 is also known as STK3) and LAT1/2 kinases phosphorylates and inactivates YAP and TAZ through their cytoplasmic translocation. YAP and TAZ are final effector proteins of the pathway acting as co-activators of TEAD transcription factors (Pan, 2010).

During homeostasis, YAP is expressed throughout the intestinal crypt (Cai et al., 2010). Genetic inhibition of the Hippo pathway increases ISC proliferation in *Drosophila* (Karpowicz et al., 2010). Imajo and colleagues report similar proliferative effects after YAP activation in the murine intestine (Imajo et al., 2014). However, overexpression of YAP-S127A, a phospho-deficient mutant that readily translocates to the nucleus, decreased proliferation and inhibited Wnt signaling *in vivo* (Barry et al., 2013). These paradoxical observations can be reconciled by postulating separate cytoplasmic and nuclear functions of YAP/TAZ. YAP/TAZ have been shown to directly bind to Axin and inhibit Wnt signaling in an overexpression system (Azzolin et al., 2014; Imajo et al., 2014). In this scenario, YAP/TAZ would thus act as part of the β-catenin destruction complex in HEK cells, and mediate the recruitment of the β-catenin E3 ligase β-TrCP, targeting it for destruction (Azzolin et al., 2014). Wnt and Lrp6 can displace YAP/TAZ from the destruction complex, inducing both YAP/TAZ nuclear translocation and β-catenin stabilization. When Wnt is inactive, YAP/TAZ is both sequestered by the destruction complex and forms an active part of it by recruiting β-TrCP. In *Apc* knockout mice, crypts become hyperplastic and produce adenomas as a result of overactivation of the Wnt pathway. This phenotype is reversed by the additional double knockout of *Yap* and *Taz*, preventing APC loss-induced lethality in mice (Azzolin et al., 2014). These observations fit a model in which Hippo and Wnt act in a concerted manner, with YAP/TAZ forming part of the Wnt response. Strikingly, the Wnt signature (high ectopic expression of Wnt targets such as Lef1 and CD44) is still present in the APC/YAP/TAZ mutant compared with loss of APC only (Azzolin et al., 2014). Knockdown of YAP/TAZ *in vivo* similarly does not affect the β-catenin/TCF4 gene signature (Imajo et al., 2014). These observations indicate that YAP/TAZ promote their own transcriptional program, or control other pathways, complementary to Wnt transcriptional targets. Indeed, YAP has been shown to activate EGF signaling independent of its interaction with the Wnt pathway, which is crucial for the progression of APC-mutant adenomas (Gregorieff et al., 2015).

The role of Hippo signaling during regeneration has been extensively studied. Upon induction of colitis using dextran sodium sulfate (DSS), YAP is initially downregulated (Cai et al., 2010).

Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

After withdrawal of DSS, YAP is dramatically increased during the regenerative phase. An intestinal knockout of *Yap* does not cause a phenotype during homeostasis, but interferes with regeneration in DSS-induced colitis (Cai et al., 2010). A similar dependence on YAP for regeneration was recently reported by Gregorieff and colleagues (Gregorieff et al., 2015). After irradiation, YAP nuclear localization increased after 2 days and returned to a predominantly cytoplasmic localization after 4 days. Knockout of *Yap* in CBCs caused increased apoptosis upon irradiation and a delayed regenerative response. In the absence of YAP, irradiation-induced recovery promotes a conversion of crypt cells to Paneth cells, which is a Wnt-dependent phenomenon (Gregorieff et al., 2015). In organoids, which mimic some aspects of regeneration, YAP deletion has been shown to result in a drastic decrease in crypt formation. Decreasing Wnt activity reverses the increase in Paneth cells, and restores the number of crypts in these YAP-deficient organoids (Gregorieff et al., 2015). By contrast, overexpression of YAP interferes with organoid formation by decreasing Paneth cell numbers and Wnt activity (Barry et al., 2013). This suggests that YAP acts in a narrow window by keeping Wnt in check during regeneration, thus preventing CBC exhaustion. High Wnt activity sensitizes CBCs to p53-induced apoptosis, which could explain the increased apoptosis upon irradiation when YAP is lost (Tao et al., 2015). In further support of this model, R-spondin injection in *Yap* knockout mice leads to a much larger increase in intestinal Wnt target gene expression – with a concomitant increase in CBCs – than that observed in wild-type mice (Barry et al., 2013). Besides regulating Wnt, YAP also apparently controls a transcriptional program required for regeneration. One of the YAP-dependent upregulated genes during regeneration is Ereg, an EGFR ligand (Gregorieff et al., 2015). Formation of YAP-deficient organoids is rescued by exogenous Ereg to a similar extent as inhibition of the Wnt pathway. Hippo signaling thus impacts on both EGF and Wnt signaling to control regeneration after irradiation.

The intricate, and still somewhat confusing, connection between Hippo and Wnt signaling could partly account for high nuclear β-catenin in the bottom of the crypt. YAP is nuclear in regions with active Wnt signaling, and cytoplasmic where Wnt is inactive (Barry et al., 2013). The 3D architecture of the bottom crypt, which is curved, has unique mechanical properties. Possibly as a consequence, YAP/TAZ expression at the crypt bottom is nuclear, compared with upper crypt and villus regions, where Hippo is active and YAP/TAZ are located in the cytoplasm. In these areas, YAP/TAZ may actively block Wnt signaling. Interestingly, YAP/TAZ are also Wnt targets (Konsavage et al., 2012), and can thus impose a negative feedback on Wnt signaling by inhibiting β-catenin. A comparable negative-feedback loop in the Wnt pathway has been described above, by the E3 ligases RNF43 and ZNRF3, which can downregulate frizzled receptors (Hao et al., 2012; Koo et al., 2012). Negative-feedback signaling on the Wnt pathway is a crucial part of the regenerative response and functions to prevent overactivation (Gregorieff et al., 2015).

Nutritional state and inflammation as regenerative cues

Nutritional state regulates intestinal stem cell activity

Nutritional state and inflammation have been described as upstream regulators of ICSs in the *Drosophila* gut (Jiang and Edgar, 2011). In snakes that are fed after a prolonged fasting period, a large amount of energy is directed to restoring the intestinal mucosa, underscoring the important link between nutritional status and intestinal homeostasis (Secor et al., 1994). The role of different stressors such as fasting, prolonged caloric restriction or nutrient availability have also recently been investigated in mammalian intestinal regeneration (Fig. 4A). Prolonged fasting has been shown to cause

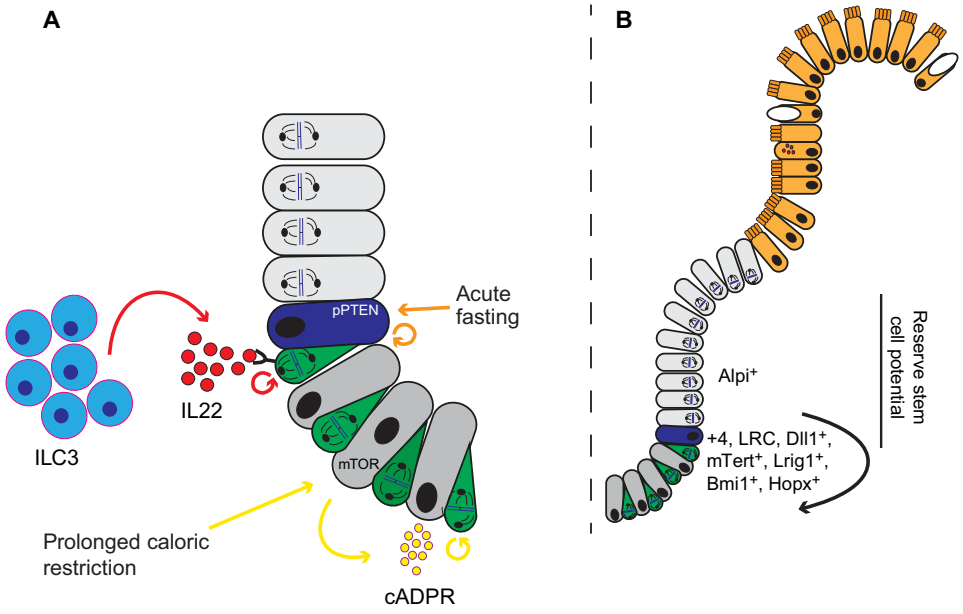

Fig. 4. Regenerative adaptations in the intestinal stem cell compartment. (A) Under normal conditions, CBSs (green) and transit-amplifying cells are continuously proliferating (indicated by mitotic spindles). When homeostasis is disturbed, various regenerative cues can result in increased proliferation of these cells (indicated by circular arrows). ILC3 cells (blue) secrete IL22, which directly increases the proliferation of CBCs that express the IL22 receptor (red arrows). Nutritional stimuli can target CBCs non-cell-autonomously. Prolonged caloric restriction (yellow arrows) augments the function of Paneth cells (gray) by inhibiting mTOR signaling. Paneth cells then secrete cyclic-ADPR (cADPR), which increases CBC self-renewal. Acute fasting (orange arrows) increases phosho-PTEN (pPTEN) levels in mTert^{+} cells (blue), directly activating their proliferation after re-feeding. (B) Stem cell potential is present along the whole crypt in dedicated Alpi^{+} cells (light gray) and cells in the +4 position (blue), which can be called upon to revert to bona fide intestinal stem cells during injury (black arrow).

Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

atrophy in the rat intestine, whereby the length and number of villi decreased (Dunel-Erb et al., 2001). Within 3 days of re-feeding, the intestinal mucosa in the rats recovered and villi were restored to normal numbers. Prolonged fasting or caloric restriction has also been shown to impinge directly on CBC activity and indirectly on the CBC niche. In mice that are calorie restricted, villi shorten and contain fewer enterocytes, and total intestinal mass decreases (Yilmaz et al., 2012). Paradoxically, CBC proliferation and number increases, whereas cell cycle activity in the transit-amplifying cell compartment decreases. Calorie restriction lowers mTOR activity in Paneth cells, which boosts both the number of Paneth cells and their function as the CBC niche cell, the latter presumably being dependent on the Paneth cell-secreted paracrine factor cyclic ADP-ribose (Yilmaz et al., 2012). When Paneth cells from calorie-restricted mice are combined with CBCs from fed mice, organoids form more efficiently. This suggests that calorie restriction increases CBCs at least in part non-cell-autonomously by hyperactivating the niche. In line with this, calorie-restricted mice resist irradiation better than their controls (Yilmaz et al., 2012).

Interestingly, a high-fat diet can increase ISC activity despite a decrease in Paneth cell number (Beyaz et al., 2016). Mice on a high-fat diet resist irradiation better than control mice, and crypts derived from these mice initiate organoid cultures more efficiently. The high-fat diet increases Wnt activity in ISCs dependent on the nuclear receptor PPARδ. Moreover, expression of the Notch ligands Jag1 and Jag2 is observed in CBCs in mice on a high-fat diet (Beyaz et al., 2016). It is possible that ISCs resist a decrease in Paneth cells by becoming independent of the niche, through expression of their own Notch ligands. These data indicate that dietary composition can promote CBC activity directly, although it is surprising that a high-fat diet and low calorie intake seem to cause similar phenotypes. Moreover, it remains to be established whether increased CBC activity is functionally relevant upon high intake of dietary fats.

Acute fasting has been shown to increase the inactive form of PTEN, pPTEN, in mTert-expressing cells (Richmond et al., 2015). mTert has been reported as marker of reserve stem cells (Montgomery et al., 2011), although it is present in Lgr5^{+} cells at the transcript level (Itzkovitz et al., 2011; Schepers et al., 2011). The number of mTert-GFP^{+} cells increases fourfold upon fasting, and lineage tracing from mTert-CreER increases accordingly in re-fed mice compared with control. This indicates activation of these cells as reserve stem cells, although crypts labeled by mTert-CreER are still rare (Richmond et al., 2015). Interestingly, lineage tracing from Lgr5^{+} cells is decreased after re-feeding suggesting inactivation or loss of these cells (Richmond et al., 2015), an important difference from the increased CBC proliferation and number observed during prolonged caloric restriction (Yilmaz et al., 2012). PTEN is a known negative regulator of AKT/mTOR signaling. When mice are fed after a 48 h fasting period, pPTEN persists in crypt cells with concomitant higher levels of active pAKT and mTOR target phospho-S6 (Richmond et al., 2015). Future studies will address the relevance of increased pAKT/mTOR signaling for recovery during re-feeding.

Inflammatory cues regulate intestinal stem cell activity

The regulation of CBC activity described so far occurs through local niche signals derived from the epithelium and mesenchyme. However, a recent seminal study by Lindemans and colleagues showed how inflammatory signals can also affect CBC behavior (Lindemans et al., 2015). This has previously been described in the *Drosophila* gut, where cytokines are secreted by enterocytes in the event of injury or infection, and activate Jak/Stat signaling in ISCs to stimulate their proliferation (Jiang et al., 2009). The mammalian intestine contains group 3 innate lymphoid cells (ILC3s), cells from the lymphoid lineage that lack antigen receptors. ILC3s reside in close proximity to intestinal crypts and are potent producers of interleukin 22 (IL22). IL22 has previously been shown to be upregulated after injury and to support subsequent epithelial regeneration (Hanash et al., 2012; Sonnenberg and Artis, 2015; Zenewicz et al., 2007). Furthermore, IL22 supports intestinal regeneration *in vivo* in a graft-versus-host disease context (Lindemans et al., 2015). In intestinal organoids, IL22 increases the phosphorylation of signal transducer and activator of transcription 3 (Stat3) in CBCs and promotes proliferation, but Wnt, Notch and EGF activities remain unchanged (Lindemans et al., 2015). Irradiated organoids upregulate the IL22 receptor, and show a higher rate of survival when treated with IL22. IL22-driven intestinal recovery occurs in the absence of Paneth cells, suggesting that it acts by directly targeting CBCs. Indeed, IL22-driven intestinal recovery depends on the presence of CBCs. When Lgr5^{+} cells are depleted using diphtheria toxin in Lgr5-DTR-EGFP organoids, IL22 fails to increase organoid size after irradiation (Lindemans et al., 2015). Pro-inflammatory signals such as IL22 can also contribute to carcinogenesis (Kirchberger et al., 2013) and are effectively counteracted by sequestering proteins such as IL22 binding protein (IL-22BP) (Huber et al., 2012).

Another recent study implies that inflammatory signals can affect symmetric division of intestinal stem cells to prevent excessive expansion during repair (Bu et al., 2016). CBCs predominantly undergo symmetric cell divisions while competing for niche space; through neutral competition clones, are eventually either lost or take over the crypt in a stochastic fashion (Snippert et al., 2010). Inflammatory signals can, however, enforce asymmetric cell divisions in CBCs. In DSS-induced colitis, the number of asymmetric cell divisions of Lgr5-GFP^{+} cells increases from 2% to 13% (Bu et al., 2016). Shen and colleagues propose that asymmetric stem cell divisions are essential to prevent inflammation-induced increase of stem cell numbers (Bu et al., 2016). Deletion of miR34a in intestinal organoids reverses this increase in asymmetric cell division following treatment with TNFα, causing a concomitant, rapid expansion of stem cell numbers. miR34a is a microRNA that negatively regulates the Notch receptor and its negative regulator Numb. By having both a negative and indirect positive effect on Notch, miR34a installs robust bimodal rather than graded Notch activity, being high in CBCs and low in their offspring (Bu et al., 2016). Deletion of Numb disrupts this binary Notch activity, creating a population of cells with intermediate Notch levels that express both stem cell and differentiated markers. It has been previously reported that miR34a is a cell fate determent that promotes differentiation after stem cell division (Bu et al., 2013). Altering the expression of miR34a can shift the balance between self-renewal and differentiation in stem cells (Bu et al., 2013). It remains to be established how differential miR34a and Notch activity are able to switch CBCs from dividing symmetrically to asymmetrically during inflammation. Future studies may utilize multi-lineage Cre reporters such as Rosa-Confetti (Snippert et al., 2010) to assess whether miR34a controls symmetric cell division *in vivo*. Upon loss of miR34a, a drift towards clonality is expected to be delayed if stem cell divisions would preferentially become asymmetric. In summary, these studies add the immune system as 'external' regulator of CBC activity by acting independently of the local niche.

Progenitor cell plasticity

Lgr5^{+} cells can be selectively removed in Lgr5-DTR-EGFP mice, which express the diphtheria toxin receptor under control of the

Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

Lgr5 promoter (Tian et al., 2012). For at least a week, depletion of CBCs does not disturb homeostasis, which is suggestive of an alternative stem cell, or alternatively a non-stem-cell source that can gain stem cell potential (Tian et al., 2012). Plasticity among progenitors would provide a large alternative source of stem cell potential (Fig. 4B).

Dedifferentiation of committed progenitors

As previously discussed, the +4 cells described by Potten and colleagues have been proposed as quiescent, reserve stem cells in the intestine (Li and Clevers, 2010). Reserve stem cell capacity of secretory progenitors has been specifically assessed using a Dll1-GFP-IRES-CREERT2 knock-in mouse (van Es et al., 2012a). Delta-like 1 (Dll1) is a Notch ligand expressed in secretory progenitors, and tracing indicates that these cells do indeed produce the entire repertoire of secretory cell types during homeostasis. Destruction of $Lgr5^+$ stem cells by irradiation can induce reversion of the committed secretory state to a stem cell state, whereby intestinal crypts are fully labeled after Dll1 tracing (van Es et al., 2012a).

Winton and colleagues have examined the reserve stem cell capacity of non-dividing cells more broadly. For this, they developed an inducible histone 2B-YFP (H2B-YFP) knock-in mouse (Buczacki et al., 2013). As expected, the long-lived Paneth cells retain the histone label for up to 8 weeks. Interestingly, a second population of label-retaining cells expresses markers of the enteroendocrine lineage, as well as the CBC marker Lgr5 and the proposed quiescent stem cell markers Bmi1 and mTert. These LRCs thus have a combined enteroendocrine and stem cell signature, and reside predominantly at the +4 position. To assess the fate of these LRCs during homeostasis and injury, an ingenious lineage-tracing strategy was used to trace cells based on their quiescence: Cre-recombinase was expressed as two fragments, one part produced ubiquitously from the *Rosa26* locus and another part inducibly, fused to histone 2B. Binding of these two fragments was dependent on a dimerizing agent (Buczacki et al., 2013). Lineage tracing of the cells that were histone label-retaining for up to 2 weeks showed that these cells could revert to the stem cell state upon injury. Moreover, $H2B\text{-}YFP^+$ cells could form organoids when isolated and stimulated with the niche signal Wnt3, further indicative of their capacity to act as stem cells. In support of these findings, quiescent Lgr5-low cells have been identified as secretory progenitors in a KI67-RFP knock-in mouse model (Basak et al., 2014).

Taken together, these studies imply that the non-dividing cells observed by Potten at the +4 location are secretory progenitors that retain 'facultative' stem cell potential upon injury, and may exclude the existence of genuine 'professional' quiescent stem cells (Table 2). When CBCs are lost, progenitor cells fall back in the stem cell niche and revert to stemness, probably through contact with Paneth cells and the availability of a potent Wnt source. Location in the stem cell zone itself is indeed linked to stemness, with $Lgr5^+$ CBC stem cells located at the border of the niche displaying a survival disadvantage over other CBCs residing in the bottom of the crypt (Ritsma et al., 2014).

Secretory progenitors retain stem cell potential although they form only a small part of the committed progenitor population. The majority of the intestinal epithelium consists of absorptive enterocytes, and their crypt progenitors are abundant and highly proliferative. If these cells could also act as stem cells when returning into the niche, the pool of 'reserve stem cell potential' would be much larger. To analyze whether potential stem cells exist among enterocyte progenitors, our lab generated an Alpi-IRES-CreERT2 knock-in mouse, based on intestinal alkaline phosphatase (Alpi), which has been widely used as marker for enterocytes and their progenitors (Tetteh et al., 2016). Clones derived from Alpi-expressing cells did indeed contain only enterocytes, and were entirely lost within days at the top of the villus under physiological conditions (Tetteh et al., 2016). Fifteen hours after tracing, the 'lowest' $Alpi^+$ cells were found around the +8 position counted from the crypt base, and did not co-express secretory markers. Upon ablation of $Lgr5^+$ CBCs, $Alpi^+$ cells contributed extensively to long-term tracing and produced all differentiated cell types, suggesting that these progenitors can readily regain stem cell potential, similar to secretory progenitor cells. Strikingly, ablation of CBCs 2-3 days after labeling $Alpi^+$ cells still resulted in rare tracing events. The 'lowest' $Alpi^+$ cells at these time points were already exiting the crypts. Apparently these cells still can act as stem cells, albeit rarely. It is likely that for these cells to return to the stem cell niche, a collapse of the entire crypt is required.

Conclusions

In this Review, we have summarized the role of different signaling pathways during crypt homeostasis and regeneration, with a particular focus on cellular dynamics within the intestinal stem cell compartment. An important question remains as to what the upstream regulators are of these signaling pathways in the injury setting. How is damage sensed and translated into the production of regenerative signals? A model that directly links injury to regenerative signals was described in the freshwater polyp *Hydra*. Here, cells undergoing apoptosis secrete Wnt3 to promote cell division of neighboring cells (Galliot, 2013). *In vitro* models such as organoids represent simple systems that may help to dissect signals that restore homeostasis after injury (Huch and Koo, 2015; Clevers, 2016). Organoids can be damaged chemically or mechanically, or can be irradiated to establish models of regeneration. Co-cultures with mesenchyme or immune cells would then be valuable for the identification of non-epithelial-derived signals that confer adaptability to damage.

Table 2. Intestinal cell types that demonstrate stem cell potential upon injury

Cell type	Marker(s)	Summary of studies	References
Secretory progenitor	Dll1	$Dll1^+$ cells produce all secretory cells during homeostasis and regain stemness upon damage.	(van Es et al., 2012a)
Enterocyte progenitor	Alpi	$Alpi^+$ cells produce enterocytes during homeostasis and regain stemness upon damage.	(Tetteh et al., 2016)
Label-retaining cells	H2B retaining, $Lgr5GFP^{low}Ki67^{low}$	Non-dividing early stem cell daughters with low Lgr5 expression are secretory progenitors that retain stemness	(Basak et al., 2014; Buczacki et al., 2013)
'Reserve' stem cells	Bmi1, Hopx, mTert, Lrig1	Bmi1, Hopx, mTert and Lrig1 mark the +4 cell, which shows stem cell potential upon lineage tracing.	(Montgomery et al., 2011; Powell et al., 2012; Sangiorgi and Capecchi, 2008; Takeda et al., 2011)

Development (2016) 143, 3639-3649 doi:10.1242/dev.133132

All progenitor cells in the intestinal crypts display a high level of plasticity upon damage, and have been shown to dedifferentiate to stem cells in mouse models in which CBCs were artificially removed. The inherent capacity of these cells to switch fates is likely to be related to their open chromatin structure (Kim et al., 2014). But does intestinal regeneration really depend on plasticity among progenitor cells? Winton and colleagues have shown how label-retaining cells can dedifferentiate in response to different kinds of perturbations, for example treatment with hydroxyurea, doxorubicin or irradiation. This dedifferentiation is a rare event and its efficiency differs depending on the type of injury (see figure 4E in Buczacki et al., 2013). Simultaneous depletion of CBCs and 6 Gy irradiation, the latter normally well-tolerated in mice, causes rapid crypt loss and disruption of the epithelial architecture (Metcalfe et al., 2014). This suggests that potential stem cells are radiosensitive and irreversibly affected, impairing their ability to revert to the stem cell state. Nevertheless, CBCs are also indispensable for irradiation-induced damage, which is a remarkable observation given their high proliferative activity. It has been previously found that CBCs are indeed very good at repairing their genome after irradiation, and do so by the low error prone homologous recombination (Hua et al., 2012). Future work will focus on teasing out the events in which regeneration is primarily driven by the differential activity of CBCs, and those in which the reserve stem cell pool of committed progenitor cells is called into action.

Acknowledgements

We thank Kim Boonekamp and Onur Basak for discussions and critically reading the manuscript, and Harry Begthel for providing immunohistochemistry figures.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was supported by the Cancer Genomics Center (CGC) via the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research (H.C.); and the European Research Council (H.C.).

References

Alexandre, C., Baena-Lopez, A. and Vincent, J.-P. (2014). Patterning and growth control by membrane-tethered Wingless. *Nature* **505**, 180-185.

Aoki, R., Shoshkes-Carmel, M., Gao, N., Shin, S., May, C. L., Golson, M. L., Zahm, A. M., Ray, M., Wiser, C. L., Wright, C. V. E. et al. (2016). Foxl1-sxpressing mesenchymal cells constitute the intestinal stem cell niche. *Cell. Mol. Gastroenterol. Hepatol.* **2**, 175-188.

Ashton, G. H., Morton, J. P., Myant, K., Phesse, T. J., Ridgway, R. A., Marsh, V., Wilkins, J. A., Athineos, D., Muncan, V., Kemp, R. et al. (2010). Focal Adhesion Kinase is required for intestinal regeneration and tumorigenesis downstream of Wnt/c-Myc signaling. *Dev. Cell* **19**, 259-269.

Azzolin, L., Panciera, T., Soligo, S., Enzo, E., Bicciato, S., Dupont, S., Bresolin, S., Frasson, C., Basso, G., Guzzardo, V. et al. (2014). YAP/TAZ incorporation in the β-catenin destruction complex orchestrates the Wnt response. *Cell* **158**, 157-170.

Barker, N., van Es, J. H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P. J. et al. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**, 1003-1007.

Barker, N., Ridgway, R. A., van Es, J. H., van de Wetering, M., Begthel, H., van den Born, M., Danenberg, E., Clarke, A. R., Sansom, O. J. and Clevers, H. (2009). Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* **457**, 608-611.

Barry, E. R., Morikawa, T., Butler, B. L., Shrestha, K., de la Rosa, R., Yan, K. S., Fuchs, C. S., Magness, S. T., Smits, R., Ogino, S. et al. (2013). Restriction of intestinal stem cell expansion and the regenerative response by YAP. *Nature* **493**, 106-110.

Basak, O., van de Born, M., Korving, J., Beumer, J., van der Elst, S., van Es, J. H. and Clevers, H. (2014). Mapping early fate determination in Lgr5+ crypt stem cells using a novel Ki67-RFP allele. *EMBO J.* **33**, 2057-2068.

Batlle, E., Henderson, J. T., Beghtel, H., van den Born, M. M. W., Sancho, E., Huls, G., Meeldijk, J., Robertson, J., van de Wetering, M., Pawson, T. et al. (2002). β-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/EphrinB. *Cell* **111**, 251-263.

Beyaz, S., Mana, M. D., Roper, J., Kedrin, D., Saadatpour, A., Hong, S.-J., Bauer-Rowe, K. E., Xifaras, M. E., Akkad, A., Arias, E. et al. (2016). High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature* **531**, 53-58.

Bu, P., Chen, K.-Y., Chen, J. H., Wang, L., Walters, J., Shin, Y. J., Goerger, J. P., Sun, J., Witherspoon, M., Rakhilin, N. et al. (2013). A microRNA miR-34a-regulated bimodal switch targets notch in colon cancer stem cells. *Cell Stem Cell* **12**, 602-615.

Bu, P., Wang, L., Chen, K.-Y., Srinivasan, T., Murthy, P. K. L., Tung, K.-L., Varanko, A. K., Chen, H. J., Ai, Y., King, S. et al. (2016). A miR-34a-Numb feedforward loop triggered by inflammation regulates asymmetric stem cell division in intestine and colon cancer. *Cell Stem Cell* **18**, 189-202.

Buczacki, S. J. A., Zecchini, H. I., Nicholson, A. M., Russell, R., Vermeulen, L., Kemp, R. and Winton, D. J. (2013). Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature* **495**, 65-69.

Cai, J., Zhang, N., Zheng, Y., de Wilde, R. F., Maitra, A. and Pan, D. (2010). The Hippo signaling pathway restricts the oncogenic potential of an intestinal regeneration program. *Genes Dev.* **24**, 2383-2388.

Cheng, H. and Leblond, C. P. (1974). Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. *Am. J. Anat.* **141**, 537-561.

Clevers, H. (2013). The intestinal crypt, a prototype stem cell compartment. *Cell* **154**, 274-284.

Clevers, H. (2015). What is an adult stem cell? *Science* **350**, 1319-1320.

Clevers, H. (2016). Modeling development and disease with organoids. *Cell* **165**, 1586-1597.

Clevers, H. and Nusse, R. (2012). Wnt/β-catenin signaling and disease. *Cell* **149**, 1192-1205.

Cordero, J. B. and Sansom, O. J. (2012). Wnt signalling and its role in stem cell-driven intestinal regeneration and hyperplasia. *Acta Physiol.* **204**, 137-143.

Dunel-Erb, S., Chevalier, C., Laurent, P., Bach, A., Decrock, F. and Le Maho, Y. (2001). Restoration of the jejunal mucosa in rats refed after prolonged fasting. *Comp. Biochem. Physiol. – A Mol. Integr. Physiol.* **129**, 933-947.

Farin, H. F., Van Es, J. H. and Clevers, H. (2012). Redundant sources of Wnt regulate intestinal stem cells and promote formation of paneth cells. *Gastroenterology* **143**, 1518-1529.e7.

Farin, H. F., Jordens, I., Mosa, M. H., Basak, O., Korving, J., Tauriello, D. V. F., de Punder, K., Angers, S., Peters, P. J., Maurice, M. M. et al. (2016). Visualization of a short-range Wnt gradient in the intestinal stem-cell niche. *Nature* **530**, 340-343.

Galliot, B. (2013). Injury-induced asymmetric cell death as a driving force for head regeneration in Hydra. *Dev. Genes Evol.* **223**, 39-52.

Gregorieff, A., Liu, Y., Inanlou, M. R., Khomchuk, Y. and Wrana, J. L. (2015). Yap-dependent reprogramming of Lgr5+ stem cells drives intestinal regeneration and cancer. *Nature* **526**, 715-718.

Grün, D., Lyubimova, A., Kester, L., Wiebrands, K., Basak, O., Sasaki, N., Clevers, H. and van Oudenaarden, A. (2015). Single-cell messenger RNA sequencing reveals rare intestinal cell types. *Nature* **525**, 251-255.

Guo, Z., Driver, I. and Ohlstein, B. (2013). Injury-induced BMP signaling negatively regulates Drosophila midgut homeostasis. *J. Cell Biol.* **201**, 945-961.

Hanash, A. M., Dudakov, J. A., Hua, G., O'Connor, M. H., Young, L. F., Singer, N. V., West, M. L., Jenq, R. R., Holland, A. M., Kappel, L. W. et al. (2012). Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. *Immunity* **37**, 339-350.

Hao, H.-X., Xie, Y., Zhang, Y., Charlat, O., Oster, E., Avello, M., Lei, H., Mickanin, C., Liu, D., Ruffner, H. et al. (2012). ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature* **485**, 195-200.

Haramis, A.-P. G. (2004). De novo crypt formation and Juvenile Polyposis on BMP inhibition in mouse intestine. *Science* **303**, 1684-1686.

Harburg, G., Compton, J., Liu, W., Iwai, N., Zada, S., Marlow, R., Strickland, P., Zeng, Y. A. and Hinck, L. (2014). SLIT/ROBO2 signaling promotes mammary stem cell senescence by inhibiting wnt signaling. *Stem Cell Rep.* **3**, 385-393.

He, X. C., Zhang, J., Tong, W.-G., Tawfik, O., Ross, J., Scoville, D. H., Tian, Q., Zeng, X., He, X., Wiedemann, L. M. et al. (2004). BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-β-catenin signaling. *Nat. Genet.* **36**, 1117-1121.

Hogan, B. L. M., Barkauskas, C. E., Chapman, H. A., Epstein, J. A., Jain, R., Hsia, C. C. W., Niklason, L., Calle, E., Le, A., Randell, S. H. et al. (2014). Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell* **15**, 123-138.

Holmberg, J., Genander, M., Halford, M. M., Annerén, C., Sondell, M., Chumley, M. J., Silvany, R. E., Henkemeyer, M. and Frisén, J. (2006). EphB receptors coordinate migration and proliferation in the intestinal stem cell niche. *Cell* **125**, 1151-1163.

Howe, J. R., Bair, J. L., Sayed, M. G., Anderson, M. E., Mitros, F. A., Petersen, G. M., Velculescu, V. E., Traverso, G. and Vogelstein, B. (2001). Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. *Nat. Genet.* **28**, 184-187.

Hua, G., Thin, T. H., Feldman, R., Haimovitz-Friedman, A., Clevers, H., Fuks, Z. and Kolesnick, R. (2012). Crypt base columnar stem cells in small intestines of mice are radioresistant. *Gastroenterology* **143**, 1266-1276.
Huber, S., Gagliani, N., Zenewicz, L. A., Huber, F. J., Bosurgi, L., Hu, B., Hedl, M., Zhang, W., O'Connor, W. Jr, Murphy, A. J. et al. (2012). IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. *Nature* **491**, 259-263.
Huch, M. and Koo, B.-K. (2015). Modeling mouse and human development using organoid cultures. *Development* **142**, 3113-3125.
Imajo, M., Ebisuya, M. and Nishida, E. (2014). Dual role of YAP and TAZ in renewal of the intestinal epithelium. *Nat. Cell Biol.* **17**, 7-19.
Itzkovitz, S., Lyubimova, A., Blat, I. C., Maynard, M., van Es, J., Lees, J., Jacks, T., Clevers, H. and van Oudenaarden, A. (2011). Single-molecule transcript counting of stem-cell markers in the mouse intestine. *Nat. Cell Biol.* **14**, 106-114.
Jiang, H. and Edgar, B. A. (2011). Intestinal stem cells in the adult Drosophila midgut. *Exp. Cell Res.* **317**, 2780-2788.
Jiang, H., Patel, P. H., Kohlmaier, A., Grenley, M. O., McEwen, D. G. and Edgar, B. A. (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the drosophila midgut. *Cell* **137**, 1343-1355.
Kabiri, Z., Greicius, G., Madan, B., Biechele, S., Zhong, Z., Zaribafzadeh, H., Edison, H., Aliyev, J., Wu, Y., Bunte, R. et al. (2014). Stroma provides an intestinal stem cell niche in the absence of epithelial Wnts. *Development* **141**, 2206-2215.
Karpowicz, P., Perez, J. and Perrimon, N. (2010). The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. *Development* **137**, 4135-4145.
Kim, K.-A., Kakitani, M., Zhao, J., Oshima, T., Tang, T., Binnerts, M., Liu, Y., Boyle, B., Park, E., Emtage, P. et al. (2005). Mitogenic influence of human R-Spondin1 on the intestinal epithelium. *Science* **309**, 1256-1259.
Kim, T.-H., Li, F., Ferreiro-Neira, I., Ho, L.-L., Luyten, A., Nalapareddy, K., Long, H., Verzi, M. and Shivdasani, R. A. (2014). Broadly permissive intestinal chromatin underlies lateral inhibition and cell plasticity. *Nature* **506**, 511-515.
Kirchberger, S., Royston, D. J., Boulard, O., Thornton, E., Franchini, F., Szabady, R. L., Harrison, O. and Powrie, F. (2013). Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *J. Exp. Med.* **210**, 917-931.
Konsavage, W. M., Jr, Kyler, S. L., Rennoll, S. A., Jin, G. and Yochum, G. S. (2012). Wnt/β-Catenin signaling regulates Yes-associated protein (YAP) gene expression in colorectal carcinoma cells. *J. Biol. Chem.* **287**, 11730-11739.
Koo, B.-K., Spit, M., Jordens, I., Low, T. Y., Stange, D. E., van de Wetering, M., van Es, J. H., Mohammed, S., Heck, A. J. R., Maurice, M. M. et al. (2012). Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature* **488**, 665-669.
Korinek, V., Barker, N., Willert, K., Molenaar, M., Roose, J., Wagenaar, G., Markman, M., Lamers, W., Destree, O. and Clevers, H. (1998). Two members of the Tcf family implicated in Wnt/β-catenin signaling during embryogenesis in the mouse. *Mol. Cell. Biol.* **18**, 1248-1256.
Kosinski, C., Li, V. S. W., Chan, A. S. Y., Zhang, J., Ho, C., Tsui, W. Y., Chan, T. L., Mifflin, R. C., Powell, D. W., Yuen, S. T. et al. (2007). Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors. *Proc. Natl. Acad. Sci. USA* **104**, 15418-15423.
Kretzschmar, K. and Watt, F. M. (2012). Lineage tracing. *Cell* **148**, 33-45.
Kuhnert, F., Davis, C. R., Wang, H.-T., Chu, P., Lee, M., Yuan, J., Nusse, R. and Kuo, C. J. (2004). Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. *Proc. Natl. Acad. Sci. USA* **101**, 266-271.
Li, L. and Clevers, H. (2010). Coexistence of quiescent and active adult stem cells in mammals. *Science* **327**, 542-545.
Li, V. S. W. and Clevers, H. (2013). Intestinal regeneration: YAP-tumor suppressor and oncoprotein? *Curr. Biol.* **23**, R110-R112.
Li, N., Yousefi, M., Nakauka-Ddamba, A., Jain, R., Tobias, J., Epstein, J. A., Jensen, S. T. and Lengner, C. J. (2014). Single-cell analysis of proxy reporter allele-marked epithelial cells establishes intestinal stem cell hierarchy. *Stem Cell Reports* **3**, 876-891.
Lindemans, C. A., Calafiore, M., Mertelsmann, A. M., O'Connor, M. H., Dudakov, J. A., Jenq, R. R., Velardi, E., Young, L. F., Smith, O. M., Lawrence, G. et al. (2015). Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* **528**, 560-564.
Metcalfe, C., Kljavin, N. M., Ybarra, R. and de Sauvage, F. J. (2014). Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration. *Cell Stem Cell* **14**, 149-159.
Mikels, A. J. and Nusse, R. (2006). Wnts as ligands: processing, secretion and reception. *Oncogene* **25**, 7461-7468.
Milano, J., McKay, J., Dagenais, C., Foster-Brown, L., Pognan, F., Gadient, R., Jacobs, R. T., Zacco, A., Greenberg, B. and Ciaccio, P. J. (2004). Modulation of Notch processing by γ-secretase inhibitors causes intestinal goblet cell metaplasia and induction of genes known to specify gut secretory lineage differentiation. *Toxicol. Sci.* **82**, 341-358.
Miyoshi, H., Ajima, R., Luo, C. T., Yamaguchi, T. P. and Stappenbeck, T. S. (2013). Wnt5a potentiates TGF-β signaling to promote colonic crypt regeneration after tissue injury. *Science* **338**, 108-113.
Montgomery, R. K., Carlone, D. L., Richmond, C. A., Farilla, L., Kranendonk, M. E. G., Henderson, D. E., Baffour-Awuah, N. Y., Ambruzs, D. M., Fogli, L. K., Algra, S. et al. (2011). Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. *Proc. Natl. Acad. Sci. USA* **108**, 179-184.
Morin, P., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B. and Kinzler, K. W. (1997). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* **275**, 1787-1790.
Muñoz, J., Stange, D. E., Schepers, A. G., van de Wetering, M., Koo, B.-K., Itzkovitz, S., Volckmann, R., Kung, K. S., Koster, J., Radulescu, S. et al. (2012). The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent "+4" cell markers. *EMBO J.* **31**, 3079-3091.
Pan, D. (2010). The hippo signaling pathway in development and cancer. *Dev. Cell* **19**, 491-505.
Pinto, D., Gregorieff, A., Begthel, H. and Clevers, H. (2003). Canonical Wnt signals are essential for homeostasis of the intestinal epithelium service Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev.* **17**, 1709-1713.
Potten, C. S., Kovacs, L. and Hamilton, E. (1974). Continuous labelling studies on mouse skin and intestine. *Cell Tissue Kinet.* **7**, 271-283.
Powell, D. W., Pinchuk, I. V., Saada, J. I., Chen, X. and Mifflin, R. C. (2011). Mesenchymal cells of the intestinal lamina propria. *Annu. Rev. Physiol.* **73**, 213-237.
Powell, A. E., Wang, Y., Li, Y., Poulin, E. J., Means, A. L., Washington, M. K., Higginbotham, J. N., Juchheim, A., Prasad, N., Levy, S. E. et al. (2012). The pan-ErbB negative regulator lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. *Cell* **149**, 146-158.
Richmond, C. A., Shah, M. S., Deary, L. T., Trotier, D. C., Thomas, H., Ambruzs, D. M., Jiang, L., Whiles, B. B., Rickner, H. D., Montgomery, R. K. et al. (2015). Dormant intestinal stem cells are regulated by PTEN and nutritional Status. *Cell Rep.* **13**, 2403-2411.
Riemer, P., Sreekumar, A., Reinke, S., Rad, R., Schäfer, R., Sers, C., Bläker, H., Herrmann, B. G. and Morkel, M. (2015). Transgenic expression of oncogenic BRAF induces loss of stem cells in the mouse intestine, which is antagonized by β-catenin activity. *Oncogene* **34**, 3164-3175.
Ritsma, L., Ellenbroek, S. I. J., Zomer, A., Snippert, H. J., de Sauvage, F. J., Simons, B. D., Clevers, H. and van Rheenen, J. (2014). Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging. *Nature* **507**, 362-365.
Sánchez Alvarado, A. and Yamanaka, S. (2014). Rethinking differentiation: Stem cells, regeneration, and plasticity. *Cell* **157**, 110-119.
Sangiorgi, E. and Capecchi, M. R. (2008). Bmi1 is expressed in vivo in intestinal stem cells. *Nat. Genet.* **40**, 915-920.
Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., van Es, J. H., Abo, A., Kujala, P., Peters, P. J. et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262-265.
Sato, T., van Es, J. H., Snippert, H. J., Stange, D. E., Vries, R. G., van den Born, M., Barker, N., Shroyer, N. F., van de Wetering, M. and Clevers, H. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* **469**, 415-418.
Schepers, A. G., Vries, R., van den Born, M., van de Wetering, M. and Clevers, H. (2011). Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. *EMBO J.* **30**, 1104-1109.
Secor, S. M., Stein, E. D. and Diamond, J. (1994). Rapid up-regulation of snake intestine in response to feeding - a new model of intestinal adaptation. *Am. J. Physiol.* **266**, G695-G705.
Seiler, K. M., Schenhals, E. L., von Furstenberg, R. J., Allena, B. K., Smith, B. J., Scaria, D., Bresler, M. N., Dekaney, C. M. and Henning, S. J. (2015). Tissue underlying the intestinal epithelium elicits proliferation of intestinal stem cells following cytotoxic damage. *Cell Tissue Res.* **361**, 427-438.
Shroyer, N. F., Helmrath, M. A., Wang, V. Y.-C., Antalffy, B., Henning, S. J. and Zoghbi, H. Y. (2007). Intestine-specific ablation of Mouse atonal homolog 1 (Math1) reveals a role in cellular homeostasis. *Gastroenterology* **132**, 2478-2488.
Snippert, H. J., van der Flier, L. G., Sato, T., van Es, J. H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A. M., van Rheenen, J., Simons, B. D. et al. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143**, 134-144.
Snippert, H. J., Schepers, A. G., Van Es, J. H., Simons, B. D. and Clevers, H. (2014). Biased competition between Lgr5 intestinal stem cells driven by oncogenic mutation induces clonal expansion. *EMBO Rep.* **15**, 62-69.
Sonnenberg, G. F. and Artis, D. (2015). Innate lymphoid cells in the initiation, regulation and resolution of inflammation. *Nat. Med.* **21**, 698-708.
Takeda, N., Jain, R., LeBoeuf, M. R., Wang, Q., Lu, M. M. and Epstein, J. A. (2011). Interconversion between intestinal stem cell populations in distinct niches. *Science* **334**, 1420-1424.

Tao, S., Tang, D., Morita, Y., Sperka, T., Omrani, O., Lechel, A., Sakk, V., Kraus, J., Kestler, H. A., Kühl, M. et al. (2015). Wnt activity and basal niche position sensitize intestinal stem and progenitor cells to DNA damage. *EMBO J.* **34**, 624-640.

Tetteh, P. W., Basak, O., Farin, H. F., Wiebrands, K., Kretzschmar, K., Begthel, H., van den Born, M., Korving, J., de Sauvage, F., van Es, J. H. et al. (2016). Replacement of lost Lgr5-positive stem cells through plasticity of their enterocyte-lineage daughters. *Cell Stem Cell* **18**, 203-213.

Tian, H., Biehs, B., Warming, S., Leong, K. G., Rangell, L., Klein, O. D. and de Sauvage, F. J. (2012). A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature* **482**, 120-120.

Ueo, T., Imayoshi, I., Kobayashi, T., Ohtsuka, T., Seno, H., Nakase, H., Chiba, T. and Kageyama, R. (2012). The role of Hes genes in intestinal development, homeostasis and tumor formation. *Development* **139**, 1071-1082.

van Es, J. H., van Gijn, M. E., Riccio, O., van den Born, M., Vooijs, M., Begthel, H., Cozijnsen, M., Robine, S., Winton, D. J., Radtke, F. et al. (2005). Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* **435**, 959-963.

van Es, J. H., de Geest, N., van de Born, M., Clevers, H. and Hassan, B. A. (2010). Intestinal stem cells lacking the Math1 tumour suppressor are refractory to Notch inhibitors. *Nat. Commun.* **1**, 18.

van Es, J. H., Sato, T., van de Wetering, M., Lyubimova, A., Yee Nee, A. N., Gregorieff, A., Sasaki, N., Zeinstra, L., van den Born, M., Korving, J. et al. (2012a). Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nat. Cell Biol.* **14**, 1099-1104.

van Es, J. H., Haegebarth, A., Kujala, P., Itzkovitz, S., Koo, B.-K., Boj, S. F., Korving, J., van den Born, M., van Oudenaarden, A., Robine, S. et al. (2012b). A critical role for the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal. *Mol. Cell. Biol.* **32**, 1918-1927.

Varelas, X. (2014). The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease. *Development* **141**, 1614-1626.

Vermeulen, L., Morrissey, E., van der Heijden, M. Nicholson, A. M., Sottoriva, A., Buczacki, S., Kemp, R., Tavaré, S. and Winton, D. J. (2013). Defining stem cell dynamics in models of intestinal tumor initiation. *Science* **342**, 995-998.

Withers, H. R. and Elkind, M. M. (1970). Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **17**, 261-267.

Wong, V. W. Y., Stange, D. E., Page, M. E., Buczacki, S., Wabik, A., Itami, S., van de Wetering, M., Poulsom, R., Wright, N. A., Trotter, M. W. B. et al. (2012). Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. *Nat. Cell Biol.* **14**, 401-408.

Yilmaz, Ö. H., Katajisto, P., Lamming, D. W., Gültekin, Y., Bauer-Rowe, K. E., Sengupta, S., Birsoy, K., Dursun, A., Yilmaz, V. O., Selig, M. et al. (2012). mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature* **486**, 490-495.

Zenewicz, L. A., Yancopoulos, G. D., Valenzuela, D. M., Murphy, A. J., Karow, M. and Flavell, R. A. (2007). Interleukin-22 but not Interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity* **27**, 647-659.

Zhang, Q.-Q., Zhou, D.-L., Lei, Y., Zheng, L., Chen, S.-X., Gou, H.-J., Gu, Q.-L., He, X.-D., Lan, T., Qi, C.-L. et al. (2015). Slit2/Robo1 signaling promotes intestinal tumorigenesis through Src-mediated activation of the Wnt/β-catenin pathway. *Oncotarget* **6**, 3123-3135.

Zhou, W.-J., Geng, Z. H., Spence, J. R. and Geng, J.-G. (2013). Induction of intestinal stem cells by R-spondin 1 and Slit2 augments chemoradioprotection. *Nature* **501**, 107-111.