Review

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Plasticity within stem cell hierarchies in mammalian epithelia

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Tissue homeostasis and regeneration are fueled by resident stem cells that have the capacity to self-renew, and to generate all the differentiated cell types that characterize a particular tissue. Classical models of such cellular hierarchies propose that commitment and differentiation occur unidirectionally, with the arrows 'pointing away' from the stem cell. Recent studies, all based on genetic lineage tracing, describe various strategies employed by epithelial stem cell hierarchies to replace damaged or lost cells. While transdifferentiation from one tissue type into another ('metaplasia') appears to be generally forbidden in nonpathological contexts, plasticity within an individual tissue stem cell hierarchy may be much more common than previously appreciated. In this review, we discuss recent examples of such plasticity in selected mammalian epithelia, highlighting the different modes of regeneration and their implications for our understanding of cellular hierarchy and tissue self-renewal.

Epithelial tissue homeostasis and regeneration

Cells lost through physiological ageing or as a result of environmental insults must be continuously replaced to preserve the 'cellular status quo' of an organism. This homeostasis is achieved by undifferentiated, self-renewing stem cells that can generate all cell types of the tissue (Figure 1). Some adult tissues, such as the epithelia of intestine, stomach, and skin, are exposed to mechanical wear-and-tear and are continuously self-renewing. Epithelia of other internal organs, such as liver, pancreas, or kidneys, are typically self-renewing at a low rate. Although some general rules may apply across these tissues, each appears to employ uniquely designed stem cell hierarchies and, correspondingly, unique tissue architectures to fulfill specific physiologic demands.

Plasticity refers to the ability of cells to adopt an alternate cellular fate in response to extrinsic or intrinsic factors. When this plasticity involves a differentiated cell changing into another differentiated cell of another lineage within a given tissue, we term this transdifferentiation. Dedifferentiation is a form of plasticity in which a differentiated cell reverts to a less differentiated cell within the same tissue lineage. Of note, this 'working definition' of plasticity excludes epithelial–mesenchymal transitions observed during embryogenesis and tumorigenesis.

Although tissue stem cells are generally viewed as the major drivers of tissue regeneration after damage, endogenous dedifferentiation and transdifferentiation of non-stem cell populations has been observed to play a key role in tissue replacement in planarians, amphibians, fishes, and even some nonepithelial tissues in mammals, (reviewed elsewhere [1–3]). Emerging evidence from mammalian systems implies that differentiated epithelial cells can function as reserve stem cells upon tissue damage, implying that this might be a universal phenomenon adopted by multicellular organisms to replenish lost cells. This has important implications for the definition of what constitutes a stem cell and for our views on cellular hierarchies in homeostasis, regeneration, and in pathologies such as cancer. In this review, we discuss recent examples of endogenous plasticity (without genetic/epigenetic manipulation) in the epithelia of organs of the gastrointestinal tract, lung, kidney, and adrenal cortex, and highlight different regenerative strategies. Notably, we focus on the intestinal epithelium as an excellent genetic model of plasticity due to the extensive characterization of stem and differentiated cell populations, which enable *bona fide* cell fate conversions to be established.

The intestine as a model of plasticity

Although the liver and pancreas are much more renowned for regeneration, their suitability as model systems to study epithelial plasticity is laden with controversies surrounding the existence of stem/progenitor cells during homeostasis and regeneration [4–7]. In the single-layered intestine epithelium, however, the localization of all stem cell populations and differentiated cells is known, all cell lineages have been extensively characterized, and multiple mouse models based on stem cell marker genes exist, as well as well-defined injury models that allow the elimination of specific cell types.

Stem cell populations in intestinal homeostasis

The intestinal epithelium is the fastest self-renewing tissue in mammals. The rapid cellular turnover of the intestinal epithelium is propelled by daily proliferation of stem cells located at crypt bottoms to generate rapidly dividing daughter cells (Figure 2A). These in turn differentiate into secretory cells (Paneth cells, goblet cells, and enteroendocrine cells) or absorptive enterocytes, which make up the bulk of the villus epithelium (Figure 2B).

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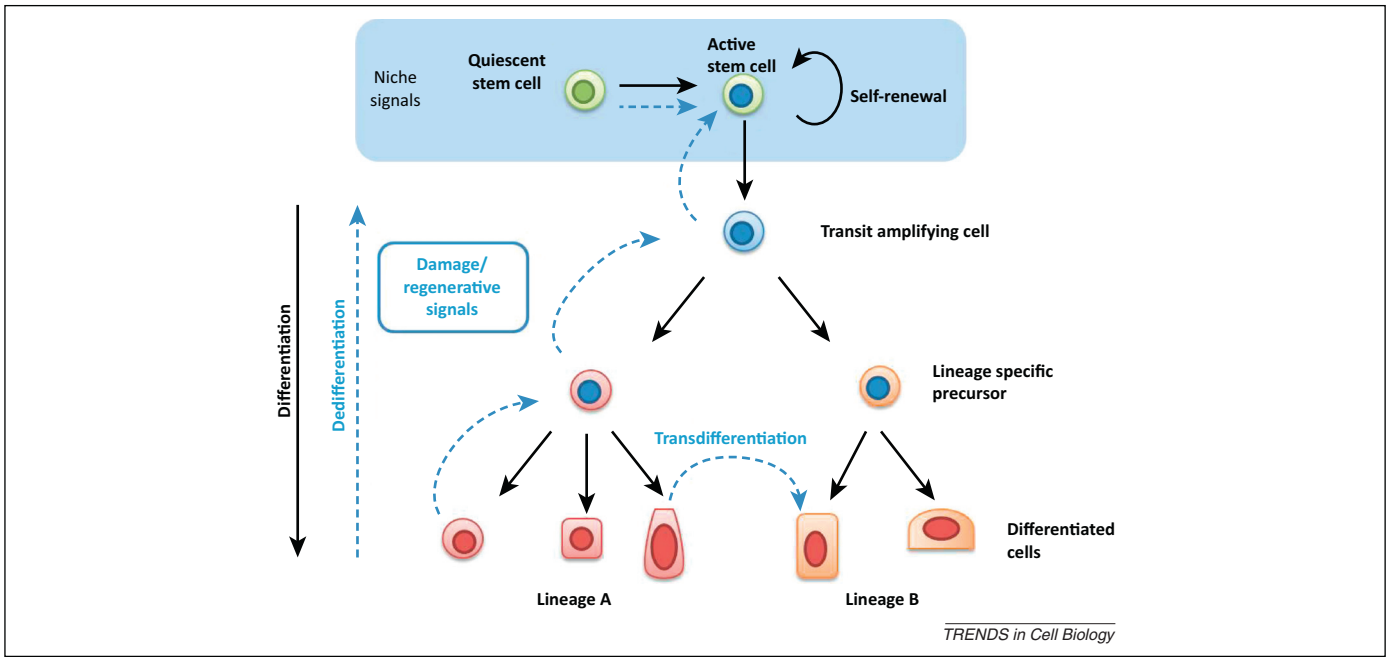

Figure 1. Stem cell hierarchy in homeostasis and regeneration. Niche signals drive stem cell self-renewal and differentiation to generate the specialized lineage populations that maintain the tissue during homeostasis. Cellular dynamics during homeostasis is indicated by black arrows. During damage and regeneration (blue arrows), cells can be replenished by mobilization of quiescent stem cells and increased proliferation of surviving stem cells. Alternatively committed cells can dedifferentiate and re-enter the cell cycle. Lost cells can also be replenished via transdifferentiation into another differentiated cell lineage. Proliferating cells are indicated with a blue nucleus, differentiated cells are represented with a red nucleus.

Cheng and Leblond proposed crypt base columnar (CBC) cells interposed between Paneth cells as the stem cells driving crypt homeostasis [8–10], which was later incorporated in the stem cell zone model of Bjerknes and Cheng [11,12]. The *leucine-rich repeat containing G-protein coupled receptor 5* gene (*Lgr5*) has been identified as a specific marker of CBCs. Genetic lineage tracing (Box 1) in mice harboring a targeted insertion of an expression cassette of

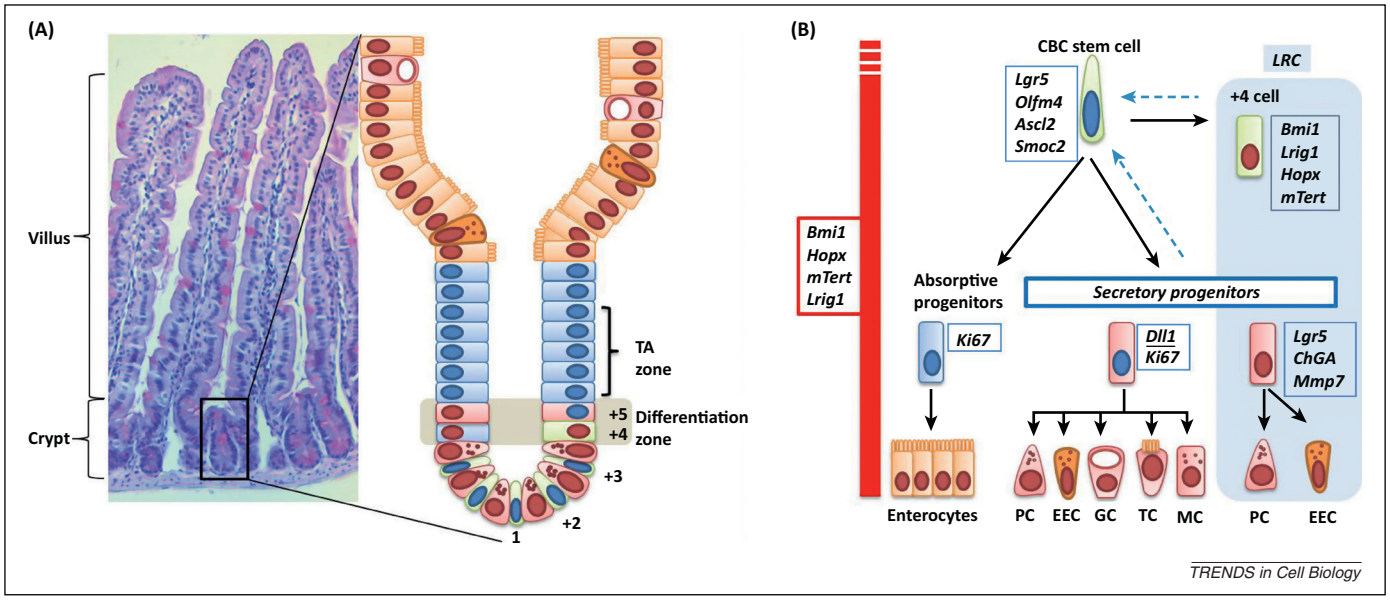

Figure 2. Current model of the stem cell hierarchies in the intestinal epithelium during homeostasis and regeneration. **(A)** The image shows a histological image of the mouse intestine [nuclei, blue hematoxylin, Goblet cells, red periodic acid–Schiff (PAS) staining], and the insert shows a schematic representation of a crypt unit and part of the villus. At the top of the hierarchy are highly proliferative crypt base columnar cells (CBCs) that are intermingled between Paneth cells at the crypt bottom. These divide to generate new CBC stem cells and daughter cells [transit amplifying (TA) cells] that are either absorptive progenitors, which proliferate and then differentiate into enterocytes that make up the bulk of the villus region, or progenitors of secretory cells. **(B)** The cell lineage relationships of diverse progenitor populations. The expression of markers, as discussed in the text, is indicated in the boxes. In homeostasis, proliferative *Lgr5*+ CBCs at the crypt base self-renew and spawn a heterogeneous population of daughter cells in the adjacent 'differentiation zone' at cell position +4/+5, where cell lineage decisions take place [see (A)]. This heterogeneous population includes transit amplifying cells (TA), and Dll1+ secretory progenitors with a limited proliferative capacity that give rise to Paneth cells (PC), enteroendocrine cells (EECs), goblet cells (GCs), and tuft cells (TC). Moreover, CBCs give rise to proliferative absorptive progenitors and quiescent label retaining cells (LRC, blue shaded region) that include *Lgr5*+ secretory precursors of PCs and EECs and an *Lgr5*- population that may represent the +4 stem cell. Proposed +4 markers such as *Bmi1*, *Lrig1*, and *mTert* have been shown to be ubiquitously expressed in all crypt cells as represented by the red line on the left. Upon damage-induced loss of CBCs, committed progenitors can dedifferentiate (broken blue arrows) to become CBCs and to restore homeostasis.

Box 1. Genetic lineage tracing and lineage ablation test to stem cell identity and function

Lineage tracing is a technique used to identify stem cells based on their capacity of multipotency and self-renewal. In mice, genetic lineage tracing is performed using the Cre-*loxP* technique. Here, Cre recombinase is expressed under the control of a cell-type specific promoter. In combination with a second transgene, such as a fluorescent or enzymatic reporter that is inactivated by a *loxP*-flanked STOP cassette, Cre activity causes recombination between *loxP* sites, excision of the STOP cassette, and reporter expression, which is also inherited by all of the progeny. Fusion of the Cre enzyme to the tamoxifen-binding domain of the estrogen receptor (ERT) further allows temporal control of recombination activity upon tamoxifen injection.

To test the requirement of a particular cell population, a knockin genetic approach of the human DTR has been developed [26]. Mice do not endogenously express DTR, but transgenic expression from a cell-type specific promoter allows selective cell ablation *in vivo* upon DT administration. DTR lineage ablation after Cre labeling of non-stem cell populations can serve as a powerful assay to reveal the potential of 'reserve' stem cell populations.

green fluorescent protein–internal ribosome entry site–Cre recombinase fused to a mutant human estrogen receptor ligand-binding domain (ERT2) (*GFP–IRES–CreERT2*) into the *Lgr5* locus has revealed that CBCs are *bona fide* actively cycling, long-lived crypt stem cells that self-renew and generate progeny comprising all the differentiated cell lineages of the intestinal epithelium [13]. With time, crypts become clonal, as indicated by the pattern of how spontaneous somatic mutations are propagated [14,15]. Multi-color lineage tracing experiments have allowed the fate of many *Lgr5*+ stem cell clones to be followed in parallel. In these experiments, it has been shown that clonal evolution follows neutral drift dynamics in which a population of effectively equipotent *Lgr5*+ stem cells stochastically compete for crypt niche occupancy [16–18]. Furthermore, *Lgr5*-GFP+ cells sorted by single fluorescence-activated cell sorting (FACS) have the capacity to initiate *ex vivo* organoid cultures ('mini-guts') in 3D matrigel [19].

An alternative crypt stem cell has been postulated, characterized by its ability to retain DNA labels and occupying the +4 position as counted from the crypt base [20]. More recent lineage tracing studies based on genes, such as polycomb complex protein 1 oncogene (*Bmi1*), mouse telomerase reverse transcriptase gene (*mTert*), leucine rich repeat protein 1 gene (*Lrig1*), and Hop homeobox (*Hopx*) [21–24] have implied that a quiescent cell population located at the +4 position does indeed play a role in intestinal homeostasis. However, whether these stem cell pools represent distinct or overlapping populations, or how they relate during homeostasis and regeneration, has been the subject of intense debate.

Plasticity of intestinal stem cell populations

Several recent papers have addressed how alternative stem cell pools or non-stem cells (identified by several cellular markers; Figure 2B), might be mobilized after perturbation of the crypt stem cell niche. Models employing irradiation and cytotoxic damage with drugs such as 5-fluorouracil (5-FU) and doxorubicin, which induce apoptosis in proliferative cells, have demonstrated a rapid repair and replenishment of crypt units, implying that plasticity of rarely dividing or nonproliferative epithelial cells contributes to the regenerative process [25].

Employing a diphtheria toxin receptor (DTR) knockin genetic strategy to selectively delete *Lgr5* expressing CBCs *in vivo* (Box 1), it was noticed that loss of actively dividing *Lgr5*-expressing CBC stem cells can be accommodated by the small intestine. Combining the *Lgr5*-DTR model with a *Bmi1–CreERT2–RosaLacZ* model, *Bmi1*-expressing cells expanded upon the elimination of CBCs to compensate for the loss of the actively cycling stem cell pool. However, following clearance of diphtheria toxin (DT), *Lgr5*+ cells rapidly reappear *in vivo* (as well as in mini-gut cultures derived from *Bmi1*+ cells), suggesting that a non-*Lgr5*+ stem cell population can convert to actively cycling *Lgr5*+ stem cells [26]. A parallel experiment involving irradiation of *Lgr5–EGFP/Bmi1–CreERT2* models arrived at a similar conclusion. Here, *Bmi1*-expressing cells expanded upon irradiation to replace the depleted *Lgr5*+ stem cells [27]. Genetic lineage tracing using a *Hopx–CreERT2/RosaLacZ* model (also marking +4 cells) provided further support for the mobilization of a quiescent stem cell pool, in the small intestine upon irradiation [22]. Re-evaluation of the mRNA expression of the purported +4 quiescent stem cell markers showed that they are rather broadly expressed in the crypt including in the *Lgr5*+ CBCs [28,29]. Moreover, cells located above Paneth cells at the +4 position also express *Lgr5* [30], indicating that lineage tracing may have originated from double *Lgr5*/*Hopx*- or *Lgr5*/*Bmi1*-expressing CBCs in these experiments. Indeed, a recent study demonstrated the importance of *Lgr5*-expressing progeny of CBCs in the *Lgr5–DTR* model during regeneration after DT injection in combination with radiation-induced damage [31]. Under this condition, depletion of *Lgr5*+ stem cells impaired the regenerative response, demonstrating that reserve stem cells such as *Bmi1*+ cells and label retaining cells (LRCs) at the +4 position are unable to mediate efficient tissue repair following irradiation exceeding a critical threshold.

Dedifferentiation of committed progenitor cells in the intestine

Within crypts, the Notch ligand Dll1 is expressed by secretory cell progenitors at cell positions just above the stem cell zone, which are immediate descendants of *Lgr5*+ cells [32,33]. Lineage tracing using a *Dll1–GFP–IRES–CreERT2* allele has shown that, during homeostasis, these cells generate small, short lived clones that comprise goblet cells, Paneth cells, tuft cells, and enteroendocrine cells. Following ablation of stem cells by irradiation, however, Dll1+ cells yield fully labeled crypt units including absorptive cells, indicating that secretory progenitors can dedifferentiate into stem cells *in vivo* [34]. This process might occur when Dll1+ cells 'fall back' to replace the deleted stem cells and re-establish contact with Paneth cells that secrete instructive niche signals [35]. The notion that contact with Paneth cells is essential for replacement of lost stem cells is also supported by conditional deletion of β-catenin mouse models. Here, replacement of lost crypt cells was critically dependent on the presence of wild type Paneth cells, while regeneration was significantly

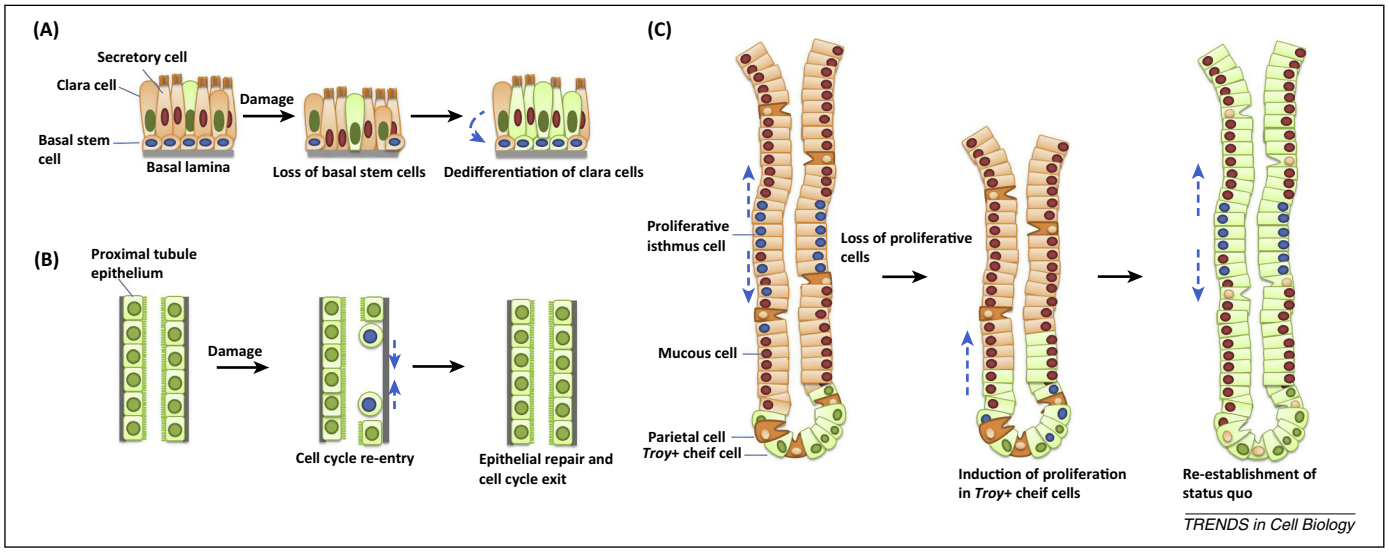

Figure 3. Dedifferentiation of epithelial cells during regeneration. **(A)** Basal stem cells generate differentiated Clara and secretory cells of the trachea during homeostasis. Damage-induced loss of basal stem cells leads to dedifferentiation of Clara cells to repair the lung epithelium; **(B)** Differentiated proximal tubule epithelial cells of the kidney become proliferative upon damage, generating new cells to repair the epithelium; **(C)** *Troy*+ differentiated chief cells populate the base of the stomach corpus epithelium. Upon damage-induced loss of proliferative isthmus cells, *Troy*+ chief cells become proliferative and are able to replenish proliferative isthmus cells as well as parietal cells, mucous cells, and neuroendocrine cells. Green color marks plastic cell populations and their offspring during regeneration. Proliferative cells are signified with blue nuclei; differentiated cells have a red nucleus.

compromised upon β-catenin deletion in all crypt cells including Paneth cells [36].

A recent elegant study re-examined the identity and function of LRCs during homeostasis and damage responses, using an inducible histone 2B–yellow fluorescent protein (H2B–YFP) knockin mouse model [37]. Two populations of cells retained the chromatin label: (i) as expected, the long-lived Paneth cells (also corroborated in another study [38]); and (ii) a more heterogeneous population that is scattered in crypts and predominantly localizes around the +3/+4 position. Intriguingly, a fraction of the heterogeneous population was positive for *Lgr5*, *Lrig1* and, prominin-1 (CD133) whereas the bulk expressed Paneth cell and enteroendocrine cell markers and might thus constitute a secretory precursor cell. Strikingly, the expression levels of proposed +4 markers such as *Bmi1*, *mTert*, *Lrig1*, and *Hopx* were similar in LRCs and CBC stem cells. Notably, YFP–LRCs could form organoids *in vitro* when stimulated by Wnt3a, implying that exposure to niche signals can indeed revert them to multipotent stem cells. To study the function of LRCs, an innovative lineage tracing strategy was employed that relied on the cell cycling status rather than on marker genes. This system hinges on a split-Cre recombinase with one split-Cre moiety expressed under the ubiquitous *Rosa26* promoter, and the second split-Cre moiety fused to H2B and expressed under the control of a drug-inducible [cytochrome P450 1A (Cyp1a)] promoter. Following H2B–Cre label incorporation into the genome, administration of a dimerizing agent allows temporal control of Cre activity. These experiments have shown that LRCs do not contribute to the stem cell pool during homeostasis. However, upon cytotoxic damage with doxorubicin, LRCs are able to give rise to multipotent stem cells supporting a role as reserve stem cells. Another recent study using a newly generated *Ki67-RFP* knockin allele has identified a quiescent *Lgr5*-low crypt population as the early secretory precursors, corroborating this data [39].

These recent studies reconcile the CBC stem cell model and the +4 model [11,12,20,40]. Actively cycling *Lgr5*+ CBCs are responsible for the day-to-day generation of new cell lineages. Among the CBC progeny are a subset of *Lgr5*-low/*Bmi1*+ quiescent cells that are precursors of Paneth and enteroendocrine cells, and are most likely equivalent to the noncycling +4 cells that can replenish the active stem cell pool upon damage-induced depletion (Figure 3). In effect, a dedicated quiescent, noncommitted stem cell pool in the intestine may not exist, as a committed progeny of *Lgr5*+ stem cells, including LRCs, can be a source of new stem cells by displaying plasticity. Since individual LRCs are relatively short-lived, they should not be considered stem cells in a strict sense. However, new LRCs are continually generated by the cycling *Lgr5*$^+$ stem cells. It has also been proposed elsewhere [38] that a pool of LRCs is, therefore, always available to be called into action as facultative reserve stem cells upon tissue damage. Whether fully differentiated intestinal cells such as enterocytes can revert to stem cells, and under which situation this might occur, will be worth investigating.

Epigenetic regulation of intestinal plasticity

Differentiation is generally associated with epigenetic changes, such as DNA methylation and histone methylation and acetylation, that regulate access to regulatory sequences for transcriptional activators and repressors [41]. Do niche signals such as Wnt, therefore, play a role in maintaining an epigenetic state that dictates 'stemness' and do epigenetic mechanisms play a role in dedifferentiation? In the intestine the loss of histone deacetylase enzymes (HDAC1/2) disrupts cell lineage commitment [42]. A recent study on histone marks that permit

chromatin accessibility surprisingly showed that many intergenic regulatory regions were prominently marked, with no significant differences, between intestinal stem cells and their progeny committed to the secretory and enterocyte lineages [43]. This implies that regulatory regions in intestinal cells are continuously accessible to lineage specifying transcription factor, upstream niche factors, and other environmental factors to alter cell fate via dedifferentiation, or perhaps, transdifferentiation.

DNA methylation is thought to be a relatively stable silencing mark and methylation surrounding transcription start sites (TSS) normally correlates with gene repression [44]. Furthermore DNA methylation is generally most dynamic at regulatory regions outside the TSS, although the functional significance of these dynamics is often unclear. A recent study showed that DNA methylation is static at TSS during small intestine stem cell differentiation, and that the minimal changes observed primarily occurred at enhancer elements [45]. In a parallel study, conditional ablation of the maintenance DNA methylating enzyme Dnmt1 led to crypt expansion, suggesting that global DNA methylation is important for differentiation, and low level DNA methylation changes were identified at regulatory regions close to genes important for stem cell maintenance and differentiation [46]. Although the disparate conclusions could be a result of difference in the analytical methods employed, these methylation changes did not affect histone marks. Given that *Dnmt3b* knockout (loss of *de novo* methylation) has no effect in the intestine [47], it remains to be seen what the functional importance is of these minimal local changes during differentiation. Future studies are needed to clarify what level of methylation change is biologically significant for lineage specification and whether other epigenetic factors such as chromatin remodelers play a role in intestinal plasticity.

Niche signals during intestinal regeneration

Niche-derived signaling cues that regulate intestinal homeostasis have been well characterized (reviewed in [48]). Slit2 has been shown to increase stem cell numbers and augments the regenerative response upon damage in an autocrine fashion [49]; however, little is known about exogenous factors that control stem cells and mobilize non-stem cell populations during regeneration. Perhaps best understood is the *Drosophila* gut where multiple extrinsic signals including nutritional, inflammatory, and physical stress signals have been described to increase stem cell turnover [50,51]. A role for diet in murine intestinal stem cell regulation, mediated by the niche, has been reported. Here, caloric restriction leads to inhibition of the mammalian target of rapamycin complex 1 (mTORC1) pathway, specifically in Paneth cells, inducing them to release the paracrine factor ADP-ribose, which enhances stem cell function. Furthermore, reduction in caloric intake improves the *ex vivo* organoid forming efficiency of isolated crypts and *in vivo* survival and proliferation of intestinal crypts after damage induced by ionizing irradiation [52].

Inflammatory cytokines released upon epithelial barrier damage act as mitogens for the epithelium and directly govern regenerative responses [53]. For example, secretion of interleukin-22 (IL-22) is induced after damage and controls intestinal repair via activation of signal transducer and activator of transcription 3 (Stat3) signaling [54]. While this pathway facilitates wound healing, its deregulation can cause hyperproliferation and cancer [55]. Not surprisingly, IL-22 signaling activity is tightly controlled by constitutive production of neutralizing IL-22 binding protein (IL22-BP) by colonic dendritic cells [56,57]. Investigating how signals facilitating plasticity yield normal cells during regeneration or induce neoplastic transformations of differentiated cells will be instrumental for their therapeutic applications.

Stem cell organization and plasticity in other epithelial organs

Depending on anatomical and physiological constraints, other organs have adopted distinct strategies of self-renewal. In this section, we discuss emerging concepts from recent literature that employ genetic lineage tracing in mice to show how stem cell hierarchies and plasticity are realized in other mammalian epithelial tissues during homeostasis and regeneration.

Dedifferentiation of committed mature airway cells in the lungs

Mammalian lungs are made up of two distinct domains: the conducting airway tubes, consisting of trachea, bronchi, and bronchioles, and the alveolar spaces where gaseous exchange takes place. Each of these regions harbors unique stem cell types and progenitor cells, which are thought to give rise to the differentiated cells that maintain the functional integrity of the lungs. In the adult mouse trachea, a scattered population of epithelial basal stem cells expressing keratin 5 (K5), keratin 14 (K14), and tumor protein p63, is responsible for cellular turnover. Lineage-tracing experiments of the basal cells using *K5–CreERT2* or *K14–CreERT2* strains has shown that these cells have the potential to self-renew and generate two differentiated cell types (Clara cells and ciliated cells) during post-natal growth and after injury, placing the basal cells at the top of the cellular hierarchy to generate and repair the tracheal epithelium [58,59].

Recently, it has been demonstrated that differentiated, secretory Clara cells in the trachea can revert into functional stem cells *in vivo* upon ablation of basal stem cells (Figure 3A). The authors concluded that the presence of basal cells inhibits dedifferentiation of Clara cells during homeostasis. Using an *ex vivo* co-culturing assay, they could demonstrate that this inhibition is not dependent on a secreted factor. Whether direct cell contact of Clara cells to basal cells inhibits dedifferentiation, or whether acquisition of contact to the underlying basement membrane promotes their dedifferentiation, remains to be seen [60]. The propensity of committed cells to dedifferentiate was inversely correlated to their state of maturity. It will be interesting to ascertain whether this plasticity of long-lived lung cells predisposes them to mutational transformation and is involved in heterogeneity of lung tumor subtypes and their varied responses to chemotherapy [60].

Dedifferentiation of mature proximal tubule epithelial cells in the kidney

In the adult mammalian kidney, the number of functional nephron units is fixed and it is critical to repair the damaged low turnover epithelium to preserve organ function. Label retaining studies [using 5-bromo-2-deoxyuridine (BrdU) incorporation] have suggested the existence of slow cycling cells in the proximal tubule (PT) that may harbor stem cell potential [61]. Regeneration of the PT epithelium following ischemic reperfusion injury has previously been suggested to involve dedifferentiation and proliferation of all surviving epithelial cells based upon expression of embryonic markers and DNA label retention [62,63]. Recent lineage tracing experiments have corroborated the involvement of dedifferentiated epithelial cells in renal repair. In this study, the researchers used a mouse model where the *CreERT2* cassette is under the control of the *SLC34a1* gene promoter, which encodes a phosphate transporter that is only expressed in fully differentiated PT epithelial cells [64]. The combination of injury with low dose tamoxifen induction to label single differentiated cells showed that the number of labeled cells increased post-ischemia, that they were highly proliferative, and thus contributed to tube elongation (Figure 3B). Interestingly, in kidneys that had undergone 'saturation labeling' with a high dose of tamoxifen followed by injury, no dilution of the label occurred post-ischemia, implying that no other progenitor population is involved in proximal renal tube repair [65]. It thus appears that dedifferentiation in the kidney may be a quicker and more efficient route to regeneration upon focal damage (such as ischemic reperfusion injury). These results, however, do not exclude the possibility that a distinct progenitor population may exist that is sensitive to ischemic damage [65,66], or that regeneration is by self-duplication of surviving differentiated cells rather than dedifferentiation. Identification of putative markers and lineage tracing procedures in combination with other injury models will be useful in clarifying these issues. Of note, this mechanism is highly reminiscent of that of the liver after hemi-hepatectomy where fully differentiated hepatocytes rapidly enter the cell cycle to rebuild the missing part of the liver [67,68].

Dedifferentiation of mature chief cells in the stomach

In the stomach, region-specific stem cell populations have been identified. In the distal pyloric antrum (also known as the pylorus), genetic lineage tracing using the *Lgr5–CreERT2* mice has shown that cells at the base of the stomach glands give rise to all the differentiated lineages [69]. Similar to the intestine, these pyloric *Lgr5*+ cells are actively cycling, self-renewing every 3–4 days, are multipotent, have a Wnt target gene expression signature, and can generate self-renewing gastric organoids *in vitro*. The existence of rare progenitor cells, marked by a villin transgene, can be found in the isthmus and base of stomach glands, and under homeostatic conditions, appear quiescent. However, upon induction by inflammatory damage, notably by interferon gamma, they become proliferative and regenerate the entire gastric epithelium [70]. It is currently unclear what the nature of these cells is, but they may represent the stomach version of intestinal LRCs. Independent genetic lineage tracing experiments have also identified a *Sox2*-expressing stem cell population composed of actively proliferating and quiescent subpopulations, just above the base of the pylorus glands [71]. However, it is unclear how these are functionally related to *Lgr5*+ pyloric stem cells.

In the corpus, Trefoil factor 2 protein gene *TFF2*+ cells located in the proliferative isthmus region have been identified as multipotent progenitor cells using genetic lineage tracing [72]. Using a *Troy–GFP–IRES–CreERT2* model, a fully differentiated cell population with stem cell potential was recently identified [73]. *Troy* (also known as *Tnfrsf19*) is a Wnt target gene that is also expressed in *Lgr5*+ stem cells in the small intestine and colon [74]. In the corpus, however, *Troy* is expressed by a subpopulation of chief cells and parietal cells located at the gland base. During homeostasis, *Troy*+ chief cells fulfill all requirements of differentiated cells. Interestingly, upon removal of proliferative isthmus cells induced by 5-FU treatment, *Troy*+ chief cells re-enter the cell cycle to replenish the lost cells [73] (Figure 3C). In support of their stem cell potential, single isolated *Troy*+ chief cells can generate stomach organoids *ex vivo* that contain mucous cells of the neck and pit. Therefore, differentiated, quiescent *Troy*+ chief cells can turn into stem cells to regenerate damaged gastric glands of the corpus upon loss of the proliferative stem cell pool. It appears that this plasticity of *Troy*+ chief cells may be regulated by Wnt signaling, because these cells express multiple Wnt target genes. However, the exact source of Wnt ligands remains to be identified.

Transdifferentiation of mature hepatocytes in the liver

The liver is undeniably the doyen of regeneration, where differentiated cells can function as facultative stem cells after injury [75]. Although lineage tracing techniques with putative marker genes have been employed to characterize various progenitor populations during homeostasis and regeneration, controversies still exist about the specificity of the cell fate, and the existence and functional characterization of liver stem cells (reviewed elsewhere [76,77]). A recent lineage tracing study using a hepatocyte specific adenovirus expressing a Cre transgenic model crossed to a *Rosa–YFP* reporter line [68] confirmed a previous study [78], showing that, upon toxin injuries and bile duct ligation, mature hepatocytes transdifferentiate into differentiated biliary epithelial cells (Figure 4A), most likely through an atypical ductal cell intermediate, under the influence of Notch signaling to replenish lost cells. This process was thought to occur via a stepwise cascade of induction of biliary markers and morphological changes, although it is unclear if the hepatocytes undergo proliferation [68]. A follow-up study using the same adenovirus Cre/reporter model corroborated previous observations that, upon partial hepatectomy and toxin injuries, hepatocytes can also undergo self-duplication to generate new hepatocytes [7]. Identification of niches and markers that will allow lineage tracing of stem cell activity during homeostasis will be useful in clarifying cell lineage specification and plasticity in the liver.

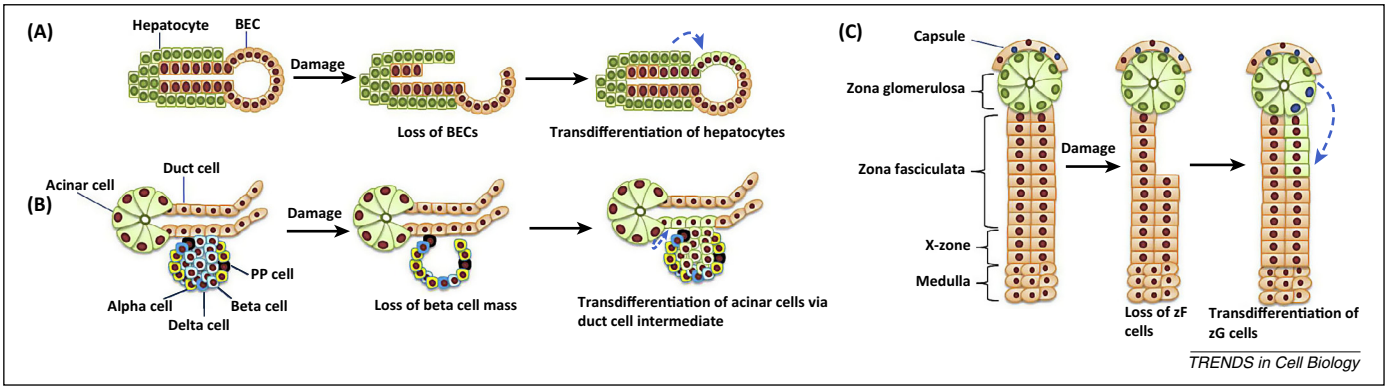

Figure 4. Transdifferentiation of epithelial cells during regeneration. **(A)** Hepatocytes in the liver transdifferentiate upon loss of biliary epithelial cells (BEC); **(B)** acinar cells of the exocrine pancreas transdifferentiate upon ablation of beta cells, which make up the bulk of the islet of Langerhans, possibly through a duct cell intermediate; **(C)** In homeostasis of adrenal cortex epithelium, proliferative cells in the capsule generate differentiated cells of zona glomerulosa (zG) and zona fasciculata (zF). Upon loss of zF cells, zG cells transdifferentiate to replenish lost zF cells concomitantly with cell cycle re-entry and cell migration. Green color marks plastic cell populations and their offspring during regeneration. Proliferative cells are signified with blue nuclei; differentiated cells have a red nucleus.

Transdifferentiation of acinar cells in the pancreas

The pancreas epithelium has a low turnover and exerts both exocrine and endocrine secretory functions. The exocrine glands consist of differentiated acinar and ductal cells, whereas the endocrine function is mediated by differentiated epithelial cells in the islets of Langerhans, which consists of glucagon secreting alpha cells, insulin secreting beta cells, somatostatin secreting delta cells, and polypeptide producing (PP) cells. Lineage tracing of alpha [79], beta [80], duct [81], and acinar cells [82] in the adult pancreas suggests that these cells self-duplicate without the need for a dedicated stem/progenitor cell population during homeostasis. Although controversies still surround the existence of pancreas stem cells and facultative progenitors, *in vivo* lineage tracing experiments have shown that transdifferentiation is utilized by the pancreas during regeneration [83]. Due to lack of space, we only highlight one recent *in vivo* study (read [4] for a detailed review). Combining acinar cell specific lineage tracing with pharmacological ablation of beta cells, it was shown that acinar cells can transdifferentiate into beta cells, most likely through a duct cell intermediate and regain of embryonic multipotency [84] (Figure 4B). Although the fraction of mature beta cells generated by this exocrine to endocrine interconversion was small, it widens the therapeutic options for *de novo* generation of beta cells in diseases such as diabetes.

Transdifferentiation in the adrenal cortex

The adrenal cortex constitutes the outer layer of the capsule-surrounded adrenal gland and physiologically secretes steroid hormones. Anatomically, it is demarcated into an outermost zona glomerulosa (zG) layer, a middle zona fasciculate (zF), and an innermost reticularis (Figure 4B). In rodents, a poorly understood X zone lies between the reticularis and the medulla. The zG and zF comprise differentiated epithelial cells secreting mineralocorticoids (aldosterone) and glucocorticoids (such as corticosterone and cortisol), respectively. Progenitor cells located in the capsule and subcapsular region, and expressing *Gli1* and *Sonic Hedgehog (Shh)*, contribute to the differentiated cell lineages in the zG and zF of the adrenal cortex [85], which has a slow turnover of approximately 12 weeks. Another population of progenitors in the X zone has also been reported to be involved in generating differentiated cells of the adrenal cortex [86].

Recently, lineage-tracing studies using a zG-specific CreERT2 line have demonstrated that postnatal adrenocortical zonation involves direct lineage conversion of zG cells into zF cells during homeostasis [87] (Figure 4C). This transdifferentiation is also observed during adrenal regeneration in older animals upon dexamethasone-induced ablation of zF cells. However, in the absence of zG cells, specification of zF cells was unperturbed implying that the adrenal cortex is highly plastic, with a preferred transdifferentiation mode of regeneration and alternate modes to generate zF cells. It will be interesting to determine in the future if progenitors in the capsule or X zone, or other differentiated progenitor cells in the cortex cell or medulla, represent alternative sources for zF cells during regeneration.

Concluding remarks

It is evident that multiple epithelial tissues in both fast-renewing as well as slow-renewing organs employ committed cells as reserve stem cells upon damage. Although there is much debate about the existence of distinct quiescent stem cells acting as reserve stems cells upon damage, it appears they may not be needed in the epithelial tissues discussed above, because differentiated cells can perform that function. In fact, it may not be easy to distinguish noncycling committed cells from genuine quiescent stem cells, unless lineage tracing strategies can be designed. It remains to be seen if the facultative employment of differentiated cells as reserve stem cells during regeneration represents a universal phenomenon in all epithelial tissues. Tissue-specific regeneration strategies that focus on defining the right conditions to 'awaken' the plasticity potential of differentiated cells (be it *in vivo* or *in vitro*) might be a valuable alternative to stem cell-focused strategies.

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References

1 Jopling, C. *et al.* (2011) Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. *Nat. Rev. Mol. Cell Biol.* 12, 79–89
2 Eguizabal, C. *et al.* (2013) Dedifferentiation, transdifferentiation, and reprogramming: future directions in regenerative medicine. *Semin. Reprod. Med.* 31, 82–94
3 Sánchez Alvarado, A. and Yamanaka, S. (2014) Rethinking differentiation: stem cells, regeneration, and plasticity. *Cell* 157, 110–119
4 Ziv, O. *et al.* (2013) The plastic pancreas. *Dev. Cell* 26, 3–7
5 Tarlow, B.D. *et al.* (2014) Clonal tracing of *Sox9*+ liver progenitors in mouse oval cell injury. *Hepatology* 60, 278–289
6 Schaub, J.R. *et al.* (2014) Evidence against a stem cell origin of new hepatocytes in a common mouse model of chronic liver injury. *Cell Rep.* 8, 933–939 http://dx.doi.org/10.1016/j.celrep.2014.07.003
7 Yanger, K. *et al.* (2014) Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. *Cell Stem Cell* http://dx.doi.org/10.1016/j.stem.2014.06.003
8 Cheng, H. *et al.* (1974) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine I. Columnar cell. *Am. J. Anat.* 141, 461–479
9 Cheng, H. and Leblond, C.P. (1974) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine III. Entero-endocrine cells. *Am. J. Anat.* 141, 503–519
10 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
11 Bjerknes, M. and Cheng, H. (1981) The stem-cell zone of the small intestinal epithelium. I. Evidence from Paneth cells in the adult mouse. *Am. J. Anat.* 160, 51–63
12 Bjerknes, M. and Cheng, H. (1981) The stem-cell zone of the small intestinal epithelium. III. Evidence from columnar, enteroendocrine, and mucous cells in the adult mouse. *Am. J. Anat.* 160, 77–91
13 Barker, N. *et al.* (2007) Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* 449, 1003–1007
14 Griffiths, D.F.R. *et al.* (1988) Demonstration of somatic mutation and colonic crypt clonality by X-linked enzyme histochemistry. *Nature* 333, 461–463
15 Winton, D.J. and Ponder, B.A.J. (1990) Stem-cell organization in mouse small intestine. *Proc. Biol. Sci.* 241, 13–18
16 Snippert, H.J. *et al.* (2010) Intestinal crypt homeostasis results from neutral competition between symmetrically dividing *Lgr5* stem cells. *Cell* 143, 134–144
17 Lopez-Garcia, C. *et al.* (2010) Intestinal stem cell replacement follows a pattern of neutral drift. *Science* 330, 822–825
18 Ritsma, L. *et al.* (2014) Intestinal crypt homeostasis revealed at single-stem-cell level by *in vivo* live imaging. *Nature* 507, 362–365 http://dx.doi.org/10.1038/nature12972 (Epub)
19 Sato, T. *et al.* (2009) Single *Lgr5* stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 459, 262–265
20 Marshman, E. *et al.* (2002) The intestinal epithelial stem cell. *Bioessays* 24, 91–98
21 Sangiorgi, E. and Capecchi, M.R. (2008) *Bmi1* is expressed *in vivo* in intestinal stem cells. *Nat. Genet.* 40, 915–920
22 Takeda, N. *et al.* (2011) Interconversion between intestinal stem cell populations in distinct niches. *Science* 334, 1420–1424
23 Powell, A.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
24 Montgomery, R.K. *et al.* (2010) Mouse telomerase reverse transcriptase (*mTert*) expression marks slowly cycling intestinal stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 179–184
25 Dekaney, C.M. *et al.* (2009) Regeneration of intestinal stem/progenitor cells following doxorubicin treatment of mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 297, G461–G470
26 Tian, H. *et al.* (2011) A reserve stem cell population in small intestine renders *Lgr5*-positive cells dispensable. *Nature* 478, 255–259
27 Yan, K.S. *et al.* (2012) The intestinal stem cell markers *Bmi1* and *Lgr5* identify two functionally distinct populations. *Proc. Natl. Acad. Sci. U.S.A.* 109, 466–471
28 Wong, V.W.Y. *et al.* (2012) *Lrig1* controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. *Nat. Cell Biol.* 14, 401–408
29 Muñoz, J. *et al.* (2012) The *Lgr5* intestinal stem cell signature: robust expression of proposed quiescent '+4' cell markers. *EMBO J.* 31, 3079–3091
30 Wang, F. *et al.* (2013) Isolation and characterization of intestinal stem cells based on surface marker combinations and colony-formation assay. *Gastroenterology* 145, 383–395.e1–21
31 Metcalfe, C. *et al.* (2013) *Lgr5*+ stem cells are indispensable for radiation-induced intestinal regeneration. *Cell Stem Cell* 14, 149–159 http://dx.doi.org/10.1016/j.stem.2013.11.008
32 Pellegrinet, L. *et al.* (2011) Dll1- and Dll4-mediated notch signaling are required for homeostasis of intestinal stem cells. *Gastroenterology* 140, 1230–1240.e1–7
33 Stamataki, D. *et al.* (2011) *Delta1* expression, cell cycle exit, and commitment to a specific secretory fate coincide within a few hours in the mouse intestinal stem cell system. *PLoS ONE* 6, e24484
34 Van Es, J.H. *et al.* (2012) Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nat. Cell Biol.* 14, 1099–1104
35 Sato, T. *et al.* (2011) Paneth cells constitute the niche for *Lgr5* stem cells in intestinal crypts. *Nature* 469, 415–418
36 Parry, L. *et al.* (2013) Evidence for a crucial role of Paneth cells in mediating the intestinal response to injury. *Stem Cells* 31, 776–785
37 Buczacki, S.J.A. *et al.* (2013) Intestinal label-retaining cells are secretory precursors expressing *Lgr5*. *Nature* 495, 65–69
38 Roth, S. *et al.* (2012) Paneth cells in intestinal homeostasis and tissue injury. *PLoS ONE* 7, e38965
39 Basak, O. *et al.* (2014) Mapping early fate determination in *Lgr5*+ crypt stem cells using a novel *Ki67-RFP* allele. *EMBO J.* http://dx.doi.org/10.15252/embj.201488017
40 Potten, C.S. (1977) Extreme sensitivity of some intestinal crypt cells to X and γ irradiation. *Nature* 269, 518–521
41 Iglesias-Bartolome, R. *et al.* (2013) Control of the epithelial stem cell epigenome: the shaping of epithelial stem cell identity. *Curr. Opin. Cell Biol.* 25, 162–169
42 Turgeon, N. *et al.* (2013) HDAC1 and HDAC2 restrain the intestinal inflammatory response by regulating intestinal epithelial cell differentiation. *PLoS ONE* 8, e73785
43 Kim, T-H. *et al.* (2014) Broadly permissive intestinal chromatin underlies lateral inhibition and cell plasticity. *Nature* 506, 511–515 http://dx.doi.org/10.1038/nature12903
44 Ehrlich, M. and Lacey, M. (2013) DNA methylation and differentiation: silencing, upregulation and modulation of gene expression. *Epigenomics* 5, 553–568
45 Kaaij, L.T. *et al.* (2013) DNA methylation dynamics during intestinal stem cell differentiation reveals enhancers driving gene expression in the villus. *Genome Biol.* 14, R50
46 Sheaffer, K.L. *et al.* (2014) DNA methylation is required for the control of stem cell differentiation in the small intestine. *Genes Dev.* 28, 652–664
47 Lin, H. *et al.* (2006) Suppression of intestinal neoplasia by deletion of *Dnmt3b*. *Mol. Cell. Biol.* 26, 2976–2983
48 Barker, N. (2014) Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. *Nat. Rev. Mol. Cell Biol.* 15, 19–33
49 Zhou, W-J. *et al.* (2013) Induction of intestinal stem cells by R-spondin 1 and Slit2 augments chemoradioprotection. *Nature* 501, 107–111
50 Jiang, H. and Edgar, B.A. (2011) Intestinal stem cells in the adult *Drosophila* midgut. *Exp. Cell Res.* 317, 2780–2788
51 Vriz, S. *et al.* (2014) Chapter five — cell death: a program to regenerate. In *Current Topics in Developmental Biology* (Vol. 108) (Galliot, B., ed.), In pp. 121–151, Academic Press
52 Yilmaz, Ö.H. *et al.* (2012) mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature* 486, 490–495
53 Peterson, L.W. and Artis, D. (2014) Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat. Rev. Immunol.* 14, 141–153
54 Sonnenberg, G.F. *et al.* (2011) Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat. Immunol.* 12, 383–390

55 Kirchberger, S. *et al.* (2013) Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *J. Exp. Med.* 210, 917–931
56 Huber, S. *et al.* (2012) IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. *Nature* 491, 259–263
57 Grivennikov, S.I. *et al.* (2010) Immunity, inflammation, and cancer. *Cell* 140, 883–899
58 Rock, J.R. *et al.* (2009) Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12771–12775
59 Hong, K.U. *et al.* (2004) *In vivo* differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 286, L643–L649
60 Tata, P.R. *et al.* (2013) Dedifferentiation of committed epithelial cells into stem cells *in vivo*. *Nature* 503, 218–223 http://dx.doi.org/10.1038/nature12777
61 Maeshima, A. *et al.* (2003) Identification of renal progenitor-like tubular cells that participate in the regeneration processes of the kidney. *J. Am. Soc. Nephrol.* 14, 3138–3146
62 Witzgall, R. *et al.* (1994) Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. *J. Clin. Invest.* 93, 2175–2188
63 Vogetseder, A. *et al.* (2007) Proximal tubular epithelial cells are generated by division of differentiated cells in the healthy kidney. *Am. J. Physiol. Cell Physiol.* 292, C807–C813
64 Biber, J. *et al.* (2009) Regulation of phosphate transport in proximal tubules. *Pflugers Arch.* 458, 39–52
65 Kusaba, T. *et al.* (2014) Differentiated kidney epithelial cells repair injured proximal tubule. *Proc. Natl. Acad. Sci. U.S.A.* 111, 1527–1532
66 Humphreys, B.D. *et al.* (2011) Repair of injured proximal tubule does not involve specialized progenitors. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9226–9231
67 Grisham, J.W. (1962) A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver; autoradiography with thymidine-H3. *Cancer Res.* 22, 842–849
68 Yanger, K. *et al.* (2013) Robust cellular reprogramming occurs spontaneously during liver regeneration. *Genes Dev.* 27, 719–724
69 Barker, N. *et al.* (2010) *Lgr5*+ve stem cells drive self-renewal in the stomach and build long-lived gastric units *in vitro*. *Cell Stem Cell* 6, 25–36
70 Qiao, X.T. *et al.* (2007) Prospective identification of a multilineage progenitor in murine stomach epithelium. *Gastroenterology* 133, 1989–1998
71 Arnold, K. *et al.* (2011) *Sox2*+ adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem Cell* 9, 317–329
72 Quante, M. *et al.* (2010) *TFF2* mRNA transcript expression marks a gland progenitor cell of the gastric oxyntic mucosa. *Gastroenterology* 139, 2018–2027.e2
73 Stange, D.E. *et al.* (2013) Differentiated *Troy*+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell* 155, 357–368
74 Fafilek, B. *et al.* (2013) *Troy*, a tumor necrosis factor receptor family member, interacts with *Lgr5* to inhibit Wnt signaling in intestinal stem cells. *Gastroenterology* 144, 381–391
75 Michalopoulos, G.K. (2011) Liver regeneration: alternative epithelial pathways. *Int. J. Biochem. Cell Biol.* 43, 173–179
76 Miyajima, A. *et al.* (2014) Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. *Cell Stem Cell* 14, 561–574
77 Tanimizu, N. and Mitaka, T. (2014) Re-evaluation of liver stem/progenitor cells. *Organogenesis* 10, 6–13
78 Michalopoulos, G.K. *et al.* (2005) Transdifferentiation of rat hepatocytes into biliary cells after bile duct ligation and toxic biliary injury. *Hepatology* 41, 535–544
79 Herrera, P.L. (2000) Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 127, 2317–2322
80 Dor, Y. *et al.* (2004) Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429, 41–46
81 Solar, M. *et al.* (2009) Pancreatic exocrine duct cells give rise to insulin-producing β cells during embryogenesis but not after birth. *Dev. Cell* 17, 849–860
82 Desai, B.M. *et al.* (2007) Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. *J. Clin. Invest.* 117, 971–977
83 Thorel, F. *et al.* (2010) Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature* 464, 1149–1154
84 Pan, F.C. *et al.* (2013) Spatiotemporal patterns of multipotentiality in *Ptf1a*-expressing cells during pancreas organogenesis and injury-induced facultative restoration. *Development* 140, 751–764
85 King, P. *et al.* (2009) Shh signaling regulates adrenocortical development and identifies progenitors of steroidogenic lineages. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21185–21190
86 Zubair, M. *et al.* (2008) Developmental links between the fetal and adult zones of the adrenal cortex revealed by lineage tracing. *Mol. Cell. Biol.* 28, 7030–7040
87 Freedman, B.D. *et al.* (2013) Adrenocortical zonation results from lineage conversion of differentiated zona glomerulosa cells. *Dev. Cell* 26, 666–673