An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control

Hans Clevers *et al.*

Science **346**, (2014);
DOI: 10.1126/science.1248012

REVIEW

STEM CELL SIGNALING

An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control

Hans Clevers,[1] Kyle M. Loh,[2] Roel Nusse[2]*

Stem cells fuel tissue development, renewal, and regeneration, and these activities are controlled by the local stem cell microenvironment, the "niche." Wnt signals emanating from the niche can act as self-renewal factors for stem cells in multiple mammalian tissues. Wnt proteins are lipid-modified, which constrains them to act as short-range cellular signals. The locality of Wnt signaling dictates that stem cells exiting the Wnt signaling domain differentiate, spatially delimiting the niche in certain tissues. In some instances, stem cells may act as or generate their own niche, enabling the self-organization of patterned tissues. In this Review, we discuss the various ways by which Wnt operates in stem cell control and, in doing so, identify an integral program for tissue renewal and regeneration.

In a 1956 review entitled "Renewal of Cell Populations," Leblond and Walker noted that multiple adult tissues, including the skin and intestines, accommodate numerous mitotic divisions but seemingly do not undergo a commensurate expansion in tissue size (*1*). The authors presciently concluded that "the cells of the tissue are said to undergo renewal" (*1*). Such tissues are perpetually being "recycled," with cells being extruded or lost and continually being replaced by newly born cells.

It is now evident that stem cells are required for continuous tissue maintenance within diverse organs. Cellular losses within these tissues (owing to either natural cellular attrition or injury) are persistently replenished by stem cells, which we define as cells that sustain continued tissue formation by generating tissue progeny while renewing themselves through division. Stem cell activity is often externally dictated by the microenvironment (the niche) so that stem cell output is precisely shaped to meet homeostatic needs or regenerative demands.

This Review details how a class of developmental signals, known as Wnt signals, control stem cell operation and are crucial for the continued renewal of multiple mammalian tissues. Such a role was presaged by a pivotal role for Wnt in the development and regeneration of the earliest animals. Although a number of signals control stem cell activity, Wnts are somewhat idiosyncratic in that they primarily seem to act as short-range cellular signals between adjacent cells. This mode of spatially constrained signaling might bear developmental and regenerative importance, communicating a directive to nearby cells without influencing a broad domain.

Signaling by lipid-modified short-range Wnt factors

A tenet of the stem cell niche model is the short range at which signals act, maintaining a limited number of stem cells near the niche. By their very nature, Wnt proteins fit the bill.

Wnts are secreted signaling proteins that by virtue of their biochemical properties, seem principally to operate over short distances. All Wnt proteins harbor a covalent lipid modification: a palmitate, appended by the palmitoyltransferase Porcupine (Fig. 1A). This lipid group renders the Wnt protein hydrophobic and tethers it to cell membranes or its cognate receptors. The transmembrane protein Wntless (Wls) exclusively binds only lipidated Wnt proteins (*2*) and conveys them to the plasma membrane for secretion. Therefore, after secretion the lipid may be pivotal in limiting Wnt dispersion and its range of biological action, a precept to which we return below.

Once secreted, how Wnt signals are conveyed to their target cells remains cryptic. Some Wnt proteins may be incorporated into secretory vesicles (*3*), in which Wls continues to bind Wnt proteins (*4*) as a chaperone (Fig. 1B), perhaps availing the presentation of lipidated Wnt proteins to their cognate receptors, known as Frizzled receptors. Wnt signaling mediated by such vesicles would operate over a short distance, such as at the neuromuscular junction (*4*) and also in stem cell niches.

Although it is sometimes assumed that Wnt signals are long-range morphogens, there is little evidence that this is the prevailing mode of Wnt action. Wnt signaling occurs mostly between cells that are touching each other. Even in the best studied example of long-range signaling by a Wnt—that is, by the Wnt ligand Wingless in *Drosophila*—recent evidence has made a case that the requirements for any function of Wingless can be largely afforded by a nondiffusible, membrane-tethered form of the protein (Fig. 1C) (*5*) and that Wingless does not act as a long-range morphogen in that context.

Once delivered to their target cells, Wnt ligands engage their cognate Frizzled receptors through their palmitate group, which extends into the lipid-binding cysteine-rich domain (CRD) of Frizzled receptors (*6*). Wnt ligands also bind the Lrp5/6 transmembrane co-receptor, inducing it to form a complex with Frizzled (Fig. 2A). This instills a conformational change in these receptors and enables phosphorylation by associated protein kinases. The phosphorylated cytoplasmic Lrp tail subsequently inhibits glycogen synthase kinase 3 (GSK3) (*7*) and also binds the Axin protein. In the absence of a Wnt signal, a destruction complex that includes Axin, adenomatous polyposis coli (APC), and GSK3 phosphorylates β-catenin, continually targeting it for degradation by the proteasome. Inhibition of the destruction complex, a consequence of Wnt–Frizzled–Lrp interactions, leads β-catenin to accumulate in the nucleus (Fig. 2B). There, β-catenin governs transcriptional programs through association with Tcf/Lef transcription factors.

In some instances, Wnt signals are transduced independently of β-catenin—for example, during morphogenetic movements in vertebrate gastrulation (*8*). In this pathway, Frizzled and an intracellular transduction component (Disheveled) are crucial, but not Lrp and β-catenin. This aspect of Wnt signaling is evolutionarily ancient and may be involved in regulating stem cell polarity and asymmetric division of stem cells within the confinement of the niche, as we discuss below.

Wnt signaling can be further augmented by secreted R-spondin proteins (*9*, *10*). R-spondins, acting through Lgr family receptors (*11*–*13*), inhibit the transmembrane E3 ubiquitin ligases Rnf43/Znrf3 that ordinarily ubiquitinate and thus degrade Frizzled receptors (*14*, *15*). By antagonizing Rnf43/Znrf3, R-spondins consequently stabilize surface Frizzled receptors and enhance Wnt signal strength (Fig. 2A) (*14*, *15*).

The fundamental core of the Wnt pathway (Wnt, Frizzled, and downstream effectors) is evolutionarily ancient and is extant in the earliest multicellular animals including ctenophores, sponge, and placozoans (*16*, *17*), in which it mediates basic axial patterning even in pre-bilateria (*18*, *19*). In contrast, the R-spondin/Lgr axis is principally a vertebrate innovation (*20*). Was the R-spondin/Lgr pathway simply collateral to vertebrate speciation? Another possibility was that it was evolutionarily co-opted to amplify Wnt signaling and thus sustain some types of adult stem cell in long-lived vertebrate species (*20*).

Wnt-driven transcriptional programs

In the nucleus, β-catenin interacts with Tcf/Lef transcriptional cofactors to regulate the transcription of Wnt target genes (Fig. 2B). Rather than

[1]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW), University Medical Centre Utrecht and CancerGenomics.nl, 3584CT Utrecht, Netherlands. [2]Department of Developmental Biology, Howard Hughes Medical Institute, Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, 265 Campus Drive, Stanford, CA 94305, USA.
*Corresponding author. E-mail: rnusse@stanford.edu

Corrected 20 October 2014; see full text.

conforming to a universal program, the transcriptional agenda imposed by β-catenin varies between lineages. However, several generalities might exist. For instance, in Wnt-responsive stem cells it seems that β-catenin can directly induce telomerase expression (*21*), causally explaining the lengthy telomeres of Wnt-driven intestinal stem cells (*22*) and pluripotent cells (*21*) and shielding them from genomic catastrophe.

Although the phenotypic consequences of Wnt signaling diverge between distinct lineages, several genes appear to represent generic Wnt transcriptional targets. *Axin2* has emerged as one such Wnt target gene (*23*) that therefore serves as a reporter of ongoing Wnt signaling (*24*). As discussed below, *Axin2* (*24*) as well as a second gene, *Lgr5* (*25*), can identify Wnt-responding lineages in diverse tissues. Genetically labeling *Lgr5*- or *Axin2*-expressing cells has revealed their participation in tissue renewal in multiple organs, compellingly nominating such cells as stem cells in specific tissues. We summarize these cell labeling experiments in Table 1 and discuss three examples in more detail.

Intestinal stem cells

The small intestinal epithelium is the fastest proliferating tissue of adult mammals, being largely made anew every 4 to 5 days (*26*). Villi protrude into the gut lumen and continually shed differentiated cells from their tips. These losses are replenished by stem cells located in proliferative intestinal crypts that surround the villus base (Fig. 3A). Wnt signals are pivotal for the perennial renewal of the intestines, as shown by disruption of the pathway—which leads to the abrupt cessation of proliferation in the intestinal crypts, consequently leading to unabated loss of intestinal tissue and often morbidity (*27*–*29*). Reciprocally, the Wnt coagonist R-spondin potently stimulates intestinal proliferation in vivo (*30*).

The crypt bottom harbors slender, cycling "crypt base columnar" (CBC) cells (*31*), which were historically proposed to represent intestinal stem cells (*32*) (Fig. 3A). Exploiting the expression of Wnt target gene *Lgr5* in CBCs, genetic labeling of *Lgr5*$^+$ crypt cells indeed demonstrated that these long-lived cells generate all differentiated intestinal cell types (*25*). Therefore, CBCs constitute multipotent intestinal stem cells (*25*) that require Wnt for proliferation (*27*, *33*), perhaps explaining why Wnt is crucial for intestinal renewal.

Residing directly above the CBC stem cell zone at the "+4" position is a potentially distinct population of slowly cycling cells [variously described by molecular markers including *Bmi1* (*34*), *Hopx* (*35*), *Lrig1* (*36*, *37*), and *Tert* (*38*, *39*)] that also can generate all intestinal lineages (Fig. 3A).

Instead of constituting irrevocably separated lineages, it seems that *Lgr5*$^+$ and +4 stem cells can interconvert. The highly proliferative *Lgr5*$^+$ CBCs appear to be the "workhorse" of daily intestinal renewal (*33*). Yet, slowly cycling "reserve" +4 stem cells can be recalled to *Lgr5*$^+$ status (*40*) and vice versa (*35*).

Adding further complexity, the two stem cell lineages may be partially overlapping. *Lgr5*$^+$ cells can coexpress +4 markers (such as *Bmi1*) (*41*–*43*). Indeed, whereas the majority of *Lgr5*$^+$ cells are proliferative stem cells, a subset of *Lgr5*$^+$ cells are nondividing secretory precursors that coexpress +4 markers (*43*). These precursors, typically confined to secretory fates, can be promoted to multipotent stem cell status upon tissue damage to effect intestinal repair (*43*). This indicates that the developmental competence of precursors is not fixed but is rather labile, as we explore further below.

Interfollicular epidermis

The interfollicular epidermis (IFE) is constantly regenerated. Differentiated cells are shed from the surface and replaced by basal layer stem cells. Most basal layer cells transduce Wnt signals, as visualized by a Wnt transcriptional reporter and expression of Wnt target gene *Axin2* (*44*, *45*). *Axin2*$^+$ basal cells continuously produce keratinocytes for over 1 year in vivo and therefore qualify as IFE stem cells (Fig. 3B) (*44*, *45*).

Certain evidence suggests that β-catenin is crucial for epidermal proliferation and maintenance of IFE stem cells, both in vivo (*44*–*46*) as well as in cell culture (*47*). However, extrapolating a role for Wnt as an IFE self-renewal signal based on these data has been complicated by the fact that β-catenin operates dually in cell adhesion (*48*) as well as Wnt/β-catenin signaling. Implying a role for Wnt signaling specifically, simultaneous loss of *Tcf3* and *Tcf4* compromises

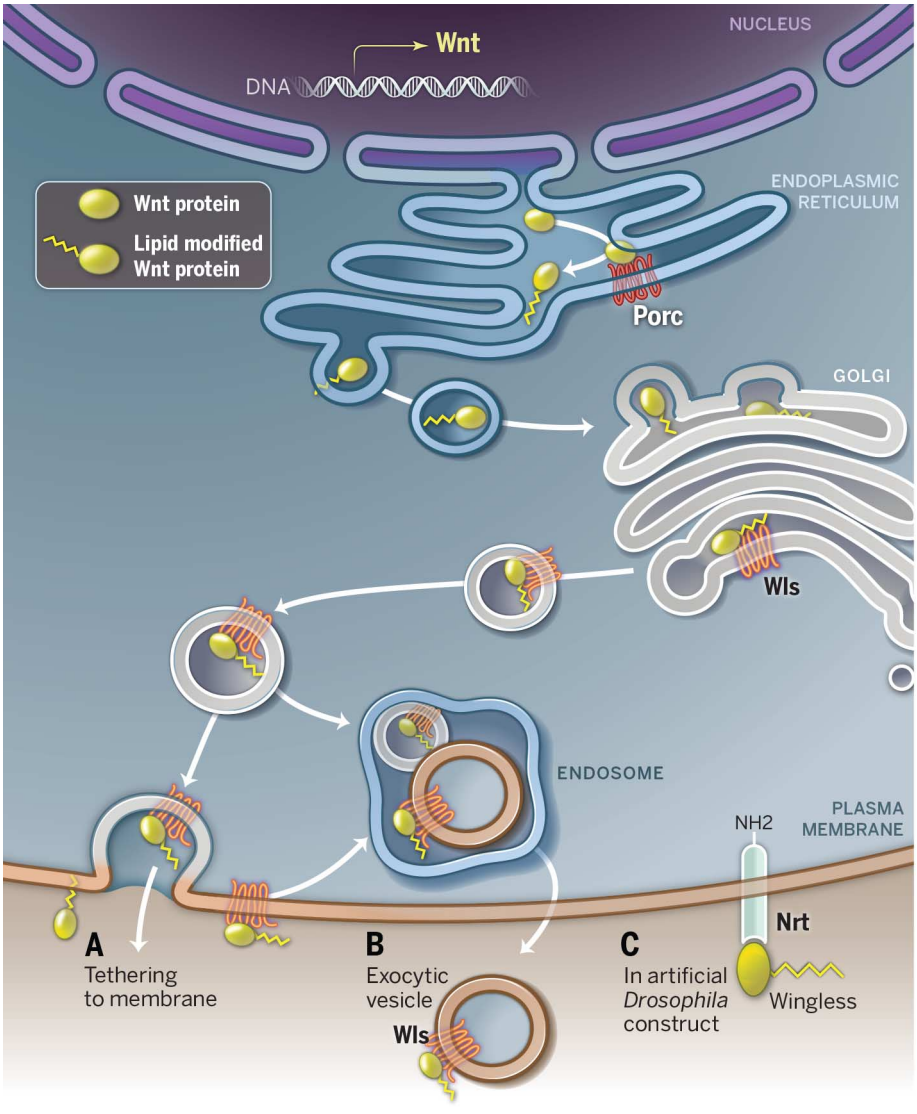

Fig. 1. Model of Wnt secretion, modification, and short-range signaling activity. Wnt proteins are lipid-modified by the Porcupine enzyme in the endoplasmic reticulum. Subsequently, lipid-modified Wnts are bound by the carrier protein Wls and might be expelled in (**B**) secretory vesicles furnishing membrane-bound Wnt ligands or (**A**) might be directly presented as cell surface–bound Wnt ligands. (**C**) In *Drosophila*, a constitutively membrane-tethered Neurotactin (Nrt)–Wingless fusion protein is able to execute all Wingless functions, implying that Wnts need not be released from the membrane in order to signal.

 Corrected 20 October 2014; see full text.

long-term IFE maintenance (*49*). Taken in collective, these findings suggest that IFE basal stem cell proliferation is controlled by Wnt signaling. Furthermore, basal cells produce their own Wnt ligands (*44*), implying autocrine (rather than niche-dependent paracrine) regulation (Fig. 3B). This concept portends a type of "developmental self-organization," considered further below.

Mammary gland

The mammary gland constitutes another venue of tissue renewal because it undergoes cycles of dynamic growth during puberty, pregnancy, and lactation. After lactation, the alveoli in the gland regress by involution and cell death, and the tissue returns to a pre-pregnancy–like state. How are these cycles of regrowth continually sustained?

Initial transplantation (*50*) and subsequent lineage-tracing experiments have established that stem cells exist in the adult mammary epithelium that and they appear to be driven by Wnt signaling (*51*) because they are designated by *Lgr5* (*52*–*55*) and *Axin2* (*24*) in vivo and can be expanded in vitro upon Wnt exposure (*56*). *Axin2*$^+$ cells self-renew and continuously fuel cellular production during multiple cycles of pregnancy, lactation, and involution (*24*), indicating that these cells (or a subset of them) are authentic stem cells.

Stochastic fate or invariant lineage?

The classical view of homeostatic stem cell self-renewal is exemplified by that of the hematopoietic stem cell, which is believed to divide rarely and invariably in an asymmetric fashion to generate one new stem cell and one differentiated daughter. However, neither the intestinal crypt nor the IFE abide by this rule of predetermined lineage choice. Each crypt contains a fixed number of stem cells, determined by the size of the niche. Each of these stem cells divides every day to generate two new "potential" stem cells. Chance decides which of these will stay within the niche at the crypt bottom and which are pushed out of the niche (*57*, *58*). This process is termed "neutral competition" and ensures that (i) the number of available stem cells is constant and (ii) that damaged or lost stem cells are immediately replaced by healthy neighbors (*59*). Also in the skin, the Wnt-responding IFE stem cells appear to divide stochastically to generate proliferating and differentiating daughter cells with equal probability (*44*, *60*). Thus, whether any given stem cell daughter will continue self-renewing is left to a throw of the dice—not destiny.

Plasticity within the stem cell hierarchy

In models of the hematopoietic hierarchy (*61*), all arrows "point away" from the stem cell, implying that once cells give up their stem cell identity, there is no way back. Intestinal cells do not abide by this rule. Although Dll1$^+$ secretory progenitors are typically short-lived precursors that are confined to secretory fates (Fig. 3A), if crypt stem cells are depleted, Dll1$^+$ secretory progenitors can regain Lgr5$^+$ stem cell status in vivo

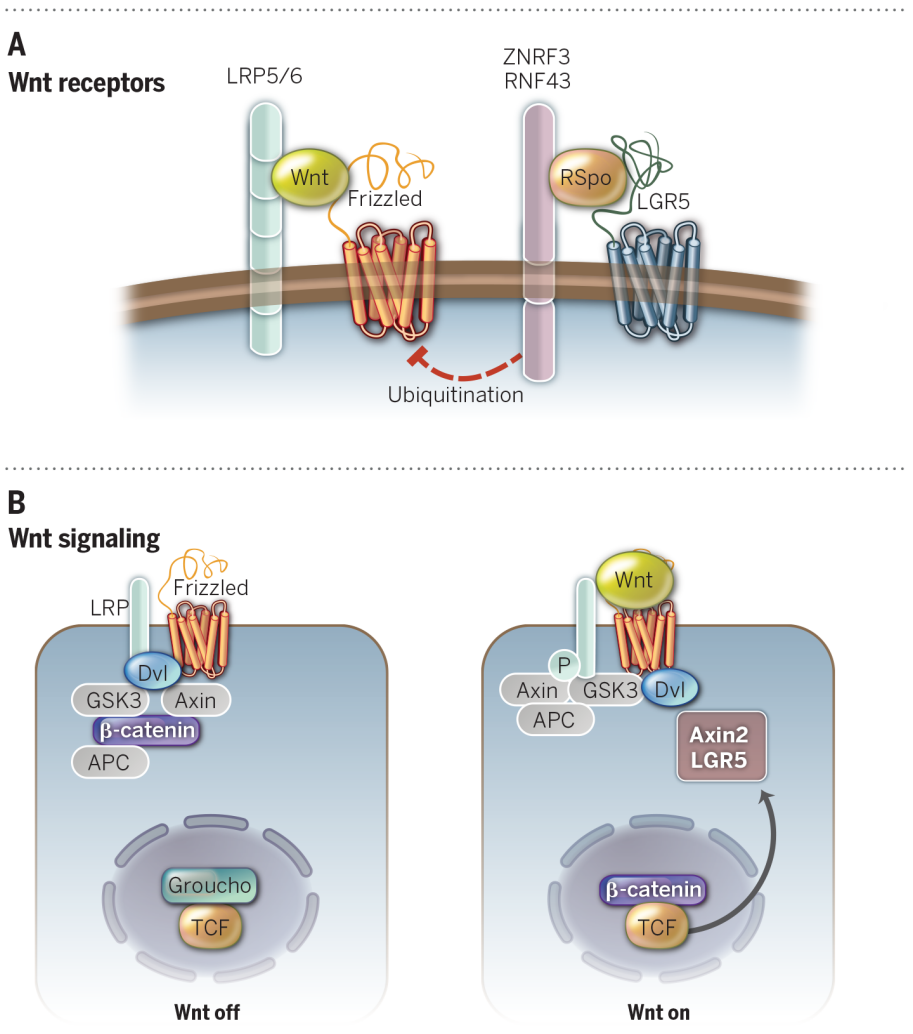

Fig. 2. Wnt signaling mechanisms. (**A**) Wnt reception on the cell surface. Wnt ligands bind to the Frizzled and Lrp5/6 receptors, activating downstream signaling. The membrane proteins Znrf3 and Rnf43 are ubiquitin ligases that continually down-regulate Frizzleds through ubiquitination. Binding of R-spondins to Znrf3 and Rnf43 and the Lgr4/5/6 receptor relieves Znrf3 and Rnf43 activity, thus stabilizing Frizzleds. (**B**). Wnt signaling in target cells. (Left) In the absence of Wnt, a destruction complex consisting of Axin, APC, and GSK3 resides in the cytoplasm, where it binds to and phosphorylates β-catenin, which is then degraded. Dvl (Disheveled) is required for activating the pathway as well. In the nucleus, T cell factor (TCF) is in an inactive state as the consequence of binding to the repressor Groucho. (Right) Binding of Wnt to its receptors induces the association of Axin with phosphorylated lipoprotein receptor-related protein (LRP). The destruction complex falls apart, and β-catenin is stabilized, subsequently binding TCF in the nucleus to up-regulate target genes, including *Axin2* and *Lgr5*.

Table 1. Wnt-responsive tissue stem cells identified by means of lineage tracing.

Tissue	Stem cell	Marked by	Reference
Intestine	Crypt base columnar cell	Lgr5	(*25*)
Mammary gland	Basal cell	Axin2, Lgr5	(*24*, *50*–*53*)
Stomach	Basal pyloric cell	Lgr5	(*85*)
Interfollicular epidermis	Basal cell	Axin2	(*44*, *45*)
Central nervous system	Radial glial cell	Axin2	(*98*)
Hair follicle	Outer bulge cell	Lgr5	(*99*)
Kidney	Nephron segment-specific stem cell	Lgr5, Axin2	(*100*, *101*)
Cochlea	Tympanic border	Axin2	(*102*)
Ovary	Hilum ovarian surface epithelial cell	Lgr5	(*103*)
Taste bud	Circumvallate papilla stem cell in posterior tongue	Lgr5	(*104*, *105*)

Corrected 20 October 2014; see full text.

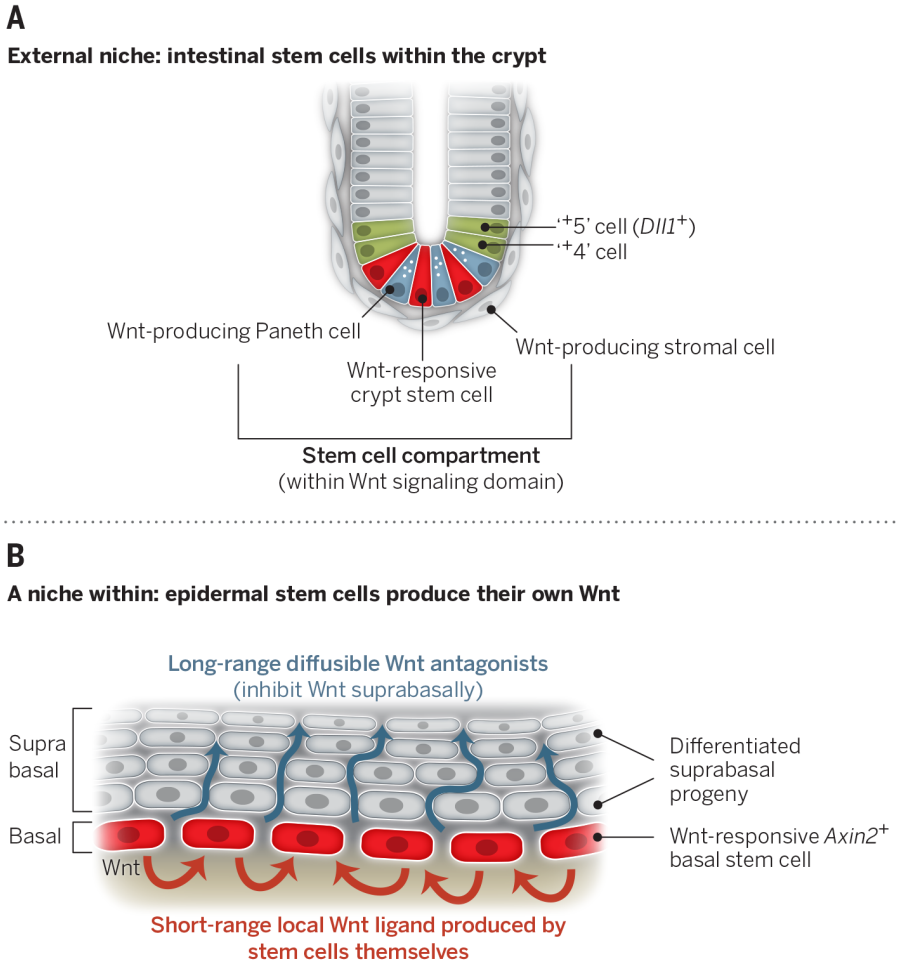

Fig. 3. The provenance of Wnt ligands in the stem cell niche. (**A**) At the intestinal crypt bottom, Paneth cells and stromal cells supply Wnt ligands to sustain the self-renewal of Lgr5$^+$ crypt stem cells, with which they are intercalated. The local Wnt signaling domain spatially delimits stem cell activity to the crypt bottom. Cells moving upward begin to differentiate, although they may be restored to stem cell status upon returning to the crypt bottom. (**B**) Within the interfollicular epidermis, basal-layer stem cells express Wnt ligands and thus continuously induce their own self-renewal and act as their own niche. Basal stem cells also express long-range Wnt antagonists that diffuse to suprabasal layers, basally limiting the Wnt signaling field and "self-organizing" the stratified epidermal architecture. (**C**) Image of Dkk3 immunostaining (red) in epidermis of Axin2-CreERT2/Rosa26-mTmG mice exposed to Tamoxifen at P21 to induce labeled clones (green) and chased for 2 months (P77). (C) is courtesy of X. Lim (*44*).

(*62*). In vitro, this process can be mimicked by a pulse of high-dose Wnt3a (*62*). Similar observations were reported for a noncycling secretory precursor (*43*). Therefore, lineage-restricted progenitors may gain an expansion of responsibility upon injury, reacquiring multipotency and long-term self-renewal to perpetuate tissue repair. The stem cell phenotype is not indelibly imprinted but may be ordained unto other cell types during the regenerative response.

Wnt and tissue regeneration in the earliest animals

Even in the earliest animals, it seems that Wnt coordinates repair after injury in certain tissues and imparts positional information crucial for shaping proper regeneration. Upon resection of their tail, planarian flatworms regenerate their tail anew. Nonetheless, upon depletion of β-catenin, a head is inappropriately regenerated in lieu of the tail, leading to the generation of multiple heads (*63*, *64*). Therefore, Wnt ensures that the original anatomic plan is faithfully restored after injury. Analogously, *Wnt10a* is upregulated upon zebrafish tail resection and is necessary for robust tail regeneration (*65*). Likewise, *Wnt3* is crucial for apical regeneration of amputated hydra (*66*). Compellingly, in hydra the Wnt source is apoptotic cells at the site of the wound, which provide Wnt3 to drive proliferation of underlying cells and thus regeneration (*67*). Therefore, Wnt elegantly links tissue loss with how such tissue might be restored.

The sources of Wnt ligands: Redefinition of the stem cell niche

Wnt signals, by virtue of their short-range nature, constitute ideal "niche factors," controlling immediately adjacent stem cells and thus permitting parsimonious command of cell fate.

For instance, Lgr5$^+$ CBCs in the crypt bottom are evenly interspersed with Paneth cells (*68*) that, together with nonepithelial lineages including mesenchymal cells (*69*–*71*), supply Wnt proteins to maintain adjacent Lgr5$^+$ CBCs (Fig. 3A). The localized spatial reach of Wnt dictates that only cells near the crypt bottom remain stem cells. Cells migrating upward out of the reach of Wnt signaling differentiate.

This "Wnt-adjacency" model can also hold true in regeneration. Upon bladder injury, stromal cells directly underlying the bladder basal epithelium up-regulate Wnt ligands, signaling to adjacent basal stem cells to initiate bladder epithelium regeneration (*72*). Therefore, stem and niche cells are paired in both spatial location and function.

Nevertheless, the past few years have seen a revision to the monolithic notion that stem cells need always be controlled by an extrinsic niche. Axin2$^+$ IFE stem cells express their own Wnt ligands, which they require for self-renewal (*44*). Therefore, they may continuously drive their own self-renewal in an autocrine fashion (Fig. 3B), akin to how Wnt3a-expressing axial stem cells in the early vertebrate embryo in essence act as their own niche (*73*) to sustain their own self-renewal during axis elongation and upon

 Corrected 20 October 2014; see full text.

serial transplantation (74, 75). In the case of the intestinal crypt, *Lgr5*$^+$ CBCs generate Wnt-producing Paneth cells (25). This underpins why single *Lgr5*$^+$ CBCs can form intestinal organoids in vitro in the absence of niche cells (76)—because stem cells can elaborate their own niche.

Developmental self-organization

These observations imply that in some contexts, stem cells can self-organize their own niche and autonomously perpetuate their activity. In this capacity, stem cells qualify as fundamental "units of development" (61) because they can incipiently seed developing tissues anew. In the developing *Drosophila* intestine, the first cell division undertaken by the earliest intestinal stem cells is to asymmetrically generate a niche cell as well as another stem cell (77). "Auto-niche generation" enables single stem cells to take root in the nascent tissue, expand to form islands of undifferentiated stem cells, and subsequently fuel intestinal development (77).

If stem cells can self-organize their own niche and continue ever-expanding in vivo, this could be easily subverted to lead to tumorigenesis. Contrary to this notion of unchecked stem cell expansion, in each intestinal crypt there exists approximately 14 Lgr5$^+$ CBCs and 10 Paneth cells per crypt bottom (57) and eight Lgr5$^+$ stem cells per stomach pylorus pit (78). How is stem cell expansion so precisely constrained in the steady state? By way of example, in the skin, IFE basal stem cells produce not only their own Wnt ligands but also diffusible Wnt antagonists, including Dkk molecules (Fig. 3C) (44). Therefore, adjacent basal stem cells signal via Wnt to sustain one another in the basal compartment, yet Dkk diffuses to the suprabasal layer to limit the Wnt signaling field and likely to induce differentiation in that domain (44). Consequently, stem cell activity is spatially confined to the basal layer, and Dkk might prevent expansion of the stem cell territory beyond that layer (Fig. 3B). In so doing, IFE stem cells might self-organize the stratified architecture of the epidermis.

Orienting asymmetric stem cell divisions by Wnt signaling within the niche

Stem cell numbers also may be numerically limited within the niche by Wnt-imposed asymmetric stem cell divisions. *Drosophila* germline stem cells divide next to neighboring hub cells. The daughter cell closest to the hub cell remains a stem cell, whereas the distal cell invariably differentiates; this asymmetric division is oriented by the Wnt signaling component APC (79). Experiments using a local source of Wnt in cell culture imply a conserved mechanism extending to mammals. A localized Wnt signal can orient a mouse embryonic stem cell (ESC) to divide asymmetrically by placing the centrosomes at opposite ends of the cell, thus orienting the mitotic spindle of the dividing cell (Fig. 4) (80). This generates a Wnt-proximal and Wnt-distal daughter cell, the latter out of contact with the signal. In the Wnt-proximal cell, Wnt signaling maintains the stem cell fate, whereas the distal daughter differentiates (80). The orientation of stem cell division is therefore coupled with the position and fate of the dividing cell through the same signal. Therefore, in some tissues Wnt signals may orient stem cell divisions within the niche in an asymmetric fashion, delimiting stem cell number and ensuring a proper ratio of stem cells to their committed progeny.

Growing Wnt-dependent stem cells

The roles of Wnt in stem cell self-renewal or lineage-specific differentiation in diverse tissues in vivo are manifold; therefore, Wnt signals have found practical use in manipulating stem cell developmental programs in vitro. From a pragmatic perspective, because Wnt induces stem cell self-renewal in certain organs, it enables the in vitro propagation of such cells. For example, mammary gland stem cells can be expanded in vitro in the presence of Wnt protein and retain their ability to reconstitute the entire mammary organ after transplantation (56).

Similarly, pluripotent naïve ESCs from the rodent blastocyst may be cultivated in vitro in defined conditions by combining Wnt agonists [either Wnt protein (81) or GSK3 inhibitors (82)] with either leukemia inhibitory factor (LIF) signals or mitogen-activated protein kinase (MAPK)/extracellular signal–regulated kinase (ERK) inhibitors (83), as exemplified by the "2i" culture regime for serum-free ESC culture (82).

Because of the primacy of Wnt in instructing the intestinal stem cell fate, Lgr5$^+$ CBC stem cells can be expanded in an R-spondin1–based three-dimensional culture system in ever-growing organoids, or "mini-guts" (76), in which crypt and villus domains are established containing normal ratios of the appropriate cell types, whereas self-renewal kinetics closely resemble the in vivo situation (84). Comparable protocols have been established for Lgr5$^+$ cells derived from the stomach (85), liver (86), and pancreas (87). When cells within organoids produce Wnt (for example, Paneth cells that secrete Wnt3 in small intestinal organoids), the addition of R-spondin suffices. When organoids harbor no endogenous source of Wnt (for example, colon organoids), exogenous Wnt3a is added in addition to R-spondin (88). Transplantation of clonal (single Lgr5$^+$ stem cell–derived) organoids derived from colon and liver has confirmed that the cultured organoids retain their physiological functions (86, 89). This again provides evidence for substantial developmental self-organization—namely, that single Lgr5$^+$ intestinal stem cells carry the morphogenetic information to create a structured tissue of complex architecture and diverse lineages.

Proper lineage differentiation and crypt-villus organization within small intestinal organoids relies on an interesting property of R-spondin1. Namely, it augments preexisting domains of Wnt signaling in the crypt bottom (68) rather than inducing Wnt signaling de novo. Thus, when cells exit the crypt bottom–like structures of mini-guts and the spatial reach of Wnt, intestinal differentiation occurs normally (76), accounting for proper organoid architecture. In contrast, spatially uniform Wnt activation by GSK3 inhibition captures a rather homogeneous population of Lgr5$^+$ stem cells in vitro in the absence of differentiated lineages (90).

That being said, Wnt does not ubiquitously instruct stem cell self-renewal and, in multiple cases, instead drives differentiation—for instance, Wnt instead stimulates primed pluripotent stem cells (including human ESCs) to differentiate into primitive streak (91, 92).

Concluding remarks

The emergent view is that lipid-modified Wnt signals predominantly act over short ranges to locally control cell behavior, economically controlling stem cells within the spatial confines of the niche. The short range of Wnt action implies a parsimonious model of niche organization and tissue physiology. Namely, in particular tissues it seems that Wnt-dependent stem cells are spatially restricted to the vicinity of the Wnt-producing niche, physically delimiting the stem cell compartment and preventing unauthorized stem cell expansion. When a stem cell divides, chance may dictate which (if any) of its successors are ousted from its niche, as in the intestines (57), stomach (78), and skin (44). In other lineages, Wnt itself may orient stem cells to divide asymmetrically (80), conveniently

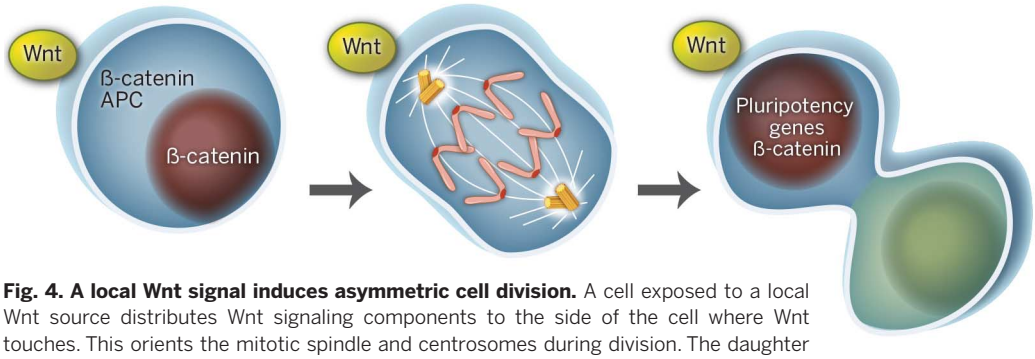

Fig. 4. A local Wnt signal induces asymmetric cell division. A cell exposed to a local Wnt source distributes Wnt signaling components to the side of the cell where Wnt touches. This orients the mitotic spindle and centrosomes during division. The daughter cell close to the Wnt source maintains nuclear β-catenin and stem cell gene expression, whereas the distal cell away from Wnt loses expression of such genes.

Corrected 20 October 2014; see full text.

anchoring Wnt-proximal stem cells to the niche and ensuring proper spatial allocation of stem cells and differentiated progeny.

In certain organs, stem cells exiting the niche become deprived of Wnt and therefore differentiate. Nonetheless, developmental plasticity may yet remain because early committed precursors can flexibly regain stem cell status upon tissue damage in vivo (*43*, *62*, *93*, *94*) or Wnt3a treatment ex vivo, in some instances (*62*). This is profound because it indicates that lineage potential is an amorphous property in vivo; lineage-restricted precursors can gain an expansion of responsibility upon injury and become fully fledged multipotent stem cells once more. Intravital microscopy has documented that upon intestinal or hair follicle damage, precursors are spatially recalled to the stem cell niche (*95*, *96*), upon which they reenter the niche signaling domain and presumably become promoted to stem cell status as a consequence, although the responsible signals remain largely elusive. Therefore, lineage barriers between stem cell and progenitor states are not always stringent in vivo and can be traversed during times of tissue damage and repair (*43*, *62*, *93*, *94*). If stem cell and progenitor fates are interconvertible upon niche contact (*97*), then stem cell status might not be an intrinsic entitlement but rather a positional privilege—reflecting whether a cell is currently in the embrace of the niche.

Nonetheless the notion of a "niche" must be refined because some stem cells may act as or establish their own niche ab initio, portending unexpected developmental self-organization. Such intrinsically programmed stem cell behavior could underpin emergence of complex patterned tissues during development and/or regeneration, as in the *Drosophila* (*77*) and mouse (*76*) intestines.

The above findings identify an integral program for tissue generation, regeneration, and renewal. In evolutionary antiquity, the core of the Wnt pathway emerged in the simplest multicellular organisms (*16*, *17*). Accruing evidence suggests that in the earliest metazoa, Wnt was an ancestral "symmetry-breaking" signal that separated otherwise-symmetric embryos into two halves (the anterior versus the posterior domain) and in so doing enabled the evolutionary emergence of axially patterned animals (*18*, *19*). Simply put, the primordial role of Wnt signaling in the earliest animals was pattern formation (during tissue generation) and pattern maintenance (during tissue regeneration), as evinced by how Wnt establishes a bodily pattern in hydra and planaria and enables the reconstitution of such pattern upon tissue regeneration (*63*, *64*, *66*). In long-lived vertebrates, this ancestral pattern maintenance program has since been extended to tissue renewal, in which Wnt permits several tissues, including the skin and intestines, to be continuously replenished and thus maintained over a lifetime.

REFERENCES AND NOTES

1. C. P. Leblond, B. E. Walker, Renewal of cell populations. *Physiol. Rev.* **36**, 255–276 (1956). pmid: 13322651
2. R. Najdi *et al*., A uniform human Wnt expression library reveals a shared secretory pathway and unique signaling activities. *Differentiation* **84**, 203–213 (2012). doi: 10.1016/j.diff.2012.06.004; pmid: 22784633
3. J. C. Gross, V. Chaudhary, K. Bartscherer, M. Boutros, Active Wnt proteins are secreted on exosomes. *Nat. Cell Biol.* **14**, 1036 (2012). doi: 10.1038/ncb2574
4. C. Korkut *et al*., Trans-synaptic transmission of vesicular Wnt signals through Evi/Wntless. *Cell* **139**, 393–404 (2009). doi: 10.1016/j.cell.2009.07.051; pmid: 19837038
5. C. Alexandre, A. Baena-Lopez, J. P. Vincent, Patterning and growth control by membrane-tethered Wingless. *Nature* **505**, 180–185 (2014). doi: 10.1038/nature12879; pmid: 24390349
6. C. Y. Janda, D. Waghray, A. M. Levin, C. Thomas, K. C. Garcia, Structural basis of Wnt recognition by Frizzled. *Science* **337**, 59–64 (2012). doi: 10.1126/science.1222879; pmid: 22653731
7. J. L. Stamos, M. L. Chu, M. D. Enos, N. Shah, W. I. Weis, Structural basis of GSK-3 inhibition by N-terminal phosphorylation and by the Wnt receptor LRP6. *eLife* **3**, e01998 (2014). doi: 10.7554/eLife.01998; pmid: 24642411
8. R. van Amerongen, R. Nusse, Towards an integrated view of Wnt signaling in development. *Development* **136**, 3205–3214 (2009). doi: 10.1242/dev.033910; pmid: 19736321
9. O. Kazanskaya *et al*., R-Spondin2 is a secreted activator of Wnt/beta-catenin signaling and is required for Xenopus myogenesis. *Dev. Cell* **7**, 525–534 (2004). doi: 10.1016/j.devcel.2004.07.019; pmid: 15469841
10. K.-A. Kim *et al*., R-Spondin family members regulate the Wnt pathway by a common mechanism. *Mol. Biol. Cell* **19**, 2588–2596 (2008). doi: 10.1091/mbc.E08-02-0187; pmid: 18400942
11. K. S. Carmon, X. Gong, Q. Lin, A. Thomas, Q. Liu, R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11452–11457 (2011). doi: 10.1073/pnas.1106083108; pmid: 21693646
12. W. de Lau *et al*., Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature* **476**, 293–297 (2011). doi: 10.1038/nature10337; pmid: 21727895
13. A. Glinka *et al*., LGR4 and LGR5 are R-spondin receptors mediating Wnt/β-catenin and Wnt/PCP signalling. *EMBO Rep.* **12**, 1055–1061 (2011). doi: 10.1038/embor.2011.175; pmid: 21909076
14. H.-X. Hao *et al*., ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature* **485**, 195–200 (2012). doi: 10.1038/nature11019; pmid: 22575959
15. B. K. Koo *et al*., Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature* **488**, 665–669 (2012). doi: 10.1038/nature11308; pmid: 22895187
16. J. F. Ryan *et al*., The genome of the ctenophore Mnemiopsis leidyi and its implications for cell type evolution. *Science* **342**, 1242592 (2013). doi: 10.1126/science.1242592; pmid: 24337300
17. M. Srivastava *et al*., The Trichoplax genome and the nature of placozoans. *Nature* **454**, 955–960 (2008). doi: 10.1038/nature07191; pmid: 18719581
18. C. P. Petersen, P. W. Reddien, Wnt signaling and the polarity of the primary body axis. *Cell* **139**, 1056–1068 (2009). doi: 10.1016/j.cell.2009.11.035; pmid: 20005801
19. T. W. Holstein, H. Watanabe, S. Ozbek, Signaling pathways and axis formation in the lower metazoa. *Curr. Top. Dev. Biol.* **97**, 137–177 (2011). doi: 10.1016/B978-0-12-385975-4.00012-7; pmid: 22074605
20. W. B. de Lau, B. Snel, H. C. Clevers, The R-spondin protein family. *Genome Biol.* **13**, 242 (2012). doi: 10.1186/gb-2012-13-3-242; pmid: 22439850
21. K. Hoffmeyer *et al*., Wnt/β-catenin signaling regulates telomerase in stem cells and cancer cells. *Science* **336**, 1549–1554 (2012). doi: 10.1126/science.1218370; pmid: 22723415
22. A. G. Schepers, R. Vries, M. van den Born, M. van de Wetering, H. Clevers, Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. *EMBO J.* **30**, 1104–1109 (2011). doi: 10.1038/emboj.2011.26; pmid: 21297579
23. B. Lustig *et al*., Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. *Mol. Cell. Biol.* **22**, 1184–1193 (2002). doi: 10.1128/MCB.22.4.1184-1193.2002; pmid: 11809809
24. R. van Amerongen, A. N. Bowman, R. Nusse, Developmental stage and time dictate the fate of Wnt/β-catenin-responsive stem cells in the mammary gland. *Cell Stem Cell* **11**, 387–400 (2012). doi: 10.1016/j.stem.2012.05.023; pmid: 22863533
25. N. Barker *et al*., Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**, 1003–1007 (2007). doi: 10.1038/nature06196; pmid: 17934449
26. C. P. Leblond, C. E. Stevens, The constant renewal of the intestinal epithelium in the albino rat. *Anat. Rec.* **100**, 357–377 (1948). doi: 10.1002/ar.1091000306; pmid: 18906253
27. J. H. van Es *et al*., A critical role for the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal. *Mol. Cell. Biol.* **32**, 1918–1927 (2012). doi: 10.1128/MCB.06288-11; pmid: 22393260
28. V. Korinek *et al*., Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* **19**, 379–383 (1998). doi: 10.1038/1270; pmid: 9697701
29. T. Fevr, S. Robine, D. Louvard, J. Huelsken, Wnt/β-catenin is essential for intestinal homeostasis and maintenance of intestinal stem cells. *Mol. Cell. Biol.* **27**, 7551–7559 (2007). doi: 10.1128/MCB.01034-07; pmid: 17785439
30. K. A. Kim *et al*., Mitogenic influence of human R-spondin1 on the intestinal epithelium. *Science* **309**, 1256–1259 (2005). doi: 10.1126/science.1112521; pmid: 16109882
31. J. Paneth, Ueber die secernirenden Zellen des Dünndarm-Epithels. *Archiv für Mikroskopische Anatomie* **31**, 113–191 (1887). doi: 10.1007/BF02955706
32. H. Cheng, C. P. Leblond, 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 (1974). doi: 10.1002/aja.1001410407; pmid: 4440635
33. K. S. Yan *et al*., The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 466–471 (2012). doi: 10.1073/pnas.1118857109; pmid: 22190486
34. E. Sangiorgi, M. R. Capecchi, Bmi1 is expressed in vivo in intestinal stem cells. *Nat. Genet.* **40**, 915–920 (2008). doi: 10.1038/ng.165; pmid: 18536716
35. N. Takeda *et al*., Interconversion between intestinal stem cell populations in distinct niches. *Science* **334**, 1420–1424 (2011). doi: 10.1126/science.1213214; pmid: 22075725
36. A. E. Powell *et al*., The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. *Cell* **149**, 146–158 (2012). doi: 10.1016/j.cell.2012.02.042; pmid: 22464327
37. V. W. Y. Wong *et al*., Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. *Nat. Cell Biol.* **14**, 401–408 (2012). doi: 10.1038/ncb2464; pmid: 22388892
38. D. T. Breault *et al*., Generation of mTert-GFP mice as a model to identify and study tissue progenitor cells. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 10420–10425 (2008). doi: 10.1073/pnas.0804800105; pmid: 18650388
39. R. K. Montgomery *et al*., Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 179–184 (2011). doi: 10.1073/pnas.1013004108; pmid: 21173232
40. H. Tian *et al*., A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature* **478**, 255–259 (2011). doi: 10.1038/nature10408; pmid: 21927002
41. S. Itzkovitz *et al*., Single-molecule transcript counting of stem-cell markers in the mouse intestine. *Nat. Cell Biol.* **14**, 106–114 (2012). doi: 10.1038/ncb2384; pmid: 22119784
42. J. Muñoz *et al*., The Lgr5 intestinal stem cell signature: Robust expression of proposed quiescent '+4' cell markers. *EMBO J.* **31**, 3079–3091 (2012). doi: 10.1038/emboj.2012.166; pmid: 22692129
43. S. J. Buczacki *et al*., Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature* **495**, 65–69 (2013). doi: 10.1038/nature11965; pmid: 23446353
44. X. Lim *et al*., Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. *Science* **342**, 1226–1230 (2013). doi: 10.1126/science.1239730; pmid: 24311688
45. Y. S. Choi *et al*., Distinct functions for Wnt/β-catenin in hair follicle stem cell proliferation and survival and interfollicular epidermal homeostasis. *Cell Stem Cell* **13**, 720–733 (2013). doi: 10.1016/j.stem.2013.10.003; pmid: 24315444
46. K. B. Jensen *et al*., Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. *Cell Stem Cell* **4**, 427–439 (2009). doi: 10.1016/j.stem.2009.04.014; pmid: 19427292
47. A. J. Zhu, F. M. Watt, Expression of a dominant negative cadherin mutant inhibits proliferation and stimulates terminal

Corrected 20 October 2014; see full text.

differentiation of human epidermal keratinocytes. *J. Cell Sci.* **109**, 3013–3023 (1996). pmid: 9004036
48. S. Beronja *et al*., RNAi screens in mice identify physiological regulators of oncogenic growth. *Nature* **501**, 185–190 (2013). doi: 10.1038/nature12464; pmid: 23945586
49. H. Nguyen *et al*., Tcf3 and Tcf4 are essential for long-term homeostasis of skin epithelia. *Nat. Genet.* **41**, 1068–1075 (2009). doi: 10.1038/ng.431; pmid: 19718027
50. M. Shackleton *et al*., Generation of a functional mammary gland from a single stem cell. *Nature* **439**, 84–88 (2006). doi: 10.1038/nature04372; pmid: 16397499
51. N. M. Badders *et al*., The Wnt receptor, Lrp5, is expressed by mouse mammary stem cells and is required to maintain the basal lineage. *PLOS One* **4**, e6594 (2009). doi: 10.1371/journal.pone.0006594; pmid: 19672307
52. K. E. de Visser *et al*., Developmental stage-specific contribution of LGR5$^+$ cells to basal and luminal epithelial lineages in the postnatal mammary gland. *J. Pathol.* **228**, 300–309 (2012). doi: 10.1002/path.4096; pmid: 22926799
53. V. Plaks *et al*., Lgr5-expressing cells are sufficient and necessary for postnatal mammary gland organogenesis. *Cell Reports* **3**, 70–78 (2013). doi: 10.1016/j.celrep.2012.12.017; pmid: 23352663
54. A. C. Rios, N. Y. Fu, G. J. Lindeman, J. E. Visvader, In situ identification of bipotent stem cells in the mammary gland. *Nature* **506**, 322–327 (2014). doi: 10.1038/nature12948; pmid: 24463516
55. A. Van Keymeulen *et al*., Distinct stem cells contribute to mammary gland development and maintenance. *Nature* **479**, 189–193 (2011). doi: 10.1038/nature10573; pmid: 21983963
56. Y. A. Zeng, R. Nusse, Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture. *Cell Stem Cell* **6**, 568–577 (2010). doi: 10.1016/j.stem.2010.03.020; pmid: 20569694
57. H. J. Snippert *et al*., Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143**, 134–144 (2010). doi: 10.1016/j.cell.2010.09.016; pmid: 20887898
58. C. Lopez-Garcia, A. M. Klein, B. D. Simons, D. J. Winton, Intestinal stem cell replacement follows a pattern of neutral drift. *Science* **330**, 822–825 (2010). doi: 10.1126/science.1196236; pmid: 20929733
59. A. M. Klein, B. D. Simons, Universal patterns of stem cell fate in cycling adult tissues. *Development* **138**, 3103–3111 (2011). doi: 10.1242/dev.060103; pmid: 21750026
60. E. Clayton *et al*., A single type of progenitor cell maintains normal epidermis. *Nature* **446**, 185–189 (2007). doi: 10.1038/nature05574; pmid: 17330052
61. I. L. Weissman, Stem cells: Units of development, units of regeneration, and units in evolution. *Cell* **100**, 157–168 (2000). doi: 10.1016/S0092-8674(00)81692-X; pmid: 10647940
62. J. H. van Es *et al*., Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nat. Cell Biol.* **14**, 1099–1104 (2012). doi: 10.1038/ncb2581; pmid: 23000963
63. C. P. Petersen, P. W. Reddien, *Smed-βcatenin-1* is required for anteroposterior blastema polarity in planarian regeneration. *Science* **319**, 327–330 (2008). doi: 10.1126/science.1149943; pmid: 18063755
64. K. A. Gurley, J. C. Rink, A. Sánchez Alvarado, β-Catenin defines head versus tail identity during planarian regeneration and homeostasis. *Science* **319**, 323–327 (2008). doi: 10.1126/science.1150029; pmid: 18063757
65. C. L. Stoick-Cooper *et al*., Distinct Wnt signaling pathways have opposing roles in appendage regeneration. *Development* **134**, 479–489 (2007). doi: 10.1242/dev.001123; pmid: 17185322
66. T. Lengfeld *et al*., Multiple Wnts are involved in Hydra organizer formation and regeneration. *Dev. Biol.* **330**, 186–199 (2009). doi: 10.1016/j.ydbio.2009.02.004; pmid: 19217898
67. S. Chera *et al*., Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. *Dev. Cell* **17**, 279–289 (2009). doi: 10.1016/j.devcel.2009.07.014; pmid: 19686688
68. T. Sato *et al*., Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* **469**, 415–418 (2011). doi: 10.1038/nature09637; pmid: 21113151
69. H. F. Farin, J. H. Van Es, H. Clevers, Redundant sources of Wnt regulate intestinal stem cells and promote formation of Paneth cells. *Gastroenterology* **143**, 1518–1529.e7 (2012). doi: 10.1053/j.gastro.2012.08.031; pmid: 22922422
70. Z. Kabiri *et al*., Stroma provides an intestinal stem cell niche in the absence of epithelial Wnts. *Development* **141**, 2206–2215 (2014). doi: 10.1242/dev.104976; pmid: 24821987
71. A. K. San Roman, C. D. Jayewickreme, L. C. Murtaugh, R. A. Shivdasani, Wnt secretion from epithelial cells and subepithelial myofibroblasts is not required in the mouse intestinal stem cell niche in vivo. *Stem Cell Rev.* **2**, 127–134 (2014). doi: 10.1016/j.stemcr.2013.12.012
72. K. Shin *et al*., Hedgehog/Wnt feedback supports regenerative proliferation of epithelial stem cells in bladder. *Nature* **472**, 110–114 (2011). doi: 10.1038/nature09851; pmid: 21389986
73. B. L. Martin, D. Kimelman, Brachyury establishes the embryonic mesodermal progenitor niche. *Genes Dev.* **24**, 2778–2783 (2010). doi: 10.1101/gad.1962910; pmid: 21159819
74. N. Cambray, V. Wilson, Axial progenitors with extensive potency are localised to the mouse chordoneural hinge. *Development* **129**, 4855–4866 (2002). pmid: 12361976
75. N. Cambray, V. Wilson, Two distinct sources for a population of maturing axial progenitors. *Development* **134**, 2829–2840 (2007). doi: 10.1242/dev.02877; pmid: 17611225
76. T. Sato *et al*., Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262–265 (2009). doi: 10.1038/nature07935; pmid: 19329995
77. D. Mathur, A. Bost, I. Driver, B. Ohlstein, A transient niche regulates the specification of *Drosophila* intestinal stem cells. *Science* **327**, 210–213 (2010). doi: 10.1126/science.1181958; pmid: 20056890
78. M. Leushacke, A. Ng, J. Galle, M. Loeffler, N. Barker, Lgr5$^+$ gastric stem cells divide symmetrically to effect epithelial homeostasis in the pylorus. *Cell Reports* **5**, 349–356 (2013). doi: 10.1016/j.celrep.2013.09.025; pmid: 24209744
79. Y. M. Yamashita, D. L. Jones, M. T. Fuller, Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. *Science* **301**, 1547–1550 (2003). doi: 10.1126/science.1087795; pmid: 12970569
80. S. J. Habib *et al*., A localized Wnt signal orients asymmetric stem cell division in vitro. *Science* **339**, 1445–1448 (2013). doi: 10.1126/science.1231077; pmid: 23520113
81. D. ten Berge *et al*., Embryonic stem cells require Wnt proteins to prevent differentiation to epiblast stem cells. *Nat. Cell Biol.* **13**, 1070–1075 (2011). doi: 10.1038/ncb2314; pmid: 21841791
82. Q.-L. Ying *et al*., The ground state of embryonic stem cell self-renewal. *Nature* **453**, 519–523 (2008). doi: 10.1038/nature06968; pmid: 18497825
83. J. Wray, T. Kalkan, A. G. Smith, The ground state of pluripotency. *Biochem. Soc. Trans.* **38**, 1027–1032 (2010). doi: 10.1042/BST0381027; pmid: 20658998
84. T. Sato, H. Clevers, Growing self-organizing mini-guts from a single intestinal stem cell: Mechanism and applications. *Science* **340**, 1190–1194 (2013). doi: 10.1126/science.1234852; pmid: 23744940
85. N. Barker *et al*., Lgr5^{+ve} stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* **6**, 25–36 (2010). doi: 10.1016/j.stem.2009.11.013; pmid: 20085740
86. M. Huch *et al*., In vitro expansion of single Lgr5$^+$ liver stem cells induced by Wnt-driven regeneration. *Nature* **494**, 247–250 (2013). doi: 10.1038/nature11826; pmid: 23354049
87. M. Huch *et al*., Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J.* **32**, 2708–2721 (2013). doi: 10.1038/emboj.2013.204; pmid: 24045232
88. T. Sato *et al*., Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* **141**, 1762–1772 (2011). doi: 10.1053/j.gastro.2011.07.050; pmid: 21889923
89. S. Yui *et al*., Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5$^+$ stem cell. *Nat. Med.* **18**, 618–623 (2012). doi: 10.1038/nm.2695; pmid: 22406745
90. X. Yin *et al*., Niche-independent high-purity cultures of Lgr5$^+$ intestinal stem cells and their progeny. *Nat. Methods* **11**, 106–112 (2014). doi: 10.1038/nmeth.2737; pmid: 24292484
91. K. M. Loh *et al*., Efficient endoderm induction from human pluripotent stem cells by logically directing signals controlling lineage bifurcations. *Cell Stem Cell* **14**, 237–252 (2014). doi: 10.1016/j.stem.2013.12.007; pmid: 24412311
92. K. C. Davidson *et al*., Wnt/β-catenin signaling promotes differentiation, not self-renewal, of human embryonic stem cells and is repressed by Oct4. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 4485 (2012).
93. T. Nakagawa, M. Sharma, Y. Nabeshima, R. E. Braun, S. Yoshida, Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. *Science* **328**, 62–67 (2010). doi: 10.1126/science.1182868; pmid: 20299552
94. J. Cheng *et al*., Centrosome misorientation reduces stem cell division during ageing. *Nature* **456**, 599–604 (2008). doi: 10.1038/nature07386; pmid: 18923395
95. L. Ritsma *et al*., Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging. *Nature* **507**, 362–365 (2014). doi: 10.1038/nature12972; pmid: 24531760
96. P. Rompolas, K. R. Mesa, V. Greco, Spatial organization within a niche as a determinant of stem-cell fate. *Nature* **502**, 513–518 (2013). doi: 10.1038/nature12602; pmid: 24097351
97. V. P. Losick, L. X. Morris, D. T. Fox, A. Spradling, *Drosophila* stem cell niches: A decade of discovery suggests a unified view of stem cell regulation. *Dev. Cell* **21**, 159–171 (2011). doi: 10.1016/j.devcel.2011.06.018; pmid: 21763616
98. A. N. Bowman, R. van Amerongen, T. D. Palmer, R. Nusse, Lineage tracing with Axin2 reveals distinct developmental and adult populations of Wnt/β-catenin-responsive neural stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 7324–7329 (2013). doi: 10.1073/pnas.1305411110; pmid: 23589866
99. V. Jaks *et al*., Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat. Genet.* **40**, 1291–1299 (2008). doi: 10.1038/ng.239; pmid: 18849992
100. N. Barker *et al*., Lgr5^{+ve} stem/progenitor cells contribute to nephron formation during kidney development. *Cell Reports* **2**, 540–552 (2012). doi: 10.1016/j.celrep.2012.08.018; pmid: 22999937
101. Y. Rinkevich *et al*., In vivo clonal analysis reveals lineage-restricted progenitor characteristics in mammalian kidney development, maintenance, and regeneration. *Cell Reports* **7**, 1270–1283 (2014). doi: 10.1016/j.celrep.2014.04.018; pmid: 24835991
102. T. A. Jan *et al*., Tympanic border cells are Wnt-responsive and can act as progenitors for postnatal mouse cochlear cells. *Development* **140**, 1196–1206 (2013). doi: 10.1242/dev.087528; pmid: 23444352
103. A. Flesken-Nikitin *et al*., Ovarian surface epithelium at the junction area contains a cancer-prone stem cell niche. *Nature* **495**, 241–245 (2013). doi: 10.1038/nature11979; pmid: 23467088
104. K. K. Yee *et al*., Lgr5-EGFP marks taste bud stem/progenitor cells in posterior tongue. *Stem Cells* **31**, 992–1000 (2013). doi: 10.1002/stem.1338; pmid: 23377989
105. N. Takeda *et al*., Lgr5 Identifies Progenitor Cells Capable of Taste Bud Regeneration after Injury. *PLOS One* **8**, e66314 (2013). doi: 10.1371/journal.pone.0066314; pmid: 23824276

ACKNOWLEDGMENTS

We thank R. van Amerongen for insightful comments. The authors are supported by the Howard Hughes Medical Institute and the California Institute for Regenerative Medicine (R.N.), the Fannie and John Hertz Foundation (K.M.L.), the U.S. National Science Foundation (K.M.L.), the Davidson Institute for Talent Development (K.M.L.), the European Union (H.C.), and the CancerGenomics.nl program (H.C.). H.C. is an inventor on several patent applications that cover culturing methods for Wnt-dependent stem cells, filed by the Royal Netherlands Academy of Arts and Sciences.

10.1126/science.1248012

Corrected 20 October 2014; see full text.