EMBO reports

review

Tracking adult stem cells

Hugo J. Snippert & Hans Clevers[+]

Hubrecht Institute, KNAW and University Medical Center Utrecht, Utrecht, The Netherlands

The maintenance of stem-cell-driven tissue homeostasis requires a balance between the generation and loss of cell mass. Adult stem cells have a close relationship with the surrounding tissue—known as their niche—and thus, stem-cell studies should preferably be performed in a physiological context, rather than outside their natural environment. The mouse is an attractive model in which to study adult mammalian stem cells, as numerous experimental systems and genetic tools are available. In this review, we describe strategies commonly used to identify and functionally characterize adult stem cells in mice and discuss their potential, limitations and interpretations, as well as how they have informed our understanding of adult stem-cell biology. An accurate interpretation of physiologically relevant stem-cell assays is crucial to identify adult stem cells and elucidate how they self-renew and give rise to differentiated progeny.

Keywords: adult stem cells; lineage tracing; stemness; multipotency; self-renewal

EMBO *reports* (2011) **12,** 113–122. doi:10.1038/embor.2010.216

See Glossary for abbreviations used in this article.

Adult stem-cell definitions

Most adult tissues are believed to contain *adult stem cells* that compensate for tissue loss by generating new cells. Two attributes of stem cells enable this regenerative capacity throughout life. First, stem cells divide, yet maintain themselves as a population over long periods of time; a property called *self-renewal*. Self-renewal is not unique to stem cells, as fully differentiated cells such as activated B and T lymphocytes can also undergo self-renewal (He *et al*, 2009). Second, stem cells supply all the cell types to the tissue in which they are found. This ability to generate daughter cells that can differentiate into all specific cell types of the pertinent tissue is called *multipotency*, or *unipotency* when a single cell type arises (He *et al*, 2009). Self-renewal and multipotency are the defining characteristics of stem cells (Potten & Loeffler, 1990). The combination of these properties is often referred to as *stemness*; the minimal set of features that all stem cells have in common (Mikkers & Frisen, 2005).

Embryonic stem cells and induced pluripotent stem cells are defined by expression of the triad OCT4, SOX2 and NANOG (Jaenisch & Young, 2008). It is not known whether a comparable molecular signature of stemness—such as a minimal core transcriptional programme—exists in (and might be shared between) adult stem cells. The absence of a defined adult stemness signature could imply that different types of adult stem cell use different mechanisms to achieve self-renewal and multipotency (Ivanova *et al*, 2002; Ramalho-Santos *et al*, 2002; Fortunel *et al*, 2003; Wong *et al*, 2008). Stem cells might be quiescent or actively cycling. Quiescent and proliferating stem-cell pools can reside in adjacent compartments, perhaps within the same tissue (Li & Clevers, 2010). Thus, it seems dangerous to extrapolate stem-cell characteristics such as marker expression or cell-cycle behaviour from one tissue to another. The dual capacity of self-renewal and multipotency should be the only criteria for stemness, independent of mechanism or signature.

The way in which stem cells balance self-renewal with the production of daughter cells is not known and might be unique to each tissue. The most prevalent view is that stem cells only divide symmetrically when their numbers need to be expanded, such as during embryonic development or after tissue injury. In the steady state, asymmetrical division—if executed perfectly—would allow stem cells to maintain their numbers while sustaining the production of transit-amplifying daughter cells (Morrison & Kimble, 2006).

Asymmetrical stem-cell division results in daughter cells with different fates. This is often shown by an asymmetrical distribution of cellular content. As a consequence of this *intrinsic* asymmetry, one daughter cell obtains the molecular cues to maintain stemness, whereas the other differentiates (Neumuller & Knoblich, 2009). The term 'asymmetrical division' also refers to division that results in two daughter cells that are positioned in different signalling environments, for example if the mitotic spindle has a pre-imposed perpendicular orientation to the original stem-cell niche (Fuller & Spradling, 2007). Although this *extrinsically* governed asymmetry has been reported in mammals (Huttner & Kosodo, 2005; Lechler & Fuchs, 2005; Kuang *et al*, 2007; Quyn *et al*, 2010), the significance of a predestined outcome is not always clear. In a *hierarchical model* of tissue homeostasis, adult stem cells follow a strict pattern of invariant asymmetry to self-renew while generating progeny.

An alternative view states that cells initially divide into intrinsically equal stem-cell daughters through *symmetrical stem-cell division*. After division, the fate of the individual daughter cells is determined independently and stochastically such that two stem cells, two daughter cells, or one stem cell and one daughter cell could result. Homeostasis is maintained at the population level through regulatory mechanisms exerted by the microenvironment in which the stem cells reside—known as the *stem-cell niche*. The

Hubrecht Institute, KNAW & University Medical Center Utrecht, Uppsalalaan 83584 CT Utrecht, The Netherlands

[+]Correspondending author. Tel: +31 (0) 30 212 1800; Fax: +31 (0) 30 251 6464; E-mail: h.clevers@hubrecht.eu

Received 10 November 2010; accepted 20 December 2010; published online 21 January 2011

Glossary

BrdU	bromodeoxyuridine
CBC	crypt base columnar
EdU	5-ethynyl-2'-deoxyuridine
EGF	epidermal growth factor
EGFP	enhanced green fluorescent protein
GFP	green fluorescent protein
FACS	fluorescence activated cell sorter
HSC	haematopoietic stem cell
IFE	interfollicular epidermis
ISC	intestinal stem cell
LRG5/6	leucine-rich repeat-containing G protein coupled receptor 5/6
LRIG1	leucine-rich repeats and immunoglobulin-like domains 1
NGN3	neurogenin 3

location of the daughter cells in relation to the stem-cell niche is important, but it is not predetermined (Morrison & Kimble, 2006). The niche has a crucial role in the homeostatic maintenance of the stem-cell pool, by supplying essential factors and adhesion anchors (Voog & Jones, 2010). In a *stochastic model* of tissue homeostasis, an equipotent population of stem cells follows a pattern of population asymmetry in which symmetrical self-renewal of stem cells compensates for the loss of neighbouring stem cells.

There are many types of adult stem cells and they might use different mechanisms to ensure homeostatic self-renewal. As argued above, the only commonality between adult stem-cell types is their capacity for self-renewal and multipotency. It is therefore essential that new candidate stem-cell populations are tested for these defining criteria, rather than for other characteristics that have proven useful in identifying a particular type of adult stem cell, such as quiescence or the expression of certain molecules.

Finally, cells might alter their characteristics due to experimentally induced stress (Potten & Loeffler, 1990). Therefore, *actual stemness*—or real stem-cell behaviour—needs to be distinguished from *stemness potential*—or the ability of a cell population to obtain actual stemness. For example, transplantation could activate stem-cell behaviour in cells that in normal situation do not behave as stem cells.

We describe four assays that are commonly used in mouse adult stem-cell biology. Examples are taken from different tissues, particularly the small intestine and the skin. We emphasize the advantages and limitations of each assay and discuss interpretation of the data (Table 1).

Quiescence and label retention

In addition to self-renewal and multipotency, a feature commonly associated with stemness is quiescence—stem cells dividing infrequently to prevent stem-cell 'exhaustion' (Arai & Suda, 2007; Orford & Scadden, 2008). Examples of slow cycling adult stem cells are hair follicle bulge cells and haematopoietic stem cells (HSCs; Cotsarelis *et al*, 1990; Tumbar *et al*, 2004; Wilson *et al*, 2008; Foudi *et al*, 2009). However, the well-defined germ stem cells in the fly actively divide (Xie *et al*, 2005). Furthermore, the best-characterized of all stem cells—mammalian embryonic stem cells—proliferate rapidly while being able to self-renew and maintain pluripotency (He *et al*, 2009). Indeed, actively proliferating stem cells have been documented in the stomach, small intestine and colon (Barker *et al*, 2007, 2010), indicating that quiescence is not a prerequisite of stemness.

The incorporation of DNA analogues—such as BrdU, tritiated thymidine or EdU—during S-phase is often used to study cell-cycle kinetics. Actively dividing stem cells can be efficiently labelled with a short pulse of such DNA labels (Barker *et al*, 2007), whereas labelling quiescent stem cells requires prolonged exposure to the DNA label or their temporary activation, for example by tissue injury (Potten *et al*, 1978). During the subsequent chase, quiescent cells slowly dilute the label, in contrast to cells that go through successive rounds of cell division. As a result, quiescence can be visualized as DNA-label retention after long chase periods (Cotsarelis *et al*, 1990). Unfortunately, the chemical DNA labels can only be visualized in fixed and permeabilized cells. The demonstration of DNA-label retention is not sufficient to identify stem cells. In many tissues, fully differentiated cells have long lifetimes and do not undergo cell division (Kiel *et al*, 2007; Foudi *et al*, 2009); such cells efficiently retain DNA labels.

As an alternative strategy, chromatin can be labelled *in vivo* with a pulse of transgenically expressed EGFP-tagged histone 2B (H2B-EGFP; Tumbar *et al*, 2004). This approach enables cell fractionation by FACS—in addition to label retention and cell visualization—and has been used in several tissues (Tumbar *et al*, 2004; Wilson *et al*, 2008; Foudi *et al*, 2009). As H2B-EGFP-retaining cells can be visualized and isolated alive, additional experimental strategies can be used to prove that stem cells were labelled.

Another possibility that would lead to the retention of DNA labels involves asymmetrical segregation of DNA strands; the 'immortal strand hypothesis' (Cairns, 1975). Dividing stem cells would retain the template DNA strands and the newly synthesized chromatids would be passed on to the daughter cells, presumably to maintain genome integrity. Potten postulated that an intestinal stem cell (ISC) is found on average at position +4 relative to the crypt bottom and divides every 24 h, but retains DNA labels, possibly through this mechanism (Potten *et al*, 2002). There are other examples of well-described adult stem cells—such as muscle satellite cells—that asymmetrically segregate their DNA strands (Shinin *et al*, 2006), whereas other adult stem cells—such as HSCs and hair follicle stem cells—have been shown not to use this mechanism (Kiel *et al*, 2007; Waghmare *et al*, 2008).

Overall, it is clear that cell-cycle properties differ between stem cells and should not be used as principal determinants of stemness.

In vitro culture

The study of isolated adult stem cells in culture has practical advantages for their study *in situ,* for example to obtain a detailed description of stem-cell behaviour or growth factor requirement (Schroeder, 2008; Lutolf *et al*, 2009). However, the main downside of studying stem cells in culture is that changes might be induced when cells are disconnected from their physiological surroundings. Despite this caveat, the development of cell-culture methods for adult stem cells has made important contributions to the stem-cell field. For example, heterogeneity was observed between epidermal cells—after extensive culture periods—when different types of clonal behaviour became apparent, which led to the definition of epidermal stem cells (Barrandon & Green, 1987). Furthermore, innovations in *in vitro* keratinocyte cultures improved long-term regeneration of human epidermis through autologous cultured skin engraftments (Pellegrini *et al*, 1999; Ronfard *et al*, 2000). More recently, the successful transplantation of clones derived from single epidermal hair follicle cells, was used to demonstrate the multipotency of the original cell (Blanpain *et al*, 2004; Claudinot *et al*, 2005).

Table 1 | Assays in adult stem-cell biology

	Advantages	Limitations
Label retention	Fluorescent label allows isolation of label-retaining cells	Not a feature of all adult stem cells Not specific to stem cells (for example, memory B and T cells) Unable to discriminate between the 'immortal strand hypothesis' and quiescence when using DNA analogues
In vitro culture	Enables manipulation of stemness potential in a controlled setting Ideal for high-throughput studies Ideal for live-imaging studies	Measures stemness potential, rather than actual stemness No physiological context Knowledge is required for culturing cells/tissues
Transplantation	Measures stem-cell behaviour *in vivo*	Measures stemness potential, rather than actual stemness Limited physiological context Knowledge is required for isolating rare cell populations
Lineage tracing	Reveals *in vivo* stem-cell behaviour in its physiological context; actual stemness Bicistronic knock-in with fluorescent marker allows isolation of cell population Reproducible experimentation/readout	Actual stem cells might be limited to a subset of the marked population Interpretation is only accurate in solid tissues Knowledge is required to find stem-cell-specific marker genes

Growing individual clones of cells derived from different progenitor populations became a widely used method to define the identity and behaviour of the relevant stem cells. Such cultures were named after the tissues they were derived from, for example neurospheres (Reynolds & Weiss, 1992), pancreatospheres (Rovira *et al*, 2010), mammospheres (Shackleton *et al*, 2006; Stingl *et al*, 2006), prostaspheres (Lawson *et al*, 2007) and tracheospheres (Rock *et al*, 2009). From muscles, single myofibres could be cultured *in vitro*, allowing the analysis of satellite-cell behaviour (Shinin *et al*, 2006; Kuang *et al*, 2007).

In addition to spheres, three-dimensional asymmetrical cell-culture conditions have been developed for the small intestine that allow the generation of long-lived organoids from adult crypts, and even from single ISCs. The resulting 'mini-guts' have all the characteristics of normal gut epithelium (Sato *et al*, 2009). The intestinal organoids do not contain mesenchymal niche elements; instead, specialized daughters of the stem cells—the Paneth cells—are interspersed between the stem cells and provide essential niche signals such as Wnt, EGF and Notch (Sato *et al*, 2010). With small adaptations, the protocol allows the robust outgrowth of stomach organoids from single stomach epithelial stem cells (Barker *et al*, 2010). Alternatively, three-dimensional intestinal organ cultures have been initiated by using neonatal tissue; in this case, both the mesenchymal niche architecture and the multilineage epithelial lining could be preserved during long-term culture (Ootani *et al*, 2009).

Interestingly, some tissues give rise to sphere formation in culture, whereas intestine and stomach cultures establish an asymmetry in which proliferative and differentiated cell types are positioned in accordance to their natural locations in the epithelial lining. It is tempting to speculate that the presence of niche cells in intestinal cultures—either mesenchymal structures and/or specialized daughter cells such as Paneth cells (Ootani *et al*, 2009; Sato *et al*, 2010)—create local morphogen gradients, thereby generating progenitor zones that are separate from areas with differentiated cells. The ability of single stem cells to grow into structures *in vitro* will facilitate analysis of the influence of growth factors on multipotency and self-renewal, and constitutes a simple and unambiguous *in vitro* test for stemness potential.

Transplantation

One of the most common assays in stem-cell biology is the transplantation of putative stem cells into recipient mice. Historically, this has been the 'gold standard', because it functionally measures the two criteria of stemness: it can directly assess if a candidate stem cell persists for long periods of time (self-renewal) and can produce all types of cell for the tissue in which it resides (multipotency).

Bone marrow stem cells were the first to be studied; the first attempt to transplant bone marrow in a clinical setting was in 1957 (Thomas *et al*, 1957). Through the enrichment of progenitor populations and sophisticated limiting dilution experiments, the hierarchical characteristics of the haematopoietic system were subsequently revealed (Spangrude *et al*, 1988; Chao *et al*, 2008). Ultimately, a single transplanted haematopoietic stem cell was successfully used to repopulate the entire haematopoietic system (Osawa *et al*, 1996).

Other adult stem-cell fields have adopted the transplantation assay to identify candidate stem cells. For example, it has long been known that injured muscle can initiate a strong regenerative response through the activation of satellite cells (Moss & Leblond, 1971). However, the self-renewal capacity and multipotency of satellite cells was only shown after transplantation of either single intact myofibres (Collins *et al*, 2005) or directly purified satellite cells (Montarras *et al*, 2005). The most stringent proof of stemness was provided when single, transplanted satellite cells repopulated new muscle fibres (Sacco *et al*, 2008). Despite this success, it remains controversial whether all satellite cells show equal stemness (Kuang *et al*, 2007). In the mammary gland, the development of the 'cleared fat pad'-transplantation assay allowed the definitive demonstration of stemness, as single, transplanted cells could grow into complete mammary glands (Shackleton *et al*, 2006; Stingl *et al*, 2006).

Although the ability of a single cell to repopulate a tissue is a dramatic example of its developmental potential, it is questionable whether the same potential could have been observed if the cell had been studied in its endogenous environment, before isolation and transplantation. Transplantation assays in cutaneous stem-cell biology illustrate this point. Label-retaining stem cells were first identified as potential stem cells in the bulge region of hair follicles (Cotsarelis *et al*, 1990). Once these cells were

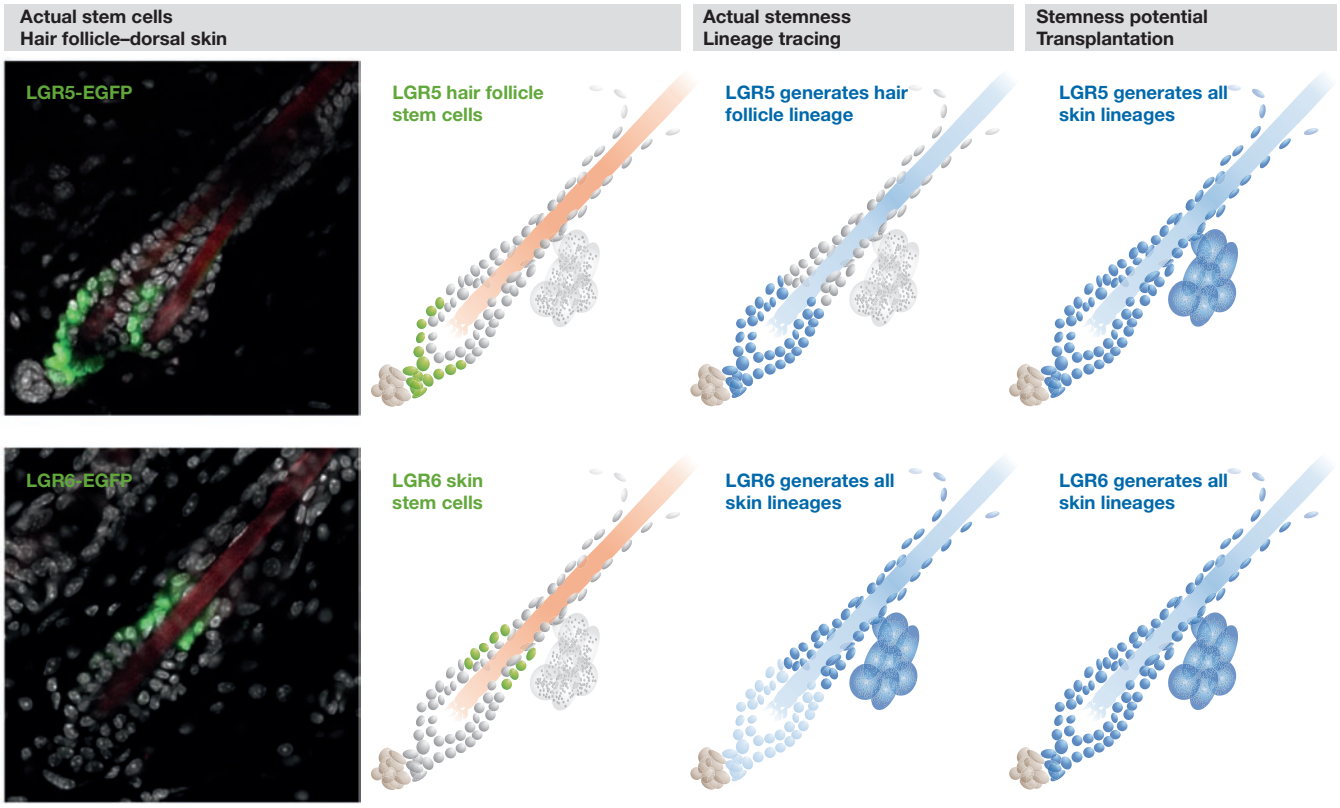

Fig 1 | Actual stemness compared with stemness potential in the skin. LGR5 and LGR6 indicate different stem-cell populations along hair follicles. Stem cells (green), nuclei (grey) and hair follicles (red) are shown in confocal pictures (left) and in the cartoon panels (second from the left). Lineage tracing reveals the actual stemness of a population. LGR5 stem cells generate progeny that repopulate the hair follicle (blue), whereas LGR6 stem cells predominantly generate progeny for sebaceous gland and IFE (blue) and, to a lesser extent, hair follicle (light blue). Transplantation of both stem-cell populations reveals equal stemness potential towards all lineages (blue). EGFP, enhanced green fluorescent protein; IFE, interfollicular epidermis.

isolated from adult mice and transplanted, they gave rise to all three of the main structures of the skin: all cell lineages of the hair follicle, sebaceous gland and interfollicular epidermis (IFE; Morris *et al*, 2004). However, when the same bulge cells were observed in normal homeostasis, the majority generated hair follicles and only occasionally sebaceous gland or IFE (Morris *et al*, 2004). These counterintuitive results were confirmed when neighbouring cell populations of the hair follicle bulge were isolated using LRIG1, LGR5 or LGR6 expression. Under normal homeostasis, LGR5$^+$ cells exclusively give rise to hair follicles (Jaks *et al*, 2008). LRIG1 and LGR6$^+$ cells predominantly give rise to sebaceous glands and IFE, whereas LGR6 hair-follicle potential diminishes with age (Jensen *et al*, 2009; Snippert *et al*, 2010a). However, after transplantation, they all generated all three components of the skin (Jaks *et al*, 2008; Jensen *et al*, 2009; Snippert *et al*, 2010a; Fig 1).

It could be argued that stemness potential, as shown after transplantation, might be fulfilled after injury. Indeed, hair follicle bulge cells demonstrate the same complete stemness potential after transplantation assays, as these cells can heal full-thickness wounds in the dorsal skin of mice by generating progeny for all three skin lineages (Morris *et al*, 2004; Blanpain *et al*, 2004; Jaks *et al*, 2008; Jensen *et al*, 2009), although their contribution to wound healing is only transient (Ito *et al*, 2007). Long-term healing of full-thickness wounds requires cells from above the hair follicle bulge, in particular LGR6$^+$ cells, which can heal wounds efficiently by generating permanent residents for all three skin lineages (Ito *et al*, 2007; Levy *et al*, 2007; Snippert *et al*, 2010a).

Similar discrepancies were observed in the testes, in which normal spermatogenesis is maintained by a small subset of undifferentiated NANOS2$^+$ spermatogonia cells that self-renew. However, during normal spermatogenesis and in regenerating tissue, a second NGN3$^+$ subpopulation that normally differentiates is able to self-renew and therefore probably has stemness potential (Nakagawa *et al*, 2007, 2010; Sada *et al*, 2009; Klein *et al*, 2010).

These studies might illustrate a more general principle in transplantation strategies. Different stem-cell populations in the skin are fully competent in the generation of all skin lineages after transplantation—stemness potential—however during normal homeostasis, their 'actual stemness' is more restricted (Watt & Jensen, 2009). In a recent example of such plasticity, thymic epithelial cells were shown to adopt adult hair-follicle stem-cell fate after translantation into the skin microenvironment (Bonfanti *et al*, 2010).

In vivo lineage tracing

In vivo lineage tracing has evolved in recent years into a powerful technique for the experimental testing of actual stemness. Central to

lineage-tracing strategies is the genetic marking of stem cells, which allows the tracing of daughter populations. Table 2 summarizes inducible genetic-tracing studies performed in adult mice that have elucidated either stem-cell identity or a mechanism of self-renewal.

In mouse intestine, such genetic marking was obtained by using the *Dlb-1* locus. Its inactivation by random mutagenesis resulted in stem-cell-derived clones lacking the ability to be stained by the lectin *Dolichos biflorus* agglutin (Winton *et al*, 1988). By inverting the original strategy, the *Dlb-1* locus could be used to draw a lineage hierarchy of intestinal progenitor compartments (Bjerknes & Cheng, 1999).

These strategies do not usually allow the exact cell-of-origin for the mutant clone to be identified. However, this limitation was circumvented in the haematopoietic system by combining clonal marking with single-cell transplantation: the stem-cell population was isolated before genetic marking and the contribution of single stem cells to various blood lineages was scored over time after transplantation (Jordan & Lemischka, 1990). In another approach, HSC mixtures from different genetic backgrounds were transplanted to determine the contribution of single HSCs to different lineages (Smith *et al*, 1991).

To minimize interference in normal physiology, Cotsarelis and colleagues introduced inducible genetic marking of a defined putative stem-cell population (Morris *et al*, 2004). A hormone-inducible version of the Cre enzyme was expressed in the putative stem-cell population of the hair follicle. The Cre enzyme remained inactive in the cytoplasm, but entered the nucleus on activation with a progesterone antagonist. When crossed with the uniform Cre-reporter mouse *R26R-LacZ* (Soriano, 1999), the active Cre enzyme could excise a transcriptional roadblock in front of the *LacZ* gene, leading to irreversible genetic marking of the Cre-expressing cell and its offspring. This could be visualized by an enzymatic (blue) staining reaction. Examination of individual blue clones over time readily revealed the growth kinetics, longevity and multipotentiality of the original marked cell (Fig 2). Intriguingly, as discussed above, marked bulge stem cells were developmentally more restricted *in situ* as scored by lineage tracing, than when analysed after transplantation (Fig 1; Morris *et al*, 2004).

A recent refinement of *in vivo* lineage tracing involves a bicistronic message being knocked into the genomic locus of a candidate gut-stem-cell gene *Lgr5* (Barker *et al*, 2007). This allows the expression of two different proteins from the same locus in the cell, such as an inducible version of Cre to start lineage tracings and a fluorescent protein for visualization of the potential stem cell (Fig 2). The ISC marker gene *Lgr5* was shown to be expressed in rare cells in several organs. By using the *Lgr5* knock-in mouse, actual stemness has been shown for LGR5$^+$ cells in the stomach, small intestine, colon and the hair follicle (Barker *et al*, 2007, 2010; Jaks *et al*, 2008). The same *in vivo* lineage-tracing strategy was used to document actual stemness for LGR6$^+$ cells, which marks cells above the bulge region where hair follicle stem cells reside (Snippert *et al*, 2010a). Intriguingly, the LGR6 and LRIG1 stem-cell populations located high in the hair follicle clearly generate IFE (Jensen *et al*, 2009; Snippert *et al*, 2010a), whereas uninjured IFE has been reported to self-maintain without the need for hair follicles (Levy *et al*, 2005; Ito *et al*, 2005; Clayton *et al*, 2007). This indicates that the LGR6 and LRIG1 stem cells are not essential in epidermis maintenance, yet both populations generate progenitors that migrate into the IFE (Jensen *et al*, 2009; Snippert *et al*, 2010a).

Although most genetic lineage tracings are initiated in defined populations, it is difficult to exclude the possibility that actual stemness is found in an even smaller subpopulation. It is therefore essential that the cell-of-origin of traced clones is carefully mapped, and that the tracing efficiencies are quantitatively measured over time, as this can indirectly score the self-renewal capacity of a population. For example, the first reported genetic-fate mappings in the small intestine were initiated in LGR5$^+$ crypt base columnar (CBC) cells at the base of intestinal crypts. The traced lineages were long-lived and included all intestinal cell types—the first identification of intestinal stem cells (Barker *et al*, 2007). However, BMI1 was subsequently reported to mark cells just above the LGR5$^+$ CBC population. These so-called '+4 cells' were capable of initiating lineage tracings with the same kinetics as LGR5$^+$ CBC cells, thereby refuelling the ISC identity debate (Sangiorgi & Capecchi, 2008). These tracing studies seem contradictory in terms of their cell-of-origin. We have since studied gene expression in crypts in greater detail and found that *Bmi1* expression levels are highest in LGR5$^+$ CBC cells (van der Flier *et al*, 2009). Thus, both tracings probably derive from overlapping—or even identical—cells at the crypt base. Another example that illustrates the need for quantitative measurements involves PROM1/ CD133, which was postulated to mark mouse ISCs. Lineage tracings initiated in PROM1$^+$ cells indicated the presence of long-lived, multilineage clones (Zhu *et al*, 2009). However, by using a comparable approach, the number of clones traced from PROM1$^+$ cells reduced by almost ten times after the first week, as PROM1/ CD133 marks not only ISCs, but also the more abundant early progenitor cells (Snippert *et al*, 2009).

A further refinement of lineage tracing has been made possible by the construction of multicolour Cre reporters by Jeff Lichtman and colleagues. The original Brainbow mice were designed to allow colour-coding of neurons with up to 90 distinguishable colour codes (Livet *et al*, 2007). Driven by a strong, ubiquitous promoter and inserted into the *Rosa* locus, this cassette yielded the *R26R-Confetti* allele, a general multicolour Cre-reporter (Snippert *et al*, 2010b). Using the *R26R-Confetti* allele, the individual behaviour of multiple stem cells in the same niche can be recorded (Fig 2). One such study showed that stem-cell fate in the intestine is stochastically determined: the observed stem-cell dynamics were consistent with a model in which the number of LGR5$^+$ stem cells doubles each day by symmetrical divisions, after which all daughters undergo a neutral competition for residency in the niche (Snippert *et al*, 2010b). We recently documented that Paneth cells constitute the niche for neighbouring LGR5$^+$ ISCs (Sato *et al*, 2010). Thus, it is likely that equal stem-cell daughters compete for available Paneth cell surface and that loss of direct Paneth cell contact—which happens to half of the stem cells each day—drives differentiation. A similar pattern of neutral drift in the intestine was deduced from quantitative analysis of the size and number of clonal tracing events on villi over time (Lopez-Garcia *et al*, 2010).

Other studies performed in unipotent-stem-cell systems similarly propose that homeostasis can be governed through a stochastic rather than a hierarchical mechanism. Lineage tracing in tail and ear epidermis revealed that basal progenitor cells are equal, but their fates are balanced such that equal numbers of progenitors and differentiated cells are produced in a population, thereby ensuring tissue homeostasis (Clayton *et al*, 2007; Doupe *et al*, 2010). Similar stochastic patterns of extinction and expansion of stem-cell clones were found in the mouse testes (Klein *et al*, 2010). We speculate

Table 2 | Inducible genetic fate mapping experiments in mice with implication to adult stem biology

Organ	Promoter	Expression pattern	Downstream lineages/implications	Longevity	Reference
Direct analysis					
Adipose tissue	*Pparγ*	Adipose vasculature	Adipocytes	30 days	Tang *et al*, 2008
Blood	*Foxp3*	Foxp3$^+$ TREG cells	Only FOXP3$^+$ Treg cells	8 months	Rubtsov *et al*, 2010
Brain	*Gli1*	SVZ and DG	Neurons, oligodendrocytes, astrocytes	1 year	Ahn & Joyner, 2005
	Nestin	SVZ and DG	Neurons, astrocytes	2 months	Lagace *et al*, 2007
Colon	*Lgr5*	Base of colonic crypts	Complete colonic epithelium	14 months	Barker *et al*, 2007
Incisor	*Gli1*	Proximal ameloblasts	Ameloblasts, str. intermedium, stel. reticulum	15 months	Seidel *et al*, 2010
Intestine	*Lgr5*	CBCs	Complete intestinal epithelium	14 months	Barker *et al*, 2007
	Bmi1	'+4' cells	Complete intestinal epithelium	9 months	Sangiorgi *et al*, 2008
	Prom1	CBCs	Presence of complete epithelium	2 months	Zhu *et al*, 2009
	Prom1	CBCs and early progenitors	Occasional complete epithelium	>2 months	Snippert *et al*, 2009
	Sox9	CBCs, Paneth cells and early prog.	Presence of complete epithelium	12 months	Furuyama *et al*, 2010
Liver	*Sox9*	SOX9$^+$ prog. in biliary duct	Biliary ducts and hepatocytes	12 months	Furuyama *et al*, 2010
Lung/ trachea	*Scgb1a1*	Clara cells, putative BASCs	Bronchiole; Clara cells, BASCs and ciliated cells	1 year	Rawlins *et al*, 2009
	Krt5	Basal cells	Basal cells, Clara cells and ciliated cells	15 weeks	Rock *et al*, 2009
Muscle	*Pax7*	Satellite cells	Myofibres + satellite cells, including 2 × injury	23 days	Lepper *et al*, 2009
Pancreas	*Insulin* (*RIP*)	β-cells	Only β-cell lineage	1 year	Dor *et al*, 2004
	Elastase	Acinar cells	Only acinar cell lineage	6 weeks	Desai *et al*, 2007
	Caii	Ductal cells	Ducts and acinar cells	3 weeks	Inada *et al*, 2008
	Bmi1	Acinar cells	Only acinar cells	1 year	Sangiorgi *et al*, 2009
	Hnf1β	HNF1β$^+$ duct/centroacinar cells	Ducts and centroacinar cells	6 months	Solar *et al*, 2009
	Sox9	SOX9$^+$ duct/centroacinar cells	Ducts, centroacinar and acinar cells	12 months	Furuyama *et al*, 2010
Prostate	*Nkx3-1*	Luminal (CARNs)	Luminal, occasionally basal after injury	10 months	Wang *et al*, 2009
Skin	*K15*	HF bulge	Predominantly HF, occasionally SG and IFE	1 month	Morris *et al*, 2004
	Lgr5	HF germ, lower bulge	HF	14 months	Jaks *et al*, 2008
	Lgr6	HF central isthmus	Predominantly SG and IFE, occasionally HF	12 months	Snippert *et al*, 2010a
Stomach	*Lgr5*	Base of pyloric glands	Complete stomach epithelium	20 months	Barker *et al*, 2010
Testis	*Ngn3*	Undif. spermatogonia A_{al}	Differentiated spermatogonia	14 months	Nakagawa *et al*, 2007
	Nanos2	Undif. spermatogonia A_s and A_{pr}	Differentiated spermatogonia	5 months	Sada *et al*, 2009
Indirect analysis					
Intestine	*v*	Epithelial cells, except Paneth cells	Neutral drift dynamics in intestinal self-renewal	12 months	Lopez-Garcia *et al*, 2010
	Cyp1a1	Epithelial cells, except Paneth cells	Neutral drift dynamics in intestinal self-renewal	7 months	Snippert *et al*, 2010b
	Lgr5	CBCs at entire crypt base	ISCs divide symmetrically	2 weeks	Snippert *et al*, 2010b
Skin/tail	*Cyp1a1*	Basal cells of tail epidermis	Homeostasis involves one progenitor cell type	12 months	Clayton *et al*, 2007
Skin/back	*K14*	Epithelial cells	HF upper isthmus clones, including SG and IFE	nd	Jensen *et al*, 2009
Skin/ear	*Cyp1a1*	Basal cells of ear epidermis	Homeostasis involves one progenitor cell type	12 months	Doupe *et al*, 2010
Testis	*Ngn3/ Nanos2*	Undif. spermatogonia A_s, A_{pr} and A_{al}	Stochastic turnover germ-line stem cells	14 months	Klein *et al*, 2010

BASC, bronchioalveolar stem cells; CARN, castration-resistant Nkx3-1-expressing cells; CBC, crypt base columnar; DG, dentate gyrus; HF, hair follicle, IFE, interfollicular epidermis; ISC, intestinal stem cell; nd, not determined; prog., progenitor; SG, sebaceous gland; str., stratum; stel., stellatum; SVZ, subventricular zone; undif., undifferentiated.

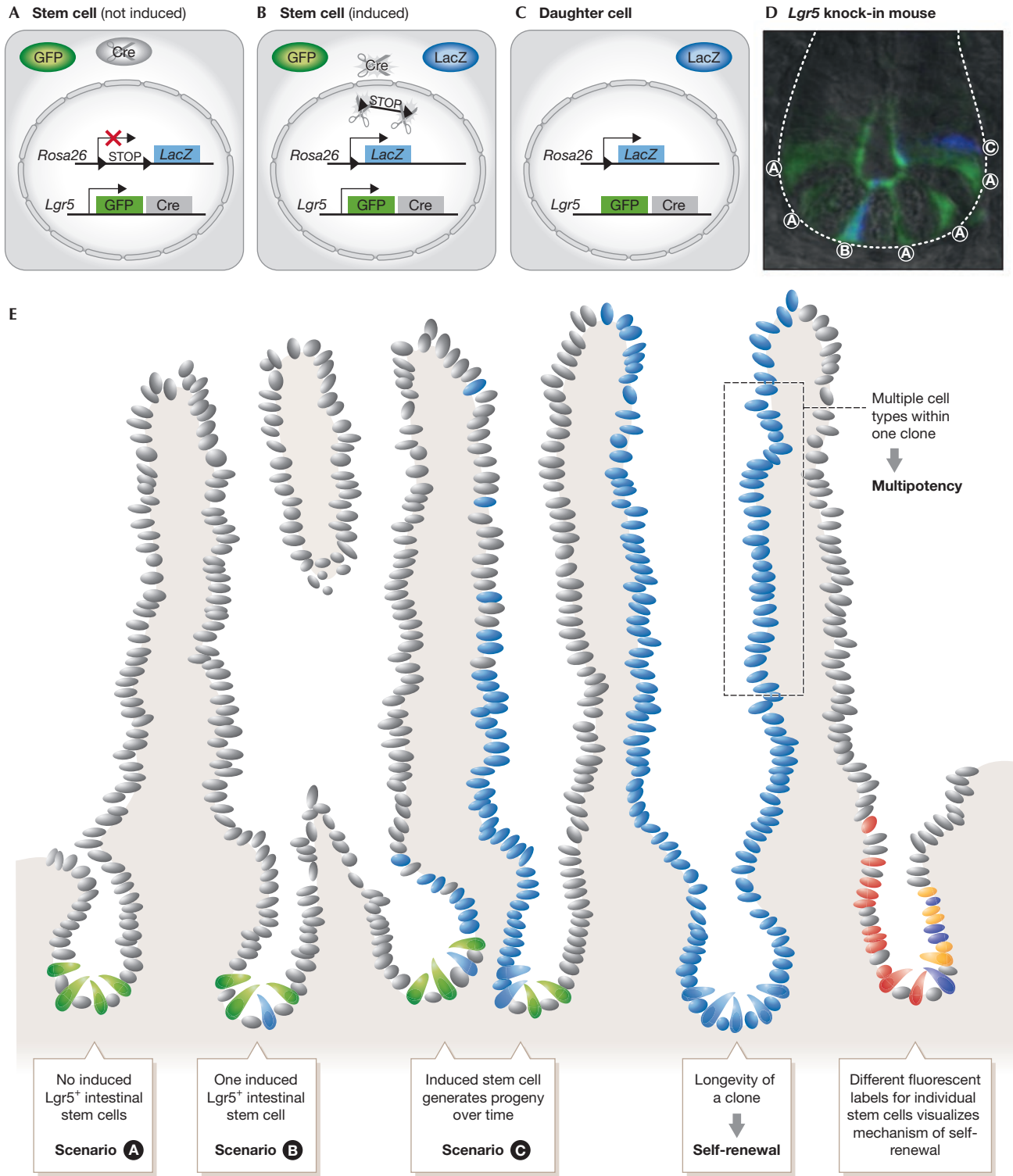

Fig 2 | *In vivo* lineage tracing in the small intestine. (**A**) Stem cells exclusively express GFP and the inactive version of Cre. (**B**) On activation of Cre, LacZ can be transcribed. (**C**) After cells differentiate, the GFP and inducible Cre are no longer produced. LacZ expression will be maintained. (**D**) *In vivo* small intestinal crypt of *Lgr5-EGFP-Ires-CreERT2* mouse with all of the above scenarios. (**E**) Cartoon of small intestine in which lineage tracings (blue) are visualized at different stages originating from LGR5$^+$ ISCs (green; Barker *et al*, 2007). Over time, true stem cells generate clones that are long-lived (self-renewal) and contain different cell types (multipotency). The crypt to the right shows lineage tracing with *R26R-Confetti* in which stem cells, and subsequently their progeny, are marked with different colours (Snippert *et al*, 2010b). EGFP, enhanced green fluorescent protein; GFP, green fluorescent protein; ISC, intestinal stem cell; LGR5, leucine-rich repeat-containing G protein coupled receptor 5.

Sidebar A | In need of answers

(i) Self-renewal and multipotency are two functionally defined criteria of stemness. Are there other characteristics common to all types of adult stem cells?

(ii) What is the relationship between the states of actual stemness and stemness potential? Are these cellular states interchangeable?

(iii) What is the role of cell populations with stemness potential *in vivo*, both during steady-state homeostasis as well as after injury?

that simultaneous tracing of multiple individual stem cells in other tissues might reveal similar unexpected insights into mechanisms of self-renewal.

Actual stemness compared with stemness potential

The defining characteristics of adult stem cells—their ability to generate all the cell types of the pertinent tissue and to do so for the lifetime of an organism—are fundamental principles in biology. Recent technical developments, especially pertaining to *in vivo* lineage-tracing strategies, have revealed discrepancies in the previous 'gold standards' of *ex vivo* culture and transplantation. Most approaches measure different aspects of stem-cell biology; lineage tracing measures the actual stemness of cells in their physiological context, whereas culture and transplantation strategies focus on stemness potential (Fig 1).

Discriminating between actual stemness and stemness potential is not always straightforward. For example, stem-cell identity and function in the endocrine pancreas are still debated, despite the fact that many lineage-tracing studies have been performed. By using a rat insulin promoter to initiate genetic-lineage tracing in differentiated islet β-cells, these cells were found to maintain their population by self-duplication during homeostasis and during regeneration after partial pancreatectomy (Dor *et al*, 2004). More recently, it was reported that new $NGN3^+$ progenitor cells arise near the ducts to give rise to new β-cells after another type of injury—pancreatic duct ligation (Xu *et al*, 2008). Although embryonic duct progenitors have the plasticity to generate exocrine as well as endocrine lineages, genetic-lineage tracing with adult duct structures showed that adult duct progenitors generate acinar cells but not new β-cells (Solar *et al*, 2009; Furuyama *et al*, 2010). Thus, although the formation of new endocrine cells occurs in the proximity of ductal structures, they are not of ductal origin.

The identity of stem cells in lung tissue is also controversial. Bronchioalveolar stem cells have been shown to have self-renewal and multipotent abilities *in vitro* (Kim *et al*, 2005), but genetic labelling of bronchioalveolar stem cells and columnar Clara cells revealed no contribution to alveoli lineages during normal homeostasis and regeneration (Rawlins *et al*, 2009). This further illustrates the differences between actual stemness—of Clara and bronchioalveolar cells—and stemness potential *in vitro*. Similar discrepancies have been reported in the mammary gland (Shackelton *et al*, 2006; Stingl *et al*, 2006; Bai & Rohrschneider, 2010) and prostate (Goldstein *et al*, 2008; Wang *et al* 2009).

As explained above, the distinction between actual stemness and stemness potential is not always clear and, in some tissues, populations with both properties could coexist and have different functions. The physiological role of cells with stemness potential is unknown; they might be able to acquire actual stemness *in situ* during tissue repair or regeneration. Future research should address the nature of plasticity in actual stemness and stemness potential. *In vitro* culture systems in which single stem cells can grow into three-dimensional tissues are likely to provide the experimental platform from which to obtain such molecular insights.

Many types of adult stem cell exist, and experimental assays reveal different aspects of stem-cell behaviour. A combinatorial approach is obviously required for a complete picture of adult stem-cell biology. Nevertheless, as adult stem cells are functionally defined by two criteria—self-renewal and multipotency—testing these should always be the primary focus when investigating new stem-cell populations. So far, genetic lineage-tracing approaches have provided the most definitive *in vivo* demonstrations of stem cells in action.

ACKNOWLEDGEMENTS

We thank Laurens van der Flier and Marc van de Wetering for helpful discussions and critical reading of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Ahn S, Joyner AL (2005) *In vivo* analysis of quiescent adult neural stem cells responding to Sonic hedgehog. *Nature* **437:** 894–897

Arai F, Suda T (2007) Maintenance of quiescent hematopoietic stem cells in the osteoblastic niche. *Ann NY Acad Sci* **1106:** 41–53

Bai L, Rohrschneider LR (2010) s-SHIP promoter expression marks activated stem cells in developing mouse mammary tissue. *Genes Dev* **24:** 1882–1892

Barker N *et al* (2007) Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* **449:** 1003–1007

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

Barrandon Y, Green H (1987) Three clonal types of keratinocyte with different capacities for multiplication. *Proc Natl Acad Sci USA* **84:** 2302–2306

Bjerknes M, Cheng H (1999) Clonal analysis of mouse intestinal epithelial progenitors. *Gastroenterology* **116:** 7–14

Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E (2004) Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* **118:** 635–648

Bonfanti P, Claudinot S, Amici AW, Farley A, Blackburn CC, Barrandon Y (2010) Microenvironmental reprogramming of thymic epithelial cells to skin multipotent stem cells. *Nature* **466:** 978–982

Cairns J (1975) Mutation selection and the natural history of cancer. *Nature* **255:** 197–200

Chao MP, Seita J, Weissman IL (2008) Establishment of a normal hematopoietic and leukemia stem cell hierarchy. *Cold Spring Harb Symp Quant Biol* **73:** 439–449

Claudinot S, Nicolas M, Oshima H, Rochat A, Barrandon Y (2005) Long-term renewal of hair follicles from clonogenic multipotent stem cells. *Proc Natl Acad Sci USA* **102:** 14677–14682

Clayton E, Doupe DP, Klein AM, Winton DJ, Simons BD, Jones PH (2007) A single type of progenitor cell maintains normal epidermis. *Nature* **446:** 185–189

Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE (2005) Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell* **122:** 289–301

Cotsarelis G, Sun TT, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* **61:** 1329–1337

Desai BM, Oliver-Krasinski J, De Leon DD, Farzad C, Hong N, Leach SD, Stoffers DA (2007) Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. *J Clin Invest* **117:** 971–977

Dor Y, Brown J, Martinez OI, Melton DA (2004) Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* **429:** 41–46

Doupe DP, Klein AM, Simons BD, Jones PH (2010) The ordered architecture of murine ear epidermis is maintained by progenitor cells with random fate. *Dev Cell* **18:** 317–323

Fortunel NO *et al* (2003) Comment on "'Stemness': transcriptional profiling of embryonic and adult stem cells" and "a stem cell molecular signature". *Science* **302:** 393; author reply 393

Foudi A, Hochedlinger K, Van Buren D, Schindler JW, Jaenisch R, Carey V, Hock H (2009) Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. *Nat Biotechnol* **27:** 84–90

Fuller MT, Spradling AC (2007) Male and female *Drosophila* germline stem cells: two versions of immortality. *Science* **316:** 402–404

Furuyama K *et al* (2010) Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet* [Epub 28 Nov] doi:10.1038/ng.722

Goldstein AS, Lawson DA, Cheng D, Sun W, Garraway IP, Witte OW (2008) Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. *Proc Natl Acad Sci USA* **105:** 20882–20887

He S, Nakada D, Morrison SJ (2009) Mechanisms of stem cell self-renewal. *Annu Rev Cell Dev Biol* **25:** 377–406

Huttner WB, Kosodo Y (2005) Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system. *Curr Opin Cell Biol* **17:** 648–657

Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J, Morita R, Sharma A, Bonner-Weir S (2008) Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. *Proc Natl Acad Sci USA* **105:** 19915–19919

Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, Cotsarelis G (2005) Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med* **11:** 1351–1354

Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE, Cotsarelis G (2007) Wnt-dependent *de novo* hair follicle regeneration in adult mouse skin after wounding. *Nature* **447:** 316–320

Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR (2002) A stem cell molecular signature. *Science* **298:** 601–604

Jaenisch R, Young R (2008) Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell* **132:** 567–582

Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, Clevers H, Toftgard R (2008) Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat Genet* **40:** 1291–1299

Jensen KB, Collins CA, Nascimento E, Tan DW, Frye M, Itami S, Watt FM (2009) Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. *Cell Stem Cell* **4:** 427–439

Jordan CT, Lemischka IR (1990) Clonal and systemic analysis of long-term hematopoiesis in the mouse. *Genes Dev* **4:** 220–232

Kiel MJ, He S, Ashkenazi R, Gentry SN, Teta M, Kushner JA, Jackson TL, Morrison SJ (2007) Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. *Nature* **449:** 238–242

Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T (2005) Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* **121:** 823–835

Klein AM, Nakagawa T, Ichikawa R, Yoshida S, Simons BD (2010) Mouse germ line stem cells undergo rapid and stochastic turnover. *Cell Stem Cell* **7:** 214–224

Kuang S, Kuroda K, Le Grand F, Rudnicki MA (2007) Asymmetric self-renewal and commitment of satellite stem cells in muscle. *Cell* **129:** 999–1010

Lagace DC *et al* (2007) Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. *J Neurosci* **27:** 12623–12629

Lawson DA, Xin L, Lukacs RU, Cheng D, Witte ON (2007) Isolation and functional characterization of murine prostate stem cells. *Proc Natl Acad Sci USA* **104:** 181–186

Lechler T, Fuchs E (2005) Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* **437:** 275–280

Lepper C, Conway SJ, Fan CM (2009) Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. *Nature* **460:** 627–631

Levy V, Lindon C, Harfe BD, Morgan BA (2005) Distinct stem cell populations regenerate the follicle and interfollicular epidermis. *Dev Cell* **9:** 855–861

Levy V, Lindon C, Zheng Y, Harfe BD, Morgan BA (2007) Epidermal stem cells arise from the hair follicle after wounding. *FASEB J* **21:** 1358–1366

Li L, Clevers H (2010) Coexistence of quiescent and active adult stem cells in mammals. *Science* **327:** 542–545

Livet J, Weissman TA, Kang H, Draft RW, Lu J, Bennis RA, Sanes JR, Lichtman JW (2007) Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature* **450:** 56–62

Lopez-Garcia C, Klein AM, Simons BD, Winton DJ (2010) Intestinal stem cell replacement follows a pattern of neutral drift. *Science* **330:** 822–825

Lutolf MP, Gilbert PM, Blau HM (2009) Designing materials to direct stem-cell fate. *Nature* **462:** 433–441

Mikkers H, Frisen J (2005) Deconstructing stemness. *EMBO J* **24:** 2715–2719

Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, Partridge T, Buckingham M (2005) Direct isolation of satellite cells for skeletal muscle regeneration. *Science* **309:** 2064–2067

Morris RJ, Liu Y, Marles L, Yang Z, Trempus C, Li S, Lin JS, Sawicki JA, Cotsarelis G (2004) Capturing and profiling adult hair follicle stem cells. *Nat Biotechnol* **22:** 411–417

Morrison SJ, Kimble J (2006) Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* **441:** 1068–1074

Moss FP, Leblond CP (1971) Satellite cells as the source of nuclei in muscles of growing rats. *Anat Rec* **170:** 421–435

Nakagawa T, Nabeshima Y, Yoshida S (2007) Functional identification of the actual and potential stem cell compartments in mouse spermatogenesis. *Dev Cell* **12:** 195–206

Nakagawa T, Sharma M, Nabeshima Y, Braun RE, Yoshida S (2010) Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. *Science* **328:** 62–67

Neumuller RA, Knoblich JA (2009) Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer. *Genes Dev* **23:** 2675–2699

Ootani A *et al* (2009) Sustained *in vitro* intestinal epithelial culture within a Wnt-dependent stem cell niche. *Nat Med* **15:** 701–706

Orford KW, Scadden DT (2008) Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. *Nat Rev Genet* **9:** 115–128

Osawa M, Hanada K, Hamada H, Nakauchi H (1996) Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* **273:** 242–245

Pellegrini G, Ranno R, Stracuzzi G, Bondanza S, Guerra L, Zambruno G, Micali G, De Luca M (1999) The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. *Transplantation* **68:** 868–879

Potten CS, Loeffler M (1990) Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* **110:** 1001–1020

Potten CS, Hume WJ, Reid P, Cairns J (1978) The segregation of DNA in epithelial stem cells. *Cell* **15:** 899–906

Potten CS, Owen G, Booth D (2002) Intestinal stem cells protect their genome by selective segregation of template DNA strands. *J Cell Sci* **115:** 2381–2388

Quyn AJ, Appleton PL, Carey FA, Steele RJ, Barker N, Clevers H, Ridgway RA, Sansom OJ, Nathke IS (2010) Spindle orientation bias in gut epithelial stem cell compartments is lost in precancerous tissue. *Cell Stem Cell* **6:** 175–181

Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA (2002) "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science* **298:** 597–600

Rawlins EL, Okubo T, Xue Y, Brass DM, Auten RL, Hasegawa H, Wang F, Hogan BL (2009) The role of Scgb1a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. *Cell Stem Cell* **4:** 525–534

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255:** 1707–1710

Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, Randell SH, Hogan BL (2009) Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci USA* **106:** 12771–12775

Ronfard V, Rives JM, Neveux Y, Carsin H, Barrandon Y (2000) Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix. *Transplantation* **70:** 1588–1598

Rovira M, Scott SG, Liss AS, Jensen J, Thayer SP, Leach SD (2010) Isolation and characterization of centroacinar/terminal ductal progenitor cells in adult mouse pancreas. *Proc Natl Acad Sci USA* **107:** 75–80

Rubtsov YP, Niec RE, Josefowicz S, Li L, Darce J, Mathis D, Benoist C, Rudensky AY (2010) Stability of the regulatory T cell lineage *in vivo*. *Science* **329:** 1667–1671

Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM (2008) Self-renewal and expansion of single transplanted muscle stem cells. *Nature* **456:** 502–506

Sada A, Suzuki A, Suzuki H, Saga Y (2009) The RNA-binding protein NANOS2 is required to maintain murine spermatogonial stem cells. *Science* **325:** 1394–1398

Sangiorgi E, Capecchi MR (2008) Bmi1 is expressed *in vivo* in intestinal stem cells. *Nat Genet* **40:** 915–920

Sangiorgi E, Capecchi MR (2009) Bmi1 lineage tracing identifies a self-renewing pancreatic acinar cell subpopulation capable of maintaining pancreatic organ homeostasis. *Proc Natl Acad Sci USA* **106:** 7101–7106
Sato T *et al* (2009) Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* **459:** 262–265
Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H (2010) Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* [Epub 28 Nov] doi:10.1038/nature09637
Schroeder T (2008) Imaging stem-cell-driven regeneration in mammals. *Nature* **453:** 345–351
Seidel K *et al* (2010) Hedgehog signaling regulates the generation of ameloblast progenitors in the continuously growing mouse incisor. *Development* **137:** 3753–3761
Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE (2006) Generation of a functional mammary gland from a single stem cell. *Nature* **439:** 84–88
Shinin V, Gayraud-Morel B, Gomes D, Tajbakhsh S (2006) Asymmetric division and cosegregation of template DNA strands in adult muscle satellite cells. *Nat Cell Biol* **8:** 677–687
Smith LG, Weissman IL, Heimfeld S (1991) Clonal analysis of hematopoietic stem-cell differentiation *in vivo*. *Proc Natl Acad Sci USA* **88:** 2788–2792
Snippert HJ, van Es JH, van den Born M, Begthel H, Stange DE, Barker N, Clevers H (2009) Prominin-1/CD133 marks stem cells and early progenitors in mouse small intestine. *Gastroenterology* **136:** 2187–2194
Snippert HJ *et al* (2010a) Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. *Science* **327:** 1385–1389
Snippert HJ *et al* (2010b) Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143:** 134–144
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
Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* **21:** 70–71
Spangrude GJ, Heimfeld S, Weissman IL (1988) Purification and characterization of mouse hematopoietic stem cells. *Science* **241:** 58–62
Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, Li HI, Eaves CJ (2006) Purification and unique properties of mammary epithelial stem cells. *Nature* **439:** 993–997
Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, Graff JM (2008) White fat progenitor cells reside in the adipose vasculature. *Science* **322:** 583–586
Thomas ED, Lochte HL Jr, Lu WC, Ferrebee JW (1957) Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med* **257:** 491–496
Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E (2004) Defining the epithelial stem cell niche in skin. *Science* **303:** 359–363
van der Flier LG *et al* (2009) Transcription factor achaete scute-like 2 controls intestinal stem cell fate. *Cell* **136:** 903–912
Voog J, Jones DL (2010) Stem cells and the niche: a dynamic duo. *Cell Stem Cell* **6:** 103–115
Waghmare SK, Bansal R, Lee J, Zhang YV, McDermitt DJ, Tumbar T (2008) Quantitative proliferation dynamics and random chromosome segregation of hair follicle stem cells. *EMBO J* **27:** 1309–1320
Wang X, Kruithof-de Julio M, Economides KD, Walker D, Yu H, Halili MV, Hu YP, Price SM, Abate-Shen C, Shen MM (2009) A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature* **461:** 495–500
Watt FM, Jensen KB (2009) Epidermal stem cell diversity and quiescence. *EMBO Mol Med* **1:** 260–267
Wilson A *et al* (2008) Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* **135:** 1118–1129
Winton DJ, Blount MA, Ponder BA (1988) A clonal marker induced by mutation in mouse intestinal epithelium. *Nature* **333:** 463–466
Wong DJ, Liu H, Ridky TW, Cassarino D, Segal E, Chang HY (2008) Module map of stem cell genes guides creation of epithelial cancer stem cells. *Cell Stem Cell* **2:** 333–344
Xie T, Kawase E, Kirilly D, Wong MD (2005) Intimate relationships with their neighbors: tales of stem cells in *Drosophila* reproductive systems. *Dev Dyn* **232:** 775–790
Xu X *et al* (2008) Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* **132:** 197–207
Zhu L, Gibson P, Currle DS, Tong Y, Richardson RJ, Bayazitov IT, Poppleton H, Zakharenko S, Ellison DW, Gilbertson RJ (2009) Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature* **457:** 603–607

