

# Compartmentalized organization: a common and required feature of stem cell niches?

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## Summary

A key question in the stem cell field is how to balance the slow cycling of stem cells with active organ growth. Recent studies of the hair follicle stem cell niche have shown that this can be achieved by organizing the stem cell niche into two compartments: one that engages in immediate, rapid new growth and one that contributes later to long-term growth that fuels hair regeneration. Based on these and other recent findings, we propose that several other adult stem cell niches, including those in the blood, intestine and brain, have a similar bi-compartmental organization and that stem cells might work cooperatively with their progeny to sustain tissue regeneration.

**Key words:** Lineage tracing, Niche, Stem cells

## Introduction

Studies from invertebrates to mammals indicate that stem cells and their niches have a key role to play in orchestrating regeneration in numerous tissues. Although the existence of a niche was first hypothesized in the mouse system (Schofield, 1978), the most compelling evidence of the niche existence has come from invertebrate work (Lin, 2002).

A stem cell niche has been described as the specialized local microenvironment in which stem cells reside. As such, the niche has to be: (1) anatomically defined, (2) composed of specific extracellular matrix components and supporting cells, and (3) enriched for growth modulating signals that together can influence stem cell self-renewal and differentiation. Adult stem cells from various tissues have been often described to be slowly cycling, a feature thought to preserve their special abilities, such that some of these stem cells enter the cell cycle only a few times during the entire life span of a mouse (Wilson et al., 2008; Foudi et al., 2009; Cotsarelis et al., 1990; Potten et al., 2002; Tumber et al., 2004; Waghmare et al., 2008). Maintaining the quiescence of stem cells is advantageous for long-lived animals as such quiescence could guard against the accumulation of oncogenic perturbations with each cell division. In addition, quiescence prevents stem cell exhaustion, which could lead to premature organ failure (Viale and Pelicci, 2009; Cheshier et al., 1999; Kiel et al., 2005b). However, stem cells have to be able to contribute rapidly to the regeneration of a tissue upon injury or to the maintenance of tissues that have a naturally high turnover rate. During homeostasis, the turnover rate of each of the cell types is rather constant and goes on for the entire lifetime of that organism. By contrast, injury is relatively acute and its nature and severity can vary greatly. Although the daily maintenance of a tissue requires a pool of stem cells to be active at any given time, it would

be dangerous to activate all of these stem cells at the same time because this could jeopardize the ability of the tissue to repair future injuries. In such a scenario, two types of cells capable of regeneration would be required. One type has to respond promptly to physiological and injury cues to reconstitute the tissue on a day-to-day basis. The other has to remain relatively inert so that its maintenance and repair functions can be invoked throughout the life of a long-lived organism.

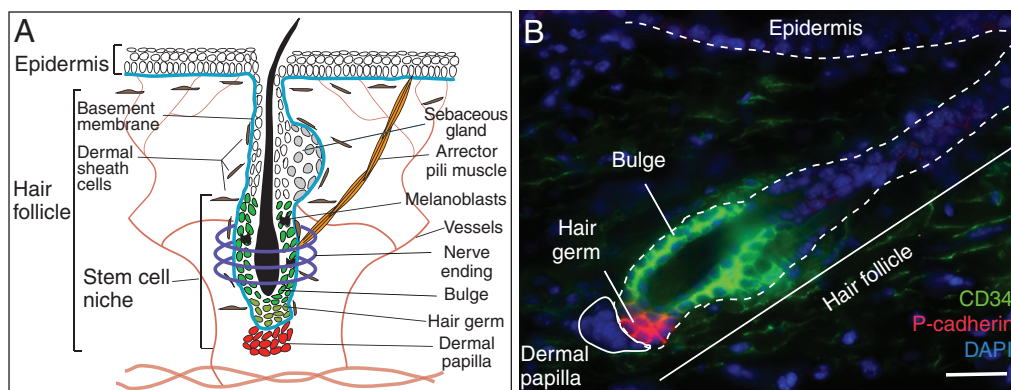
The mouse hair follicle system is an ideal system in which to address the question of how a stem cell pool can maintain itself while responding to situations of injury and tissue turnover owing to its particular amenability and tight spatial organization (Fig. 1A). The hair follicle undergoes continuous regeneration and alternates between quiescent and active phases of growth (Muller-Rover et al., 2001). Grafting and transplantation studies have revealed that two components of this system are crucial for hair regeneration to occur: (1) an epithelial stem cell pool localized within a specialized niche, called the bulge (Cotsarelis et al., 1990; Oshima et al., 2001; Taylor et al., 2000), and (2) a cluster of mesenchymal cells that have hair-inducing abilities called dermal papilla (Jahoda et al., 1984) (Fig. 1). On the basis of these findings, a bulge-centric model was invoked to explain hair regeneration. According to this model, crosstalk occurs between the mesenchymal cells of the dermal papilla and the epithelial stem cells of the bulge that is sufficient to activate hair follicle growth during regeneration (Morris et al., 2004; Blanpain et al., 2004; Tumber et al., 2004). Our recent studies, however, have changed this classic bulge-centric view to a two-step model, which is based on the recent discovery that the hair follicle stem cell niche has a bi-compartmental organization (Greco et al., 2009). During quiescence, the bulge cells coexist with their progeny, called the hair germ, in the bottom of the follicle (Fig. 1; Box 1). Once growth starts, the bulge and the hair germ contribute to the new hair follicle but their contribution differs temporally. The expansion of the follicle appears to occur initially at the expense of the hair germ, with the bulge contributing later to hair follicle regeneration during active hair growth (Greco et al., 2009). This bi-compartmental organization accounts for both the fast new hair growth response, which occurs under physiological conditions, and the need to preserve the stem cell pool.

A number of questions arise from these studies. How are the components of the niche established? Do both bulge and hair germ possess multipotentiality over an indefinite period of time? Most importantly, is this compartmentalized architecture of the stem cell niche utilized by other tissue stem cells as well?

In this article, we start by reviewing the literature on a number of adult mammalian stem cell types and their niche organization and propose, based on published data, that a compartmentalized stem cell organization might be a common structure that is a prerequisite of the biological functions of a niche. We discuss potential methodologies, together with their limitations, to extend and ascertain such an argument in various types of stem cell niches.

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**Fig. 1. The hair follicle as a model system to study stem cells.** (A) A schematic of the hair follicle stem cell niche, which resides in the lower part of the follicle and comprises epithelial cells (the bulge and the hair germ) and mesenchymal cells (dermal papilla). Many cells are in contact with this niche. A basement membrane (blue) surrounds the hair follicle, along with a dermal sheath made of fibroblasts, connective tissue and macrophages. Nerve endings (purple) surround the bulge (dark green) and the arrector pili muscle (orange) inserts into the bulge. The melanocyte stem cells (melanoblasts; black) are also located in the bulge. For simplicity, the immune cells have been excluded. (B) The lower part of the hair follicle is organized into two different epithelial compartments, the hair germ (red) and the bulge (green), and the mesenchymal dermal papilla. The bulge contains bona fide epithelial stem cells and is positive for epithelial markers, such as integrin alpha 6 (not shown) and the cell surface glycoprotein CD34 (green). The hair germ is enriched for the adhesion molecule P-cadherin (red) and contains the epithelial progenitors that initiate a new round of regeneration. The epithelial cells are delineated by a dashed line and the mesenchymal dermal papilla cells by a solid line. All nuclei are marked in blue with DAPI. Scale bar: 50  $\mu$ m.

### Evidence for a bi-compartmental organization of the hair follicle niche

The hair follicle stem cell niche is composed of two epithelial compartments during quiescence – the bulge and its progeny, the hair germ (Panteleyev et al., 2001; Ito et al., 2004; Greco et al., 2009; Zhang et al., 2009). Although grafting and single-cell labeling experiments have shown that bulge cells are bona fide stem cells, there is no such definitive evidence that hair germ cells are stem cells. Gene expression analysis shows that the hair germ possesses features that are distinct from the dormant bulge compartment (Greco et al., 2009; Fuchs, 2009). During hair follicle regeneration, new hair follicle growth is accomplished in two distinct activation steps. First, the hair germ cells are activated in response to mesenchymal signals coming from the dermal papilla (Fig. 1; Box 1); this step initiates hair follicle growth. Second, once the growth phase has advanced, the bulge stem cells become activated to contribute to hair growth (Greco et al., 2009).

### Evidence for a bi-compartmental organization of other stem cell niches

Although the hair follicle system provides an example of tight temporal and spatial control of stem cells, evidence suggests that a similar compartmentalized organization of other tissue stem cell niches might also exist.

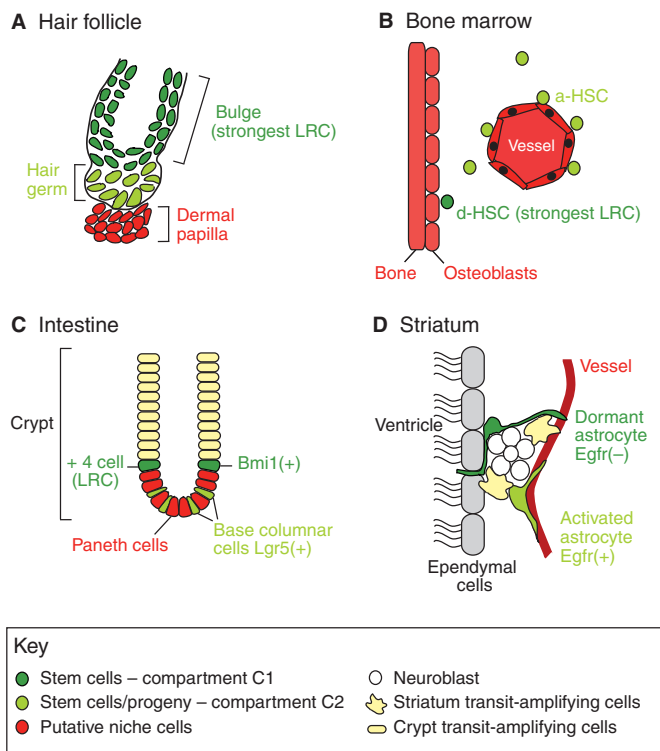
### The hematopoietic stem cell niche

Owing to the ease with which the components of the hematopoietic system can be transplanted, it has been long recognized that cells with differing repopulating capability exist in the adult bone marrow. The identification of the regulatory elements of this repopulating behavior, both intrinsic and extrinsic to the cells possessing such abilities, has far-reaching implications for understanding disease and developing new therapies. Aided by the development of multicolor fluorescence-activated sorting (FACS), our ability to identify and purify hematopoietic stem cells (HSCs) has advanced greatly over the past decade or so (Spangrude et al., 1988; Ogata et al., 1992; Osawa et al., 1996; Kiel et al., 2005b; Wilson et al., 2008). The stereotypical

depiction of hematopoietic development was that of a linear hierarchy comprising long-term hematopoietic stem cells (LT-HSC) that give rise to short-term (ST) HSCs, which in turn give rise to multipotent progenitors (MPPs) (Passegue et al., 2003). Recently, the LT-HSCs population, which is defined using a stringent marker panel (negative for the hematopoietic differentiated lineages, c-Kit+Sca+CD150+CD48–CD34–), has been further divided into two compartments: the d-HSCs and a-HSCs, for dormant and activated HSCs, respectively (Fig. 2B) (Wilson et al., 2008; Foudi et al., 2009; Glauche et al., 2009; Raaijmakers and Scadden, 2008). So far, no simple marker or marker combination can prospectively distinguish these two populations. The existence of d-HSC and a-HSC was

#### Box 1. Hair growth and regeneration in the mouse

The hair follicle undergoes continuous regeneration, alternating between quiescent and growth phases. During quiescence, both the bulge stem cells and its progeny hair germ are dormant; however, during hair growth, these two compartments have distinct behaviors. First, bulge and hair germ cycling behavior is different at different times. When the hair follicle starts to grow, only the hair germ cells are rapidly engaged in proliferation. The bulge becomes actively cycling only once the hair has considerably grown in size. Second, the bulge and the hair germ cells respond differently to activating signals. At the beginning of a new growth, the hair germ proliferate in response to fibroblast growth factor (Fgf) 7 and 10 and Bmp inhibitors such as Bambi and Sostdc1, which come from the adjacent dermal papilla (Fig. 1B) (Plikus et al., 2008; Greco et al., 2009). Interestingly, when these stimuli are ectopically added during quiescence, they are able to activate the hair germ but not the bulge (Greco et al., 2009). Furthermore, little is known about the source and signals that regulate bulge activation. It is conceivable that the two compartments have different niches. The dermal papilla could serve as the hair germ niche, whereas the activated hair germ could be the bulge niche. In addition, there are many other cell types located close to the bulge and the hair germ (see Fig. 1), and future studies are needed to address their potential roles as niche components.



**Fig. 2. A bi-compartmental organization of different adult mammalian stem cell niches.**

A schematic of stem cells in many tissues. Because stem cells have a slow cell cycle, they can be identified by their ability to retain nucleotide analogs such as bromodeoxyuridine (BrdU) longer than other cells, resulting in their name as label retaining cells (LRCs). (A) The hair follicle stem cell niche is organized into two different epithelial compartments, the hair germ and the bulge. The hair germ (light green) is located close to the mesenchymal dermal papilla (red), which acts as a signaling center. The bulge (dark green) is located further away from the dermal papilla. The cells with the most label retention are found in the bulge. (B) In the hematopoietic system, dormant hematopoietic stem cells (d-HSCs, dark green) and activated (a-) HSCs (light green) have been identified. The d-HSCs retain label better than do the a-HSCs and are probably located closer to the endosteal niche of osteoblasts (red), whereas the a-HSCs are probably further away from the bone and closer to the vasculature (red vessel). (C) In the intestine, stem cells are positive for *Bmi1* (dark green) and are located in position +4 in the intestinal crypt. *Lgr5*-labelled stem cells (light green) have also been identified at the base of the crypts. The differentiated Paneth cells (red) could possibly function as a niche for the *Lgr5*<sup>+</sup> stem cells. (D) In the subventricular zone of the striatum, two groups of astrocytic stem cells have been identified: *Gfap*<sup>+</sup> *Egfr*<sup>-</sup> astrocytes (dark green) and *Gfap*<sup>+</sup> *Egfr*<sup>+</sup> astrocytes (light green). These cells presumably rely on the ventricle and the blood vessels as a niche (red). *Bmi1*, B lymphoma Mo-MLV insertion region 1; *Egfr*, epidermal growth factor receptor; *Lgr5*, leucine-rich repeat-containing G-protein-coupled receptor 5; LRC, label retaining cells.

deduced from the kinetics of long-term label retention [of bromodeoxyuridine (BrdU) and of histone-GFP] in phenotypic HSCs together with mathematical modeling. One exception to this might be N-cadherin expression, which seems to be capable of marking HSCs into 'reserved' and 'primed' pools (Haug et al., 2008), a concept that is roughly equivalent to quiescent and active stem cells. However, the ability of this marker to distinguish a more quiescent pool of HSCs is under debate (Kiel et al., 2007; Foudi et al., 2009).

d-HSCs account for about 15-20% of immuno-phenotypically identifiable LT-HSCs in young adult bone marrow (Wilson et al., 2008; Foudi et al., 2009). d-HSCs display reduced DNA replication machinery and are estimated to divide only about five times over the entire lifetime of a mouse (Wilson et al., 2008). Importantly, these d-HSCs can be activated by physiopathological cues, such as interferon and granulocyte colony-stimulating factor (G-CSF) (Wilson et al., 2008; Essers et al., 2009; Sato et al., 2009a). Upon tissue repair, d-HSCs return to dormancy. The adult HSC niche itself consists of distinctive cellular entities, namely the endosteal niche and the vasculature niche, which are proposed to be associated with the dormant and activated HSCs, respectively (Wilson et al., 2007; Kopp et al., 2005; Scadden, 2006). Sophisticated three-dimensional imaging studies have revealed that intimate contacts exist between these two types of niche elements with osteoblasts enmeshed in microvessels (Lo Celso et al., 2009; Xie et al., 2009). How the endosteal and the vasculature niche elements communicate with each other and with the resident HSCs to regulate the relative abundance of HSCs in dormancy and cycling remains to be answered.

The recent descriptions of the dormant and active HSCs add to our existing appreciation of stem cell heterogeneity (Venezia et al., 2004; Passegue et al., 2005; Zhang et al., 2003), which can manifest in multiple ways. For example, in long-lived large animals, the activation of HSC clones is successive and stochastic (Abkowitz et al., 1990). In addition, HSCs in G0 possess much higher long-term engraftment ability, indicating that phenotypically similar stem cells can differ in their function depending on the exact time-point when they are assayed (Fleming et al., 1993; Passegue et al., 2005). Furthermore, studies of a large cohort of single-cell-repopulated animals have shown that there is a consistent difference among the repopulating behaviors of HSCs as reflected by their reconstitution kinetics and lineage outcome (Dykstra et al., 2007; Sieburg et al., 2006). It is yet to be determined to what extent the relative dormancy of HSCs could account for these heterogeneous behaviors. As stem cells are tightly regulated by their niches, it is probable that there are also considerable differences in their niches in terms of their local cellular and matrix components and topologies.

### The intestinal stem cell niche

The mammalian intestine is one of the tissues that turns over most rapidly. The lining of the intestine is organized into crypts, which comprise proliferative cells, a few specialized cells and villi, where most of the differentiated cells reside (Clevers, 2009; Scoville et al., 2008). Slowly cycling cells have been localized in the crypts between the specialized Paneth cells and the transit-amplifying cells in what is referred to as position +4 (on average, 4 cells from the bottom of the crypt; Fig. 2C) (Potten et al., 2002). Recently, it has been shown that cells positive for the polycomb gene *Bmi1* are found in the +4 position and can sustain intestinal turnover for at least 4 months. *Bmi1*<sup>+</sup> cells can generate all of the differentiated lineages in the crypts and villi (Sangiorgi and Capecchi, 2008). Interestingly, cells with similar characteristics to the *Bmi1*<sup>+</sup> cells have also been identified at the bottom of the crypts. These cells, called crypt base columnar (CBC) cells, were proposed to be the intestinal stem cells several decades ago (Cheng and Leblond, 1974) and have recently been shown to be positive for the orphan G-protein-coupled receptor *Lgr5* and the stem-cell-associated antigen CD133 (prominin 1) (Barker et al., 2007; Zhu et al., 2009). Definitive evidence now shows that CBC cells are multipotent and contribute to intestinal regeneration for months both in vivo (Barker et al., 2007; Zhu et al., 2009) and in vitro (Sato et al., 2009b). Interestingly, whereas the *Bmi1*<sup>+</sup> cells might be the slowly proliferating cells described by Potten, at least some of the *Lgr5*<sup>+</sup> cells



are actively cycling (Barker et al., 2007). Thus, the  $Bmi1^+$  and  $Lgr5^+$  cells could represent two distinct stem cell compartments with different molecular identities and anatomic locations, both of which can share an apparently similar ability to sustain tissue regeneration. However, current experiments have not addressed whether the kinetics of their contribution to intestinal regeneration are different.

### The subventricular zone stem cell niche

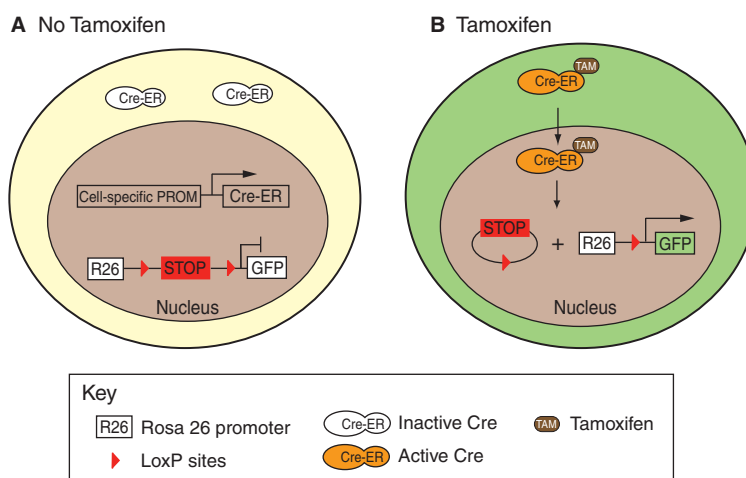
Although the blood and intestine are both high turnover tissues, evidence to support that a tissue with much slower turnover also has a stem cell niche with a bi-compartmental organization is beginning to emerge. The largest adult neurogenic niche in the brain is found in the subventricular zone (SVZ, also known as the subependymal zone) of the striatum. In this region, stem cells have been characterized as being glial fibrillary acidic protein (Gfap)-positive astrocytes on the basis of their slow cycling properties and their potential to give rise to neurons in vivo and in vitro (Doetsch et al., 1999; Laywell et al., 2000; Alvarez-Buylla and Garcia-Verdugo, 2002). Recently, two groups of Gfap<sup>+</sup> astrocytes have been identified on the basis of epidermal growth factor receptor (*Egfr*) expression: Gfap<sup>+</sup> Egfr<sup>-</sup> and Gfap<sup>+</sup> Egfr<sup>+</sup> astrocytes (Fig. 2D) (Pastrana et al., 2009). The Egfr<sup>-</sup> astrocytes are quiescent and give rise to the actively cycling Egfr<sup>+</sup> astrocytes, which in turn give rise to transit-amplifying cells and neuroblast cells. Both Gfap<sup>+</sup> astrocytes are presumably required during physiological regeneration and injury processes (Pastrana et al., 2009). However, formal proof will be required to definitively establish to what extent each population contributes to regeneration under homeostatic and regenerative conditions in vivo.

### Lineage tracing: testing the kinetics of tissue regeneration

On the basis of the data we have discussed, we propose that a bi-compartmental organization might be a general paradigm that applies to the stem cell niches of multiple adult tissues. In order to verify whether this is the case, assays are required that: (1) test the stemness of the cells in a physiological context and (2) allow the

results of such tests to be analyzed in the context of all the other cells in the tissue. To date, approaches such as grafting assays have been utilized in a variety of tissues (such as skin and blood, among others) to test the regenerative potential of the cells (Lichti et al., 1993; McCulloch and Till, 1960; Lo Celso and Scadden, 2007). However, what we learn from these experiments is often the potential of these cells beyond their normal physiological roles. For instance, in the skin, the epidermis and the hair follicle appear to have two separate stem cell pools, which are responsible for making either the epidermis or the hair follicle in a physiological context (Levy et al., 2005; Ito et al., 2005). Nevertheless, in a grafting assay, hair follicle bulge stem cells are able to make hair follicles, as well as epidermis. Furthermore, epidermal cells that are committed to differentiation can give rise to both undifferentiated and differentiated epidermal cells, as well as to hair follicles in a graft assay (Mannik et al., 2009). Rather than assaying the physiological role of stem cells during normal homeostasis and regeneration, these data suggest that we might be over-extrapolating from these assays the potential of such cells in response to severe injury. Conversely, grafting assays might also underestimate the quantity and quality of stem cells. For example, cell cycle status of HSCs at the time of transplantation crucially affects the graft outcome, with G0 HSCs grafting the best (Venezia et al., 2004; Fleming et al., 1993). Approaches that address tissue regeneration under normal homeostasis have been largely lacking in the stem cell field, particularly in studies of the blood and brain, where injury models have predominated.

As such, there is a need to employ a more rigorous approach to studying stem cells during homeostasis in vivo. One such approach uses a Cre recombinase enzyme-mediated lineage tracing to track mouse cells in vivo by permanently marking them without disrupting the normal physiology of the tissue under study (Lakso et al., 1992). The power of this system lies in the ability to restrict the Cre recombinase expression patterns both spatially, by utilizing cell-type-specific promoters, and temporally, by utilizing an inducible version of Cre (such as tamoxifen-responsive Cre, Cre-ER<sup>TAM</sup>; Fig. 3). Such Cre is obtained by generating a Cre fusion protein with an altered



**Fig. 3. Cre-recombinase-based lineage tracing system.** Cre recombinase expression can be spatially restricted by expressing it under the control of a tissue-specific promoter. Temporal restriction is achieved by fusing it to the tamoxifen-responsive hormone-binding domain of the estrogen receptor (Cre-ER<sup>TAM</sup>). The Cre enzyme is in an inactive state in the absence of the ligand tamoxifen. Once tamoxifen is added, the Cre is active and can translocate to the nucleus. When these Cre constructs are used in conjunction with reporter genes, such as green fluorescent protein (GFP) ubiquitously expressed under the control of the ROSA26 (R26) promoter for example, and placed downstream of a STOP codon flanked by Cre recombinase recognition (loxP) sites, reporter gene expression can be activated in specific cell types at defined time-points. **(A)** In the absence of tamoxifen, no expression of the reporter gene is observed because of the presence of the stop signal upstream of the reporter gene. **(B)** When tamoxifen is administered, the Cre is activated and mediates recombination between the loxP sites in cells. As a consequence, the STOP codon is excised and the cells are permanently marked by the reporter gene. ER, estrogen receptor; GFP, green fluorescent protein.

hormone-binding domain of the mouse estrogen receptor ER<sup>TAM</sup>, which fails to bind estrogen but instead responds to the synthetic ligand 4-hydroxytamoxifen (Littlewood et al., 1995). In the absence of the ligand, the Cre-ER fusion protein is inactive. Upon tamoxifen binding, the receptor is released from the inhibited state, allowing the translocation of the activated Cre enzyme to the nucleus. Therefore, the marking of cells, which is controlled by the activation of the Cre enzyme, can be tightly regulated by the administration of the ligand tamoxifen. Temporal control of Cre alleles can also be achieved by using STOP signals in conjunction with constitutively active regulatory sequences upstream of a reporter gene (such as *lacZ* or GFP). In this approach, the reporter gene lies downstream of two Cre recombinase recognition (*loxP*) sites that are separated by a STOP codon. In a cell that has not experienced Cre activation, the STOP codon prohibits the production of the reporter protein (Fig. 3A). Upon Cre importation into the nucleus in the presence of tamoxifen, the STOP codon is excised and the reporter gene is expressed (Fig. 3B) (Danielian et al., 1998). A cell that undergoes such a Cre-mediated recombination event during its developmental history will remain permanently marked by the expression of the activated reporter gene. Importantly, this mark can be stably passed onto all of its progeny. Therefore, this method enables one to specifically mark the cells of interest at defined time-points to test the multipotency and longevity of a putative stem cell population, given that promoters specifically expressed in these populations are known (Fig. 4; Box 2).

The proposed model for stem cell bi-compartmental organization raises a number of key questions, which can be extensively addressed by the lineage-tracing approach. First, what is the relationship between the two compartments? Is there a continuous cellular flux between the two compartments? Is the flux uni-directional or bi-directional? Second, how do both compartments contribute to tissue regeneration, to what extent and with similar or different kinetics? Third, what is the functional role for both compartments? If one compartment was ablated, how would that impair tissue regeneration? Alternatively, can the two compartments functionally substitute for each other? In a tissue with a bi-compartmental organization, assuming the exchange is uni-directional between these two compartments, the labeling of the progeny of a stem cell population would reveal a mosaic pattern with some of the differentiated lineage being labeled and some not (Fig. 4B). We examine these different possibilities below.

### How lineage tracing can solve the puzzle

We think that the lineage-tracing approach could be utilized across a variety of tissues, despite their strikingly different features. Solid tissues such as hair follicle and intestine have distinctive and repetitive functional units that are well-defined anatomically, whereas such units are lacking in the adult bone marrow (Kiel et al., 2005a). In addition, bone marrow cellular components are highly dynamic, with cells continuously exiting and migrating throughout the bone marrow, either in response to physiological cues or upon reaching a certain differentiation status. Despite these differences that could make the approach more challenging in some tissues versus others (Box 2), lineage tracing can offer a more stringent approach to investigating stem cell compartments in both fluid and solid types of tissues, as we discuss in more detail below.

Although some efforts have been invested in these approaches already (Morris et al., 2004; Sangiorgi and Capecchi, 2008; Barker et al., 2007), the field is still far from having access to the cell-type-specific *Cre* alleles that are needed to discern stem cells from progenitors and/or from more differentiated cells in different tissues.

### Box 2. Limitations of lineage tracing for studying niche architecture

Although Cre-recombinase-mediated lineage tracing is a powerful approach, it is also subject to a number of technical and biological limitations that we discuss below.

It requires extensive knowledge about the cellular and molecular identifiers of specific stem cells. Promoter-driven Cre expression should be highly cell-type-specific and should not be leaky.

It assumes a unidirectional cellular flow and anticipates developmental hierarchy. If cells can revert to a more immature state, as has been proposed in the mouse testis (Barroca et al., 2009), the interpretation of the data becomes significantly more difficult.

The data can be more easily interpreted in solid tissues, in which cell migration is limited. This is in contrast to the hematopoietic system, in which migration is integral to cell differentiation. In this system, lineage tracing can only be achieved at a population, rather than clonal, level.

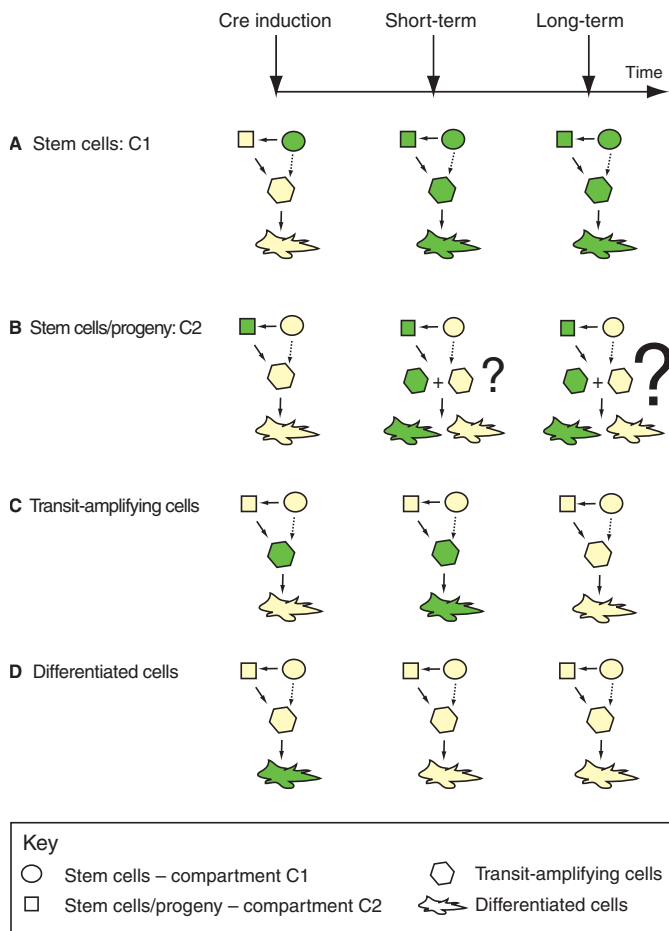
Niche structure might be dynamic (Nystul and Spradling, 2007). Unless the same niche and/or stem cells can be followed over a sufficient period of time, the information obtained might be relevant only for the specific temporal points under study.

Depending on the biology of the particular stem cells studied, it might be technically challenging to visualize the label-positive progeny. For the dormant HSCs, in which cell division occurs about every 145 days, such an approach will be extremely time consuming.

Gene expression profiling and/or deep sequencing can be used to identify coding and non-coding gene promoter elements to use in generating cell-type-specific Cre-ER lines in multiple different tissues, including tissues such as kidney or liver, for which there is still a limited amount of information on stem cell identity and organization. To date, there are very few tissues in which differential gene expression has been examined in detail in relation to distinct stem cell compartments (Greco et al., 2009; Tumber et al., 2004; Sato et al., 2009b; Passegue et al., 2005; Venezia et al., 2004; Wilson et al., 2008). The genes we have discussed that are markers of different stem cell compartments, such as *Bmi1* and *Lgr5*, could represent a starting point for identifying specific stem cell promoters as they are highly enriched in stem cells versus their progeny. In addition, the search for a stem-cell-specific gene expression signature could be extended to non-coding genes, for example microRNAs, which are known to be highly faithful in marking cell states and in influencing cell fate decisions (Lu et al., 2005; Lu et al., 2008; Yi et al., 2008). When microRNA expression profiles were compared across multiple tissue types including neuronal stem cells (Smirnova et al., 2005; Wulczyn et al., 2007), hair follicles and HSCs, multiple miRNAs are seen to be common to all of these stem cell types (Yi et al., 2008).

### Relationship between the two compartments

The putative stem cell progeny that might exist within a bi-compartmental architecture of a niche might represent a point of no return in the differentiation of stem cells. Alternatively, it is possible that these two compartments represent two different states of the same cells. Stem cells could therefore alternate between these two states, according to tissue physiology. If true, this would imply that bi-directional cell exchange between the two compartments could occur. Although normal development usually is uni-directional, observations of *Drosophila* ovaries and of mammalian testes have suggested otherwise (Nystul and Spradling, 2007; Barroca et al., 2009). These studies support the evidence that a stem cell character might not be associated with a specific cell but rather be determined by the niche



**Fig. 4. Testing cell potential with lineage tracing.** The inducible Cre system can be utilized to specifically mark different subsets of cells within a tissue. The persistence of labeled cells within a tissue is influenced by the turnover rate of the tissue, as well as by the cell cycling properties of the labeled cells. If cell-specific promoters are available (for stem cells, transit-amplifying cells or differentiated cells), several different labeling scenarios can be predicted. **(A)** If the stem cell population is marked, the whole tissue will be labeled and this label will persist for a long time, as stem cells are multipotent and long-lived. **(B)** If stem cell progeny are marked, and stem cells directly contribute to the organ regeneration, the transit-amplifying and differentiated cells will have a mosaic pattern (labeled and non-labeled cells) in the short term. If the progeny is also long-lived, this mosaic pattern will be sustained long term as well. **(C)** If transit-amplifying cells are marked, their differentiated daughter cells will also be marked in the short-term. However, as the transit-amplifying cells are short-lived, both labeled transit and differentiated cells will be replaced by unlabelled cells in the long term. **(D)** Conversely, if differentiated cells are marked, the label will be quickly lost, as differentiated cells are, in most tissues, short-lived and replaced by new stem cell progeny (there are exceptions of long-lived differentiated cells, such as memory B and T cells). By using this scheme, it is possible to address the regenerative potential of each of the cellular compartments without disrupting normal tissue physiology.

environment. In particular, the dynamic niche concept that has arisen from *Drosophila* stem cell studies (Nystul and Spradling, 2007) highlights the importance of utilizing a developmental model in mammals to address the role of the niche, as injury and transplantation models might not reflect physiological circumstances.

In the hair follicle, the most restricted promoters available for lineage tracing are expressed in both the bulge and the hair germ (Jaks et al., 2008; Morris et al., 2004). However, gene signatures for the bulge and the hair germ are now available to develop bulge-specific or hair-germ-specific promoter Cre-ER mouse transgenic lines (Greco et al., 2009). By specifically marking all bulge stem cells prior to hair germ formation during quiescence, we could learn about the temporal origin of their putative hair germ cell progeny. In addition, the frequency with which the bulge stem cell compartment gives rise to the hair germ compartment can be revealed by monitoring the percentage of labeled hair germ cells (i.e. bulge-derived cells). Similarly, lineage tracing under homeostatic conditions can elucidate the contribution of the d-HSC to the a-HSC pools and of the *Egfr*<sup>-</sup> to the *Egfr*<sup>+</sup> astrocyte pools if we could specifically label all d-HSCs or *Egfr*<sup>-</sup> astrocytes. Such an approach could inform us about the rate of contribution from one compartment to the other, and about any bi-directional cellular exchange between the two compartments. Such exchange could be revealed by monitoring the presence of unlabelled cells in the d-HSC and *Egfr*<sup>-</sup> astrocyte pool of cells (or alternatively, by using promoters that label all cells in the hair germ, a-HSC and *Egfr*<sup>-</sup> compartments). Interestingly, the *Bmi1* and *Lgr5* lineage-tracing experiments suggest that there might be a bi-directional cellular flux between these two compartments, although this has yet to be proven formally (Barker et al., 2007; Sangiorgi and Capecchi, 2008). It is important to point out that a clear interpretation of the results will only be ensured by a highly efficient labeling system (Box 2).

#### Relative contribution of compartments to tissue regeneration

Lineage tracing can also shed light on whether the two compartments both have stem cell properties. In particular, it can address whether both compartments are multipotent and long-lived and, if so, whether the kinetics with which they sustain tissue turnover is comparable. For example, by specifically marking hair germ cells, we could observe the extent to which differentiated label-positive cells are generated during growth and whether these labeled hair germ progeny are long-lived and can survive multiple hair regenerative cycles. In addition, provided that all the hair germ cells are labeled, the relative ratio of labeled to non-labeled differentiated cells could provide insights into the differences or similarities of the contributions of the two compartments to hair homeostasis.

In the case of the intestine, a lineage-tracing experiment approach has been performed by using the *Lgr5* and the *Bmi1* promoters to drive the expression of the Cre recombinase specifically in the CBC and +4 cells, respectively, thus specifically marking these cells. These studies have demonstrated that both crypt cell populations marked by these two genes can make all the cells of the intestinal crypt (Barker et al., 2007; Sangiorgi and Capecchi, 2008). Possibly, a dual lineage-tracing method that adopts an alternative system, such as the flippase recombination enzyme in combination with the existing Cre recombinase could address the differences or similarities in the contribution of the *Bmi1*<sup>+</sup> and *Lgr5*<sup>+</sup> compartments to intestine regeneration in the same mouse at the same time.

As Cre activation is a functional indicator of stem cell states, the expression of the reporter gene could serve as a functional identifier of HSCs, leading to a more accurate characterization of the elements that constitute dormant and activating niches. In such a scenario, a true HSC should fulfill all the phenotypic definitions of this cell type, while being positive for the reporter immediately after the administration of tamoxifen. Under homeostatic conditions, reporter-positive HSCs should be mostly in the dormant niche. If tamoxifen is co-administered with HSC activating agents such as



chemotherapy compounds, G-CSF or interferon, the reporter-positive HSCs should be in association with the activated niche. Similarly for the subventricular zone, lineage tracing could also help to explain how the balance between the two Gfap<sup>+</sup> astrocyte populations has an impact on the generation of adult neurons in vivo (Alvarez-Buylla and Garcia-Verdugo, 2002). The ability to distinguish between the contribution of the Egfr<sup>+</sup> versus Egfr<sup>-</sup> astrocytes, together with the identification of their niches, will be important for understanding how stem cells work with their progeny in sustaining tissue regeneration in a physiological context.

### Functional roles of the bi-compartmental niche

It is not clear whether the above-mentioned tissues rely on the presence of both stem cell compartments to sustain regeneration. Would ablation of either compartment impair regeneration? Experiments performed in the hair follicle have suggested that the hair germ could compensate for bulge loss in hair-plucking assays (Ito et al., 2004). However, a genetic approach could provide formal proof and an unequivocal answer to this question. In addition, the cell-specific promoter system can be utilized to drive a suicidal gene (Visnjic et al., 2001) to ablate cells in a compartment-specific manner. This could reveal whether one compartment can compensate for the loss of the other. Similar approaches have been successful in elucidating the distinct systems that are in place to sustain the epidermis and the hair follicle (Ito et al., 2005; Levy et al., 2005).

Finally, lineage tracing could improve our understanding of the stem cell compartmentalization during normal development and aging. For instance, fetal and neonatal HSCs actively cycle until they abruptly switch to a largely quiescent behavior around 3–4 weeks after birth (Bowie et al., 2006; Kim et al., 2007). Would this be the time for the HSCs to segregate into the d-HSC and a-HSC compartments? Evidence from the hair follicle suggests, for instance, that slowly cycling cells are established during embryonic skin development (Nowak et al., 2008). In addition, the expression pattern of adult hair germ and bulge markers is recapitulated embryonically, which indicates that the bi-compartmental organization might be set up early during embryogenesis (Nowak et al., 2008; Greco et al., 2009). Conversely, how does ageing influence the size of these compartments and their relative proportions? HSCs increase in number during aging with a concomitant decline in function (Janzen et al., 2006). Is this a reflection of a change of d-HSCs toward a-HSCs under cell-intrinsic, as well as extrinsic, regulations? It is conceivable that the two compartments have a rather stable homeostatic structure that can be modified by physiology and injuries. How is the balance between these two compartments maintained and which genes/pathways can disrupt this balance? Such questions could be explored using a lineage-tracing approach in various tissues. This approach will be particularly insightful when combined with models in which normal stem cell compartmentalization can be genetically manipulated, such as in *Pten*- and *p53*-null mice, in which HSC quiescence is disrupted (Liu et al., 2009; Yilmaz et al., 2006; Zhang et al., 2006).

### Conclusions

Under repeated injury and in rapidly regenerating tissues, stem cells face an exhaustion risk. In addition, for tissues in which stem cells are slowly cycling, their participation in homeostasis and repair might impose a severe stress on the cells, requiring them to switch rapidly from a quiescent status to an actively proliferating one. We propose that a compartmentalized organization of the stem cell niche could be a general mechanism by which stem cells could rapidly respond to growth/injury, without risking depletion. The canonical view envisions the path from stem cells to differentiated cells in two

dimensions, where cells transit uni-directionally from an immature state to a more differentiated one. In our view, the bi-compartmental model suggests that at, any given time, quiescent and activated stem cell states might coexist as different compartments within a tissue. In addition, these stem cell functional states might reach an equilibrium and might be interchangeable, depending on the physiological context. Thus, the rate of short-term tissue regeneration could rely on the quality and quantity of the cells in the active pool (e.g. hair germ, a-HSCs, Gfap<sup>+</sup> Egfr<sup>+</sup> astrocytes and Lgr5<sup>+</sup> cells), and disease and aging would affect the long-term tissue regeneration capacity of a stem cell pool by limiting the size of the quiescent stem cell compartment (e.g. the bulge, d-HSCs, Gfap<sup>+</sup> Egfr<sup>-</sup> astrocytes and Bmi1<sup>+</sup> cells). Importantly, it is probable that the bi-compartmental model represents a simplified view given the discussed heterogeneity in the HSCs and the observed heterogeneity in the expression of bulge markers such as integrin alpha 6, and of the orphan G-protein-coupled receptor Lgr5 in the hair follicle niche (Blanpain et al., 2004; Jaks et al., 2008). Rigorous experimental approaches, such as those we have proposed, are required to prove the general existence of a compartmentalized organization of stem cell niches and the relationship between such compartments. The answers to these key questions are likely to be provided by developmental models, which can reveal more faithfully the physiological role of stem cells, particularly when compared with injury models.

### Acknowledgements

We thank Rebecca Ihrie, Fiona Doetsch, Diane Krause, Jun Lu, Amalia Pasolli, Leonard Milstone and Charles Radding for critical discussions. We apologize to those colleagues whose work could not be referenced directly due to space constraints. S.G. is funded by the NIH. Deposited in PMC for release after 12 months.

### Competing interests statement

The authors declare no competing financial interests.

### References

- Abkowitz, J. L., Linenberger, M. L., Newton, M. A., Shelton, G. H., Ott, R. L. and Guttrop, P. (1990). Evidence for the maintenance of hematopoiesis in a large animal by the sequential activation of stem-cell clones. *Proc. Natl. Acad. Sci. USA* **87**, 9062–9066.
- Alvarez-Buylla, A. and Garcia-Verdugo, J. M. (2002). Neurogenesis in adult subventricular zone. *J. Neurosci.* **22**, 629–634.
- Barker, N., van Es, J. H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P. J. et al. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**, 1003–1007.
- Barroca, V., Lassalle, B., Coureuil, M., Louis, J. P., Le Page, F., Testart, J., Allemand, I., Riou, L. and Fouchet, P. (2009). Mouse differentiating spermatogonia can generate germinal stem cells in vivo. *Nat. Cell. Biol.* **11**, 190–196.
- Blanpain, C., Lowry, W. E., Geoghegan, A., Polak, L. and Fuchs, E. (2004). Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* **118**, 635–648.
- Bowie, M. B., McKnight, K. D., Kent, D. G., McCaffrey, L., Hoodless, P. A. and Eaves, C. J. (2006). Hematopoietic stem cells proliferate until after birth and show a reversible phase-specific engraftment defect. *J. Clin. Invest.* **116**, 2808–2816.
- Cheng, H. and Leblond, C. P. (1974). Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cell. *Am. J. Anat.* **141**, 461–479.
- Cheshier, S. H., Morrison, S. J., Liao, X. and Weissman, I. L. (1999). In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA* **96**, 3120–3125.
- Clevers, H. (2009). Searching for adult stem cells in the intestine. *EMBO Mol. Med.* **1**, 255–259.
- Cotsarelis, G., Sun, T. T. and Lavker, R. M. (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.
- Danielian, P. S., Muccino, D., Rowitch, D. H., Michael, S. K. and McMahon, A. P. (1998). Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. *Curr. Biol.* **8**, 1323–1326.

- Doetsch F, Caillé I, Lim D. A., García-Verdugo J. M. and Alvarez-Buylla A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703-716.
- Dykstra, B., Kent, D., Bowie, M., McCaffrey, L., Hamilton, M., Lyons, K., Lee, S. J., Brinkman, R. and Eaves, C. (2007). Long-term propagation of distinct hematopoietic differentiation programs in vivo. *Cell Stem Cell* **1**, 218-229.
- Essers, M. A., Offner, S., Blanco-Bose, W. E., Waibler, Z., Kalinke, U., Duchosal, M. A. and Trumpp, A. (2009). IFN $\alpha$  activates dormant haematopoietic stem cells in vivo. *Nature* **458**, 904-908.
- Fleming, W. H., Alpurn, E. J., Uchida, N., Ikuta, K., Spangrude, G. J. and Weissman, I. L. (1993). Functional heterogeneity is associated with the cell cycle status of murine hematopoietic stem cells. *J. Cell Biol.* **122**, 897-902.
- Foudi, A., Hochedlinger, K., Van Buren, D., Schindler, J. W., Jaenisch, R., Carey, V. and Hock, H. (2009). Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. *Nat. Biotechnol.* **27**, 84-90.
- Fuchs, E. (2009). The tortoise and the hare: slow-cycling cells in the stem cell race. *Cell* **137**, 811-819.
- Glauche, I., Moore, K., Thielecke, L., Horn, K., Loeffler, M. and Roeder, I. (2009). Stem cell proliferation and quiescence—two sides of the same coin. *PLoS Comput. Biol.* **5**, e1000447.
- Greco, V., Chen, T., Rendl, M., Schober, M., Pasolli, H. A., Stokes, N., Dela Cruz-Racelis, J. and Fuchs, E. (2009). A two-step mechanism for stem cell activation during hair regeneration. *Cell Stem Cell* **4**, 155-169.
- Haug, J. S., He, X. C., Grindley, J. C., Wunderlich, J. P., Gaudenz, K., Ross, J. T., Paulson, A., Wagner, K. P., Xie, Y., Zhu, R. et al. (2008). N-cadherin expression level distinguishes reserved versus primed states of hematopoietic stem cells. *Cell Stem Cell* **2**, 367-379.
- Ito, M., Kizawa, K., Hamada, K. and Cotsarelis, G. (2004). Hair follicle stem cells in the lower bulge form the secondary germ, a biochemically distinct but functionally equivalent progenitor cell population, at the termination of catagen. *Differentiation* **72**, 548-557.
- Ito, M., Liu, Y., Yang, Z., Nguyen, J., Liang, F., Morris, R. J. and 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.
- Jahoda, C. A., Horne, K. A. and Oliver, R. F. (1984). Induction of hair growth by implantation of cultured dermal papilla cells. *Nature* **311**, 560-562.
- Jaks, V., Barker, N., Kasper, M., van Es, J. H., Snippert, H. J., Clevers, H. and Toftgard, R. (2008). Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat. Genet.* **40**, 1291-1299.
- Janzen, V., Forkert, R., Fleming, H. E., Saito, Y., Waring, M. T., Dombkowski, D. M., Cheng, T., DePinho, R. A., Sharpless, N. E. and Scadden, D. T. (2006). Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature* **443**, 421-426.
- Kiel, M. J., Iwashita, T., Yilmaz, O. H. and Morrison, S. J. (2005a). Spatial differences in hematopoiesis but not in stem cells indicate a lack of regional patterning in definitive hematopoietic stem cells. *Dev. Biol.* **283**, 29-39.
- Kiel, M. J., Yilmaz, O. H., Iwashita, T., Terhorst, C. and Morrison, S. J. (2005b). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* **121**, 1109-1121.
- Kiel, M. J., He, S., Ashkenazi, R., Gentry, S. N., Teta, M., Kushner, J. A., Jackson, T. L. and Morrison, S. J. (2007). Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. *Nature* **449**, 238-242.
- Kim, I., Saunders, T. L. and Morrison, S. J. (2007). Sox17 dependence distinguishes the transcriptional regulation of fetal from adult hematopoietic stem cells. *Cell* **130**, 470-483.
- Kopp, H. G., Avezilla, S. T., Hooper, A. T. and Rafii, S. (2005). The bone marrow vascular niche: home of HSC differentiation and mobilization. *Physiology* **20**, 349-356.
- Lakso, M., Sauer, B., Mosinger, B., Jr, Lee, E. J., Manning, R. W., Yu, S. H., Mulder, K. L. and Westphal, H. (1992). Targeted oncogene activation by site-specific recombination in transgenic mice. *Proc. Natl. Acad. Sci. USA* **89**, 6232-6236.
- Laywell E. D., Rakic P., Kukekov V. G., Holland E. C. and Steindler D. A. (2000). Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc. Natl. Acad. Sci. USA* **97**, 13883-13888.
- Levy, V., Lindon, C., Harfe, B. D. and Morgan, B. A. (2005). Distinct stem cell populations regenerate the follicle and interfollicular epidermis. *Dev. Cell* **9**, 855-861.
- Lichti, U., Weinberg, W. C., Goodman, L., Ledbetter, S., Dooley, T., Morgan, D. and Yuspa, S. H. (1993). In vivo regulation of murine hair growth: insights from grafting defined cell populations onto nude mice. *J. Invest. Dermatol.* **101**, 1245-1295.
- Lin, H. (2002). The stem-cell niche theory: lessons from flies. *Nat. Rev. Genet.* **3**, 931-940.
- Littlewood, T. D., Hancock, D. C., Danielian, P. S., Parker, M. G. and Evan, G. I. (1995). A modified oestrogen receptor ligand-binding domain as an improved switch for the regulation of heterologous proteins. *Nucleic Acids Res.* **23**, 1686-1690.
- Liu, Y., Elf, S. E., Miyata, Y., Sashida, G., Liu, Y., Huang, G., Di Giandomenico, S., Lee, J. M., Deblasio, A., Menendez, S. et al. (2009). p53 regulates hematopoietic stem cell quiescence. *Cell Stem Cell* **4**, 37-48.
- Lo Celso, C. and Scadden, D. (2007). Isolation and transplantation of hematopoietic stem cells (HSCs). *J. Vis. Exp.* **25**, 157.
- Lo Celso, C., Fleming, H. E., Wu, J. W., Zhao, C. X., Miake-Lye, S., Fujisaki, J., Cote, D., Rowe, D. W., Lin, C. P. and Scadden, D. T. (2009). Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. *Nature* **457**, 92-96.
- Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B. L., Mak, R. H., Ferrando, A. A. et al. (2005). MicroRNA expression profiles classify human cancers. *Nature* **435**, 834-838.
- Lu, J., Guo, S., Ebert, B. L., Zhang, H., Peng, X., Bosco, J., Pretz, J., Schlanger, R., Wang, J. Y., Mak, R. H. et al. (2008). MicroRNA-mediated control of cell fate in megakaryocyte-erythrocyte progenitors. *Dev. Cell* **14**, 843-853.
- Mannik, J., Alzayady, K. and Ghazizadeh, S. (2009). Regeneration of multilineage skin epithelia by differentiated keratinocytes. *J. Invest. Dermatol.* **130**, 388-397.
- McCulloch, E. A. and Till, J. E. (1960). The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. *Radiat. Res.* **13**, 115-125.
- Morris, R. J., Liu, Y., Marles, L., Yang, Z., Trempus, C., Li, S., Lin, J. S., Sawicki, J. A. and Cotsarelis, G. (2004). Capturing and profiling adult hair follicle stem cells. *Nat. Biotechnol.* **22**, 411-417.
- Muller-Rover, S., Handjiski, B., van der Veen, C., Eichmuller, S., Foitzik, K., McKay, I. A., Stenn, K. S. and Paus, R. (2001). A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. *J. Invest. Dermatol.* **117**, 3-15.
- Nowak, J. A., Polak, L., Pasolli, H. A. and Fuchs, E. (2008). Hair follicle stem cells are specified and function in early skin morphogenesis. *Cell Stem Cell* **3**, 33-43.
- Nystul, T. and Spradling, A. (2007). An epithelial niche in the *Drosophila* ovary undergoes long-range stem cell replacement. *Cell Stem Cell* **1**, 277-285.
- Ogata, H., Taniguchi, S., Inaba, M., Sugawara, M., Ohta, Y., Inaba, K., Mori, K. J. and Ikehara, S. (1992). Separation of hematopoietic stem cells into two populations and their characterization. *Blood* **80**, 91-95.
- Osawa, M., Hanada, K., Hamada, H. and Nakauchi, H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* **273**, 242-245.
- Oshima, H., Rochat, A., Kedzia, C., Kobayashi, K. and Barrandon, Y. (2001). Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* **104**, 233-245.
- Panteleyev, A. A., Jahoda, C. A. and Christiano, A. M. (2001). Hair follicle predetermination. *J. Cell Sci.* **114**, 3419-3431.
- Passage, E., Jamieson, C. H., Ailles, L. E. and Weissman, I. L. (2003). Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? *Proc. Natl. Acad. Sci. USA* **100 Suppl.** **1**, 11842-11849.
- Passage, E., Wagers, A. J., Giuriato, S., Anderson, W. C. and Weissman, I. L. (2005). Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. *J. Exp. Med.* **202**, 1599-1611.
- Pastrana E., Cheng L. C. and Doetsch F. (2009). Simultaneous prospective purification of adult subventricular zone neural stem cells and their progeny. *Proc. Natl. Acad. Sci. USA* **106**, 6387-6392.
- Plikus, M. V., Mayer, J. A., de la Cruz, D., Baker, R. E., Maini, P. K., Maxson, R. and Chuong, C. M. (2008). Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. *Nature* **451**, 340-344.
- Potten, C. S., Owen, G. and Booth, D. (2002). Intestinal stem cells protect their genome by selective segregation of template DNA strands. *J. Cell Sci.* **115**, 2381-2388.
- Raaijmakers, M. H. and Scadden, D. T. (2008). Divided within: heterogeneity within adult stem cell pools. *Cell* **135**, 1006-1008.
- Sangiorgi, E. and Capecchi, M. R. (2008). Bmi1 is expressed in vivo in intestinal stem cells. *Nat. Genet.* **40**, 915-920.
- Sato, T., Onai, N., Yoshihara, H., Arai, F., Suda, T. and Ohteki, T. (2009a). Interferon regulatory factor-2 protects quiescent hematopoietic stem cells from type I interferon-dependent exhaustion. *Nat. Med.* **15**, 696-700.
- Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., van Es, J. H., Abo, A., Kujala, P., Peters, P. J. et al. (2009b). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262-265.
- Scadden, D. T. (2006). The stem-cell niche as an entity of action. *Nature* **441**, 1075-1079.
- Schofield, R. (1978). The relationship between the spleen colony-forming cell and the haematopoietic stem cell. *Blood Cells* **4**, 7-25.



- Scoville, D. H., Sato, T., He, X. C. and Li, L. (2008). Current view: intestinal stem cells and signaling. *Gastroenterology* **134**, 849-864.
- Sieburg, H. B., Cho, R. H., Dykstra, B., Uchida, N., Eaves, C. J. and Muller-Sieburg, C. E. (2006). The hematopoietic stem compartment consists of a limited number of discrete stem cell subsets. *Blood* **107**, 2311-2316.
- Smirnova, L., Grafe, A., Seiler, A., Schumacher, S., Nitsch, R. and Wulczyn, F. G. (2005). Regulation of miRNA expression during neural cell specification. *Eur. J. Neurosci.* **21**, 1469-1477.
- Spangrude, G. J., Heimfeld, S. and Weissman, I. L. (1988). Purification and characterization of mouse hematopoietic stem cells. *Science* **241**, 58-62.
- Taylor, G., Lehrer, M. S., Jensen, P. J., Sun, T. T. and Lavker, R. M. (2000). Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell* **102**, 451-461.
- Tumbar, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W. E., Rendl, M. and Fuchs, E. (2004). Defining the epithelial stem cell niche in skin. *Science* **303**, 359-363.
- Venezia, T. A., Merchant, A. A., Ramos, C. A., Whitehouse, N. L., Young, A. S., Shaw, C. A. and Goodell, M. A. (2004). Molecular signatures of proliferation and quiescence in hematopoietic stem cells. *PLoS Biol.* **2**, e301.
- Viale, A. and Pelicci, P. G. (2009). Awaking stem cells from dormancy: growing old and fighting cancer. *EMBO Mol. Med.* **1**, 88-91.
- Visnjic, D., Kalajzic, I., Gronowicz, G., Aguila, H. L., Clark, S. H., Lichtler, A. C. and Rowe, D. W. (2001). Conditional ablation of the osteoblast lineage in Col2.3deltak transgenic mice. *J. Bone. Miner. Res.* **16**, 2222-2231.
- Waghmare, S. K., Bansal, R., Lee, J., Zhang, Y. V., Mc Dermitt, D. J. and Tumbar, T. (2008). Quantitative proliferation dynamics and random chromosome segregation of hair follicle stem cells. *EMBO J.* **27**, 1309-1320.
- Wilson, A., Oser, G. M., Jaworski, M., Blanco-Bose, W. E., Laurenti, E., Adolphe, C., Essers, M. A., Macdonald, H. R. and Trumpp, A. (2007). Dormant and self-renewing hematopoietic stem cells and their niches. *Ann. New York Acad. Sci.* **1106**, 64-75.
- Wilson, A., Laurenti, E., Oser, G., van der Wath, R. C., Blanco-Bose, W., Jaworski, M., Offner, S., Dunant, C. F., Eshkind, L., Bockamp, E. et al. (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* **135**, 1118-1129.
- Wulczyn, F. G., Smirnova, L., Rybak, A., Brandt, C., Kwidzinski, E., Ninnemann, O., Strehle, M., Seiler, A., Schumacher, S. and Nitsch, R. (2007). Post-transcriptional regulation of the let-7 microRNA during neural cell specification. *FASEB J.* **21**, 415-426.
- Xie, Y., Yin, T., Wiegraebe, W., He, X. C., Miller, D., Stark, D., Perko, K., Alexander, R., Schwartz, J., Grindley, J. C. et al. (2009). Detection of functional haematopoietic stem cell niche using real-time imaging. *Nature* **457**, 97-101.
- Yi, R., Poy, M. N., Stoffel, M. and Fuchs, E. (2008). A skin microRNA promotes differentiation by repressing 'stemness'. *Nature* **452**, 225-229.
- Yilmaz, O. H., Valdez, R., Theisen, B. K., Guo, W., Ferguson, D. O., Wu, H. and Morrison, S. J. (2006). Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* **441**, 475-482.
- Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W. G., Ross, J., Haug, J., Johnson, T., Feng, J. Q. et al. (2003). Identification of the hematopoietic stem cell niche and control of the niche size. *Nature* **425**, 836-841.
- Zhang, J., Grindley, J. C., Yin, T., Jayasinghe, S., He, X. C., Ross, J. T., Haug, J. S., Rupp, D., Porter-Westpfahl, K. S., Wiedemann, L. M. et al. (2006). PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature* **441**, 518-522.
- Zhang, Y. V., Cheong, J., Ciapurin, N., Mc Dermitt, D. J. and Tumbar, T. (2009). Distinct self-renewal and differentiation phases in the niche of infrequently dividing hair follicle stem cells. *Cell Stem Cell* **5**, 267-278.
- Zhu, L., Gibson, P., Curre, D. S., Tong, Y., Richardson, R. J., Bayazitov, I. T., Poppleton, H., Zakharenko, S., Ellison, D. W. and Gilbertson, R. J. (2009). Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature* **457**, 603-607.