Dynamic stem cell heterogeneity

Teresa Krieger[1,2] and Benjamin D. Simons[1-3]

[1]The Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK.

[2]Cavendish Laboratory, Department of Physics, J.J. Thomson Avenue, University of Cambridge, Cambridge CB3 0HE, UK.

[3]Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge, UK.

Corresponding author: bds10@cam.ac.uk

Summary

Recent lineage tracing studies based on inducible genetic labeling have emphasized a crucial role for stochasticity in the maintenance and regeneration of cycling adult tissues. These studies have revealed that stem cells are frequently lost through differentiation and that this is compensated by the duplication of neighbors, leading to the consolidation of clonal diversity. Through the combination of long-term lineage tracing assays with short-term *in vivo* live-imaging, the cellular basis of this stochastic stem cell loss and replacement has begun to be resolved. With a focus on mammalian spermatogenesis, intestinal maintenance, and the hair cycle, we review the role of dynamic heterogeneity in the regulation of adult stem cell populations.

Introduction

In multicellular organisms, groups of cells specialize within tissues and organs to perform particular tasks and functions. In the course of adult life, these functional cells can become exhausted and progressively lost. To compensate for the ongoing loss of differentiated cells, new functional cells must be generated so that tissues remain in homeostasis. The maintenance and repair of cycling adult tissues usually rely upon the turnover of a small population of cells – termed adult stem cells - that possess the ability to self-renew, giving rise to differentiated cells while maintaining their number (1, 2).

The capacity of self-renewal has long been considered the defining feature of adult stem cells (3-5). To achieve homeostasis, stem cell proliferation and differentiation must be perfectly balanced such that, following division, one daughter cell stays in the stem cell compartment, while the other differentiates either directly or through a limited series of divisions. Such fate asymmetry can be achieved as the invariant

result of each and every stem cell division (termed “invariant asymmetry”). Alternatively, fate asymmetry may be orchestrated at the level of the population (termed “population asymmetry”), such that cell fate following each stem cell division is unpredictable or “stochastic”, and is specified only up to some defined probability (6, 7). These alternative models (Fig. 1A), both of which may be instructed by intrinsic (cell-autonomous) or extrinsic (environmental) cues, suggest very different regulatory mechanisms.

To address the factors that regulate stem cell self-renewal in adult tissues, attention has focused on defining the molecular mechanisms that control fate behavior. By combining static marker-based assays with the transcriptional profiling of fixed samples, significant progress has been made in resolving key elements of the gene regulatory networks and signaling pathways that control stem cell activity and fate behavior (8-13). However, it is becoming evident that stem cells function in dynamic and noisy environments in which levels of gene expression may adjust or fluctuate in response to promoter activity and extrinsic signals from the local microenvironment or niche (14). Therefore, to discriminate tissue stem cells from their more differentiated cell progeny and define their functional behavior, it is essential to address *dynamic* as well as static measures. Historically, the importance of such an approach was recognized prior to the genomics revolution. Charles Philippe Leblond, considered by many as the father of modern stem cell biology, emphasized the ‘time dimension in histology’, and did much to advance early lineage tracing methods using autoradiography and the incorporation of thymidine analogues (15). However, it was not until the advent of transgenic animal models that it became possible to reliably trace the lineage of individual cells and their labeled progeny over time (16).

In recent years, pioneering studies using *in vivo* live-imaging platforms have begun to provide access to continuous-time lineage data (17-22), while methods based on single-cell deep sequencing now offer the potential to resolve individual phylogenies, even in human tissues (23, 24). By combining these lineage tracing approaches with static marker-based assays, snapshots of clonal evolution over time can be integrated with population-level measures to reveal how stem and progenitor cells contribute to tissue maintenance. Efforts have also been made to develop statistical and mathematical methods that can resolve (conserved) strategies of progenitor cell fate in development and tissue maintenance (6). Applied to actively cycling adult tissues in both human and model organisms, including the epidermis (25, 26), oesophagus (27), intestine (7, 28-31), and germline (32), these studies show a preference for population asymmetric self-renewal, in which stem cells are continuously and stochastically lost and replaced by neighbors. This pattern of self-renewal results in “neutral drift” dynamics, with the continual and stochastic loss of clones through differentiation compensated by the expansion of others so that the overall stem cell population remains constant in size. In some cases, these studies have overturned long-held paradigms and refocused the search for the molecular regulatory mechanisms that underpin stochastic fate behavior. In particular, they have prompted the question of how the balance of stem cell proliferation and differentiation is regulated within dynamically changing environments (7).

These studies have also begun to question our understanding of stem cell identity in adult tissues. When considering the identity of stem cells, two key assumptions are usually implicit, but rarely challenged. First, it is presumed that stem cells are defined by the signature expression of molecular markers, distinct from their more differentiated cell progeny. Second, in the course of tissue turnover, stem cells and their progenitor cell progeny are thought to move *irreversibly* through a differentiation hierarchy (Fig. 1B). However, with the advent of more refined lineage tracing approaches, both of these assumptions have been called into question. Increasing evidence suggests that expression levels of key fate determinants are not fixed, but drift over time or fluctuate in response to transcriptional activity and extrinsic cues from the local microenvironment (33-37). Furthermore, progenitors expressing the same putative stem cell marker can exhibit heterogeneous fate choices (38), while stem cells with different expression profiles may behave similarly in the long term. Finally, recent studies in disparate tissues have also shown that cells normally committed to differentiation can, in the course of regeneration following the targeted ablation of endogenous stem cell populations, reacquire the hallmark properties of tissue stem cells, including the potential for long-term self-renewal (22, 39-42).

Together, these findings question the traditional view of adult stem cell populations as discrete entities comprising functionally equivalent cells. Instead, gathering evidence suggests that, in some tissues, stem cells may transit *reversibly* between discrete or a continuum of states in which they become temporarily biased towards particular fates, but the final decision is made stochastically or governed locally by cell-extrinsic factors (43-45). In this way, a transcriptionally heterogeneous cell population may function, long-term, as a single equipotent stem cell pool (Fig. 1C). Here, we review case studies from three canonical cycling adult tissues types – the mammalian germline, intestine, and hair follicle – that exemplify the role of heterogeneity and stochasticity in the regulation of adult stem cell behavior, as well as the conservation of self-renewal strategies between seemingly disparate tissue types. These studies highlight the value of a multifaceted approach to the study of tissue maintenance that places emphasis on quantitative and dynamic measures of fate behavior.

Examples of dynamic stem cell heterogeneity

Mammalian spermatogenesis

In mammals, spermatogenesis takes place in the seminiferous tubules of testes (46). In common with other cycling adult tissues, the testes contain adult stem cells – termed germline stem cells (GSCs) – that continually self-renew throughout adult life and are capable of rapid regeneration following injury. Throughout all stages of their development, germ cells are nourished by large somatic Sertoli cells, which support a network of tight junctions that separate the basal and adluminal compartments of the seminiferous tubule (Fig. 2A). Spermatogonia (mitotic germ cells that include GSCs) lie in close association with the basement membrane of the seminiferous tubule, and form the basal germ cell compartment. When meiosis begins, cells

detach from the basement membrane, and translocate across the tight junctions. They then undergo meiotic divisions and spermiogenesis (Fig. 2B) before their release into the lumen as mature sperm. In mice, spermatogonia are subdivided into 'undifferentiated' and 'differentiating' populations, with the differentiated cells expressing the receptor tyrosine kinase c-Kit. Furthermore, undifferentiated spermatogonia can exist as singly isolated cells (termed A_{single} or A_s) or as syncytial chains of cells connected by cellular bridges, consisting mainly of 2 (A_{pair} or A_{pr}), 4 ($A_{aligned\text{-}4}$ or $A_{al\text{-}4}$), 8 ($A_{al\text{-}8}$), or 16 ($A_{al\text{-}16}$) cells (Fig. 2C) (47).

In early studies, detailed analyses of fixed specimens led to the conjecture that stem cell activity is limited to the population of A_s spermatogonia, while interconnected A_{pr} and A_{al} syncytia were irreversibly committed to differentiation, a hypothesis known as the 'A_s model' (48, 49). Consistent with this model, post-transplantation colony formation and regeneration assays confirmed that the vast majority of stem cell activity is restricted to the population of undifferentiated (Kit-negative) spermatogonia (50). More recently, the identification of genetic markers that are enriched in or restricted to A_s spermatogonia, including the transcriptional repressor ID4 the polycomb complex protein Bmi1, and the paired-box protein Pax7, allowed the potency of individual A_s cells to be assessed (51-53). These studies confirm that at least a fraction of A_s cells retains long-term self-renewal potential, lending further support to the A_s model paradigm.

However, recent lineage tracing studies have questioned the validity of the A_s model and offer a new perspective on the identity and function of adult stem cell populations, in germline and indeed in other adult tissues. These studies focused on the fate and behavior of two separate compartments of undifferentiated spermatogonia, characterized by the expression of glial cell-derived neurotrophic factor (GDNF) family receptor alpha-1 (GFRα1) and the transcription factor Neurogenin 3 (Ngn3). In undisturbed testes, these factors are expressed heterogeneously, with GFRα1 expressed more widely in A_s cells and shorter syncytia (A_{pr} and a few A_{al}), while Ngn3 is expressed in a complementary manner (Fig. 2C). By developing an inducible genetic labeling transgenic mouse model based on the Cre-loxP recombination system with a Ngn3 promoter, studies by the Yoshida lab showed that the vast majority of Ngn3-expressing cells proceed rapidly to differentiation, maturation and loss, but that a small minority of cells retains long-term self-renewal potential (54). Further, through the development of long-term "scaling" properties of the measured clone size distribution, a follow-up study showed that GSCs are not individually long-lived, but are stochastically lost through differentiation and replaced by neighboring GSCs, leading to neutral drift dynamics of the surviving clone size (55). While these results are seemingly compatible with the A_s model, a subsequent *in vivo* live-imaging study by the same group revealed that the cellular bridges that connect cells within syncytia can break down, leading to the infrequent "fragmentation" of Ngn3-expressing syncytia into single cells or shorter syncytia (56). Such flexible behavior of Ngn3-expressing spermatogonia questions the premise of the A_s model that syncytia are irreversibly committed to differentiation. Instead, these results suggest that the entire pool of undifferentiated spermatogonia may contribute to stem cell activity.

To address this question, Yoshida and colleagues combined detailed *in vivo* live-imaging with long-term genetic lineage tracing using a pulse-labeling assay to follow the fate of individual GFRα1+ spermatogonia and their differentiating progeny (30). Continuous live-imaging data totaling more than 1 year of filming revealed that just 5% of GFRα1-expressing A_s cell divisions are complete, with the vast majority leading to the generation of A_{pr} syncytia. Therefore, if the transition from A_s to A_{pr} indeed signaled commitment to differentiation, as conjectured by the A_s model, the GFRα1+ A_s population would become rapidly depleted over time. However, alongside the cell division rate of around once per 10 days for GFRα1-expressing cells (independent of syncytial length), the live-imaging study also revealed fragmentation of GFRα1-expressing syncytia at a rate of around once per 20 days per interconnecting bridge, providing a possible route to replenish the A_s compartment.

Together, these findings suggest a revised model of GSC maintenance in which a morphologically heterogeneous cell population, comprised predominantly of GFRα1-expressing spermatogonia (including A_s and syncytial chains), functions long-term as a single stem cell pool. In this paradigm, germ cell production involves a coordinated process in which the commitment of cells to differentiation (signaled by the down-regulation of GFRα1 expression and up-regulation of Ngn3) is perfectly compensated by the fragmentation of neighboring GFRα1-expressing syncytia (Fig 2D). To test this hypothesis, the measured rates of cell division and syncytial fragmentation were used to predict the medium (weeks to months) and long-term (months to over a year) clonal evolution of labeled GFRα1-expressing cells and their differentiating progeny. By collecting clone size and compositional data at single cell resolution, compelling quantitative evidence was obtained in support of the new model for germline maintenance. Through continual GSC loss and replacement, clones undergo a neutral drift process in which their chance expansion through syncytial fragmentation is perfectly compensated by the contraction or loss of others through differentiation. At the same time, this study established a cellular basis to understand the process of GSC loss and replacement that was revealed by the long-term scaling behavior of the clone size distribution reported in the earlier Ngn3 lineage tracing study.

Although the cellular organization of the mammalian germline is of course unusual, these studies highlight several important features of stem cell dynamics that may translate to other stem cell-supported cycling adult tissues. First, maintenance of the stem cell compartment involves the continual stochastic loss and replacement of stem cells, leading to a progressive consolidation of clonal diversity. Second, stem cell competence is not restricted to a homogeneous cell population, defined by a signature expression of molecular markers. Instead, through the reversible transfer of cells between morphologically and genetically distinct states with differential survival probability, a heterogeneous population is able to function long-term as a single equipotent stem cell pool (cf. Fig. 1C). Third, it is only through *quantitative* analysis that “neutral” competition between equipotent stem cells can be discriminated from “non-neutral” clonal dominance associated with engrained (i.e. long-term) heterogeneity of fate potential. Fourth, the elucidation of short-term heterogeneity, and the cellular basis for GSC loss and replacement, is facilitated by access to continuous time *in vivo* live-imaging.

As well as presenting a new perspective on the identity of GSCs and the cellular basis for stem cell self-renewal, these studies also raise new mechanistic questions. What is the molecular regulatory basis for stochastic GSC loss and replacement? What role is played by the periodic seminiferous cycle that orchestrates the progression of spermatogonia and spermatocytes through the differentiation pathway? How is the fragmentation of GFRα1+ syncytia so exquisitely correlated with the commitment to differentiation of neighbors when GSCs are separated by legions of differentiating progeny? And, functionally, given the singular role of the germline in the propagation of genetic information to the next generation, what are the implications of neutral drift clone dynamics for the inheritance of congenital disorders due to the acquisition and spread of *de novo* mutations in GSCs (57, 58)? But before speculating on potential regulatory mechanisms and their implications, it is instructive to look for parallels of these dynamics. To this end, we turn now to consider a second mammalian epithelial tissue that is characterized by a high degree of turnover.

Intestinal maintenance

The epithelium of the mammalian small intestine is organized into large numbers of self-renewing crypt-villus units (Fig. 3A). Villi form finger-like protrusions of the gut wall that project into the lumen to maximize available absorptive surface area. The villi are covered by a simple post-mitotic epithelium, beneath which capillaries and lymph vessels mediate transport of absorbed nutrients into the body. The base of each villus is surrounded by multiple epithelial invaginations, termed crypts of Lieberkühn. The crypts play host to a population of rapidly proliferating intestinal stem cells (ISCs), which fuel the active self-renewal of the epithelium throughout adult life (59). These multipotent cells give rise to lineage restricted transit-amplifying cell progeny, which migrate along the walls of the intestinal crypt and generate the various differentiated absorptive and secretory cell types.

The identity, multiplicity, and behavior of ISCs have remained the subjects of continuing debate and controversy. Beginning with the early pioneering studies of Leblond, which were based on incorporation of thymidine analogues, ISCs have been localized to the base region of the crypt (60, 61). However, these early studies could not assess the long-term potency or heterogeneity of individual crypt base progenitors. Subsequent lineage tracing studies of individual clones marked by a chemical mutagen (ethylnitrosourea) showed that, in the course of turnover, the entire intestinal epithelium could be derived from a single marked cell (62). This result provided strong evidence that the intestinal epithelium is maintained by multipotent progenitor cells, which reside at the apex of a proliferative hierarchy. Later, with the advent of transgenics, long-term lineage tracing studies based on the clonal marking of targeted cell populations identified several putative ISC markers including the leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5), the polycomb complex protein Bmi1, and the homeodomain protein Hopx as (63-65). These markers have been associated with different subpopulations of ISCs (Fig. 3B). Lgr5 expression is enriched in “crypt base columnar cells”, which lie interspersed between large mature secretary cells known as Paneth cells (63). By contrast, Bmi1 is

more widely expressed in the crypt base region, with a peak of expression around the boundary of the Paneth cell compartment (row +4 from the base of the crypt), while Hopx is more tightly expressed in the same region (66).

Although these studies confirm that the ISC compartment contains cells expressing Lgr5, Bmi1, and/or Hopx, such qualitative studies cannot define the range, potential short-term heterogeneity, and fate behavior of ISCs. Once again, long-term lineage tracing studies, allied with short-term *in vivo* live imaging, have provided the means to address the identity and functional properties of the ISC compartment. The first of these studies, a long-term lineage tracing investigation based on the inducible genetic labeling of intestinal cells using a Cre-loxP recombinase system under the control of a ubiquitous promoter, showed that, in common with GSCs, ISCs follow a pattern of population asymmetric self-renewal (as evidenced by scaling behavior of the clone size distribution) in which ISC loss through differentiation is perfectly compensated by the duplication of a neighboring ISC (28). Through this process of stochastic ISC loss and replacement, stem cell-derived clones undergo neutral drift dynamics, expanding or contracting around the crypt base until individual clones become lost, or the crypt becomes monoclonal.

While this study provided insight into the functional behavior of the ISC compartment, by focusing on medium- (weeks) and long-term (months to a year) clonal dynamics, the size, molecular identity, and short-term potential of the ISC compartment could not be resolved. However, subsequent pulse-chase lineage tracing studies based on Lgr5 expression (29), combined with studies of the colony forming efficiency of Lgr5-expressing cells co-cultured with Paneth cells (67), led to the conjecture that stem cell competence may be linked to Wnt factors, which signal through Lgr5, associated with Paneth cells (68). Thus, through “neutral” competition for Paneth cell contact following cell division, ISCs become displaced from the niche environment, and enter into a differentiation pathway (Fig. 3C).

Although, in principle, the short-term potency of crypt base progenitors can be assessed through the use of targeted promoters, difficulties associated with the toxicity and delayed action of the Cre recombinase, effects of the inducing agent, and the slow acquisition of fluorescent reporters make a definitive assessment problematic. Instead, to resolve potential heterogeneity of the stem cell compartment, medium- and long-term lineage tracing assays were combined with short-term *in vivo* live imaging of clonally labeled tissue (69). By following the fate of marked Lgr5-expressing cells and their differentiating progeny over several days of time-lapse imaging, van Rheenen and colleagues showed that cells positioned at the base of the crypt (rows 0 to +2) experience a survival advantage over cells positioned near the border of the Paneth cell niche (rows +3 to +4). Yet, through the reversible transfer of cells between the border and base regions, the heterogeneous population of ISCs function long-term as a single equipotent stem cell pool (cf. Fig. 1C) (28, 70). Whether the short-term potency of ISCs correlates with the expression of the putative stem cell markers remains an intriguing open question.

Together these findings highlight the fact that, despite obvious differences in anatomy and cellular organization, the dynamics and behaviors of GSCs and ISCs, as

well as the means through which they were elucidated, show striking and unexpected parallels. In both cases, the stem cell compartment is heterogeneous, with cells transferring reversibly between "states" temporarily biased or "primed" for proliferation or differentiation. Yet, once a clone comprised of an individually labeled stem cell and its progeny has become representative by composition of the heterogeneous stem cell pool (a situation that will prevail on time scales comparable to the time of transfer between different primed states), the subsequent clonal evolution will become statistically indistinguishable from that of an effective single *equipotent* stem cell pool (Fig. 1C). In this limit, quantitative clonal analyses of both the germline and intestine reveal a process of stochastic stem cell loss and replacement, leading to (one-dimensional) neutral drift dynamics in which continual clonal contraction and loss is compensated by the expansion of neighboring clones (6).

Hair follicle

We have emphasized the functional similarities of stem cell maintenance in the germline and intestine. But to what extent does their behavior provide insight into other cycling tissue types? As mentioned above, quantitative clonal analyses based on genetic lineage tracing approaches have provided evidence that population asymmetric stem cell self-renewal may be a ubiquitous feature of adult tissue maintenance, at least in actively cycling epithelial tissues (6, 7, 25-31). In each documented case in which quantitative data on long-term clonal evolution has been available, its analysis is seen to be consistent with the steady turnover of an equipotent stem cell population. However, as illustrated by the examples of the mammalian testis and intestinal crypt, long-term steady-state behavior may mask the presence of short-term dynamic heterogeneity and fate priming of the stem cell pool.

In this context, the mammalian hair follicle provides an interesting case study. The hair follicle is unusual as a cycling tissue because it is not generated at a constant steady rate, but undergoes periodic bouts of regression and regeneration throughout adult life. On the basis of label-retaining assays and lineage tracing studies using targeted promoters (71, 72), stem cell identity has been localized to a permanent and discrete region of the hair follicle known as the bulge (Fig. 4A). Otherwise dormant stem cells residing in the bulge region enter sporadically into cell cycle in response to signals derived from the base of the niche, and give rise to progeny that repopulates the hair follicle. Alongside putative stem cell markers such as Keratin 15 and the transcription factor Sox9, which are expressed throughout the bulge region, other markers are expressed heterogeneously such as the Hematopoietic progenitor cell antigen CD34, which is expressed more strongly in the distal region, while Lgr5 is enriched proximally (73) (Fig. 4A).

To trace the dynamics of hair follicle stem cells during the phase of regeneration, the Greco lab has recently employed a novel two-photon *in vivo* live-imaging approach, allowing deep penetration into the tissue (22). When combined with genetic lineage tracing, this method has enabled individual stem cell lineages to be followed from

their exact place of origin throughout the process of regeneration (74). This study found that stem cells located in the upper half of the hair follicle niche were more likely to remain quiescent, or proliferate without committing to a specific fate (Fig. 4A). In contrast, stem cells situated in the lower bulge region were more likely to proliferate in response to activating stimuli from the niche base, undergoing limited amplification before differentiating. These observations suggest that, in common with intestinal crypt, the location of a stem cell within the niche at the onset of a new regeneration cycle dictates its fate during the cycle (74). Whether stem cells in the lower bulge region continue to harbor long-term self-renewal potential, as do stem cells at the niche border of crypt, or whether they have irreversibly entered a differentiation pathway remains unclear. However, the flexibility and regenerative capacity of the progenitor populations have been tested under injury conditions.

Using laser-induced cell ablation to specifically remove either the bulge stem cells or the hair germ (stem cell progeny) at the onset of hair growth (74), further studies by the Greco lab showed that, remarkably, in both cases the hair follicle niche recovered the lost cell population, restored its anatomical features, and proceeded normally through the hair cycle (Fig. 4C). Differentiating hair follicle cells can thus regain stem cell competence in response to injury. Surprisingly, distant epithelial cells located above the bulge were also observed to become proliferative, and some descended rapidly into the niche (Fig. 4C). By limiting genetic labeling to epithelial cells outside of the niche, it was confirmed that loss of the stem cell pool due to injury can mobilize cells that do not normally participate in hair regeneration to re-populate the niche and sustain hair growth. Indeed, once these cells entered the niche, they displayed characteristics consistent with the fate of endogenous stem cells in their new locations.

The hair follicle study therefore provides evidence for both stem cell heterogeneity and flexibility under conditions of stress. While, in this case, the recruitment of differentiating cells to the stem cell niche has not yet been confirmed under normal physiological conditions, the conversion of epithelial cells to bulge stem cells in response to crisis suggests that cells seemingly committed to a differentiation lineage are able to “reprogramme” and assume long-term stem cell fate identity. Future studies will reveal whether stem cell heterogeneity and the flexibility of differentiating progeny represent a more ubiquitous feature of this and other adult stem cell populations.

Questioning stem cell identity

The emergence of stochastic stem cell fate behavior, stem cell heterogeneity and priming in tissue maintenance questions our understanding of adult stem cell identity and the definition of commitment. Even within an equipotent stem cell population, while all cells retain long-term self-renewal potential, chance stem cell loss and replacement mean that only a diminishing minority of clones actually persist long-term. Yet it would make no sense to segregate cells prospectively according to their eventual long-term fate. Similarly, in a dynamic heterogeneous stem cell population, the long-term survival potential of individual cells may itself vary over

time. For example, in the intestinal crypt, an ISC positioned at the border of the niche has a long-term survival probability that is several times smaller than an ISC positioned towards the crypt base (69). However, if the border ISC or its ISC progeny transfers to the base region, the survival probability is proportionately readjusted. It would therefore seem inappropriate to designate only the base population as a stem cell type; instead, the entire compartment functions as just *one* heterogeneous population.

Further, in defining stem cell behavior, much of the discussion in the literature has centered on the mode of division, and in particular on whether the fate outcome is symmetric or asymmetric (1, 75-77). However, this designation is useful only if fate behavior is defined shortly prior to, or on, division. If fate outcome is linked to the proximity of daughter cells to the niche following division, as implicated in the germline and intestine, the division mode may not be the primary determinant of daughter cell fate. In the search for mechanism, it would therefore be expedient to focus more on local environmental cues instructing fate behavior.

The potential for ambiguity in the definition of an adult stem cell doesn't end there. Alongside the innate regenerative capacity of the endogenous stem cell population, evidence from regenerative studies of hair follicle shows that cells normally committed to differentiation in steady-state are able to repopulate the stem cell compartment and reacquire long-term self-renewal potential in response to injury or stress. Indeed, such behavior is far from unique (42). Following the ablation of spermatogonia through busulphan administration, studies have shown that the recovery of the GSC compartment involves the large-scale transfer of Ngn3-expressing cells to the GFRα1-expressing stem cell compartment, as well as the expansion of the surviving GFRα1-expressing cell population (30, 56). Similarly, the targeted genetic ablation of Lgr5-expressing cells following exposure to diphtheria toxin leads to the transfer of differentiating cells back into the stem cell compartment, and the regeneration of the stem cell pool (78). Further, independent studies show that cells positive for the Notch ligand Dll1 as well as quiescent Lgr5-expressing cells, which are both largely committed to differentiation into the secretory cell lineage in conditions of normal homeostasis, can reestablish multipotency and contribute to long-term self-renewal following the ablation of ISCs through radiation damage (39, 79). Finally, the regeneration of trachea following the genetic ablation of basal cells (which includes the resident stem cell population) involves the de-differentiation of club cells (40). Together, these results suggest that the entry of cells into a differentiation pathway may not involve an abrupt 'binary' decision but may occur progressively, with cells retaining stem cell *potential* ready to be mobilized under appropriate cues. Such flexibility may strengthen the resilience of tissues to crisis or injury, enabling the ensemble of differentiating progeny to function as a "reserve" stem cell population (cf. (80)).

Taken together, these studies suggest that the fate potential of stem and progenitor cells may not be organized into a strict classical "one-way" proliferative hierarchy involving functionally discrete cell populations. Rather, the arrangement of cell types may be more accurately represented as a continuum, in which both the proliferative and fate potential becomes gradually restricted. In such cases, transitions between

different cell "states" may occur reversibly even under physiological conditions, in response to niche-dependent factors, and can be promoted through injury or stress.

Stem cell-niche interactions

The intestinal crypt and hair follicle bulge highlight the crucial role played by interactions with the local microenvironment in defining the proliferative capacity and fate behavior of stem cells. In the intestinal crypt, the balance between stem cell loss and replacement, as well as the size of the stem cell pool, are regulated by exposure to Paneth cells as well as factors from the adjacent stromal tissue. Through competition for limited niche access, ISCs are able to self-renew, and they can recover their number during the regeneration of tissue following the partial ablation of the stem cell compartment by radiation damage or other forms of injury (78, 81).

A similar strategy to regulate the size of the stem cell compartment may operate in the germline. Although studies have not yet identified a localized niche structure in the mammalian testis, the association of undifferentiated spermatogonia with the vasculature (82) suggests that intratubular domains may play host to a somatic cell type (or types) that create a niche environment to support GSCs, much as Paneth cells do in crypts. Competition of spermatogonia for access to these limited niche domains may provide a simple and robust mechanism to regulate both the balance between syncytial fragmentation and differentiation, and the total size of the stem cell pool. Furthermore, if GFRα1 expression is linked to proximity to the niche, then the fragmentation of GFRα1+ syncytia may displace their neighbors from niche-maintaining sites, leading to loss of GFRα1 expression and the upregulation of differentiation markers such as Ngn3.

In contrast to the mammalian testis, the function of localized niche factors on stem cell regulation have been defined in the *Drosophila* ovary and testis. In these cases, stem cell identity is traditionally thought to be restricted to the population of GSCs that directly contact a central hub of stromal cells (83, 84). These cells remain closely associated with their niche during the cell cycle through cadherin-mediated cell adhesion (85). Through a regulated process of spindle orientation, GSC division leads to predominantly asymmetric fate outcome in which one daughter cell remains anchored to the hub and retains GSC identity while another is displaced from the niche and enters into a differentiation pathway (83). However, static lineage tracing studies and *ex vivo* live-imaging in the *Drosophila* testis and ovary show that, even under normal physiological conditions, sporadic stem cell loss from the hub may be compensated by the symmetric duplication of neighboring stem cells, and vice versa, leading to neutral drift dynamics of the clonal population (32, 86). Whether these rare events are associated with chance loss or active displacement of "inferior" GSCs, or whether infrequent symmetric divisions are a routine part of the normal program of homeostatic turnover remains unclear. In this context, it is interesting to note that the second resident stem cell population in *Drosophila* testis, the somatic cyst stem cells (CySC) that give rise to the cyst cells ensheathing developing germ, undergo loss and replacement at a much higher rate (31, 87).

Alongside the ability of GSCs to undergo symmetric as well as asymmetric cell divisions in normal homeostasis, differentiating germ cells also retain the ability to reestablish contact with the hub and reacquire stem cell function in the course of regeneration following the depletion of the GSC pool by protein starvation or genetic ablation (88, 89). Although such behavior has been traditionally associated with a process of de-differentiation, and distinct from the GSC renewal through symmetric cell division, it is interesting to note that the process of spindle orientation and division asymmetry may not be essential for germline maintenance. In particular, studies based on the targeted depletion of *stat* in GSCs, which lead to their detachment from the hub, show that contact with CySCs alone is sufficient to maintain GSC self-renewal and spermatogenesis (87). Indeed, under these conditions, the maintenance of *Drosophila* germ line may in fact parallel the process of dynamic heterogeneity that characterizes the mammalian system.

Dynamic interactions with the niche may thus serve to both constrain stem cell identity under physiological conditions, and orchestrate the regeneration of the stem cell compartment following injury. Future studies might address the extent to which recruitment to the stem cell pool upon injury is a reflection of underlying cell fate heterogeneity, or instead is the consequence of active cell fate reprogramming following catastrophic stem cell loss.

The role of stem cell quiescence

Although lineage tracing assays provide a powerful read-out of the behavior and dynamics of cycling cells, they are notoriously insensitive to the existence and potential function of long-term quiescent (slow-cycling) or dormant cell populations. Indeed, both the germline and intestinal crypt have been associated with a quiescent progenitor cell population. In humans and other primates, detailed analyses of fixed specimens have identified a subpopulation of singly isolated spermatogonia, termed A_{dark} on the grounds of their appearance in histological (47). It has been speculated that this minority cell population may play a special role in the long-term maintenance of tissue, supporting the more rapidly cycling but transient spermatogonial cell population (90). Similarly, studies of the mouse intestinal crypt have identified a population of quiescent cells marked by the expression of telomerase reverse transcriptase (mTert) or Lgr5 (66, 91).

It is difficult to identify the potential function and significance of these minority quiescent cell populations, particularly in tissues such as the germline and intestinal crypt, where active cycling cells are seen to maintain life-long self-renewal (at least in mice). As shown by a recent study of intestinal crypt, quiescence may not in itself be a signature of stem cell function, at least under conditions of normal maintenance (79). However, in long-lived organisms, it may be advantageous to hold a dedicated slow-cycling or dormant stem cell population in reserve so that it may “drip-feed” the cycling stem cell pool to compensate for progressive chance loss or ageing.

Alternatively, the reversible transfer of stem cells between an active and quiescent state under physiological conditions (Fig. 1C), itself a manifestation of dynamic

heterogeneity, may provide a robust mechanism to maintain a stem cell pool where the overall turnover rate of the tissue is steady but slow. Equally, the sporadic entry of stem cells into a quiescent or dormant state in a cycling tissue may provide an insurance mechanism to shield the wider population from demands experienced by actively cycling cells, and thereby protect the long-term integrity of tissue. Such behavior would mirror the strategy of phenotypic switching observed in bacterial populations (92).

Conclusion

Taken together, these observations highlight the requirement to develop an extended definition of stem cell identity, one that adequately captures the heterogeneity of tissue stem cells and the flexibility of their progeny. In the studies discussed above, stem cell identity consistently emerges not as the property of a discrete population, but as a functional state that a wider population of cells may enter, exit, and re-enter according to the demands of the tissue. All cells belonging to this wider population therefore have the capacity for long-term self-renewal, but their proliferative potential at any given time may depend on their precise location, signals from the niche environment, and other extrinsic and intrinsic factors.

If stem cell identity is indeed a state that is accessible to a wide population of progenitors, it becomes crucial to ask how recruitment to and exit from the stem cell pool is regulated at the molecular level. The dependence of self-renewal potential on the position of a cell within the niche that was observed in intestine and hair follicle suggests that spatially localized signals may play an important role in determining the state of progenitors; these could result from direct interactions with surrounding cells of the same or different types, extracellular matrix components, or soluble paracrine mediators (93). Non-local signals, including metabolic and endocrine factors, may furthermore contribute to aligning overall niche stem cell activity with the requirements of distant tissues (94).

In maintaining the stem cell pool at a constant size, it is not clear whether the aberrant loss of a stem cell triggers recruitment of a differentiating progenitor back to the niche, or whether stem cells exit the compartment in response to the fate reversal of differentiating cells. While the mechanisms that govern regeneration after injury may differ substantially from those operative in homeostasis, the examples above, however, suggest that stem cell recruitment may occur in response to stem cell loss from the niche. As repopulation of the niche has been observed even following the ablation of the entire stem cell pool, it is conceivable that stem cell identity can be initiated by factors derived from supporting cells or the extracellular matrix, rather than other stem cells. The physical structure of the niche, which is independently maintained by the extracellular matrix, associated vasculature and supporting cells, may therefore play a more active role in regulating stem and progenitor cell fate than previously appreciated.

The observation that the recruitment of differentiating progenitors back to the stem cell pool occurs under normal physiological conditions opens up an intriguing new

possibility for the mechanism of ageing in tissues with high cellular turnover. While the accumulation of mutations in nuclear and mitochondrial DNA is considered to be the most fundamental and irreversible cause of ageing, declining homeostasis in cycling tissues has also been associated with changes in the numbers or properties of stem cells and their niches (95, 96). In particular, tissue ageing may be accompanied by a gradual loss of functional stem cells, which has been thought to result from increased rates of stem cell death, quiescence, or differentiation (97, 98). However, paradigms that could explain how the global process of ageing leads to the gradual loss of only a small fraction of stem cells at any one time have so far been lacking. Following the discoveries of heterogeneity within stem cell compartments and the ongoing interconversion between stem cells and their differentiating progeny, it may be that ageing is a result not (only) of an increase in the active loss of stem cells, but (also) of a decrease in the recruitment of differentiating cells back to the niche. This would be consistent with a number of studies showing that stem cells from ageing animals can continue to function normally when maintained in a young niche or provided with young systemic factors (99, 100).

Over the past few years, the coexistence of distinct progenitor cell behaviors has been reported across a wide range of tissues, often through genetic lineage tracing approaches (101-103). It remains an open question whether these observations reflect an underlying heterogeneity within a single stem cell compartment, and whether the continual inter-conversion of long-term stem cells and their differentiating progeny occurs in these tissues under physiological conditions. Static lineage tracing alone can provide important clues to the degree of progenitor cell plasticity in some tissues, but owing to the lack of tools for the targeted labeling of particular subpopulations of cells, it is frequently impossible to identify unambiguously the source of labeled clones. To unravel stem cell heterogeneity, lineage hierarchies, and the de-differentiation capacity of differentiating cells, *in vivo* live imaging approaches will therefore be indispensable. In resolving the biological significance of heterogeneity within the stem cell compartment, we expect that stem cell diversity and lineage plasticity will emerge as ubiquitous features of adult tissue stem cell populations.

Acknowledgements

We are grateful to Cedric Blanpain, Hans Clevers, Tariq Enver, Valentina Greco, Kenshiro Hara, Allon Klein, Anna Philpott, Jacco van Rheenen, Shahragim Tajbakhsh, Doug Winton, Shosei Yoshida and all members of the Simons group for illuminating discussions. BDS acknowledges the financial support of the Wellcome Trust (grant number 098357/Z/12/Z) as well as core grants from the Wellcome Trust (092096) and Cancer Research UK (C6946/A14492).

References

1. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science (New York, NY). 2000;287(5457):1427-30.
2. Fuchs E, Chen T. A matter of life and death: self-renewal in stem cells. EMBO reports. 2013;14(1):39-48.
3. Clermont Y, Leblond CP. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann NY Acad Sci. 1952:548-73.
4. Clermont Y, Leblond CP. Renewal of spermatogonia in the rat. Am J Anat. 1953;93:475-502.
5. Leblond CP, Stevens CE. The constant renewal of the intestinal epithelium in the albino rat. Anat Rec. 1948;100:357-71.
6. Klein AM, Simons BD. Universal patterns of stem cell fate in cycling adult tissues. Development (Cambridge, England). 2011;138:3103-11.
7. Simons BD, Clevers H. Strategies for homeostatic stem cell self-renewal in adult tissues. Cell. 2011;145(6):851-62.
8. Clements WK, Traver D. Signalling pathways that control vertebrate haematopoietic stem cell specification. Nature reviews Immunology. 2013;13(5):336-48.
9. Guruharsha KG, Kankel MW, Artavanis-Tsakonas S. The Notch signalling system: recent insights into the complexity of a conserved pathway. Nature reviews Genetics. 2012;13(9):654-66.
10. Holland JD, Klaus A, Garratt AN, Birchmeier W. Wnt signaling in stem and cancer stem cells. Current opinion in cell biology. 2013;25(2):254-64.
11. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012;149(2):274-93.
12. Singh AM, Reynolds D, Cliff T, Ohtsuka S, Mattheyses AL, Sun Y, et al. Signaling network crosstalk in human pluripotent cells: a Smad2/3-regulated switch that controls the balance between self-renewal and differentiation. Cell stem cell. 2012;10(3):312-26.
13. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell. 2012;149(6):1192-205.
14. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008;132(4):598-611.
15. Leblond CP. The time dimension in histology. Am J Anat. 1965:1-27.
16. Kretzschmar K, Watt FM. Lineage tracing. Cell. 2012;148(1-2):33-45.
17. Bertrand JY, Chi NC, Santoso B, Teng S, Stainier DY, Traver D. Haematopoietic stem cells derive directly from aortic endothelium during development. Nature. 2010;464(7285):108-11.
18. Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. Nature. 2010;464(7285):116-20.
19. Lam EY, Hall CJ, Crosier PS, Crosier KE, Flores MV. Live imaging of Runx1 expression in the dorsal aorta tracks the emergence of blood progenitors from endothelial cells. Blood. 2010;116(6):909-14.
20. Yaniv K, Isogai S, Castranova D, Dye L, Hitomi J, Weinstein BM. Live imaging of lymphatic development in the zebrafish. Nature medicine. 2006;12(6):711-6.

21. Ritsma L, Steller EJ, Ellenbroek SI, Kranenburg O, Borel Rinkes IH, van Rheenen J. Surgical implantation of an abdominal imaging window for intravital microscopy. Nature protocols. 2013;8(3):583-94.
22. Rompolas P, Deschene ER, Zito G, Gonzalez DG, Saotome I, Haberman AM, et al. Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration. Nature. 2012;487(7408):496-9.
23. Shapiro E, Biezuner T, Linnarsson S. Single-cell sequencing-based technologies will revolutionize whole-organism science. Nature reviews Genetics. 2013;14(9):618-30.
24. Treutlein B, Brownfield DG, Wu AR, Neff NF, Mantalas GL, Espinoza FH, et al. Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. Nature. 2014;509(7500):371-5.
25. Clayton E, Doupé DP, Klein AM, Winton DJ, Simons BD, Jones PH. A single type of progenitor cell maintains normal epidermis. Nature. 2007;446:185-9.
26. Mascré G, Dekoninck S, Drogat B, Youssef KK, Brohée S, Sotiropoulou PA, et al. Distinct contribution of stem and progenitor cells to epidermal maintenance. Nature. 2012;489:257-64.
27. Doupé DP, Alcolea MP, Roshan A, Zhang G, Klein AM, Simons BD, et al. A single progenitor population switches behavior to maintain and repair esophageal epithelium. Science (New York, NY). 2012;337:1091-3.
28. Lopez-Garcia C, Klein AM, Simons BD, Winton DJ. Intestinal stem cell replacement follows a pattern of neutral drift. Science (New York, NY). 2010;330:822-5.
29. Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, et al. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell. 2010;143(1):134-44.
30. Hara K, Nakagawa T, Enomoto H, Suzuki M, Yamamoto M, Simons BD, et al. Mouse Spermatogenic Stem Cells Continually Interconvert between Equipotent Singly Isolated and Syncytial States. Cell stem cell. 2014;14:658-72.
31. Amoyel M, Simons BD, Bach EA. Neutral competition of stem cells is skewed by proliferative changes downstream of Hh and Hpo. The EMBO journal. 2014;33(20):2295-313.
32. Sheng XR, Matunis E. Live imaging of the Drosophila spermatogonial stem cell niche reveals novel mechanisms regulating germline stem cell output. Development (Cambridge, England). 2011;138(16):3367-76.
33. Chambers I, Silva J, Colby D, Nichols J, Nijmeijer B, Robertson M, et al. Nanog safeguards pluripotency and mediates germline development. Nature. 2007;450(7173):1230-4.
34. Chang HH, Hemberg M, Barahona M, Ingber DE, Huang S. Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. Nature. 2008;453(7194):544-7.
35. Pina C, Fugazza C, Tipping AJ, Brown J, Soneji S, Teles J, et al. Inferring rules of lineage commitment in haematopoiesis. Nature cell biology. 2012;14(3):287-94.
36. Levine JH, Lin Y, Elowitz MB. Functional roles of pulsing in genetic circuits. Science (New York, NY). 2013;342(6163):1193-200.
37. Imayoshi I, Isomura A, Harima Y, Kawaguchi K, Kori H, Miyachi H, et al. Oscillatory control of factors determining multipotency and fate in mouse neural progenitors. Science (New York, NY). 2013;342(6163):1203-8.

38. Graf T, Stadtfeld M. Heterogeneity of embryonic and adult stem cells. Cell stem cell. 2008;3(5):480-3.
39. van Es JH, Sato T, van de Wetering M, Lyubimova A, Nee AN, Gregorieff A, et al. Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. Nature cell biology. 2012;14(10):1099-104.
40. Tata PR, Mou H, Pardo-Saganta A, Zhao R, Prabhu M, Law BM, et al. Dedifferentiation of committed epithelial cells into stem cells in vivo. Nature. 2013;503(7475):218-23.
41. Yanger K, Knigin D, Zong Y, Maggs L, Gu G, Akiyama H, et al. Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. Cell stem cell. 2014;15(3):340-9.
42. Tetteh PW, Farin HF, Clevers H. Plasticity within stem cell hierarchies in mammalian epithelia. Trends in cell biology. 2015;25(2):100-8.
43. Enver T, Pera M, Peterson C, Andrews PW. Stem cell states, fates, and the rules of attraction. Cell stem cell. 2009;4(5):387-97.
44. Enver T, Heyworth CM, Dexter TM. Do stem cells play dice? Blood. 1998;92(2):348-51; discussion 52.
45. Chalancon G, Ravarani CN, Balaji S, Martinez-Arias A, Aravind L, Jothi R, et al. Interplay between gene expression noise and regulatory network architecture. Trends in genetics : TIG. 2012;28(5):221-32.
46. Russell LD, Ettlin RA, Hikim APS, Clegg ED. Histological and Histopathological Evaluation of the Testis. International Journal of Andrology. 1993;16(1):83-.
47. de Rooij DG, Russell LD. All you wanted to know about spermatogonia but were afraid to ask. Journal of andrology. 2000;21(6):776-98.
48. Huckins C. The spermatogonial stem cell population in adult rats. I. Their morphology, proliferation and maturation. The Anatomical record. 1971;169(3):533-57.
49. Oakberg EF. Spermatogonial stem-cell renewal in the mouse. The Anatomical record. 1971;169(3):515-31.
50. Shinohara T, Orwig KE, Avarbock MR, Brinster RL. Spermatogonial stem cell enrichment by multiparameter selection of mouse testis cells. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(15):8346-51.
51. Aloisio GM, Nakada Y, Saatcioglu HD, Pena CG, Baker MD, Tarnawa ED, et al. PAX7 expression defines germline stem cells in the adult testis. The Journal of clinical investigation. 2014;124(9):3929-44.
52. Komai Y, Tanaka T, Tokuyama Y, Yanai H, Ohe S, Omachi T, et al. Bmi1 expression in long-term germ stem cells. Scientific reports. 2014;4:6175.
53. Oatley MJ, Kaucher AV, Racicot KE, Oatley JM. Inhibitor of DNA binding 4 is expressed selectively by single spermatogonia in the male germline and regulates the self-renewal of spermatogonial stem cells in mice. Biology of reproduction. 2011;85(2):347-56.
54. Nakagawa T, Nabeshima Y, Yoshida S. Functional identification of the actual and potential stem cell compartments in mouse spermatogenesis. Developmental cell. 2007;12(2):195-206.
55. Klein AM, Doupe DP, Jones PH, Simons BD. Mechanism of murine epidermal maintenance: cell division and the voter model. Physical review E, Statistical, nonlinear, and soft matter physics. 2008;77(3 Pt 1):031907.

56. Nakagawa T, Sharma M, Nabeshima Y, Braun RE, Yoshida S. Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. Science (New York, NY). 2010;328(5974):62-7.
57. Goriely A, McVean GA, Rojmyr M, Ingemarsson B, Wilkie AO. Evidence for selective advantage of pathogenic FGFR2 mutations in the male germ line. Science (New York, NY). 2003;301(5633):643-6.
58. Giannoulatou E, McVean G, Taylor IB, McGowan SJ, Maher GJ, Iqbal Z, et al. Contributions of intrinsic mutation rate and selfish selection to levels of de novo HRAS mutations in the paternal germline. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(50):20152-7.
59. Clevers H. The intestinal crypt, a prototype stem cell compartment. Cell. 2013;154(2):274-84.
60. Cheng H, Leblond CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cell. The American journal of anatomy. 1974;141(4):461-79.
61. Cheng H, Leblond CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian Theory of the origin of the four epithelial cell types. The American journal of anatomy. 1974;141(4):537-61.
62. Winton DJ, Blount MA, Ponder BA. A clonal marker induced by mutation in mouse intestinal epithelium. Nature. 1988;333(6172):463-6.
63. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007;449(7165):1003-7.
64. Sangiorgi E, Capecchi MR. Bmi1 is expressed in vivo in intestinal stem cells. Nature genetics. 2008;40(7):915-20.
65. Takeda N, Jain R, LeBoeuf MR, Wang Q, Lu MM, Epstein JA. Interconversion between intestinal stem cell populations in distinct niches. Science (New York, NY). 2011;334(6061):1420-4.
66. Munoz J, Stange DE, Schepers AG, van de Wetering M, Koo BK, Itzkovitz S, et al. The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent '+4' cell markers. The EMBO journal. 2012;31(14):3079-91.
67. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459(7244):262-5.
68. de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature. 2011;476(7360):293-7.
69. Ritsma L, Ellenbroek SI, Zomer A, Snippert HJ, de Sauvage FJ, Simons BD, et al. Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging. Nature. 2014;507(7492):362-5.
70. Kozar S, Morrissey E, Nicholson AM, van der Heijden M, Zecchini HI, Kemp R, et al. Continuous clonal labeling reveals small numbers of functional stem cells in intestinal crypts and adenomas. Cell stem cell. 2013;13(5):626-33.
71. Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, et al. Defining the epithelial stem cell niche in skin. Science (New York, NY). 2004;303(5656):359-63.

72. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell. 1990;61(7):1329-37.
73. Rompolas P, Greco V. Stem cell dynamics in the hair follicle niche. Seminars in cell & developmental biology. 2014;25-26:34-42.
74. Rompolas P, Mesa KR, Greco V. Spatial organization within a niche as a determinant of stem-cell fate. Nature. 2013;502(7472):513-8.
75. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature. 2006;441(7097):1068-74.
76. Fuchs E, Horsley V. Ferreting out stem cells from their niches. Nature cell biology. 2011;13(5):513-8.
77. Watt FM, Huck WT. Role of the extracellular matrix in regulating stem cell fate. Nature reviews Molecular cell biology. 2013;14(8):467-73.
78. Tian H, Biehs B, Warming S, Leong KG, Rangell L, Klein OD, et al. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature. 2011;478(7368):255-9.
79. Buczacki SJ, Zecchini HI, Nicholson AM, Russell R, Vermeulen L, Kemp R, et al. Intestinal label-retaining cells are secretory precursors expressing Lgr5. Nature. 2013;495(7439):65-9.
80. Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development (Cambridge, England). 1990;110(4):1001-20.
81. Metcalfe C, Kljavin NM, Ybarra R, de Sauvage FJ. Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration. Cell stem cell. 2014;14(2):149-59.
82. Yoshida S, Sukeno M, Nabeshima Y. A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis. Science (New York, NY). 2007;317(5845):1722-6.
83. Hardy RW, Tokuyasu KT, Lindsley DL, Garavito M. The germinal proliferation center in the testis of Drosophila melanogaster. Journal of ultrastructure research. 1979;69(2):180-90.
84. Kiger AA, White-Cooper H, Fuller MT. Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature. 2000;407(6805):750-4.
85. Song X, Xie T. DE-cadherin-mediated cell adhesion is essential for maintaining somatic stem cells in the Drosophila ovary. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(23):14813-8.
86. Kronen MR, Schoenfelder KP, Klein AM, Nystul TG. Basolateral junction proteins regulate competition for the follicle stem cell niche in the Drosophila ovary. PloS one. 2014;9(7):e101085.
87. Leatherman JL, Dinardo S. Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in Drosophila testes. Nature cell biology. 2010;12(8):806-11.
88. Brawley C, Matunis E. Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. Science (New York, NY). 2004;304(5675):1331-4.
89. Kai T, Spradling A. Differentiating germ cells can revert into functional stem cells in Drosophila melanogaster ovaries. Nature. 2004;428(6982):564-9.

90. Hermann BP, Sukhwani M, Hansel MC, Orwig KE. Spermatogonial stem cells in higher primates: are there differences from those in rodents? Reproduction (Cambridge, England). 2010;139(3):479-93.
91. Montgomery RK, Carlone DL, Richmond CA, Farilla L, Kranendonk ME, Henderson DE, et al. Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(1):179-84.
92. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. Bacterial persistence as a phenotypic switch. Science (New York, NY). 2004;305(5690):1622-5.
93. Jones DL, Wagers AJ. No place like home: anatomy and function of the stem cell niche. Nature reviews Molecular cell biology. 2008;9(1):11-21.
94. Scadden DT. The stem-cell niche as an entity of action. Nature. 2006;441(7097):1075-9.
95. Liu L, Rando TA. Manifestations and mechanisms of stem cell aging. The Journal of cell biology. 2011;193(2):257-66.
96. Jones DL, Rando TA. Emerging models and paradigms for stem cell ageing. Nature cell biology. 2011;13(5):506-12.
97. Nijnik A, Woodbine L, Marchetti C, Dawson S, Lambe T, Liu C, et al. DNA repair is limiting for haematopoietic stem cells during ageing. Nature. 2007;447(7145):686-90.
98. Inomata K, Aoto T, Binh NT, Okamoto N, Tanimura S, Wakayama T, et al. Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation. Cell. 2009;137(6):1088-99.
99. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science (New York, NY). 2014;344(6184):630-4.
100. Ryu BY, Orwig KE, Oatley JM, Avarbock MR, Brinster RL. Effects of aging and niche microenvironment on spermatogonial stem cell self-renewal. Stem cells (Dayton, Ohio). 2006;24(6):1505-11.
101. Challen GA, Boles NC, Chambers SM, Goodell MA. Distinct hematopoietic stem cell subtypes are differentially regulated by TGF-beta1. Cell stem cell. 2010;6(3):265-78.
102. Lu CP, Polak L, Rocha AS, Pasolli HA, Chen SC, Sharma N, et al. Identification of stem cell populations in sweat glands and ducts reveals roles in homeostasis and wound repair. Cell. 2012;150(1):136-50.
103. Van Keymeulen A, Rocha AS, Ousset M, Beck B, Bouvencourt G, Rock J, et al. Distinct stem cells contribute to mammary gland development and maintenance. Nature. 2011;479(7372):189-93.

Figures

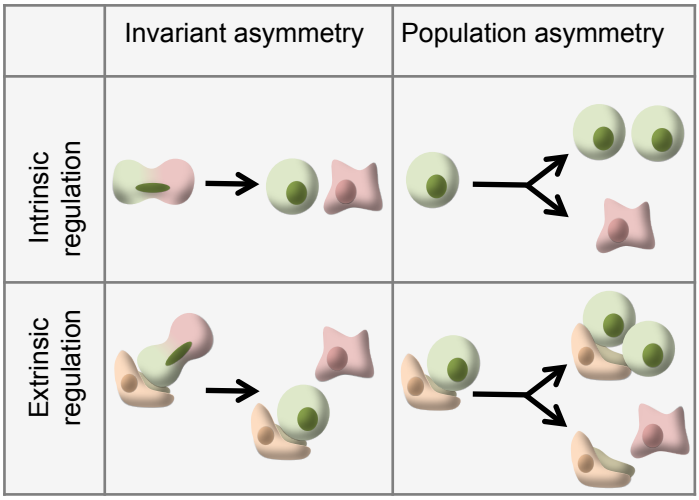
Fig. 1. Proliferative hierarchies and patterns of stem cell self-renewal. (A) During tissue homeostasis, patterns of adult stem cell self-renewal can be grouped into four generic classes depending on whether stem cell fate is regulated intrinsically (cell-autonomously), or whether it relies on extrinsic signals associated with the niche/ microenvironment, and whether fate asymmetry is enforced at each and every stem cell division, or whether it is achieved only at the level of the population. (B) Traditionally, adult stem cell populations are thought to reside at the apex of linear (i.e. "one-way") proliferative hierarchies in which they give rise to one or more types of transit-amplifying cell progeny with strictly limited proliferative potential. (C) Recent studies suggest a more flexible organization in which long-term self-renewal potential, fate bias, and proliferative activity may be moderated by niche location and/or dynamical changes in transcriptional activity. In this scheme, stem cells form a "dynamically heterogeneous" pool in which cells may transfer reversibly between "states" of variable survival and fate potential. In addition, progenitors that are normally committed to differentiation may reacquire long-term self-renewal potential in crisis or injury, following exposure to niche factors.

Fig. 2. Stem cell dynamics during mammalian spermatogenesis. (A) Schematic showing the architecture and cellular organization of the mammalian testis. Spermatogonia lie in close association with the basement membrane of the seminiferous tubule. When meiosis begins, they detach from the basement membrane, translocate across the tight junctions between supporting Sertoli cells and undergo meiotic divisions and differentiation before their release into the lumen as mature sperm. (B) Spermatogonia progress through a differentiation hierarchy while migrating from the basement membrane to the lumen. (C) In the undifferentiated compartment, spermatogonia can exist as singly isolated cells (termed A_{single} or A_s) or as syncytial chains of 2 (A_{pair} or A_{pr}), 4 ($A_{aligned\text{-}4}$ or $A_{al\text{-}4}$), 8 ($A_{al\text{-}8}$), or 16 ($A_{al\text{-}16}$) cells. Undifferentiated spermatogonia are characterized by heterogeneous and complementary expression of GFRα1 and Ngn3, with A_s and smaller syncytial chains biased towards GFRα1. Following up-regulation of Ngn3, spermatogonia are competent to transfer to the differentiated Kit+ compartment, in concert with the periodic seminiferous cycle. (D) Whole-mount (top panels) of a seminiferous tubule showing GFRα1 expression (magenta) and GFP-labelled clones (green) at 14 days post clonal induction. Fragmentation of an $A_{al\text{-}4}$ syncytial chain results in two A_{pr} chains. (Courtesy of Ref. (34).) Schematic (bottom) showing the cellular basis for germ line stem cell maintenance: chance stem cell loss through differentiation, signaled by the down-regulation of GFRα1, is perfectly compensated by stem cell duplication achieved through the fragmentation of neighboring GFRα1+ syncytia. Through this ongoing process of stem cell loss through differentiation and replacement, stem cell-derived clones follow a "quasi" one-dimensional pattern of "neutral drift" where their chance extinction is compensated by the expansion of neighbors along the seminiferous tubule.

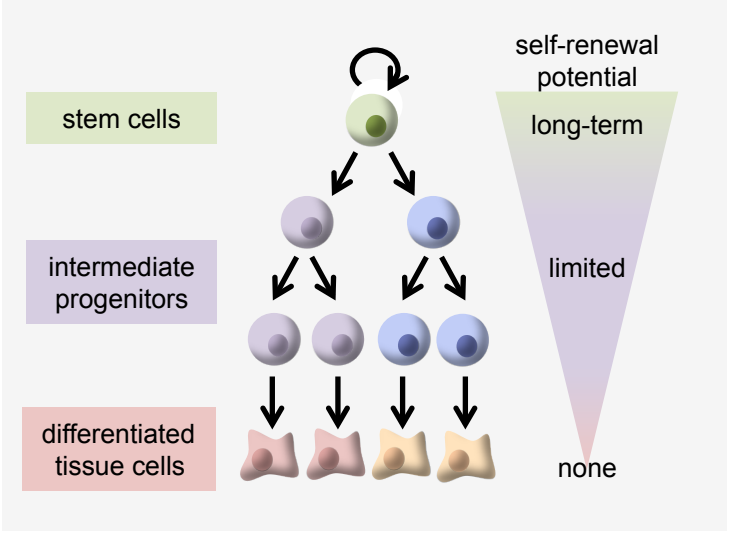
Fig. 3. Stem cell dynamics during intestinal maintenance. (A) Schematic showing the cellular organization of the mammalian small intestine. In adults, stem cells at the intestinal crypt base exhibit multi-lineage potential, giving rise to transit-amplifying cell progeny, which migrate along the walls of the crypt and differentiate into functional secretory and absorptive cell types. (B) On the basis of genetic lineage tracing assays, the intestinal stem cell compartment has been associated with several molecular markers (including Lgr5, Bmi1, and Hopx), which are expressed heterogeneously within the crypt. (C) Time-lapse *in-vivo* clonal data depicting the process of dynamic heterogeneity. Upper panels: Following genetic labeling, a clone marked by RFP containing two Lgr5-positive cells (GFP), both at the niche border, expands over the next 3 days to occupy both border and niche base regions. Lower panels: A clone containing 3 Lgr5+ cells all at the niche base expands to occupy both border and base regions. (Courtesy of Ref. (67).) Through this process of loss and replacement, stem cell-derived clones follow a quasi one-dimensional pattern of neutral drift in which their chance extinction is perfectly compensated by the expansion of neighbors around the collar of the crypt, leading to scaling of the clone size distribution.

Fig. 4. Stem cell dynamics in the hair follicle. (A) Schematic of mouse hair follicle during the resting phase of the hair cycle. Stem cells reside in the bulge and hair germ, while other epithelial cell populations occupy the compartments located above the bulge. Clones derived from stem cells situated in different parts of the niche have been observed to follow different fates, with cells in the hair germ primed for differentiation while cells in the upper part of the bulge are mostly quiescent. (B) Hair growth and stem cell self-renewal in the bulge is driven by a hetereogenous progenitor population. While cells express Keratin 15 and Sox9 throughout the compartment, CD34 is expressed more strongly towards the upper end of the bulge, and Lgr5 is enriched proximally. (C) Following ablation of either the hair germ or the bulge region, the entire niche is repopulated through proliferation and migration of the remaining stem cells. In response to ablation of the entire stem cell niche, non-hair epithelial cells migrate downwards to regenerate the stem cell compartment.

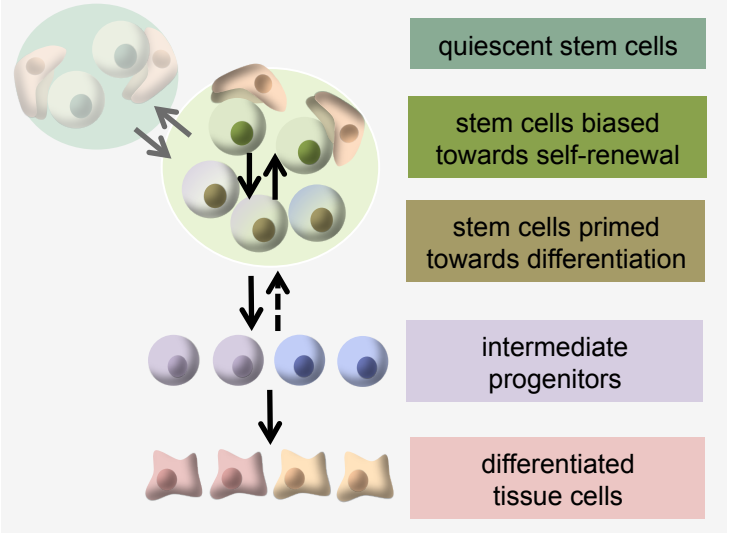
A

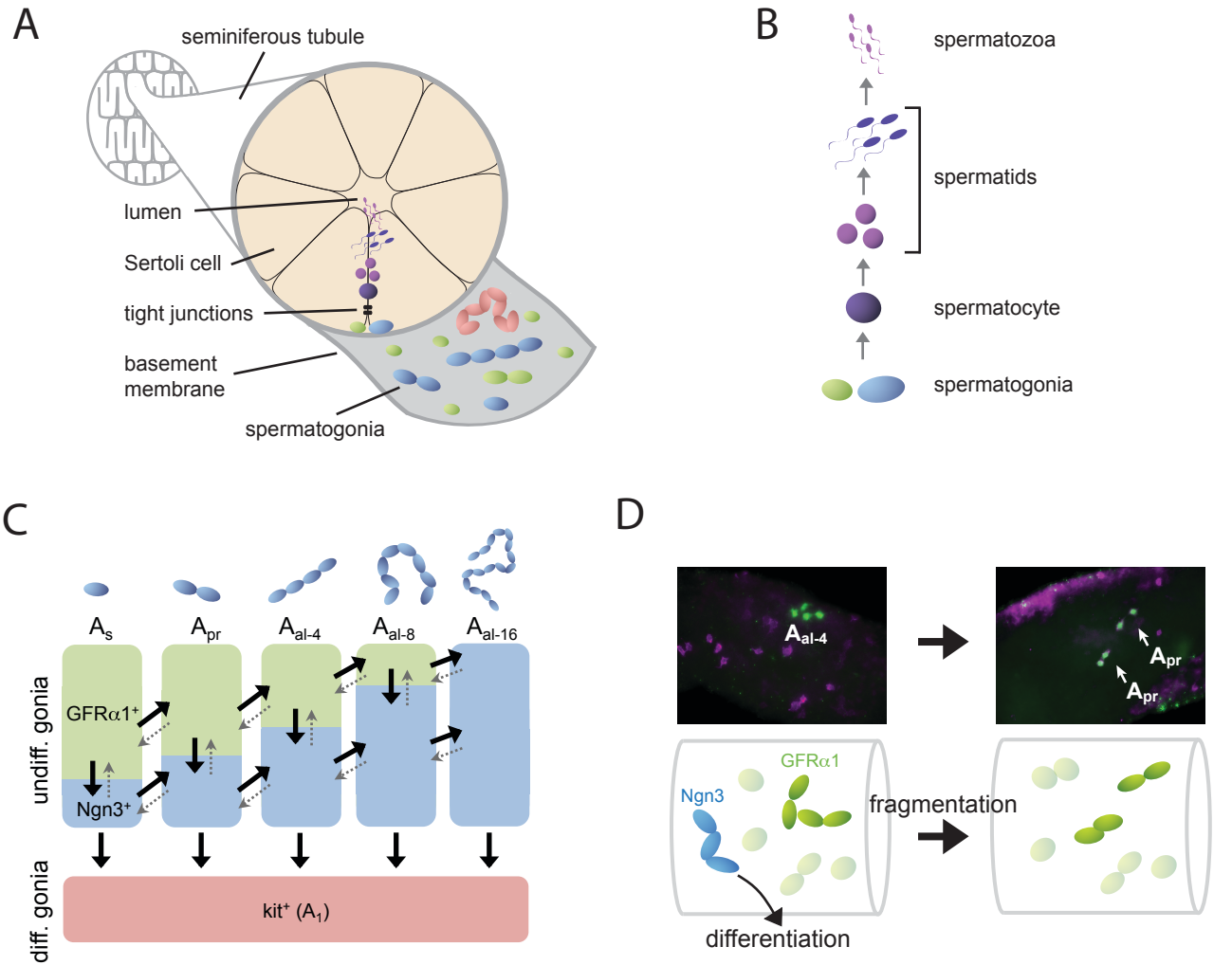

Invariant asymmetry
Population asymmetry
Intrinsic regulation
Extrinsic regulation

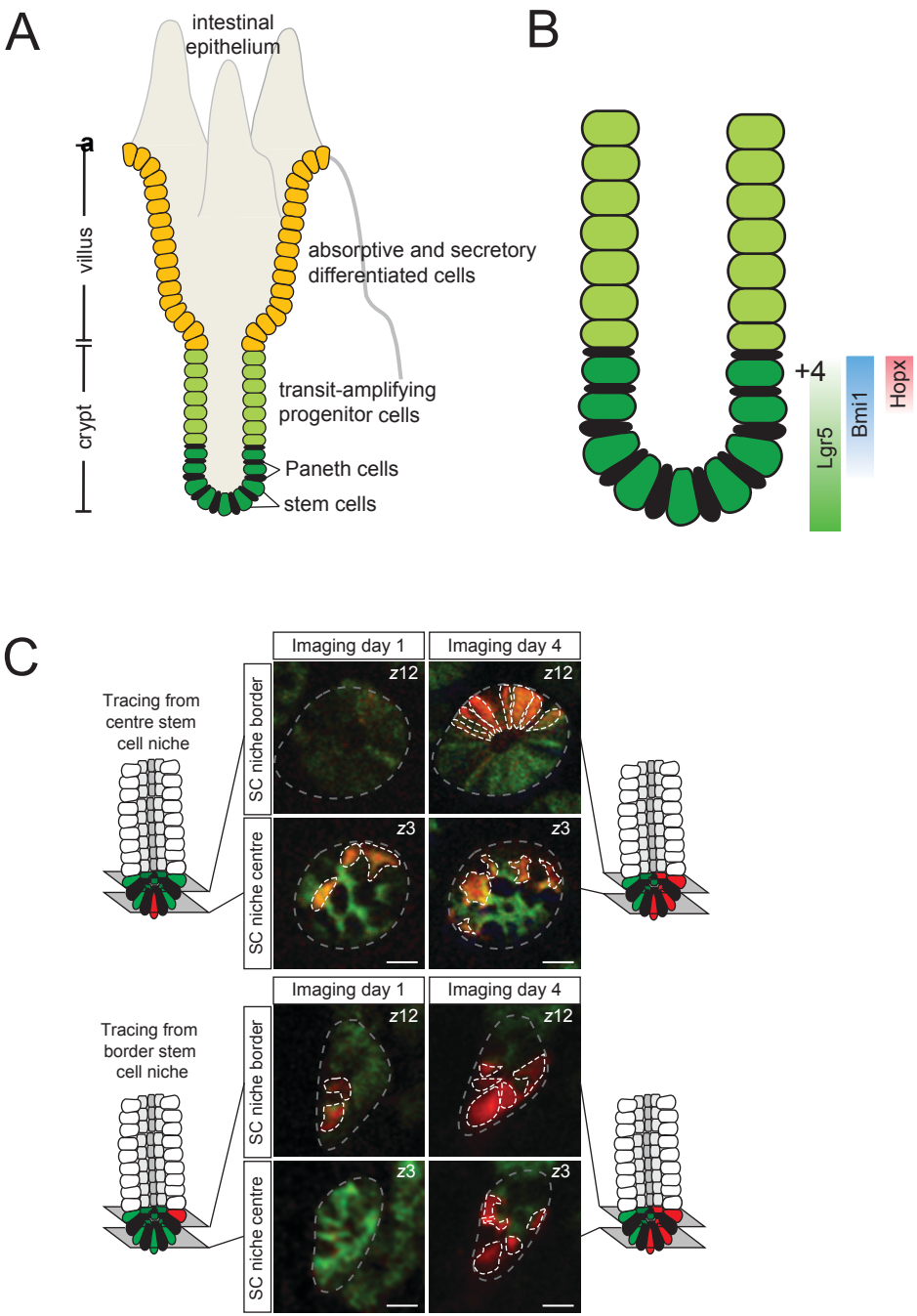
B

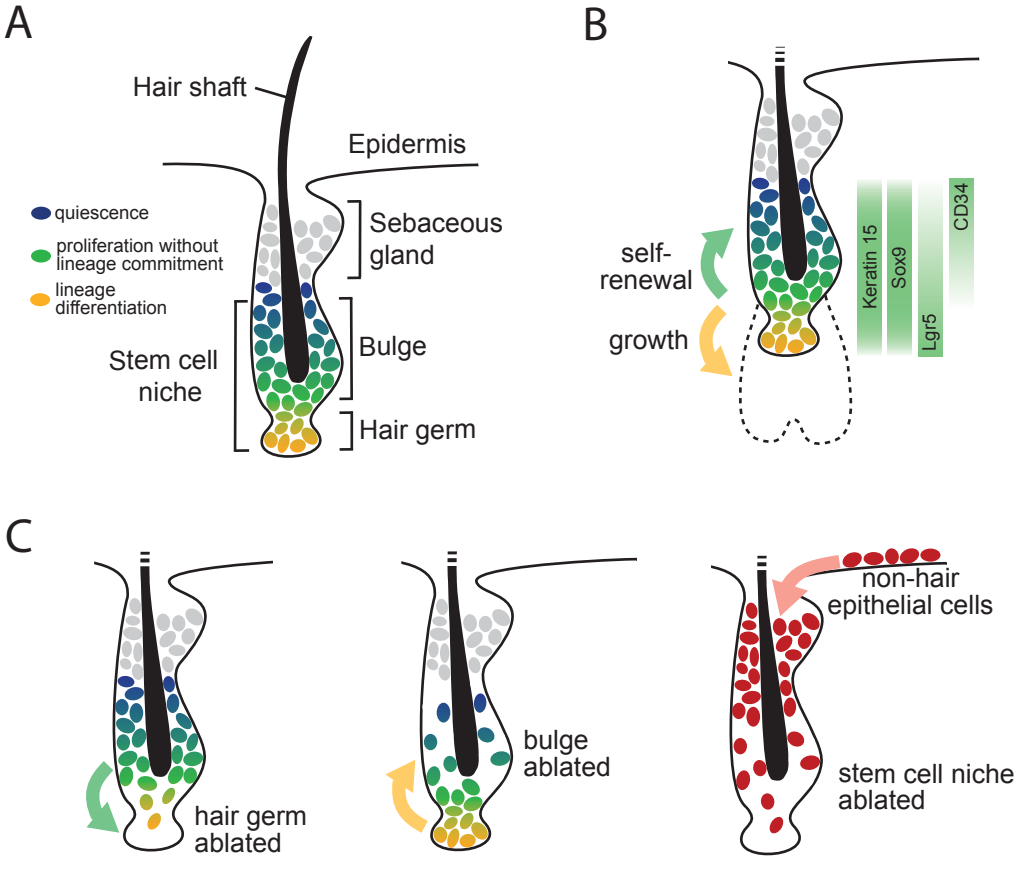

self-renewal potential
stem cells
long-term
intermediate progenitors
limited
differentiated tissue cells
none

C


quiescent stem cells
stem cells biased towards self-renewal
stem cells primed towards differentiation
intermediate progenitors
differentiated tissue cells


A
seminiferous tubule
lumen
Sertoli cell
tight junctions
basement membrane
spermatogonia
B
spermatozoa
spermatids
spermatocyte
spermatogonia
C
A_s
A_{pr}
A_{al-4}
A_{al-8}
A_{al-16}
GFRα1+
Ngn3+
undiff. gonia
diff. gonia
kit+ (A_1)
D
A_{al-4}
A_{pr}
A_{pr}
GFRα1
Ngn3
fragmentation
differentiation


A
intestinal epithelium
villus
crypt
absorptive and secretory differentiated cells
transit-amplifying progenitor cells
Paneth cells
stem cells
B
+4
Lgr5
Bmi1
Hopx
C
Imaging day 1
Imaging day 4
Tracing from centre stem cell niche
SC niche border
SC niche centre
z12
z3
Tracing from border stem cell niche


A
Hair shaft
Epidermis
quiescence
proliferation without lineage commitment
lineage differentiation
Sebaceous gland
Bulge
Stem cell niche
Hair germ
B
self-renewal
growth
Keratin 15
Sox9
Lgr5
CD34
C
hair germ ablated
bulge ablated
non-hair epithelial cells
stem cell niche ablated