

Switching roles: the functional plasticity of adult tissue stem cells

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Abstract

Adult organisms have to adapt to survive, and the same is true for their tissues. Rates and types of cell production must be rapidly and reversibly adjusted to meet tissue demands in response to both local and systemic challenges. Recent work reveals how stem cell (SC) populations meet these requirements by switching between functional states tuned to homeostasis or regeneration. This plasticity extends to differentiating cells, which are capable of reverting to SCs after injury. The concept of the niche, the micro-environment that sustains and regulates stem cells, is broadening, with a new appreciation of the role of physical factors and hormonal signals. Here, we review different functions of SCs, the cellular mechanisms that underlie them and the signals that bias the fate of SCs as they switch between roles.

Keywords differentiation; niche; regeneration; signal transduction; stem cells
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Introduction

Tissues are diverse not only in their structure and function but also in the dynamics of their constituent cells (Leblond & Walker, 1956). SC turnover rates in adult mouse lineages vary by orders of magnitude (Fig 1). Epithelial and male germ SCs cycle rapidly, while in tissues such as liver, kidney and muscle, proliferation is scarcely detectable. In homeostasis, on average, one SC and one differentiating cell are produced per SC division, and the rate of SC proliferation is matched with the rate of loss of cells from the differentiated lineage(s) it supports. Following damage, however, SCs must rapidly and reversibly switch to produce an excess of proliferating cells to regenerate the lost tissue. Here, we will define SCs in purely functional terms as cell populations that sustain long-term tissue turnover and/or contribute to tissue repair after injury. We first survey SC behaviour in high turnover and quiescent tissues in homeostasis, then consider how SCs respond to injury and conclude by considering the physical and molecular signals which switch SC behaviour to meet the requirements of the organism.

The functional repertoire of SCs

There are two cellular mechanisms by which SCs may maintain homeostasis (Watt & Hogan, 2000). A population of SCs may behave so that on average, 50% of their progeny are themselves SCs and 50% differentiating cells. Such ‘population asymmetry’ may result from the cell autonomous properties of SCs or external regulation, for example by a niche of restricted size in which SCs can only divide when a cell differentiates and leaves the niche. Alternatively, each individual SC may divide asymmetrically. Advances in lineage tracing and live imaging reveal population asymmetry emerges as the predominant mechanism in tissues with a high rate of turnover (Alcolea & Jones, 2013; Rompolas & Greco, 2014). We will focus on tissues in mice where robust evidence on SC behaviour is available, before turning to other species.

Some of the first insights into SC dynamics *in vivo* came from studies of squamous epithelia, which cover the skin, mouth and oesophagus and predominantly consist of layers of keratinocytes. Proliferation is confined to the deepest, basal cell layer, which forms the SC niche (Fig 2A). When dividing cells commit to differentiation, they withdraw from the cell cycle and migrate out of the basal layer (Alcolea & Jones, 2014). Large-scale lineage tracing has revealed that cellular homeostasis is achieved by a single population of keratinocyte SCs in the basal cell layer (Clayton *et al*, 2007; Doupe *et al*, 2010, 2012; Mascre *et al*, 2012; Lim *et al*, 2013). The outcome of individual cell divisions is unpredictable, generating two SCs, two differentiating cells or one cell of each type (Alcolea & Jones, 2013) (Fig 2A). However, the probabilities of each division outcome are balanced, so an equal number of SCs and differentiating cells are generated across the basal layer, an example of ‘population asymmetry’ (Watt & Hogan, 2000; Clayton *et al*, 2007). The random element in SC fate appears at odds with histological features such as the ordered columns of differentiating cells seen in mouse epidermis, but these arise simply from the physical packing properties of suprabasal cells rather than reflecting deterministic SC behaviour (Doupe *et al*, 2010). Analysis of lineage-tracing data argues that the balanced stochastic fate of squamous SCs is a cell-intrinsic property rather than a consequence of external regulation, but the molecular machinery that achieves this is unknown (Clayton *et al*, 2007; Jones & Simons, 2008).

The epidermis is punctuated by appendages in the form of hair follicles (HFs) and sweat ducts, each a self-maintaining mini-organ

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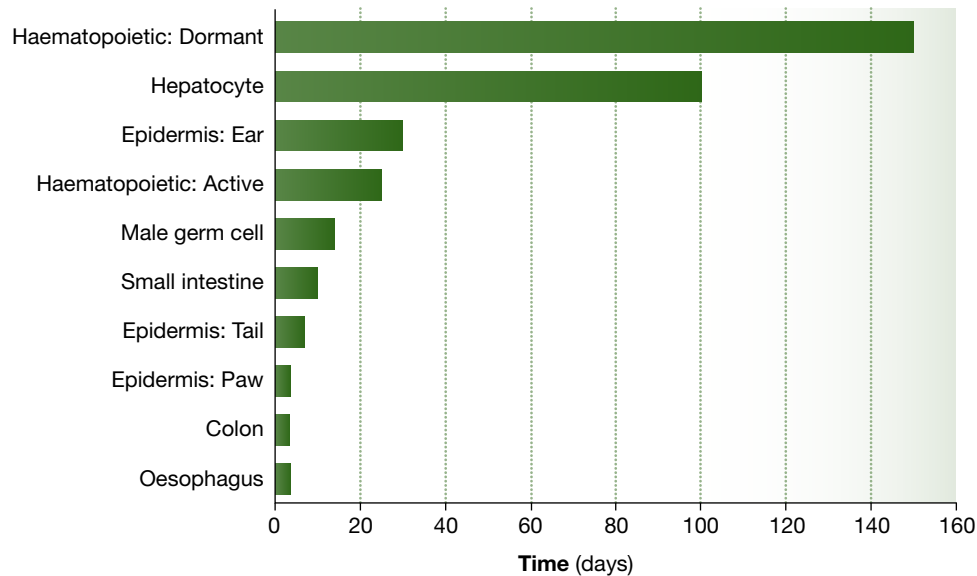


Figure 1. SC turnover across mouse tissues.
Average cycle times for SCs in the lineages indicated.

containing multiple cell lineages (Schepeler *et al*, 2014). HF are split into functionally discrete compartments (Page *et al*, 2013). The lower hair follicle undergoes cyclical expansion (anagen) and contraction (catagen) followed by a period of quiescence (telogen) (Fig 2B). The HF is homeostatic in SC numbers, and tissue organisation remains the same at telogen phase of each successive hair cycle. HF are sustained by SC populations in a region known as the

bulge and the adjacent hair germ (Rompolas & Greco, 2014). Lineage tracing and cell culture studies have revealed a bewildering range of apparently overlapping candidate SCs in HF, though it is unclear whether variations in gene expression mark functionally distinct SC populations (Rompolas & Greco, 2014; Schepeler *et al*, 2014).

The HF bulge, which marks the upper limit of the lower follicle, was first identified as a distinct SC niche as it contains slow-cycling

Figure 2. SC dynamics across lineages.

(A) Squamous epithelia consist of layers of keratinocytes, SCs (green) reside in the basal cell layer, along with post-mitotic cells waiting to stratify (red). When a differentiating cell leaves the niche (arrow), a nearby SC divides with one of the three division outcomes shown to maintain constant cell density in the niche. The probabilities of each outcome (expressed as per cent, oesophageal epithelium shown) are balanced, so equal numbers of SCs and differentiated cells are produced across the population. (B) The hair follicle cycles between a resting stage (telogen) and expansion of the lower follicle (anagen). Multiple SCs have been identified, in the junctional zone (purple), the bulge (green) and the hair germ (blue), which lie in contact with the mesenchymal cells of the dermal papilla (blue). The hair shaft (black) is surrounded by concentric layers of inner root sheath cells that have been omitted for clarity. In the transition into anagen, hair germ and upper bulge cells self-duplicate. In the bulge, divisions are aligned parallel with the axis of the hair shaft (inset). Lower bulge cells contribute differentiating progeny to greatly expand the root sheath. Later in anagen, hair germ cells assemble around the dermal papilla and then generate inner root sheath cells (arrows). Self-renewal and differentiation are balanced, so the numbers of SCs in each compartment is maintained at a constant level across multiple hair cycles. (C) Intestinal epithelium contains four lineages sustained by SCs (green) that lie between Paneth cells in the crypt base. Differentiating cells migrate through a progenitor compartment in the upper crypt from which post-mitotic cells populate the villus, from which they are shed. Inset shows a simplified top-down view of the niche. As a differentiating SC exits the niche, it is replaced by the self-duplicating division of an immediately adjacent SC. (D) Haematopoietic SCs reside close to blood vessels in the bone marrow. Dormant SCs (green) lie close to arterioles, receiving paracrine signals from endothelial cells (red), perivascular cells expressing *Ng2* and sympathetic nerve endings (blue). On activation, SCs migrate to be close to venous sinusoids that support *Lepr*-expressing perivascular cells. (E) Asymmetric fate and symmetric fate of haematopoietic SCs. Transplantation of daughter cells of a single SC reveals self-duplicating and asymmetric divisions: dark green: long-term-reconstituting SCs; light green: short- or intermediate-term-reconstituting SCs; yellow: megakaryocyte progenitor; and blue: common myeloid progenitor. (F) Native haematopoiesis. Lineage tracing in homeostasis suggests that myeloid lineages may be maintained by self-sustaining progenitor cells (yellow) exhibiting 'population asymmetry' by generating equal proportions of progenitor and differentiating cells (grey) (see inset). Haematopoietic SCs (green) make negligible contribution to myelopoiesis in homeostasis, but function as 'reserve' cells. (G) Male germ cell SCs are diverse in appearance but functionally equivalent. Male germ cells expressing *GFRα1* reside in the outermost layer of the seminiferous tubule. SCs (green) exist as singles or 2–4 cell syncytia connected by cytoplasmic bridges, which may self-duplicate (blue arrows) to generate two SCs or undergo fragmentation (short red arrows). Upon differentiation (pink arrow) into *Ngn3*-positive cells (red), the same behaviour continues, but *Ngn3*⁺ cells are unlikely to revert to *GFRα1*⁺ SCs in homeostasis. Once in the *Ngn3* compartment, syncytia larger than 4 cells form which may undergo further differentiation into *ckit*-expressing cells (not shown), which are even less likely to revert to *GFRα1*-positive cells. (H) Neural SCs exhibit symmetric and asymmetric cell divisions. Quiescent neural SCs (light green) reside in the subventricular zone niche, extending processes into cerebrospinal fluid (blue) that fills the lateral ventricles (LV) and underlying endothelial cells (red) lining blood vessels (BV). Endothelial cells that line the LV are shown in grey. Differentiating transit amplifying cells (pink) and neural precursor cells (orange) lie adjacent to the SCs. Figure after Silva-Vargas *et al* (2013). (I) Division outcomes of neural SCs in the dentate gyrus inferred from lineage tracing. Neural SCs interconvert between quiescent (light green) and proliferating (dark green) states. Division of SCs has a range of symmetric and asymmetric outcomes as shown, generating neural SCs, neuroblasts (orange) or differentiated astrocytes (blue).

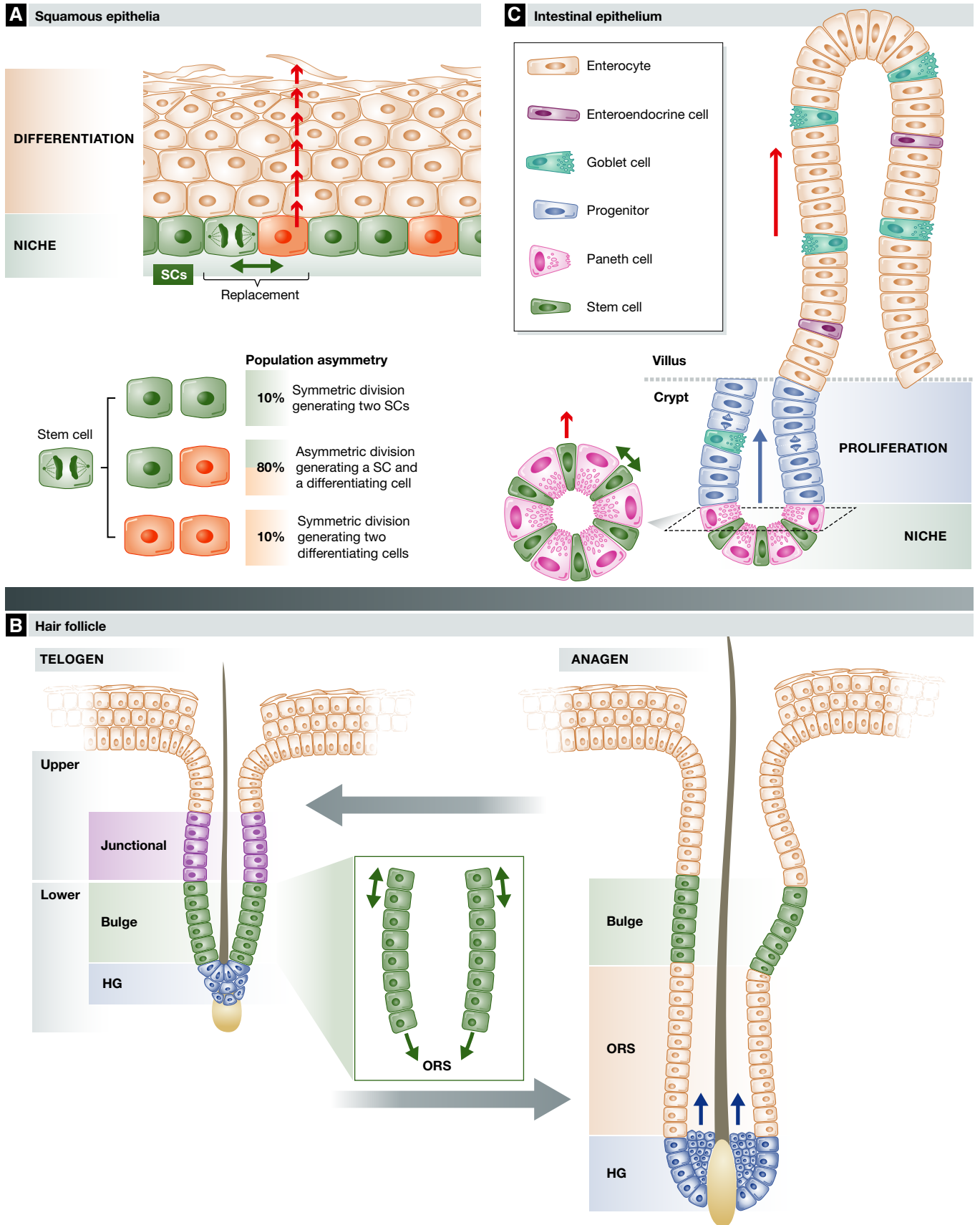


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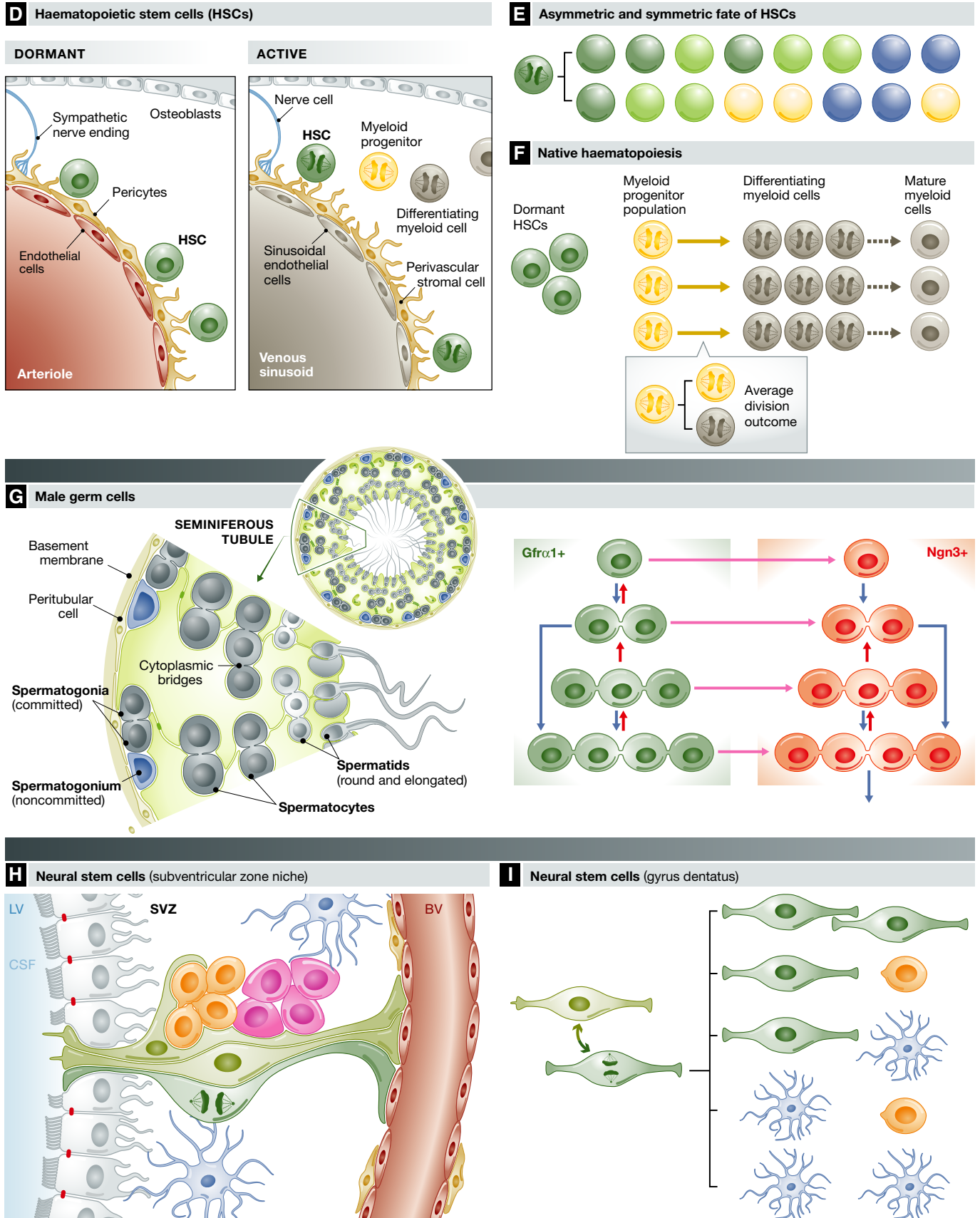


Figure 2.

cells (Cotsarelis *et al.*, 1990; Tumber *et al.*, 2004) (Fig 2B). Bulge SCs differentiate into multiple lineages when sorted and transplanted, and undergo both symmetric and asymmetric divisions (Blanpain *et al.*, 2004; Waghmare *et al.*, 2008; Zhang *et al.*, 2009). More recently, lineage tracing and intra-vital imaging have shown that SCs in the hair germ are the first cells to be mobilised as HF enter anagen, undergoing apparently symmetric self-duplicating divisions aligned with the long axis of the HF (Greco *et al.*, 2009; Rompolas *et al.*, 2012). Only when the exponentially expanding hair germ has morphed to envelop a bud of mesenchyme called the dermal papilla (DP) SCs differentiate into 'transit amplifying' cells that will form the inner root sheath that surrounds the hair shaft (Rompolas *et al.*, 2012). Signals from the DP are essential for the progression of the hair cycle; indeed, the number of cells in the DP determines hair size and shape (Rompolas *et al.*, 2012; Chi *et al.*, 2013). In the bulge, SCs remain in slow cycling or quiescent, until a proportion of cells are recruited into division by signals from TA cells. These induce SCs to first contribute to the outer root sheath and then undergo self-renewal until the bulge is repopulated with SCs, when quiescence resumes (Hsu *et al.*, 2014) (Fig 2B). HF expansion is followed by involution (catagen), and by the time the telogen phase is reached, bulge and hair germ SCs are restored, achieving homeostasis over the entire cycle (Hsu *et al.*, 2014).

The glandular epithelium that lines the intestine is also rapidly turned over but has a distinctive tissue organisation that contributes to SC regulation. The differentiated cells on the finger-like villi are shed rapidly from the villus tip. Cell replacement is achieved by SCs and progenitor cells located in pits known as crypts (Clevers, 2013) (Fig 2C). Cycling SCs lie at the crypt base among cells expressing the Wnt target gene *Lgr5*, maintaining four lineages of differentiated cells via the lineage-committed progenitor cells that populate the upper crypt (Barker *et al.*, 2007). Differentiated Paneth cells lie next to SCs and secrete signals to sustain SCs in the niche (Sato *et al.*, 2011b). The requirement for paracrine signals restricts the size of the SC pool and ensures homeostasis. Lineage tracing indicates that as a SC differentiates, it migrates out of the niche and is replaced by the self-duplicating division of a neighbouring SC (Fig 2C) (Lopez-Garcia *et al.*, 2010; Snippert *et al.*, 2010). It was initially thought that all *Lgr5*⁺ cells were cycling functionally equivalent SCs, but a recent study has shown only 5 of the 14–15 *Lgr5*⁺ cells in each crypt base are proliferating at any one time (Kozar *et al.*, 2013). Live imaging further suggests the likelihood of a SC differentiation depends on its location, increasing in the uppermost part of the niche (Ritsma *et al.*, 2014). Analysis of spontaneous clones carrying mitochondrial mutations argues similar SC behaviour maintains human colonic epithelium (Baker *et al.*, 2014). Intestinal homeostasis is thus achieved by linking differentiation to self-duplicating SC division within a niche of restricted size.

It has long been argued that the prodigious cell turnover of the haematopoietic system is sustained by rare SCs, found both in the circulation and in a specific niche environment in the bone marrow. Haematopoietic SCs (HSCs) are assayed by their ability to reconstitute the blood system in the long term following transplantation into irradiated host animals (Babovic & Eaves, 2014; Ema *et al.*, 2014). Highly purified HSCs expressing cell surface identical markers may be cycling or dormant, but only the latter group has long-term transplant potential (Fig 1) (Wilson *et al.*, 2008; Foudi *et al.*, 2009; Catlin *et al.*, 2011; Anjos-Afonso *et al.*, 2013; Oguro *et al.*, 2013). Dormant

HSCs reside in a distinct niche environment, next to arterioles, and migrate to lie close to venous sinusoids on activation (Fig 2D) (Kunisaki *et al.*, 2013; Mendelson & Frenette, 2014). Transplant assays argue that haematopoiesis is a hierarchy, with HSCs continually generating progenitors that become progressively restricted in both lineage and self-renewal potentials as they differentiate, but recent discoveries are challenging this view. Transplantation of daughters from a single HSC division reveals self-duplicating and asymmetric divisions which generate a variety of differentiating progenitors directly from HSCs, challenging the view of an ordered differentiation programme (Yamamoto *et al.*, 2013) (Fig 2E). More remains to be discovered about the apparent heterogeneity among HSCs with long-term transplant potential. Some of this may result from HSCs commuting between different states that vary in their ability to survive the rigours of transplantation (Ema *et al.*, 2014; van Galen *et al.*, 2014). Recently, transposon-based lineage tracing has revealed native haematopoiesis is very different from that in transplanted animals. Rather than myeloid lineages deriving from a few HSCs, as is seen after transplantation, numerous short-lived progenitor clones are observed, consistent with myelopoiesis being maintained by a self-sustaining progenitor pool exhibiting population asymmetry (Fig 2F) (Sun *et al.*, 2014b). This argues for a separation of roles in haematopoiesis, with a pool of progenitors maintaining the myeloid lineage in homeostasis and quiescent HSCs functioning as a 'reserve', mobilised to expand the progenitor population when the demand for myeloid cells rises (Wilson *et al.*, 2008; Sun *et al.*, 2014b). Different SC functions may thus be devolved to discrete populations in some tissues.

The final high turnover lineage we will consider is that of male germ cells. Spermatogenesis continues throughout life, sustained by a population of SCs, which reside in the basal cell layer of the seminiferous tubule, express *GFR α 1* and are negative for the differentiation marker *Ngn3*. Spermatogonia undergo incomplete cell division, generating syncytial clusters of cells linked by cytoplasmic bridges (Yoshida, 2012) (Fig 2G). It was thought that only single *GFR α 1*⁺ cells were SCs, but combined live imaging and lineage tracing have revealed that all *GFR α 1*⁺ cells, whether single or in syncytia of up to 4 cells, are functionally equivalent SCs (Hara *et al.*, 2014). Quantitative modelling argues SCs divide symmetrically producing two SC daughters, while the stochastic differentiation of a SC into a *Ngn3*⁺ cell appears linked to syncytial fragmentation by an as yet unknown mechanism. In combination, these mechanisms achieve population asymmetry as SC division is balanced with differentiation across the *GFR α 1*⁺ compartment (Klein *et al.*, 2010; Hara *et al.*, 2014). *Ngn3*⁺ cells have a high probability of undergoing differentiation into cKit-expressing cells, but infrequently revert back to *GFR α 1* SC. Almost all cKit cells undergo terminal differentiation. The spermatogenesis paradigm supports the concept that SC function may reside in a pool of heterogeneous cells which interconvert between different states, so that there are multiple reversible paths by which a given SC may differentiate (Nakagawa *et al.*, 2010) (Hara *et al.*, 2014).

Turning to tissues with a low rate of turnover, it is now clear that both rodent and human brains, once thought to be post-mitotic, continue to produce new neurons throughout life which contribute to specific forms of learning and memory and responding to stress (Snyder *et al.*, 2011; Spalding *et al.*, 2013; Aimone *et al.*, 2014; Ernst *et al.*, 2014). Adult neural SCs are found in the subventricular zone (SVZ) lining the lateral ventricles and in the dentate gyrus (DG) of

the hippocampus and are largely quiescent (Fig 2H). Lineage tracing in transgenic mice reveals that proliferating DG SCs are capable of both asymmetric and symmetric divisions generating two SCs or two differentiating astroglial or neural progenitor daughters (Bonaguidi *et al*, 2011) (Fig 2I). After division, some SCs return to a quiescent state, negative for proliferation markers, paralleling the transition between dormant and active states seen in haematopoietic SCs.

Even establishing the existence of SCs in adult tissues with a very low rate of turnover is challenging. Studies in mouse and human support a slow rate of turnover of differentiated hepatocytes in the liver and glomerular and tubular cells in the kidney, though the existence of a discrete stem cell population in these tissues is controversial (Magami *et al*, 2002; Rodins *et al*, 2002; Fellous *et al*, 2009; Kusaba *et al*, 2014; Miyajima *et al*, 2014). In contrast, adult skeletal muscle contains a well-defined quiescent SC population, residing among the satellite cells that lie beneath the plasma membrane of terminally differentiated multinucleate muscle fibres (Brack & Rando, 2012). Muscle SCs typify the 'reserve' role of SCs, actively maintained in a quiescent state until they are mobilised following muscle injury (Cheung *et al*, 2012; Cheung & Rando, 2013).

In summary, there are two cellular mechanisms of population asymmetry in mammalian tissues with a high turnover (Watt & Hogan, 2000; Klein & Simons, 2011). The first, exemplified by squamous epithelia, is SCs within an 'open plan' niche with an apparently cell-intrinsic mechanism which balances the probabilities of generating SCs or differentiating progeny (Clayton *et al*, 2007; Alcolea & Jones, 2014). An alternative strategy seen in the intestine is having a niche of limited size in which self-duplicating SC division occurs as a differentiating cell exits the niche (Lopez-Garcia *et al*, 2010; Vermeulen & Snippert, 2014). In both these examples, the robust linkage of division and the exit of a nearby cell maintain a constant cellular density within the niche. Another common theme is that while the fate of an individual SC is unpredictable, homeostasis is achieved across the SC population. The male germ cell lineage illustrates the unreliability of using marker gene expression or cellular morphology to define SCs, as such characteristics may vary within a functionally equivalent pool of SCs. Likewise, active SCs may coexist with those within a dormant state in the hair follicle, bone marrow and intestinal crypt.

While the discussion above has focussed on mammalian systems, population asymmetry has also been observed in *Drosophila*, where neutral competition for a niche of fixed size is seen in ovarian SCs, while a balanced three way division outcome reminiscent of mammalian squamous epithelia is a feature of midgut SCs (de Navascues *et al*, 2012; Kronen *et al*, 2014). Intriguingly transgenic lineage tracing reveals that neural retina SCs of the medaka fish, *Orizias latipes*, have fixed asymmetric cell division in homeostasis, the first observation of such behaviour in an adult tissue (Centanin *et al*, 2014).

In summary, for the mammalian and *Drosophila* lineages where SC dynamics has been resolved, cellular homeostasis is achieved by population asymmetry, which may be cell intrinsic or niche specified. The fate of individual cells is unpredictable, but the probabilities of self-renewal and differentiation are balanced across the SC population, so equal proportions of SCs and differentiating cells are generated. Evolution has also sampled another mechanism of tissue maintenance, fixed asymmetric SC division, but to date, this has only been observed in fish retina.

Regeneration: using all the options

Tissue injury is an inevitable part of life. It is becoming clear that following damage, SCs and their differentiating progeny exhibit hitherto unexpected plasticity in their behaviour (Doupe & Jones, 2013). We first consider how cycling SC populations respond to injury before turning to the activation of quiescent SCs in low turnover tissues.

Wounding is frequent in surface epithelia. In the squamous epithelium of the oesophagus and the epidermis of the paw, SCs adjacent to a wound rapidly and reversibly switch to producing an excess of SCs, reverting to homeostatic behaviour once the defect is closed (Fig 3A) (Doupe *et al*, 2012; Lim *et al*, 2013). The need for such flexibility may be one of the reasons for three division outcomes in squamous epithelial SCs. Pure asymmetric division achieves cellular homeostasis but is inflexible, while adjusting the probability of the two types of symmetric division allows rapid modulation of cell production.

Most of the epidermis has a dense array of HFs, which normally make negligible contribution to homeostasis. Following injury, however, the progeny of bulge and upper follicle stem cells migrate into the epidermis and contribute extensively to wound repair (Ito *et al*, 2005; Levy *et al*, 2005; Page *et al*, 2013) (Fig 3B). In the specialised tail epidermis, which is sparse in HFs, rare, slow-cycling 'reserve' SCs are mobilised, while in hairless skin, sweat duct-derived cells contribute to wound healing (Lu *et al*, 2012; Mascré *et al*, 2012; Roshan & Jones, 2012). In humans, similar principles apply, but sweat ducts make a larger contribution to epidermal repair than HFs (Rittie *et al*, 2013).

Injury also induces the appearance of new HFs by a non-cell autonomous route. The infiltration of specialised gamma delta T cells following epidermal injury activates Wnt signalling via Fgf9 signalling (Gay *et al*, 2013). *Wnt* has been shown to alter the axis of division of bulge stem cells to generate a new hair follicle alongside an existing one (Deschene *et al*, 2014).

Recent developments in live imaging combined with laser ablation to delete cell subpopulations have also revealed unexpected plasticity in HFs. Following ablation, bulge SCs are replaced by the progeny from cells in the upper HF which normally make no contribution to the hair cycle (Fig 3C; Rempel *et al*, 2013; Rempel & Greco, 2014). Similarly, the hair germ can be replaced if the bulge SCs remain intact. Only destruction of the niche cells surrounding the bulge or separation of the DP from the hair germ halts hair regeneration, indicating the niche plays an essential role in hair regeneration.

Cellular plasticity extends beyond SCs crossing normal compartmental barriers. Following ablation of the Lgr5-positive cells in the intestinal crypt, differentiating lineage-committed precursor cells above the crypt base dedifferentiate into functional SCs which repopulate the crypt (Fig 3D) (van Es *et al*, 2012; Buczacki *et al*, 2013; Ritsma *et al*, 2014). In lung and gastric epithelia, reversion of post-mitotic differentiated cells into SCs is observed following injury (Stange *et al*, 2013; Tata *et al*, 2013). In non-epithelial tissues, evidence for dedifferentiation is more limited, but following cytotoxic drug treatment, the depleted male germ cell GFR α 1⁺ SC pool is regenerated by the combination of decreasing the probability of reversion of GFR α 1⁺ differentiation and reversion of Ngn3⁺ cells, most of which differentiate in

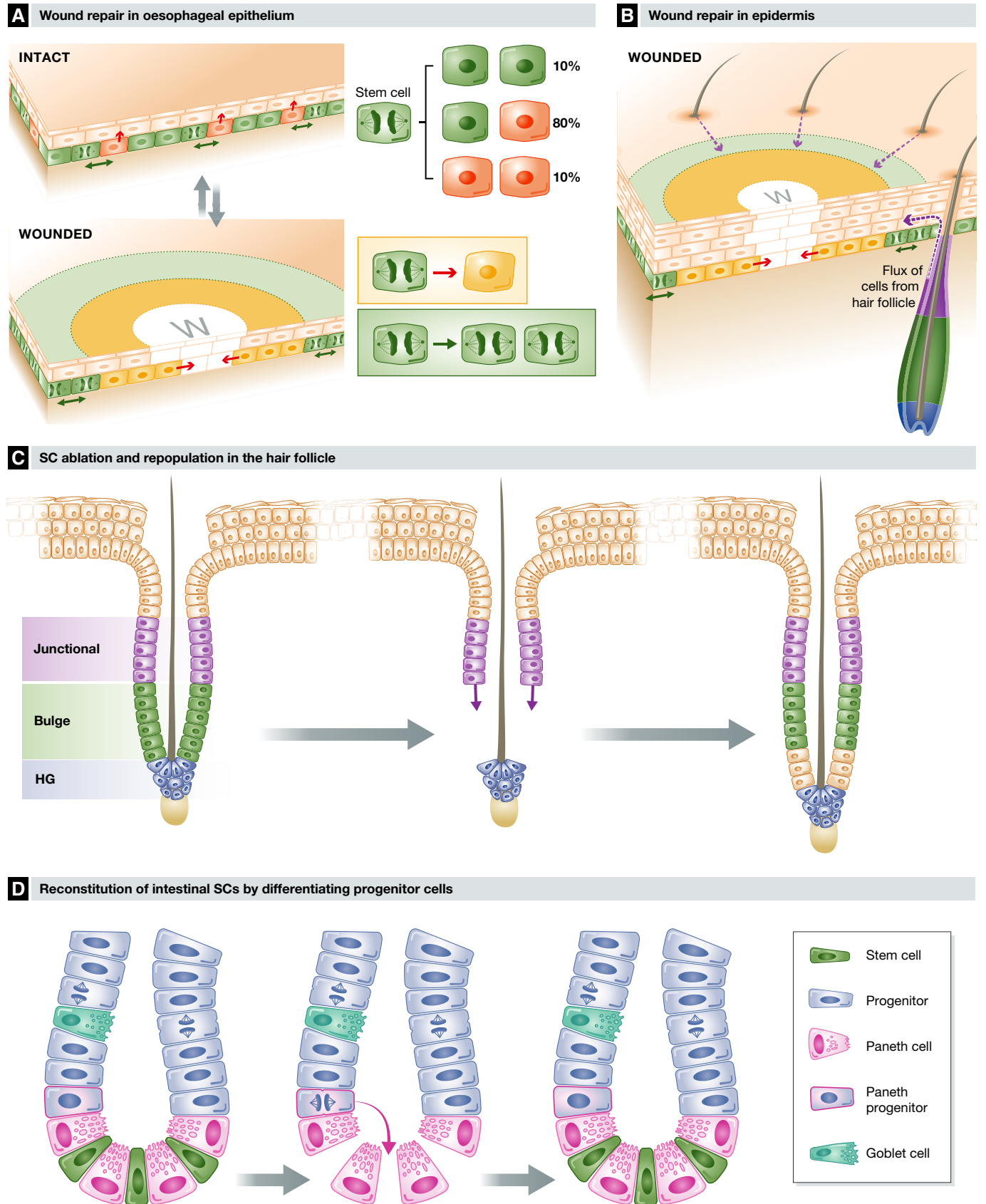


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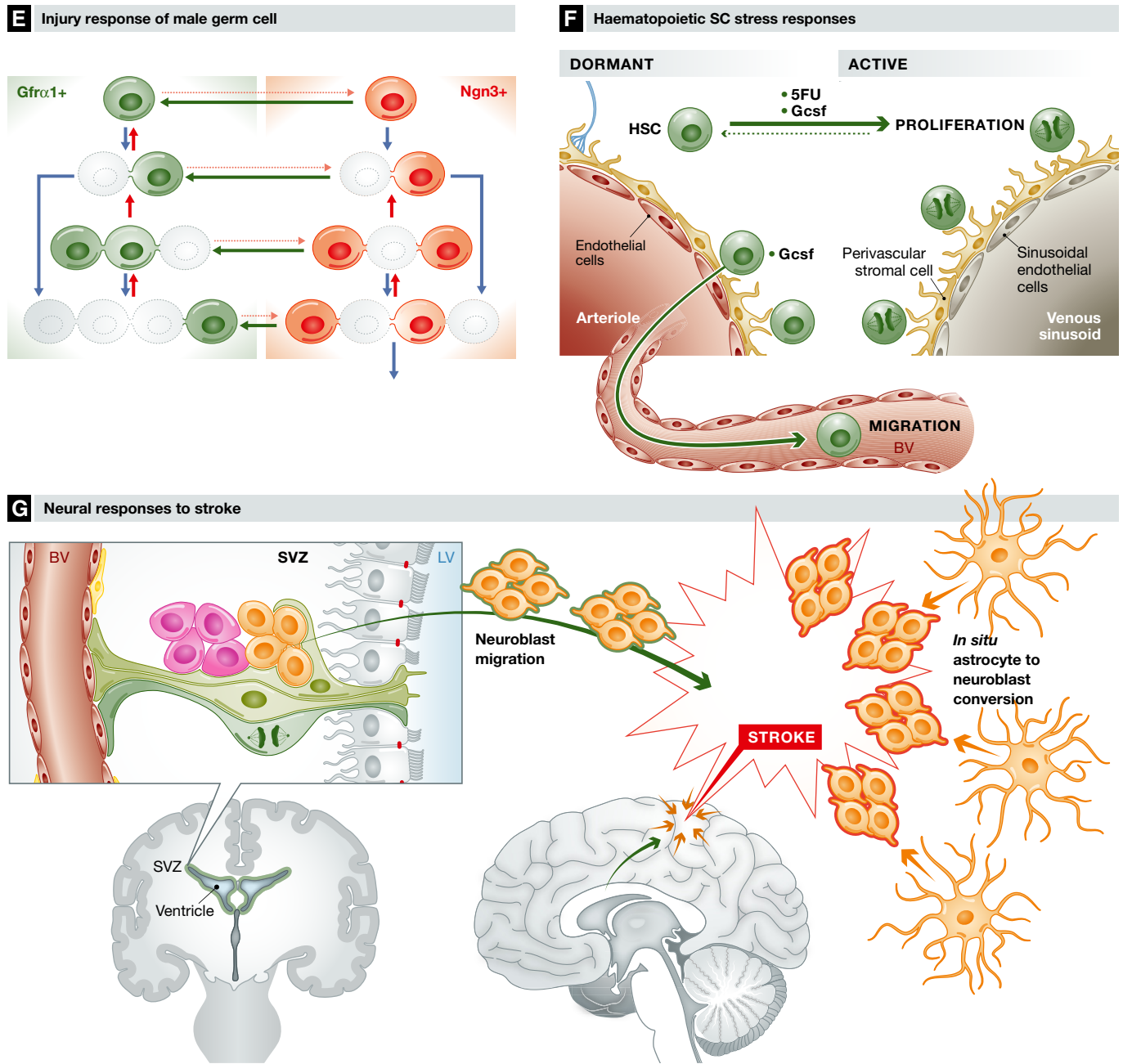


Figure 3. Stem cell responses to stress.

(A) Wound repair in oesophageal epithelium. Following wounding, progenitor cells next to the wound (w) exit the cell cycle and migrate towards the defect (yellow area and inset). Behind this 'migrating front', cycling progenitors undergo divisions heavily biased to self-duplication (green area and inset), expanding the progenitor compartment to generate the excess cells required to repair the epithelium. Once the wound has closed, progenitor behaviour switches back to homeostasis. (B) Wound repair in epidermis. In the epidermis, progenitor cells change their behaviour as in the oesophagus, but wound repair is supported by a flux of cells into the epidermis from hair follicles (purple arrows) and, in tail skin, mobilisation of quiescent reserve cells in the epidermis. Migration explains the appearance of radial clones around a healed wound seen in lineage-tracing experiments. (C) HF ablation and repopulation. Following ablation of SCs in the bulge (green) of telogen HFs, cells in the upper follicle (purple) migrate and proliferate (purple arrows) to reconstitute the bulge SC population, enabling the hair cycle to proceed normally. (D) Reconstitution of intestinal SCs by differentiating progenitor cells. Following ablation of Lgr5-expressing SCs in the crypt base (green), Paneth cell precursors (blue with pink border) dedifferentiate and colonise the crypt base niche with SCs functionally equivalent to the original population. (E) Injury response of male germ SCs. Following treatment with the cytotoxic agent busulphan, the GFR α 1⁺ SC population is depleted (grey outlines) but then reconstituted by surviving Ngn3⁺ cells reverting into GFR α 1⁺ cells (green arrows) and a decrease in the probability of GFR α 1⁺ cells transferring to the Ngn3⁺ compartment (pink dotted arrows). (F) Haematopoietic SC stress responses. Dormant SCs are recruited into cycle by treatment after damage to the haematopoietic system from a cytotoxic drug (5FU) or cytokines, such as Gcsf, released after infection. Gcsf also triggers dormant SCs to migrate into the circulation. (G) Neural SC responses to stroke. Following a CNS stroke, which results in cell death due to ischaemia, clusters of neuroblasts (orange) are seen in the injured region. Lineage tracing argues these derive both from mobilisation of neural SCs in the SVZ followed by migration to the site of injury (orange with green border) and from differentiated astrocytes within the area of the stroke entering a neurogenic programme (orange with red borders).

homoeostasis, back into GFR α 1⁺ SCs (Fig 3E; Nakagawa *et al*, 2010)(Hara *et al*, 2014).

Tissue damage or stress mobilises slow-cycling or quiescent SCs, which function as 'reserve' cells, such as dormant haematopoietic SCs that are rapidly recruited into cycle following treatment with the cytotoxic drug 5 fluorouracil or the cytokine Gcsf, the latter also triggering SC migration into the circulation (Wilson *et al*, 2008; Cheung & Rando, 2013) (Fig 3F). The liver is an intriguing organ, capable of extensive regeneration after a variety of insults (Miyajima *et al*, 2014). Lineage tracing in different models of injury indicates surviving hepatocytes re-enter the cell cycle to restore their own lineage (Schaub *et al*, 2014; Tarlow *et al*, 2014; Yanger *et al*, 2014). Tissue repair can thus be achieved by dedifferentiation alone in the absence of a reserve stem cell population. Differentiated cells have also been observed to generate cycling progenitors of a different lineage. Following vascular stroke in mouse brain, lineage tracing provides evidence for differentiated astrocytes generating proliferating neuroblasts, supplementing neuroblasts derived from mobilised neural SCs in the SVZ which have migrated into the zone of injury (Magnusson *et al*, 2014) (Fig 3G).

The ultimate test of the regenerative potential of a SC is to reconstitute a tissue from a single cell, a feat that requires an exponential increase in SC number and the generation of differentiated cells. Transplantation into immune-suppressed mice has long been a standard SC assay, but more recently, SC culture methods have been developed which reveal the ability of a single SC to reconstitute a tissue-like 'organoid' in defined culture conditions (Sato *et al*, 2009; Sato & Clevers, 2013). This was first demonstrated with human epidermal SCs that generate large colonies containing multiple SCs and differentiating keratinocytes, which fuse into stratified sheets (Rheinwald & Green, 1975; Jones *et al*, 1995). When this cultured epidermis is grafted back onto burnt patients, it persists over many years (Gallico *et al*, 1984; Compton *et al*, 1989). More recently, 3-dimensional culture systems were established for intestinal and prostatic SCs from both mice and humans (Sato *et al*, 2009, 2011a; Chua *et al*, 2014; Karthaus *et al*, 2014). Disaggregation and re-culturing reveal each organoid contains self-renewing SCs capable of multiple rounds of organoid formation (Sato *et al*, 2009). The ability of SCs and their progeny to assemble themselves into a niche within a tissue-like array of differentiated cells in the absence of external spatial cues is remarkable and is most graphically illustrated in the formation of complex brain-like structures in 3D cell culture (Lancaster *et al*, 2013; Lancaster & Knoblich, 2014).

If this technology can be used to expand SC populations *ex vivo* which successfully reconstitute and sustain damaged tissues *in vivo*, it will have great potential for regenerative medicine (Huch *et al*, 2013a,b; Sato & Clevers, 2013; Dorrell *et al*, 2014). In combination with genome editing, *ex vivo* stem cell expansion may be particularly powerful for treating genetic diseases with tissue-restricted phenotypes, where engrafted SCs can compete with host SCs on at least even terms (Schwank *et al*, 2013). The factors required to culture organoids give insight into the nature of niche signals for SC survival and proliferation, an area we will explore below (Sato *et al*, 2011b).

To summarise, recent *in vivo* studies have revealed dramatic plasticity following tissue damage. SCs are recruited into cycle and/or change their fate to increase cell production. Progenitors and even differentiated cells may dedifferentiate to speed repair. Spatial

compartments and lineage boundaries are crossed. Advances in cell culture are further revealing the self-organising ability of SCs and their progeny in generating organ-like structures in the absence of tissue cues.

Autonomy and instruction: regulating SC states

We now turn to examine recent insights into the regulation of SC dynamics in homoeostasis and regeneration, critical in tuning cell production to the requirements of the tissue and whole organism. Potentially adjustable parameters include the rate of SC division and the balance of the resulting SC and differentiating progeny. In tissues with minimal cell turnover, SCs are actively maintained in a quiescent state and conditionally activated. We will consider the relative contributions of cell-intrinsic factors, paracrine signalling in the SC microenvironment, the physical characteristics of the niche and 'remote' signals such as hormones and neural regulation in switching SC behaviour.

For squamous SCs, population asymmetry requires balancing the probabilities of three potential division outcomes (Clayton *et al*, 2007; Doupe *et al*, 2012). This process requires Notch signalling, which was long known to drive the differentiation of post-mitotic keratinocytes (Lowell *et al*, 2000; Rangarajan *et al*, 2001; Nicolas *et al*, 2003; Blanpain *et al*, 2006). Transgenic inhibition of Notch signalling in individual oesophageal SCs accelerates the rate of cell division and blocks the symmetric differentiation division outcome (Alcolea *et al*, 2014). As a result, eventually, the entire epithelium is replaced by Notch-inhibited cells. Intriguingly, as this occurs, the basal layer becomes crowded and the symmetric differentiation division outcome is restored (Alcolea *et al*, 2014). In culture, cell crowding promotes keratinocyte differentiation, suggesting the reinstatement of homoeostasis may be a consequence of increased cell density within the basal layer (Watt *et al*, 1988). Regulation of cell density is a robust and widespread phenomenon, and potentially is a defence against SCs carrying any mutation that enhances proliferation and results in crowding in the niche (Eisenhoffer *et al*, 2012; Marinari *et al*, 2012; Frede *et al*, 2014).

The regulation of squamous SC proliferation also involves highly conserved cell-cell and paracrine signalling pathways. Epidermal SCs secrete both Wnt ligands and Wnt inhibitory Dkk proteins, resulting in the activation of Wnt target genes in basal cells (Choi *et al*, 2013; Lim *et al*, 2013). Blockade of Wnt signalling in SCs inhibits their proliferation, resulting in a thinned epidermis (Choi *et al*, 2013; Lim *et al*, 2013). Autocrine Wnt signalling is thus an essential part of the squamous SC niche, providing permission to proliferate (Frede & Jones, 2013).

The remodelling of HF during the hair cycle makes dissection of SC regulation in the fast-changing niche especially challenging, but cell autonomous factors and paracrine signalling again emerge as crucial SC regulators. Expression of the transcription factor *Sox9* in HF SCs is essential for their survival and normal differentiation, but this is achieved at least in part by regulation of paracrine activation/TGF β signalling by *Sox9* target genes (Kadaja *et al*, 2014). Wnt signalling again plays a key role. If Wnt signals are inhibited, HF SCs enter a state of 'suspended animation' in which they persist long term but do not proliferate, re-entering the cell cycle when the Wnt activity is reinstated (Choi *et al*, 2013). In contrast,

activating the Wnt pathway triggers SC expansion and tilts the axis of cell division to generate new HFs (Deschene *et al*, 2014). As with the epidermis, there is evidence for paracrine signalling, as SCs with high Wnt activity recruit adjacent SCs by secreting Wnt ligands (Deschene *et al*, 2014). Hedgehog signalling mediates the activation of quiescent SCs by differentiating TA cells (Hsu *et al*, 2014).

The short range of Wnt signals from Paneth cells, essential for SCs remaining in an undifferentiated state, restricts the number of SCs in the intestinal niche (Sato *et al*, 2011b). Expansion of the Paneth cell population is a feature of benign intestinal tumours, whose formation is driven by Wnt-activating *Apc* mutations (Schepers *et al*, 2012). *Apc*-mutant SCs eventually colonise the crypt through their enhanced fitness over wild-type cells, as a differentiating cell is more likely to be replaced by a mutant than a wild-type cell (Vermeulen *et al*, 2013). Loss of *Lrig1*, which negatively regulates ErbB family growth factor signalling, causes crypt expansion, while activation of *Ras*, which lies downstream of ErbB receptors, in intestinal SCs can trigger a process called crypt fission in which a mutant crypt splits into two allowing a mutant stem cell clone to spread through the intestine (Wong *et al*, 2012; Amoyel *et al*, 2014). Thus, while limiting niche size is a simple means of achieving homeostasis, it is not invulnerable to mutations that confer a clonal fitness advantage on SCs.

The morphology of the niche has long been recognised as of pivotal importance in plants, whose architecture is determined by the spatial arrangement of the meristem (Heidstra & Sabatini, 2014). The size and shape of hair can be manipulated by altering the number of cells in the dermal papilla that forms the niche for hair germ SCs (Chi *et al*, 2013). Within the bone marrow, HSCs reside in close proximity to stromal cells adjacent to blood vessels and the bone surface (Mendelson & Frenette, 2014). Each of these cell types makes a distinct paracrine contribution to the niche (Ding *et al*, 2012). For example, the chemokine Cxcl12, which is essential for maintaining HSCs, derives primarily from the perivascular stromal cells that lie directly adjacent to quiescent HSCs (Ding & Morrison, 2013; Greenbaum *et al*, 2013). Paracrine niche signals also regulate neural SCs in the SVZ, which extend cellular processes into the cerebrospinal fluid (CSF) that fills the ventricles and forms contacts with underlying endothelial cells (Fig 2H) (Silva-Vargas *et al*, 2013; Codega *et al*, 2014). These contacts allow regulation of neural SCs via both secreted factors in the CSF and signals from the vasculature. For example, endothelium-derived Ntf3 and G protein-coupled receptor ligands in the CSF promote SC quiescence (Codega *et al*, 2014; Delgado *et al*, 2014).

Seemingly at odds with SCs residing in an ordered niche is their ability to recapitulate the morphology of a tissue in culture (Rheinwald & Green, 1975; Sato *et al*, 2009; Stange *et al*, 2013; Karthaus *et al*, 2014). Feeder cells or exogenous growth factors and ligands in the media are able to substitute for the paracrine environment provided by the niche, but in isotropic 3-dimensional culture systems, morphogenic gradients are initially absent. A common feature of many protocols is the requirement for the Rock kinase inhibitor Y26732 during establishment of the culture. This suggests that manipulation of cytoskeletal signalling is required for isolated SCs to enter a state that allows survival and promotes proliferation in the absence of niche cells (Sato *et al*, 2009; Huch *et al*, 2013a,b; Karthaus *et al*, 2014). Rather than

being determined by SCs, the resemblance of organoids to their parent tissue may rest on the ability of differentiating cells to self-assemble into a tissue-like array by virtue of cell shape-defined packing considerations and differential adhesion (Watt & Green, 1982; Honda *et al*, 1996).

Thus, recent studies are revealing more of the micro-architecture of the SC niche and the highly conserved paracrine signals, such as Wnt, which regulate cell behaviour across a wide range of lineages. While the niche may not ‘micromanage’ the outcome of each individual SC division, it has an essential role in sustaining SC populations in a proliferating or quiescent state. Understanding how cell-intrinsic factors, autocrine signals and exogenous factors combine to sustain SCs in culture will likely give new understanding of the niche *in vivo*.

Remote control: integrating SC dynamics and whole body physiology

Stem cell behaviour must not only match local requirements in the tissue, but also needs across the organism (Fig 4). One example of such distant regulation is the circadian control of SCs. Proliferation varies dramatically in a diurnal cycle. For example in the mouse oesophagus, 10-fold more SCs undergo mitosis in the early diurnal than in the early nocturnal phase (Burns *et al*, 1976). In the epidermis, hair follicle bulge SCs vary in their transcription of the *Per1* gene, arguing they are in different phases of the circadian cycle. Cells high in *Per1* expression have increased clonogenicity *in vitro*, suggesting variation in circadian phase may contribute to functional heterogeneity in bulge SCs (Janich *et al*, 2011). In the hematopoietic system, SCs regularly leave the bone marrow and enter the circulation before returning to their niche. This migration also has a circadian rhythm with twice as many circulating SCs in the diurnal than in the nocturnal phase. The exit of SCs is controlled centrally from the suprachiasmatic nucleus in the brain, via release of noradrenaline from nerve endings in the bone marrow, which in turn regulates the transcription of Cxcl12, a chemokine that directs HSC migration (Mendez-Ferrer *et al*, 2008).

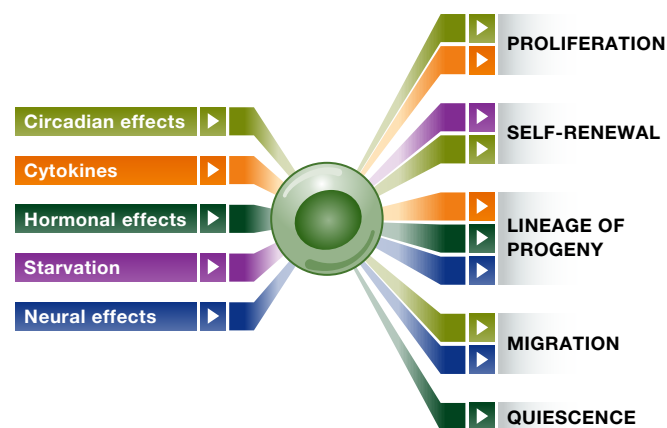


Figure 4. SC regulation from outside the niche.

Summary of systemic influences on SC fate. Fate outcome is mapped by colour to distinct systemic factors. See the text for more detail.

Sex hormone regulation of reproductive and mammary SCs is well documented, but recently has been found to extend to SCs in other tissues. For example, HSCs are subject to positive regulation of proliferation and self-renewing cell divisions by oestrogen in female mice. In pregnancy, oestrogen levels rise, the number of cycling HSCs increases and more erythroid progenitor cells are produced, meeting the physiological requirement of the animal for increased oxygen delivery to tissues (Nakada *et al*, 2014). Another example is the hair cycle, which is arrested during in pregnancy by prolactin driving HF SCs into a quiescent state (Goldstein *et al*, 2014).

A significant evolutionary pressure on organisms and SCs is starvation, which induces a body-wide adaptive response that includes effects on SCs (Longo & Mattson, 2014; Mihaylova *et al*, 2014). The midgut of fasted *Drosophila* expands dramatically after feeding, underpinned by a switch in the mode of SC division from balanced cell production to self-duplication in response to insulin (O'Brien *et al*, 2011). In the murine small intestine, fasting reduces Mtorc1 signalling in the Paneth cells that form the SC niche (Yilmaz *et al*, 2012). In turn, this increases paracrine signalling to SCs via cyclic ADP ribose, resulting in an increased efficiency of organoid formation in cultures from fasted animals (Yilmaz *et al*, 2012). While not definitive, this result suggests starvation may expand the SC population by acting on the niche. Starvation also impacts haematopoiesis, acting via IGF1 signalling to increase the number of transplantable HSCs consistent with an enhanced probability of self-renewing division (Cheng *et al*, 2014).

Another route for physiological SC regulation is via the nervous system, recently revealed as having a key role in HSC regulation. Chronic psychological stress induces increased levels of leucocytes. As with circadian control, this effect is mediated by noradrenaline release from sympathetic neurons in the bone marrow, which decreases production of Cxcl12 in niche cells triggering HSC proliferation (Heidt *et al*, 2014). The importance of this mechanism is illustrated in the neoplastic myeloproliferative disorder, which is caused by JAK2 mutations in HSCs (Arranz *et al*, 2014). Secretion of interleukin 1b by the mutant HSCs induces neuropathy in bone marrow, decreasing CXCL12 levels and triggering HSC proliferation and disease progression. The development of the disease is halted by restoring adrenergic signalling by treatment with β 3-adrenergic agonists (Arranz *et al*, 2014).

HSCs also illustrate how systemic signals interact with cell-intrinsic factors to promote the generation of a specific lineage from a multipotent SC population. For example, the cytokine M-CSF, which is released in high levels during infection, induces the master myeloid transcription factor PU-1, increasing the probability of HSCs differentiating into myeloid progenitors to meet the increased requirement for leucocytes (Mossadegh-Keller *et al*, 2013).

Drawing these studies together reveals a new aspect of SC biology that goes beyond single lineage or tissue. Understanding how neural, endocrine and cytokine signals regulate cell production to meet the requirements of an organism is a key challenge for SC research.

Sleeping, waking and sleeping again: regulating SC quiescence

Maintaining the quiescence of SCs is an active process (Cheung & Rando, 2013). For example, muscle satellite cells are maintained in

a quiescent state by specific miRNAs and transcription factors downstream of Notch, loss of which results in depletion of the SC population (Fukada *et al*, 2011; Cheung *et al*, 2012; Crist *et al*, 2012). In the liver, the hepatocytes that fulfill the role of 'reserve' SCs are actively maintained in a quiescent and differentiated state by Hippo signalling acting via the Notch pathway (Yimlamai *et al*, 2014).

When a tissue is stressed or injured, quiescent cells must be mobilised. Entry into cycle may require specific transcription factors, such as *Ascl1*, which is required for neural SCs to exit the quiescent state (Andersen *et al*, 2014). The transition from quiescence also involves the induction of cellular biogenesis. In muscle SCs, the rapid upregulation of translation is achieved by post-translational mechanisms involving the sequestration of regulatory miRNAs in mRNP granules (Crist *et al*, 2012). Following a remote injury, circulating factors acting via HGF signalling and the mTorc1 signalling node prime quiescent SCs enhancing their regenerative potential. Once activated, some SCs contribute to differentiated muscle, while others re-enter the quiescent state to maintain the SC population (Shea *et al*, 2010). Return to quiescence requires multiple factors including *Sprouty1*, a negative regulator of receptor tyrosine kinase signalling and active Notch signalling (Shea *et al*, 2010; Gopinath *et al*, 2014).

It is becoming clear that the quiescent state is far from being a protected state as used to be thought. While cell competition results in the loss of mutant SCs in proliferating tissues, quiescent SCs accumulate epigenetic and genetic damage, resulting in impaired function as they age (Liu *et al*, 2013; Martins *et al*, 2014; Sun *et al*, 2014a). In quiescent HSCs, the error-prone non-homologous end-joining DNA repair alterations progressively accumulate (Mohrin *et al*, 2010; Beerman *et al*, 2014). Recruitment of HSCs into cycle is accompanied by induction of a broad range of DNA repair genes mobilised to remedy genomic alterations (Beerman *et al*, 2014). As well as intrinsic factors, age-associated inflammatory signalling acting via the Jak/Stat pathway impairs muscle SC function (Price *et al*, 2014; Tierney *et al*, 2014). Intriguingly, administration of a single circulating growth factor, Gdf11, seems able to reverse the defects in ageing muscle SCs (Sinha *et al*, 2014). Gdf11 also benefits neurogenesis by ageing neural SCs, possibly acting via effects on the vasculature in the SVZ (Katsimpardi *et al*, 2014). Understanding how quiescent SCs accumulate molecular damage and the ways in which this may be reversed is a central question in the biology of ageing.

Conclusion

Adult SCs have evolved a diverse repertoire of SC behaviours to meet tissue-specific requirements. In cycling tissues, homeostatic balance between self-duplication and differentiation may be achieved by cell-intrinsic mechanisms, a spatially constrained niche, or exceptionally invariant asymmetric cell division. In each case, SC division is restrained by the requirement to maintain a constant cell density within the niche. Repair is also achieved by diverse strategies, with mobilisation of reserve SCs, plasticity of SC behaviour and dedifferentiation emerging as common themes. While specific growth factors may bias cell-intrinsic lineage selection, a shared paracrine signalling toolkit sustains SCs across multiple lineages

(Clevers *et al.*, 2014). Stochastic cell-intrinsic processes may be biased by extrinsic signals, including ‘long-distance’ signals from nerves and hormones. The application of powerful imaging and transgenic tools to reveal the dynamics of SCs within their niche offers the prospect of integrating molecular and cellular mechanisms to gain a broader perspective on the functional plasticity of SCs in the context of the whole organism.

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Conflict of interest

The authors declare that they have no conflict of interest.

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