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 SC 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 SC, the cellular mechanisms that underlie them and the signals that bias SC fate as they switch between roles.

Keywords:

Stem cells, niche, differentiation, regeneration, signal transduction

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 (**Figure 1**). Epithelial and male germ SC 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 is 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, SC must rapidly and reversibly switch to produce an excess of proliferating cells to regenerate the lost tissue. Here, we will define SC 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 SC 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 SC

There are two cellular mechanisms by which SC may maintain homeostasis (Watt & Hogan, 2000). A population of SC may behave so that on average, 50% of their progeny are themselves SC and 50% differentiating cells. Such 'population asymmetry' may result from the cell autonomous properties of SC or external regulation, for example by a niche of restricted size in which SC 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 **(Figure 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 SC in the basal cell layer (Clayton et al, 2007; Doupe et al, 2012; Doupe et al, 2010; Lim et al, 2013; Mascre et al, 2012). The outcome of individual cell divisions is unpredictable, generating two SC, two differentiating cells or one cell of each type (Alcolea & Jones, 2013) (**Figure 2A)**. However the probabilities of each division outcome are balanced so an equal number of SC and differentiating cells are generated across the basal layer, an example of 'population asymmetry' (Clayton et al, 2007; Watt & Hogan, 2000). 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 SC 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 (HF) and sweat ducts, each a self-maintaining mini-organ 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) **(Figure 2B)**. The HF is homeostatic in SC numbers and tissue organisation remain 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 SC 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 cells (Cotsarelis et al, 1990; Tumbar et al, 2004) (**Figure 2B**). Bulge SC differentiate into multiple lineages when sorted and transplanted, and undergo both symmetric and asymmetric division (Blanpain et al, 2004; Waghmare et al, 2008; Zhang et al, 2009). More recently lineage tracing and intra-vital imaging have shown that SC 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) do SC 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 (Chi et al, 2013; Rompolas

et al, 2012). In the bulge, SC remain in slow cycling or quiescent, until a proportion of cells are recruited into division by signals from TA cells. These induce SC to first contribute to the outer root sheath and then undergo self renewal until the bulge is repopulated with SC, when quiescence resumes (Hsu et al, 2014) (**Figure 2B)**. HF expansion is followed by involution (catagen) and by the time the telogen phase is reached, bulge and hair germ SC 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 SC and progenitor cells located in pits known as crypts (Clevers, 2013) (**Figure 2C)**. Cycling SC 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 which populate the upper crypt (Barker et al, 2007). Differentiated Paneth cells lie next to SC and secrete signals to which sustain SC 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 (**Figure 2C)** (Lopez-Garcia et al, 2010; Snippert et al, 2010). It was initially thought that all Lgr5+ cells were cycling functionally equivalent SC, 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 differentiating 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. Heamopoietic SC (HSC) 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 HSC expressing cell surface identical markers may be cycling or dormant, but only the latter group have long term transplant potential **(Figure 1)** (Anjos-Afonso et al, 2013; Catlin et al, 2011; Foudi et al, 2009; Oguro et al, 2013; Wilson et al, 2008). Dormant HSC reside in a distinct niche environment, next to arterioles, and migrate to lie close to venous sinusoids on activation **(Figure 2D)**(Kunisaki et al, 2013; Mendelson & Frenette, 2014). Transplant assays argue that haematopoiesis is a hierarchy, with HSC continually generating progenitors which become progressively restricted in both lineage and self renewal potential 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 HSC, challenging the view of an ordered differentiation programme (Yamamoto et al, 2013) (**Figure 2E)**. More remains to be discovered about the apparent heterogeneity among HSC with long term transplant potential. Some of this may result from HSC commuting between different states which vary in their ability to survive the rigors 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 HSC, 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 (**Figure 2F**) (Sun et al, 2014b). This argues for a separation of roles in haemopoiesis, with a pool of progenitors maintaining the myeloid lineage in homeostasis and quiescent HSC functioning as a 'reserve', mobilised to expand the progenitor population when the demand for myeloid cells rises (Sun et al, 2014b; Wilson et al, 2008). 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 SC, which reside in the basal cell layer of the seminiferous tubule, express *Gfr1α* and are negative for the differentiation marker *Ngn3*. Spermatogonia undergo incomplete cell division, generating syncytial clusters of cells linked by cytoplasmic bridges (Yoshida, 2012) (**Figure 2G)**. It was thought that only single *Gfr1α*$^{+}$cells were SC, but combined live imaging and lineage tracing have revealed that all *Gfr1α*$^{+}$ cells, whether single or in syncytia of up to 4 cells, are functionally equivalent SC (Hara et al, 2014). Quantitative modelling argues SC 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 *Gfr1α*$^{+}$ compartment (Hara et al, 2014; Klein et al, 2010). *Ngn3*$^{+}$ cells have a high probability of undergoing differentiation into cKit expressing cells, but infrequently revert back to *Gfr1α 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 (Aimone et al, 2014; Ernst et al, 2014; Snyder et al, 2011; Spalding et al, 2013). Adult neural SC are found in the subventricular zone (SVZ) lining the lateral ventricles and in the dentate gyrus (DG) of the hippocampus and are largely quiescent (**Figure 2H**). Lineage tracing in transgenic mice reveals that proliferating DG SC are capable of both asymmetric and symmetric divisions generating two SC cells or two differentiating astroglial or neural progenitor daughters (Bonaguidi et al, 2011) (**Figure 2I**). After division some SC return to a quiescent state, negative for proliferation markers, paralleling the transition between dormant and active states seen in heamatopoietic SC.

Even establishing the existence of SC 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 (Fellous et al, 2009; Kusaba et al, 2014; Magami et al, 2002; Miyajima et al, 2014; Rodins et al, 2002). In contrast, adult skeletal muscle contains a well defined quiescent SC population, residing amongst the satellite cells which lie beneath the plasma membrane of terminally differentiated multinucleate muscle fibres (Brack & Rando, 2012). Muscle SCs typify the 'reserve' role of SC, 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 (Klein & Simons, 2011; Watt & Hogan, 2000). The first, exemplified by squamous epithelia, is that SC within an 'open plan' niche with an apparently cell intrinsic mechanism which balances the probabilities of generating SC or differentiating progeny (Alcolea & Jones, 2014; Clayton et al, 2007). 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 maintains 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 SC, as such characteristics may vary within a functionally equivalent pool of SC. Likewise, active SC may co-exist 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

SC, while a balanced three way division outcome reminiscent of mammalian squamous epithelia is a feature of mid-gut SC (de Navascues et al, 2012; Kronen et al, 2014). Intriguingly transgenic lineage tracing reveals that neural retina SC 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 SC 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, SC 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 SC in low turnover tissues.

Wounding is frequent in surface epithelia. In the squamous epithelium of the oesophagus and the epidermis of the paw, SC adjacent to a wound rapidly and reversibly switch to producing an excess of SC, reverting to homeostatic behaviour once the defect is closed (**Figure 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 SC. 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 HF, 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) (**Figure 3B**). In the specialised tail epidermis, which is sparse in HF, rare, slow cycling 'reserve' SC are mobilised, while in hairless skin sweat duct derived cells contribute to wound healing (Lu et al, 2012; Mascre et al, 2012; Roshan & Jones, 2012). In humans, similar principles apply but sweat ducts make a larger contribution to epidermal repair than HF (Rittie et al, 2013).

Injury also induces the appearance of new HF 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 HF. Following ablation, bulge SC are replaced by the progeny from cells in the upper HF which normally make no contribution to the hair cycle (**Figure 3C**) (Rompolas & Greco, 2014; Rompolas et al, 2013). Similarly, the hair germ can be replaced if the bulge SC, 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 SC 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 SC which repopulate the crypt (**Figure 3D**) (Buczacki et al, 2013; Ritsma et al, 2014; van Es et al, 2012). In lung and gastric epithelia, reversion of post mitotic differentiated cells into SC 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 *Gfra1*+ SC pool is are regenerated by the combination of decreasing the probability of reversion of *Gfra1*+ differentiation and reversion of *Ngn3*$^{+}$ cells, most of which differentiate in homeostasis, back into *Gfra1*+ SC (**Figure 3E**) (Nakagawa et al, 2010)(Hara et al, 2014).

Tissue damage or stress mobilises slow cycling or quiescent SC, which function as 'reserve' cells, such as dormant haematopoietic SC which are rapidly recruited into cycle following treatment with the cytotoxic drug 5 Fluorouracil or the cytokine Gcsf, the later also triggering SC migration into the circulation (Cheung & Rando, 2013; Wilson et al, 2008) (**Figure 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 SC in the SVZ which have migrated into the zone of injury (Magnusson et al, 2014) (**Figure 3G**).

The ultimate test of the regenerative potential of a SC is to reconstitute a tissue from a single cell, a feat which 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 & Clevers, 2013; Sato et al, 2009). This was first demonstrated with human epidermal SC, which generate large colonies containing multiple SC and differentiating keratinocytes, which fuse into stratified sheets (Jones et al, 1995; Rheinwald &

Green, 1975). When this cultured epidermis is grafted back onto burnt patients, it persists over many years (Compton et al, 1989; Gallico et al, 1984). More recently, 3 dimensional culture systems were established for intestinal and prostatic SC from both mice and humans (Chua et al, 2014; Karthaus et al, 2014; Sato et al, 2011a; Sato et al, 2009). Disaggregation and re-culturing reveals each organoid contains self-renewing SC capable of multiple rounds of organoid formation (Sato et al, 2009). The ability of SC 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 & Knoblich, 2014; Lancaster et al, 2013).

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 (Dorrell et al, 2014; Huch et al, 2013a; Huch et al, 2013b; Sato & Clevers, 2013). In combination with genome editing, *ex vivo* stem cell expansion may be particularly powerful for treating genetic diseases with tissue restricted phenotypes, where engrafted SC can compete with host SC 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. SC 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 SC 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 homeostasis 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 of SC and differentiating progeny. In tissues with minimal cell turnover, SC 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 SC, population asymmetry requires balancing the probabilities of three potential division outcomes (Clayton et al, 2007; Doupe et al, 2012). This process requires Notch signaling, which was long known to drive the differentiation of post mitotic keratinocytes (Blanpain et al, 2006; Lowell et al, 2000; Nicolas et al, 2003; Rangarajan et al, 2001). Transgenic inhibition of Notch signalling in individual oesophageal SC accelerates the rate cell of 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 homeostasis 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 SC carrying any mutation that enhances proliferation and results in crowding in the niche (Eisenhoffer et al, 2012; Frede et al, 2014; Marinari et al, 2012).

The regulation of squamous SC proliferation also involves highly conserved cell-cell and paracrine signalling pathways. Epidermal SC 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 signaling in SC inhibits their proliferation, resulting in a thinned epidermis (Choi et

al, 2013; Lim et al, 2013). Autocrine Wnt signaling 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 signaling again emerge as a crucial SC regulators. Expression of the transcription factor *Sox9* in HF SC is essential for their survival and normal differentiation, but this is achieved at least in part by regulation of paracrine activin/TGFβ signaling by *Sox9* target genes (Kadaja et al, 2014). Wnt signalling again plays a key role. If Wnt signals are inhibited, HF SC 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 HF (Deschene et al, 2014). As with the epidermis, there is evidence for paracrine signalling, as SC with high Wnt activity recruit adjacent SC by secreting Wnt ligands (Deschene et al, 2014). Hedgehog signaling mediates the activation of quiescent SC by differentiating TA cells (Hsu et al, 2014).

The short range of Wnt signals from Paneth cells, essential for SC remaining in an undifferentiated state, restricts the number of SC in 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 SC eventually colonize 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 SC 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

(Amoyel et al, 2014; Wong et al, 2012). Thus while limiting niche size is a simple means of achieving homeostasis it is not invulnerable to mutations which confer a clonal fitness advantage on SC.

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 which forms the niche for hair germ SC (Chi et al, 2013). Within the bone marrow, HSC 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 HSC derives primarily from the perivascular stromal cells which lie directly adjacent to quiescent HSC (Ding & Morrison, 2013; Greenbaum et al, 2013). Paracrine niche signals also regulate neural SC in the SVZ, which extend cellular processes into the cerebrospinal fluid (CSF) which fills the ventricles and form contacts with underlying endothelial cells (**Figure 2H**)(Codega et al, 2014; Silva-Vargas et al, 2013). These contacts allow regulation of neural SC both via secreted factors in the CSF and signals from the vasculature. For example, endothelial 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 SC residing in an ordered niche is their ability to recapitulate the morphology of a tissue in culture (Karthaus et al, 2014; Rheinwald & Green, 1975; Sato et al, 2009; Stange et al, 2013). 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 signaling is required for isolated SC to enter a state that allows survival and promotes proliferation in the absence of niche cells (Huch et al, 2013a; Huch et al, 2013b;

Karthaus et al, 2014; Sato et al, 2009). Rather than being determined by SC, the resemblance of organoids to their parent tissue may rest on ability of differentiating cells to self assemble into a tissue like array by virtue of cell shape defined packing considerations and differential adhesion (Honda et al, 1996; Watt & Green, 1982).

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 SC in culture will likely give new understanding of the niche *in vivo*.

Remote control: integrating SC dynamics and whole body physiology

SC behaviour must not only match local requirements in the tissue, but also needs across the organism (**Figure 4**). One example of such distant regulation is the circadian control of SC. Proliferation varies dramatically in a diurnal cycle. For example in the mouse oesophagus, 10 fold more SC undergo mitosis in the early diurnal than the early nocturnal phase (Burns et al, 1976). In the epidermis, hair follicle bulge SC 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 SC (Janich et al, 2011). In the hematopoietic system, SC 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 SC in the diurnal than the nocturnal phase. The exit of SC 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).

Sex hormone regulation of reproductive and mammary SC is well documented, but recently has been found to extend to SC in other tissues. For example HSC 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 HSC 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 SC into a quiescent state (Goldstein et al, 2014).

A significant evolutionary pressure on organisms and SC is starvation, which induces a body wide adaptive response which includes effects on SC (Longo & Mattson, 2014; Mihaylova et al, 2014). The mid gut 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 SC 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 signaling to increase the number of transplantable HSC 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 leukocytes. 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 HSC (Arranz et al, 2014). Secretion of interleukin 1b by the mutant HSC 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 signaling by treatment with β3-adrenergic agonists (Arranz et al, 2014).

HSC 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 HSC differentiating into myeloid progenitors to meet the increased requirement for leukocytes (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 SC 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 (Cheung et al, 2012; Crist et al, 2012; Fukada et al, 2011). In the liver, the hepatocytes which fulfil the role of 'reserve' SC 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 SC to exit the quiescent

state (Andersen et al, 2014). The transition from quiescence also involves the induction of cellular biogenesis. In muscle SC 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 SC enhancing their regenerative potential. Once activated some SC 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 signaling (Gopinath et al, 2014; Shea et al, 2010).

It is becoming clear that far from being a protected state as used to be thought the quiescent state is. While cell competition results in the loss of mutant SC in proliferating tissues, quiescent SC 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 HSC, the error prone non homologous end joining DNA repair alterations progressively accumulate (Beerman et al, 2014; Mohrin et al, 2010). Recruitment of HSC 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 signaling 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 aging muscle SC (Sinha et al, 2014). Gdf11 also benefits neurogenesis by aging neural SC, possibly acting via effects on the vasculature in the SVZ (Katsimpardi et al, 2014). Understanding how quiescent SC accumulate molecular damage and the ways in which this may be reversed is a central question in the biology of aging.

Conclusion

Adult SC 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 SC, 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 SC 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 SC within their niche offers the prospect of integrating molecular and cellular mechanisms to gain a broader perspective on the functional plasticity of SC in the context of the whole organism.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Figure Legends

Fig 1: SC turnover across mouse tissues

Average cycle times for SC in the lineages indicated.

Fig 2: SC dynamics across lineages

A: Squamous epithelia consist of layers of keratinocytes, SC (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 percent, oesophageal epithelium shown) are balanced so equal numbers of SC 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 SC have been identified, in the junctional zone (purple), the bulge (green) and the hair germ (blue) which lies 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 which 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 SC in each compartment are maintained at a constant level across multiple hair cycles.

C: Intestinal Epithelium contains four lineages sustained by SC (green) which 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: Heamatopoietic SC reside close to blood vessels in the bone marrow. Dormant SC (green) lie close to arterioles, receiving paracrine signals from endothelial cells (grey), perivascular cells expressing *Ng2* (orange) and sympathetic nerve endings (Blue). On activation, SC migrate to be close to venous sinusoids which support *Lepr* expressing perivascular cells (purple).

E: Asymmetric and symmetric fate of Haematopoietic SC. Transplantation of single daughter cells SC division reveals self duplicating and asymmetric divisions; dark green: Long term reconstituting SC; light green: short or intermediate term reconstituting SC; yellow: megakaryocyte progenitor; 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 cell (grey) progeny (see inset). Haematopoietic SC (green) make negligible contribution to myelopoiesis in homeostasis, but function as 'reserve' cells.

G: Male germ cell SC are diverse in appearance but functionally equivalent. Male germ cells expressing Gfr1a reside in the outermost layer of the seminiferous tubule. SC (green) exist as singles or 2-4 cell synctia connected by cytoplasmic bridges, which may self duplicate (blue arrows) to generate two SC or undergo fragmentation. Upon differentiation (pink arrow) into Ngn3 positive cells (pink), the same behaviour continues, but $Ngn3^{+}$ cells are unlikely to revert to $Gfr1a^{+}$ SC in homeostasis. Once in the Ngn3 compartment, syncitia larger than 4 cells form which may undergo further differentiation into cKit expressing cells (not shown), which are even less likely to revert to Gfr1a positive cells.

H: Neural SC exhibit symmetric and asymmetric cell division. Quiescent Neural SC (purple) reside in the sub ventricular zone niche, extending processes into cerebrospinal fluid (blue) which fills the lateral ventricles (LV) and underlying endothelial cells (orange) lining blood vessels (BV). Endothelial cells which line the LV are shown gray. Differentiating transit amplifying cells (pink) and neural precursor cells (red) lie adjacent to the SC. Figure after (Silva-Vargas et al, 2013).

I: Division outcomes of neural SC in the dentate gyrus inferred from lineage tracing. Neural SC interconvert between quiescent (purple) and proliferating (green) states. Division of SC has a range of symmetric and asymmetric outcomes as shown, generating neural SC, neuroblasts (pink) or differentiated astrocytes (blue).

Fig 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 (red 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 tissue 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 (blue 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 SC in the bulge (green) of telogen HF, cells in the upper follicle (purple) migrate and proliferate (red arrows) to reconstitute the bulge SC population, enabling the hair cycle to proceed normally.

D: Reconstitution of Intestinal SC by differentiating progenitor cells

Following ablation of Lgr5 expressing SC in the crypt base (green) Paneth cell precursors (grey with pink border) dedifferentiate and colonise the crypt base niche with SC functionally equivalent to the original population.

E: Injury response of Male germ SC

Following treatment with the cytotoxic agent Busulphan the $Gfr1a^+$ SC population is depleted (grey outlines) but then reconstituted by surviving $Ngn3^+$ cells reverting into $Gfr1a^+$ cells (green arrows) and a decrease in the probability of $Gfr1a^+$ cells transferring to the $Ngn3^+$ compartment (pink arrows).

F: Haematopoietic SC stress responses

Dormant SC 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 SC 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 SC in the SVC followed by migration to the site of injury (orange with blue border) and from differentiated astrocytes within the area of the stroke entering a neurogenic programme (orange with green border).

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. Please see the text for more detail.

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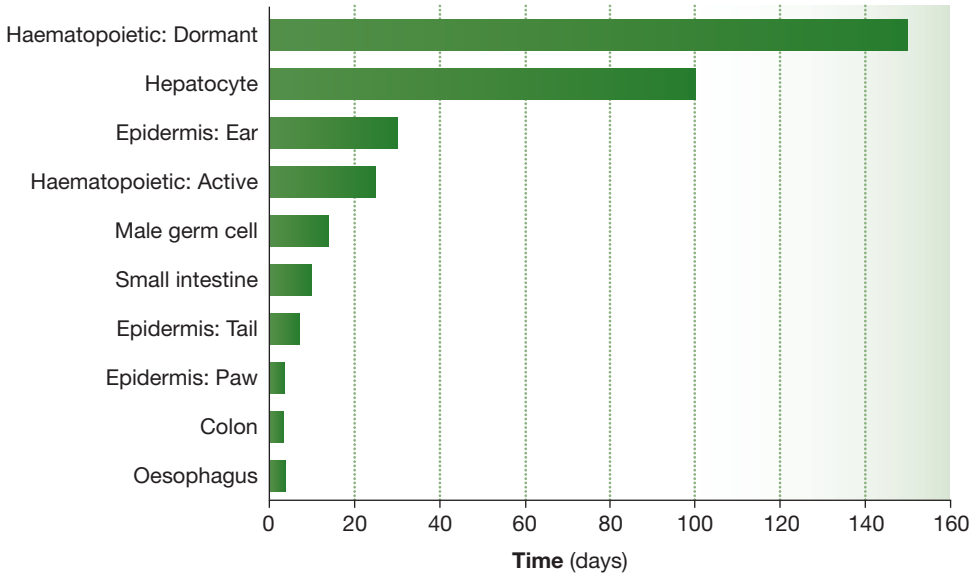
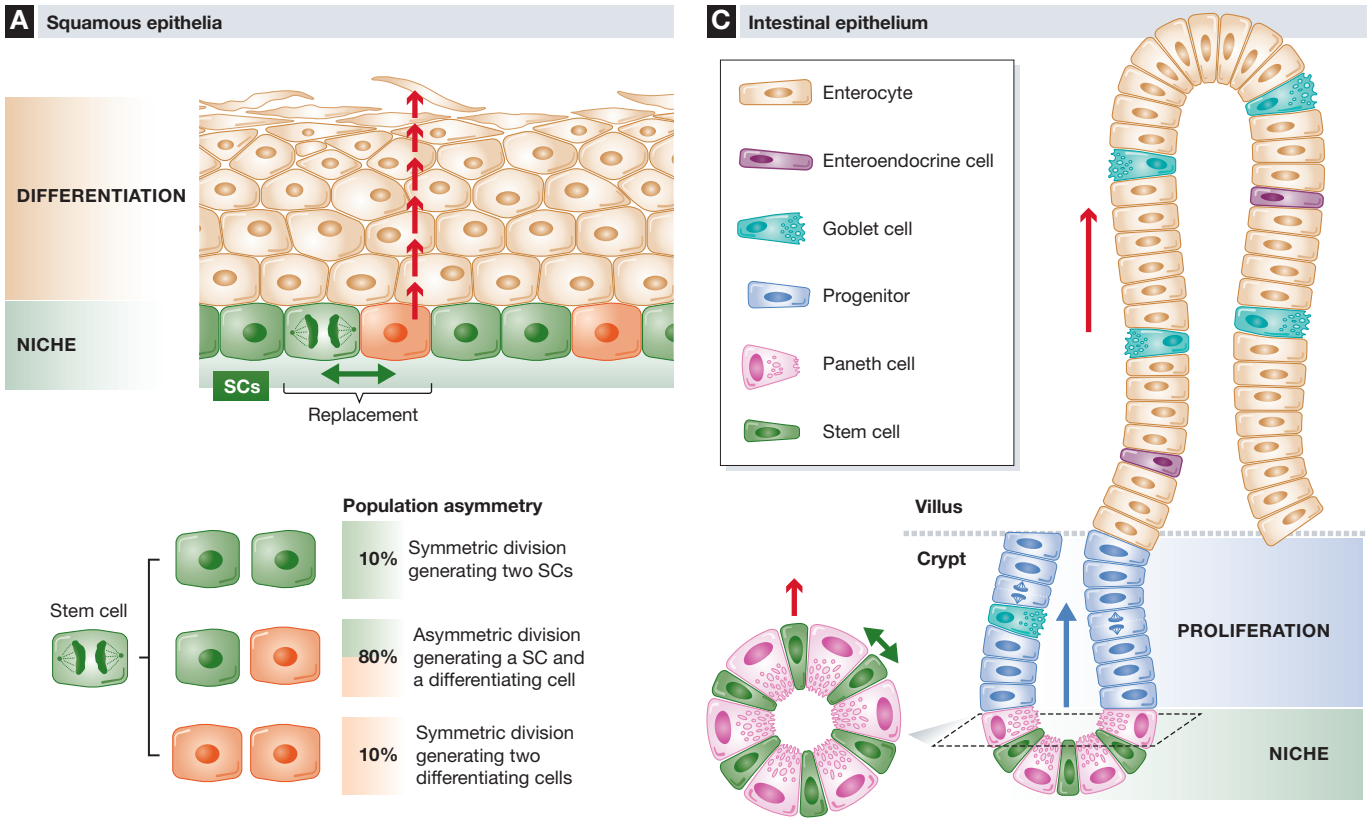
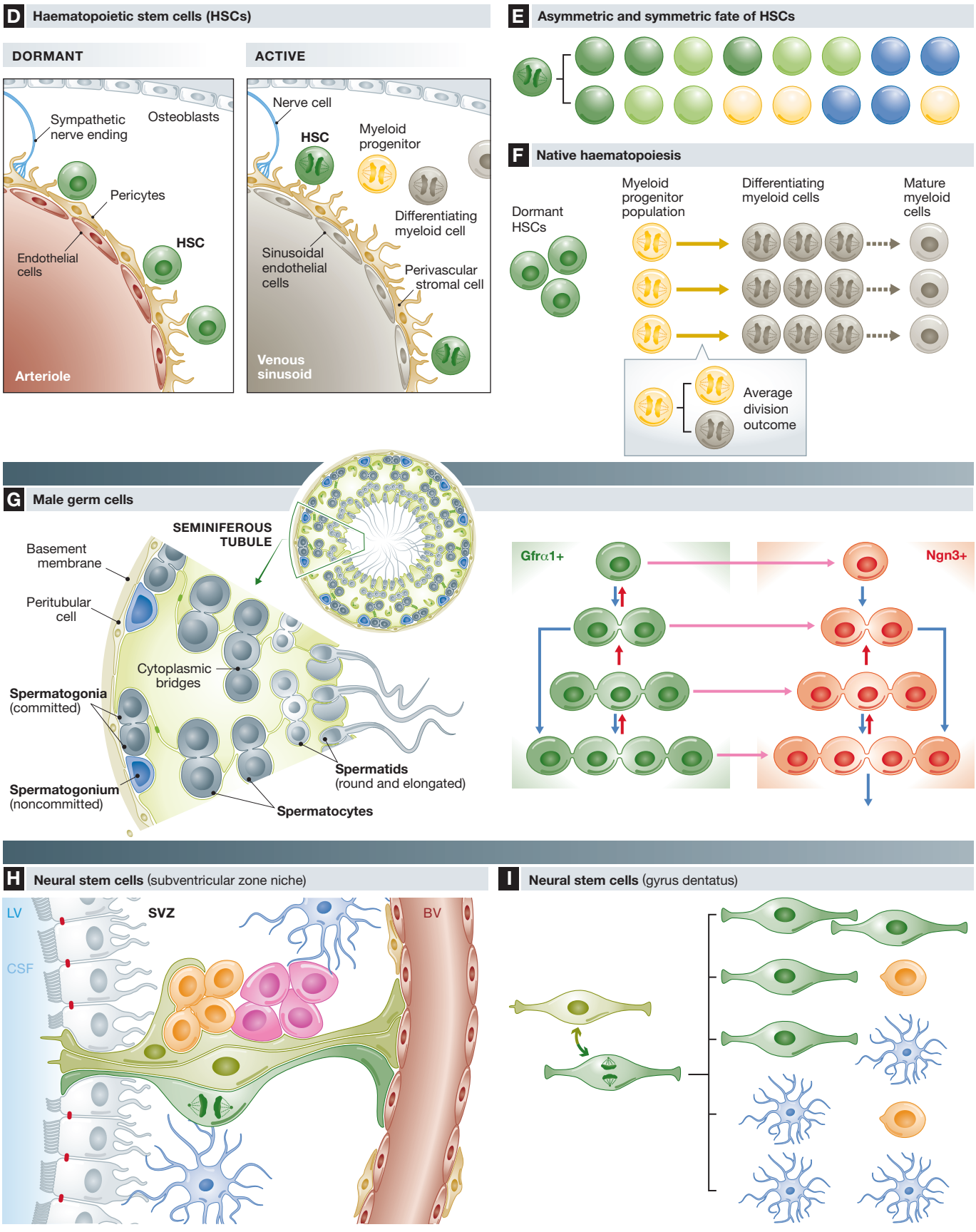
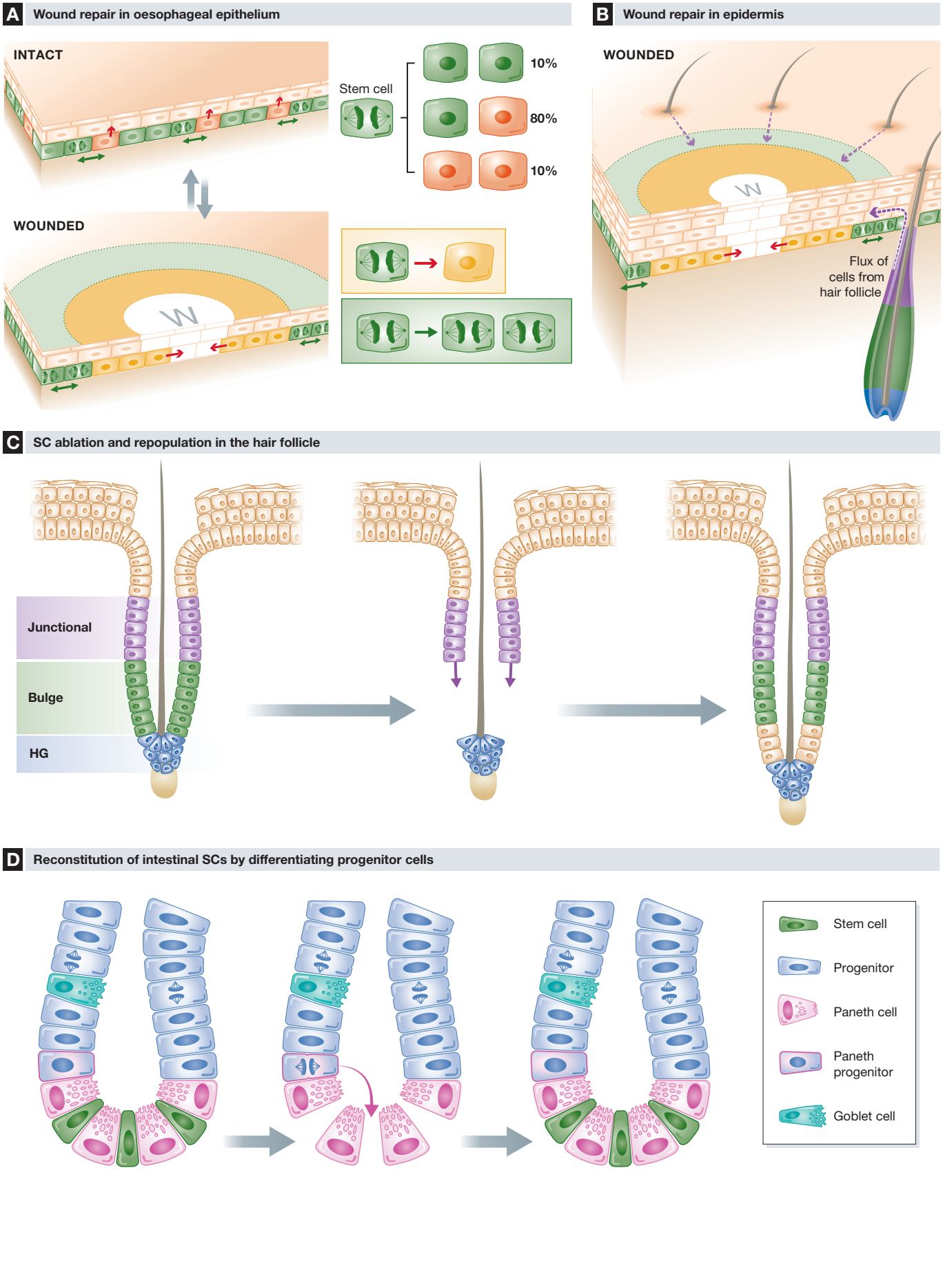
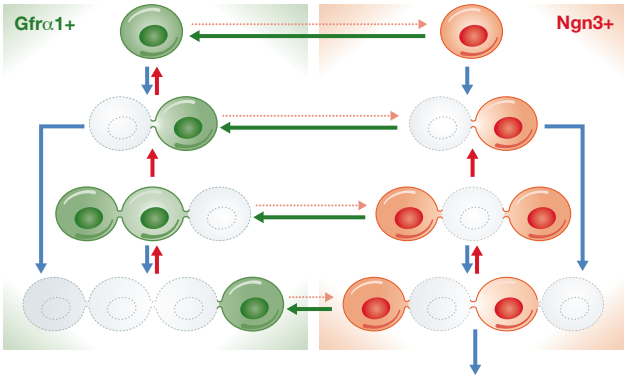

Haematopoietic: Dormant
Hepatocyte
Epidermis: Ear
Haematopoietic: Active
Male germ cell
Small intestine
Epidermis: Tail
Epidermis: Paw
Colon
Oesophagus
0
20
40
60
80
100
120
140
160
Time (days)


A
Squamous epithelia
DIFFERENTIATION
NICHE
SCs
Replacement
Population asymmetry
Stem cell
10%
Symmetric division generating two SCs
80%
Asymmetric division generating a SC and a differentiating cell
10%
Symmetric division generating two differentiating cells
C
Intestinal epithelium
Enterocyte
Enteroendocrine cell
Goblet cell
Progenitor
Paneth cell
Stem cell
Villus
Crypt
PROLIFERATION
NICHE
B
Hair follicle
TELOGEN
Upper
Junctional
Lower
Bulge
HG
ORS
ANAGEN
Bulge
ORS
HG

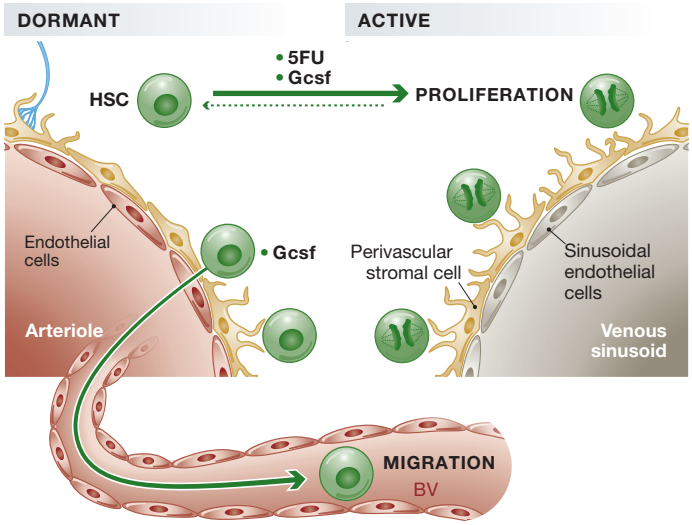

D Haematopoietic stem cells (HSCs)
DORMANT
ACTIVE
Sympathetic nerve ending
Osteoblasts
Pericytes
HSC
Endothelial cells
Arteriole
Nerve cell
HSC
Myeloid progenitor
Differentiating myeloid cell
Sinusoidal endothelial cells
Perivascular stromal cell
Venous sinusoid
E Asymmetric and symmetric fate of HSCs
F Native haematopoiesis
Dormant HSCs
Myeloid progenitor population
Differentiating myeloid cells
Mature myeloid cells
Average division outcome
G Male germ cells
SEMINIFEROUS TUBULE
Basement membrane
Peritubular cell
Cytoplasmic bridges
Spermatogonia (committed)
Spermatogonium (noncommitted)
Spermatids (round and elongated)
Spermatocytes
Gfrα1+
Ngn3+
H Neural stem cells (subventricular zone niche)
LV
CSF
SVZ
BV
I Neural stem cells (gyrus dentatus)


A
Wound repair in oesophageal epithelium
INTACT
Stem cell
10%
80%
10%
WOUNDED
B
Wound repair in epidermis
WOUNDED
Flux of cells from hair follicle
C
SC ablation and repopulation in the hair follicle
Junctional
Bulge
HG
D
Reconstitution of intestinal SCs by differentiating progenitor cells
Stem cell
Progenitor
Paneth cell
Paneth progenitor
Goblet cell

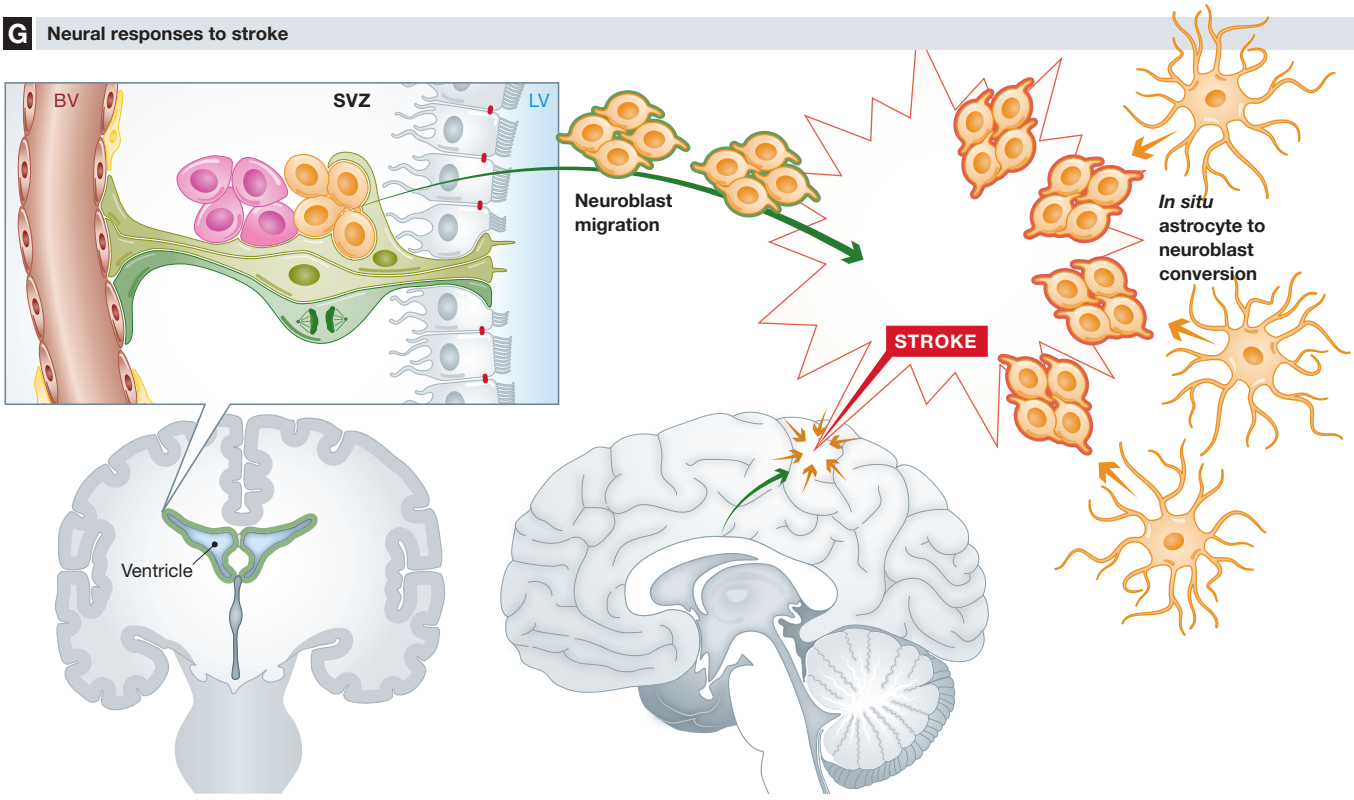
E Injury response of male germ cell

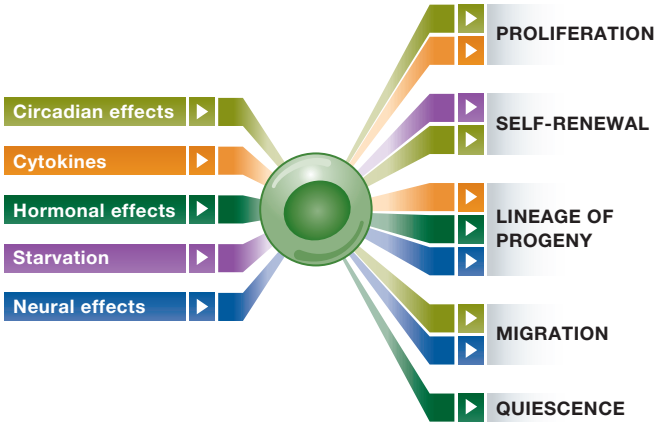


F Haematopoietic SC stress responses



G Neural responses to stroke




Circadian effects
Cytokines
Hormonal effects
Starvation
Neural effects
PROLIFERATION
SELF-RENEWAL
LINEAGE OF PROGENY
MIGRATION
QUIESCENCE