Stem cells find their niche

Allan Spradling, Daniela Drummond-Barbosa & Toshie Kai

HHMI/Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210, USA

The concept that stem cells are controlled by particular microenvironments known as 'niches' has been widely invoked. But niches have remained largely a theoretical construct because of the difficulty of identifying and manipulating individual stem cells and their surroundings. Technical advances now make it possible to characterize small zones that maintain and control stem cell activity in several organs, including gonads, skin and gut. These studies are beginning to unify our understanding of stem cell regulation at the cellular and molecular levels, and promise to advance efforts to use stem cells therapeutically.

Stem cells fascinate us from both a theoretical and a practical viewpoint. Their defining characteristics — extensive proliferative potential and an ability to give rise to one or more differentiated cell types — are common in early mammalian embryos. But embryonic cells lose these properties as differentiation ensues and growth-promoting signals decline. By adulthood, the few remaining stem cells are dispersed and virtually invisible; however, the surviving adult stem cells have achieved something remarkable. They can operate at 'steady state' — that is, they can generate on average one replacement stem cell and one tissue cell at each division with no apparent limit.

The mechanisms that stem cells use to accomplish self-renewal promise fundamental insight into the origin and design of multicellular organisms. These pathways make it possible to repair damage and extend organismal life beyond that of component cells, and probably preceded the evolution of complex metazoans. Can knowledge of stem cells be used to develop a cell-based medicine to repair malformed, damaged or ageing tissues? We are drawn to the subject of stem cells as we are to stories of perpetual motion machines or the fountain of youth; understanding the inner workings of stem cells might help to realize their mythic promise.

The true nature of stem cells can be learned only by discovering how they are regulated. One possibility is that internal mechanisms in the stem cell control a stereotypic pattern of unequal division that generates daughters with different fates. Such autonomously acting stem cells would probably exhibit specialized, tissue-specific patterns of gene expression. It is becoming increasingly clear, however, that the specialized functions required to ensure proper stem cell function are vested in neighbouring differentiated cells. By signals and other intercellular interactions, these cells control the behaviour of adjacent stem cells that may themselves be relatively unspecialized. Consequently, location rather than special patterns of gene expression may characterize stem cells.

The realization that stable, custom microenvironments might control haematopoietic stem cells led Schofield[1] to call such regions 'niches'. For our purposes, a niche (Fig. 1) is considered to be a subset of tissue cells and extracellular substrates that can indefinitely house one or more stem cells and control their self-renewal and progeny production *in vivo*. Here we review our current understanding of stem cell niches, focusing on those systems that are best characterized at the cellular and mechanistic levels. Haematopoietic and neural stem cells are considered separately in the respective reviews by Weissman and colleagues (pages 105–111) and Temple (pages 112–117) in this issue.

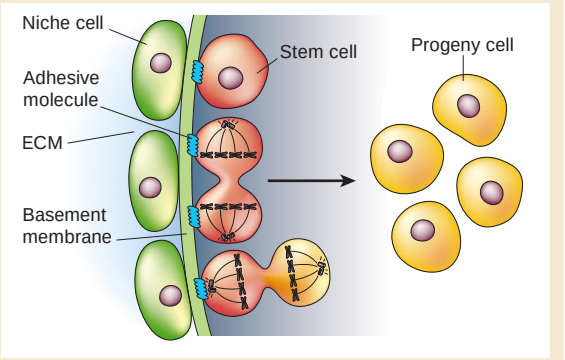

Figure 1 Niche structure. Niche cells (green) underlying a basement membrane signal to stem cells (red) to block differentiation and regulate division. When a lineage mechanism prevails (lower mitotic cell), the stem cell divides such that one daughter retains its connections to the niche, while the other (yellow) becomes untethered and begins to differentiate. When a population mechanism prevails (upper mitotic cell), stem cell division may be either symmetric (shown) or asymmetric (not shown), as determined by local factors. ECM, extracellular matrix.

Locating and classifying niches

Identifying niches and determining how they are regulated is experimentally challenging. To look systematically for niches in a target tissue, stem cells first need to be identified by marking cells and following their lineages. When individual stem cells have been localized, evidence that they reside in a common microenvironment is sought by characterizing cellular neighbours, expression patterns of signalling molecules and local environmental factors such as extracellular matrices. If commonalities are found, then the microenvironment can be perturbed to learn which aspects, if any, affect stem cell behaviour.

Showing that a niche is present requires ultimately that stem cells be removed and added back (Fig. 2a). A niche should persist in the absence of resident stem cells and support the stem cell activity of competent exogenous cells. By contrast, the fate of the removed stem cells is irrelevant to the niche issue. Sometimes exogenous cells can be added directly to intact tissues and then function as stem cells, presumably by occupying empty, partially filled or ectopic niches, or by displacing endogenous stem cells. But endogenous stem cells may have to be depleted before a niche accepts donor cells. The limitations of this reconstitution

assay include the difficulty of interpreting negative results, and the uncertain biological significance of niches defined by reconstitution when information on the tissue's normal stem cells is lacking.

Niches are thought to operate in one of two basic ways. Lineage niches specify precisely individual stem cell divisions and daughter cell fates. For example, a stem cell in such a niche might contact the stroma asymmetrically and orientate its division plane to ensure that only one daughter inherits adhesive contacts with the basement membrane (Fig. 1, bottom). The daughter inheriting these contacts is held in the niche, where it will continue to be maintained as a stem cell, whereas the other daughter becomes untethered and relocates away from the stromal cells and their signals. By contrast, when a population mechanism operates (Fig. 1, middle cells), both daughters may remain as stem cells or both may differentiate. These two mechanisms can be distinguished by lineage studies in which single labelled stem cells and their progeny are followed (Fig. 2b): the number of labelled stem cells will remain unchanged by a lineage mechanism, but a population mechanism will eventually homogenize the labelling of all the niche's stem cells.

Germline niches

Mammalian testis

The strongest evidence for niche-based regulation in any mammalian tissue probably comes from studies of spermatogenesis. In the testis, germ cell development is maintained by germline stem cells (GSCs) known as A_{single} (A_s) spermatogonia that lie in contact with the basement membrane of the seminiferous tubule (Fig. 3a). After stem cell division, a large collection of A- and B-type spermatogonia joined by intercellular bridges forms through a series of roughly ten synchronous, incomplete divisions. The spermatogonia can move laterally along the basement membrane and are encased by Sertoli cells that mediate many aspects of male germ cell development. As germ cells enter meiosis and begin to differentiate into sperm, they lose contact with the basement membrane and move towards the lumen of the seminiferous tubule, while maintaining their association with Sertoli cells[2,3].

The existence of niches in the testis was demonstrated directly when early germ cells were transplanted into the seminiferous tubules of host males whose GSCs had been depleted[4]. Introduced cells colonize sites on the wall of the tubule and found growing clones of developing sperm that can restore host fertility. Each colony seems to be established by a single GSC that proliferates and differentiates in a regular manner, as it expands in both directions along a segment of the host tubule[5–7]. Most of the founder cell's initial progeny become stem cells. These cells probably occupy empty niches that lie in abundance along the depleted basal layer. Only when the colony has expanded greatly are differentiating sperm seen near the centre of the colony. Thus, the number of colonies represents a minimum estimate of the number of niches, which must in reality be much larger. Although a reconstitution assay has long been available for haematopoietic stem cell niches[8], the simple morphology and accessibility of the testis offer experimental advantages for studying the cellular and molecular regulation of a normal niche *in vivo*. For example, the reconstituted stem cells and their surroundings can be compared with the situation in the normal testis — information that has been lacking for haematopoietic stem cells.

The reconstitution assay not only reveals the presence of thousands of germline stem cell niches along the wall of the seminiferous tubules, but also provides information on their developmental properties. For example, niche number and quality seem to change during the development of mouse testes[9]. Testes from W^- mice lack normal c-Kit function and are deficient in endogenous spermatogenesis. As hosts, they support 8–10 times more colonies when derived from pups than from adults, and the colonies are larger in pups. This suggests that niches may be more abundant or more accessible in young than in adult seminiferous tubules. A caveat is that there may be developmental differences in the number of unoccupied niches in

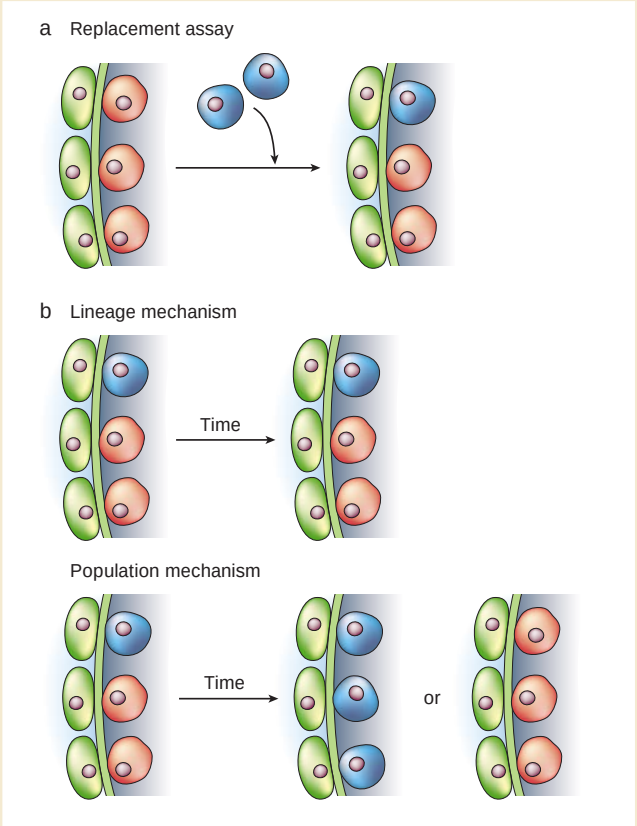

Figure 2 Manipulating niches. **a**, Replacement assay to identify niches. Labelled stem cells (blue) are introduced into a tissue. If one or more of the introduced cells becomes associated stably with a particular tissue region and subsequently functions as a stem cell, then the existence and location of a stem cell niche (green) is inferred. **b**, Cell marking experiments. Marking a single stem cell *in vivo* (blue), and following the number and location of marked progeny cells reveals its regulatory behaviour. When the stem cell follows a lineage mechanism, the number of labelled stem cells remains relatively constant over many stem cell cycles. When the initially labelled stem cell follows a population mechanism (bottom), initially mosaic niches will sort out stochastically into niches that have all labelled or all unlabelled cells.

W^- testes, because the assay depends on depleting endogenous niches[10]. Other studies show that compared with adult spermatogonial cells, many fewer colonies per input cell are obtained using prospermatogonia — cells that have not yet become associated with the basement membrane or Sertoli cells — from newborn mice. Thus, germ cells seem to become more competent to populate a niche as a result of developmental events that correlate with the establishment of niches *in vivo*.

Several candidate niche components and signals have been analysed. The $\alpha_6\beta_1$ integrin is enriched in the basal layer and may help to attach spermatogonial stem cells to laminin in the basement membrane[11]. Consistent with this idea, transplant assays show that crude cell populations can be enriched for male GSCs by binding to laminin, the target of $\alpha_6\beta_1$, but not to collagen IV or fibronectin. Bone morphogenetic protein (BMP)-4 produced by the extraembryonic ectoderm stimulates the growth and development of early germ cells[12]. BMP-8a and BMP-8b also are needed for germ cell development[13], but an ongoing function for BMPs in germline stem cells has not been established as yet[14]. Sertoli cells produce another transforming growth factor (TGF)-β-related factor, GDNF, that affects the proliferation of pre-meiotic germline cells, including perhaps stem cells[15].

Several other candidate signals seem to act at a slightly later stage. Signals mediated by SCF, a Sertoli cell-product encoded by the *Steel*

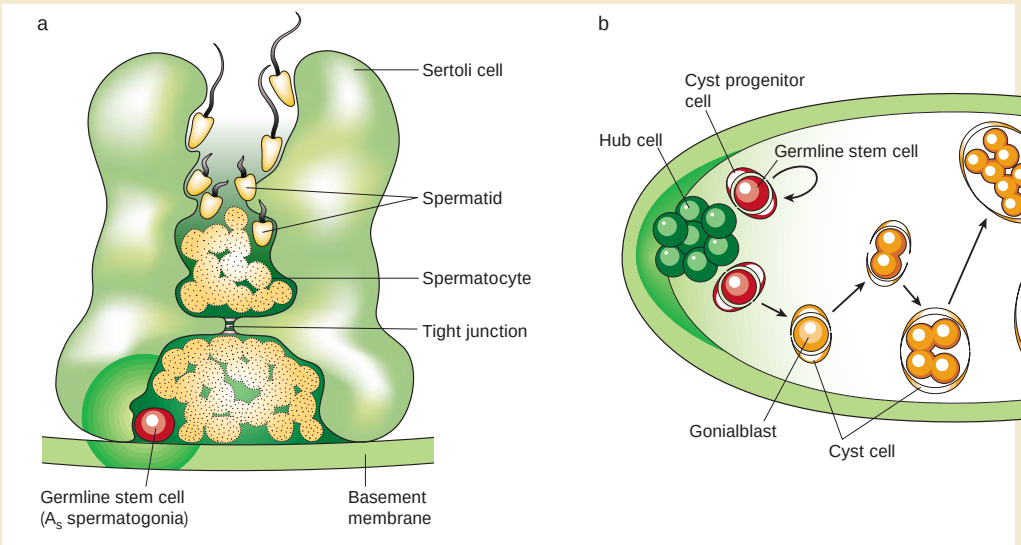

Figure 3 Male germ cell niches. **a**, A cross-section of part of a seminiferous tubule of a mouse testis is shown to illustrate the approximate location and properties of the mammalian germline stem cell niche. The stem cells also known as A_{single} (A_s) cells (red) constitute a minority of the basal germ cells in contact with the basement membrane (green). Most other basal cells (yellow) are part of growing germ cell cysts, whose members are interconnected by ring canals. As development proceeds, the cysts leave the basement membrane and move in a highly ordered manner towards the lumen of the tubule (top). All developing germ cells are surrounded by Sertoli cells (green). It is not known whether the region of the Sertoli cell or basement membrane near the stem cells are specialized (dark green). **b**, A sagittal section of the distal tip of the *Drosophila* testis is drawn schematically and leaves out most of the cells for clarity. The hub cells (green) are attached to 5–9 groups comprising one germline stem cell and two cyst progenitor stem cells (red). The basement membrane (green) may be thinner and specialized (dark green) near the hub. Germline stem cell daughters called gonialblasts are encased by two cyst cells, begin to differentiate, and move posteriorly away from the hub. After four more divisions, the cyst cells (yellow) cease division and synchronously enter meiosis.

(*Sl*) locus, are received by the germ cell c-Kit receptor and required for germ cells to develop beyond type A spermatogonial stages. Wild-type germ cells transplanted to Sl^- testes can still form colonies[10], but they fail to differentiate, which places the requirement for SCF downstream from the stem cell stage. The RNA helicase Vasa is expressed in the developing germ cells of many different species, including mice. In the absence of Vasa, male germ cell proliferation is reduced severely in spermatogonial stages[16]. The timing of the defect argues against a requirement for Vasa to maintain germline stem cells in the niche.

Further studies at the level of individual stem cells are needed to increase our understanding of testis niches and their regulation. Do endogenous A_s spermatogonia reside at special positions along the wall of the seminiferous tubule, or does the entire membrane, in collaboration with local Sertoli cells, constitute a stem cell niche? If there are special sites, how are they distributed, and do transplanted A_s cells have to occupy them, or can they colonize 'new' niches not used by normal stem cells? Do male germline stem cells maintain themselves using a population mechanism, or do the two daughter cells normally acquire different fates?

Drosophila testis

Germline stem cells in model invertebrates are located near the blind end of simple tubes. The ends of both male and female *Drosophila* gonads contain germline stem cells, whose behaviour has been verified by cell marking[17,18] and which have been proposed to reside in niches[19–21]. In the adult testis (Fig. 3b), a cluster of 10–12 cells form the 'hub' distally, in contact with the basement membrane. About 5–9 GSCs and 10–18 cyst progenitor stem cells (somatic stem cells or SSCs) are interspersed around the hub in a ring, with part of each cell directly contacting the hub.

Both GSCs and SSCs divide radially outwards from the hub. As expected for a lineage niche, one GSC daughter remains in the same environment adjacent to the hub and continues as a stem cell, whereas the other enters a different environment, acquires new cell contacts, and differentiates as a gonialblast. In larval testes, both the number of hub cells and the number of associated stem cells are twice as large as in young adults[22]. In older adults, the number of these cells decreases further[21]. This decrease may parallel the developmental decline in the ability of the mammalian testis to support the growth of transplanted stem cells[9]. But proof that the *Drosophila* testis functions as a stem cell niche will require reconstituting stem cell function using exogenous cells.

Signalling by JAK–STAT (Janus kinase/signal transducer and activator of transcription) has been shown to be important in controlling male GSCs (E. Matunis, personal communication). The *unpaired* (*upd*) gene encodes a putative ligand that activates this pathway through an unidentified receptor. Upd is produced by hub cells and can stimulate stem cells when expressed ectopically in the testis.

Other intercellular signals are also likely to regulate stem cells and their progeny. When newly formed somatic cyst cells contain somatic clones of *raf*[21], differentiation of nascent gonialblast and germ cell cysts is slowed, causing these early stages to accumulate at the testis tip. Under these conditions, associations between somatic cyst cells and germ cells are abnormal, and the number of GSCs associated with the hub does not decline as fast as in wild-type males of the same age. Reductions in epidermal growth factor receptor (*Egfr*) activity produce similar effects through an action on somatic cells[3]. This shows that a somatic Egfr and Raf-mediated signal is important for gonialblast and spermatogonial development, but it is less clear whether the signals or the disruptions in germ cell development are responsible for the reduced turnover of stem cells.

Drosophila ovariole

The tip of each ovariole contains specialized cells that contribute to its ability to act as a niche (Fig. 4a). Two germline stem cells associate closely with a stack of about ten terminal filament cells. Like the dermal papilla of a mammalian hair follicle (see below), the terminal filament first contributes to ovariole development[23] and then supports stem cell activity[24,25]. Five or six cap cells located asymmetrically at the base of the terminal filament directly contact the GSCs, forming junctions that associate preferentially with the stem cell's specialized organelle known as the fusome[26]. GSCs divide along the anterior–posterior axis[27], which ensures that the anterior germ cell daughter inherits a cap cell contact and the stem cell fusome, and remains a stem cell. The posterior daughter is blocked from cap cell access, and is drawn away from the ovariole tip where it differentiates into a cystoblast. Although GSCs divide asymmetrically with respect to the fusome, this inequality can be eliminated by mutating a fusome structural component without affecting stem cell activity[28]. Thus,

GSCs utilize a lineage mechanism, but their unequal partitioning of cytoplasmic components is either unimportant or redundant for stem cell function.

To prove that ovarioles contain a stem cell niche, one would ideally remove the existing stem cells, reintroduce exogenous stem cells, and show they can re-occupy and come under the control of the remaining stromal cells. 'Empty niches' can be created genetically by eliminating maternally required genes such as *oskar* or *tudor*, but a method for introducing exogenous cells is not available. Nonetheless, the presence of a niche has been shown directly by a different approach[29]. One GSC was marked and genetically destabilized so that it would begin to differentiate and move away. Ovarioles were then identified in which the marked GSC had recently differentiated and opened-up space adjacent to the cap cells. In all cases, a new germ cell was found to arise by symmetric division of an adjacent stem cell, and to fill such a vacancy almost immediately. Replacement also occurs at a low level in wild-type adults. These observations suggest that conditions inside a niche, such as the availability of a cap cell-binding site, influence whether a lineage-based or population-based mechanism is used.

The BMP homologue encoded by *decapentaplegic* (*dpp*) functions as a chief signal for female GSCs[19,29]. Loss of Dpp reception causes stem cell differentiation, whereas excess Dpp blocks differentiation and leads to stem cell tumours. Several somatic cell types near the stem cells express *dpp* messenger RNA, but it remains unclear whether the final active signal is distributed locally or is widespread. Stem cells are the only germ cells located near the niche that require *dpp* signalling, but this pathway is involved in several additional processes later in gametogenesis[19,30].

Several other genes have been identified that may contribute to operating the ovariolar niche. The *piwi* gene encodes a phylogenetically conserved nucleoplasmic protein that is expressed in both somatic niche cells and germ cells[31]. Somatically, *piwi* is needed to maintain stem cells, while loss of *piwi* in GSCs slows their division and disrupts germ cell development; the production of male germ cells is also greatly reduced and males are sterile. Genes related to *piwi* are known or likely to function in a wide variety of stem cells, making it one of the best current candidates for a 'stem cell' gene[32–34]. The *fs(1)Yb* gene is also needed for continuing female GSC activity, and may act primarily to maintain *piwi* expression in terminal filament and cap cells[25]. The putative translational regulators Pumilio and Nanos are specifically required to maintain female GSCs[28,35]. The activity of these genes may be modulated in response to niche signals.

Developing male and female germ cells both require the *bag-of-marbles* (*bam*) and *benign gonial cell neoplasm* (*bgcn*) genes[36,37], but whereas these genes function in cystoblasts in females, they are needed to terminate cyst growth in males. Expressing Bam in female GSCs causes them to differentiate, but the same treatment does not perturb male GSCs. The striking differences between male and female GSC may reflect their different evolutionary origins. The male niche may be evolutionarily old, as active stem cells are found in adults from a wide range of species. In contrast, most female gonads, even among insects, do not retain stem cells in adulthood. The female stem cell niche might have arisen relatively recently during Dipteran evolution, perhaps by maintaining into adulthood mechanisms that stimulate germ cell growth in the embryo.

Caenorhabditis elegans

In the hermaphroditic gonad of *C. elegans* adults, a cluster of actively cycling syncytial cells at the distal end maintains the production of germ cells (Fig. 4b). A somatic distal-tip cell that expresses the Lag-2 ligand[38] extends processes that contact these cells and stimulates mitotic division by interacting with the Glp-1 receptor[39–41]. Asymmetric stem cell divisions have not been observed[41], and the worm gonad represents a strong candidate for a niche that is controlled by a population mechanism. But appropriate lineage studies to unambiguously distinguish these mechanisms have not been carried out, nor have stem cell replacement experiments been reported.

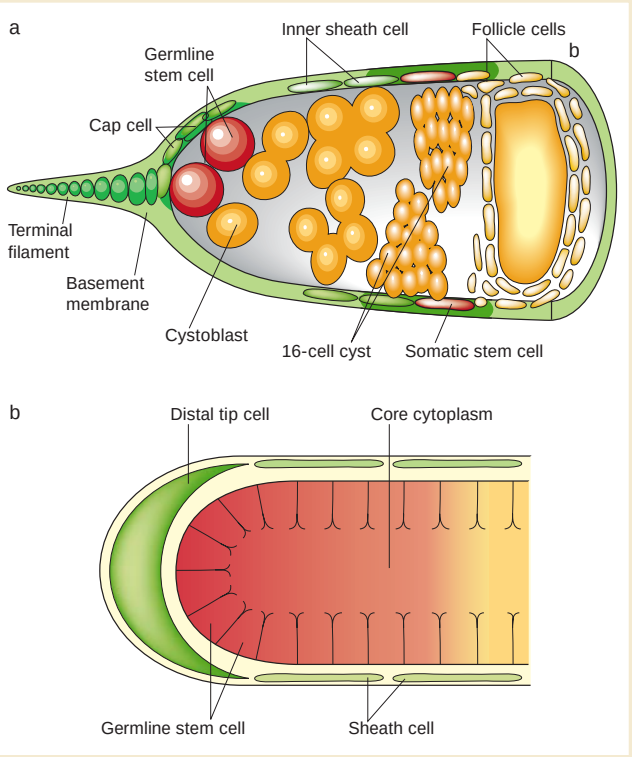

Figure 4 Female and hermaphrodite gonads. **a**, Sagittal section of a *Drosophila* germarium, showing the location of germline stem cells (red cells on left) and somatic stem cells (red cells on top and bottom). The terminal filament and cap cells (green) that make up the germ cell niche lie against a basement membrane. The cystoblast and developing cysts (yellow) move away from the anterior tip while contacting inner sheath cells (green). At the posterior zone of inner sheath cells lie 2–3 somatic stem cells (SSCs; red). SSCs contact the basement membrane (green), which may be locally specialized (dark green). The progeny of the SSCs (yellow) divide and then migrate inwards to surround the germline cysts where they become follicle cells. The SSCs receive signals from the terminal filament and cap cells that control stem cell number and proliferation rate. **b**, The distal end of the *C. elegans* gonad. The distal tip cell (green) contacts syncytial germline stem cells (red) in the mitotic zone near the distal end of the gonad. More proximally, germ cells (yellow) enter meiosis and eventually bud-off as growing oocytes.

Epithelial niches

Mammalian skin

Many different stem cells maintain mammalian skin and its associated structures, such as hair follicles, sebaceous glands and sweat glands (Fig. 5a). Away from the follicles, a steady flow of differentiating keratinocytes is supplied by relatively rare epidermal stem cells located in the basal layer[42,43] and in the deep rete ridges[44]. Hair follicles contain two zones of stem-like cells. The hair shaft and its surrounding sheaths are produced by matrix cells located on a basement membrane overlying the protruding piece of dermis know as the dermal papilla. Longer-lived stem cells reside more apically within the 'bulge' region[45,46]. Cell marking and follicle transplantation experiments have shown that the bulge contains stem cells that repopulate the matrix region, form new sebaceous glands, and replace epidermal stem cells[47,48].

A stem cell replacement assay is not available for mammalian epidermis to test directly whether niches exist. Because bulge stem cells have not been mapped with single-cell resolution, they have yielded few clues concerning their mode of regulation. However, the normal developmental cycle of hair growth (anagen) and degrowth (catagen)[49]

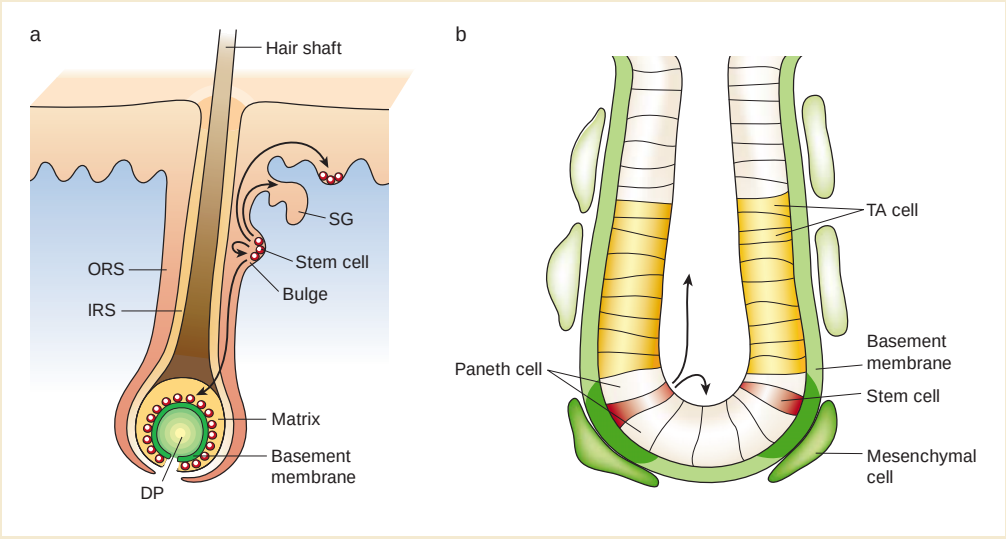

Figure 5 Epithelial stem cell niches. **a**, Mammalian epidermal stem cells. A hair follicle and a segment of adjacent skin is illustrated. The dermal papilla (DP) signals to matrix stem cells (red) located across a basement membrane (green). Matrix cell daughters (yellow) differentiate into a variety of cell types, including the medulla, cortex and cuticle of the hair shaft (brown), the inner root sheath (IRS) and the outer root sheath (ORS). About two-thirds of the way up an anagen follicle lies the bulge — an expanded region that contains long-term stem cells (red). These cells periodically replenish (arrows) the matrix cells, and also help maintain the sebaceous gland (SG) and the epidermal stem cells (red, top layer) that lie against the basement membrane (not shown) overlying the basal layer in interfollicular regions. **b**, A mammalian gut crypt is a tube of cells arrayed on a basement membrane (green). Stem cells (red) are located in the basal region along with Paneth cells, but their exact location is variable and both types account for only a fraction of the cells present in the regions shown. A portion of the basement membrane in the stem cell region may be specialized (dark green). Stem cell progeny (yellow) known as transit amplifying cells (TA) move upwards and differentiate. Underlying mesenchymal cells (green) send signals that help regulate stem cell activity.

provides a natural replacement assay, and leads us to regard the matrix region of the follicle as a stem cell niche. During catagen, the lower two-thirds of the follicle gradually disappears and the dermal papilla reaches the level of the bulge. When the next round of anagen begins, daughter cells derived from the bulge stem cells move onto the dermal papilla, become new matrix cells and re-initiate a hair shaft. Other bulge cell derivatives move upwards to maintain the sebaceous gland, and probably replenish surrounding epidermal stem cells.

The dermal papilla seems to be a key niche component and a source of signals that stimulate the activity of matrix stem cells. Hair follicles do not develop, persist or function without a dermal papilla. Fibroblast growth factor (FGF)-7 is produced in the dermal papilla and stimulates keratinocyte growth[50]. Targeted disruption of β1-integrin in matrix and outer root sheath cells retards matrix cell proliferation and hair growth without grossly disrupting matrix cell attachment to the dermal papilla during the first hair cycle[51,52]. Disruption of α-catenin leads to hyperproliferation[53]. BMP-4 is also expressed in dermal papilla cells, and mice expressing the BMP inhibitor Noggin in matrix cells and early hair precursors have altered proliferation of hair shaft precursors[54].

Additional studies are uncovering the roles played by two other signalling pathways. Sonic hedgehog (Shh) is required in developing hair follicles[55], and like Krox-20 and TGFβRII, it is heavily expressed in the matrix along only one side of the hair bulb[56]. Activating mutations in *shh* or *ptc1* frequently cause basal cell carcinomas in humans and mice, but the cellular origin of these carcinomas remains in dispute and it is unclear whether Shh normally stimulates matrix cell proliferation in its niche.

Genetic studies indicate that Wnt signalling is also required to form hair follicles[57,58] and for matrix-derived cells to differentiate into follicular rather than epidermal keratinocytes[59]. Overactivation of Wnt signalling causes mice to develop more hair follicles[60], and to yield skin- and hair-derived tumours[61]. Wnt3a and Wnt5a probably mediate a reciprocal maintenance signal from the follicle's epidermal components to the dermal papilla[62]. Stimulation of bulge stem cells, which express Tcf3, may explain some of these observations[58].

Drosophila follicle cells

The SSCs in the *Drosophila* ovariole seem to reside in a relatively simple niche (Fig. 4a). Two or three SSCs lie against the basement membrane at the posterior edge of a monolayer of inner sheath cells, which they resemble both morphologically and in their patterns of gene expression. Lineage-marking experiments[17] verify that the SSCs are stem cells and that their progeny give rise to at least ten different subtypes of ovarian follicle cells. The SSC division rate is coordinated with that of downstream cells and is regulated by nutrition over a fourfold range[63]. The main evidence for an SSC niche is a low level of natural stem cell replacement[17,64], similar to what occurs in the germ cell niche. It has not been shown, however, that a replacement stem cell occupies the same position as the lost SSC.

SSC division is strongly influenced by the activity of the Hedgehog (Hh) pathway[64–67]. Hedgehog is highly expressed in the terminal filament and cap cells that lie a short distance from the SSCs. Mutations in several genes that downregulate the Hh pathway decrease somatic cell production, whereas upregulation of the Hh signalling by various means causes a two- to threefold overproduction of follicle cell precursors[64–67]. Unlike nutritional changes, altering Hh signalling affects cell output by changing the number of stem cells[64]. Some of the extra SSCs are located very close to existing stem cells, suggesting that an individual niche has expanded in size to accommodate two rather than one SSC. In other cases, however, the stem cells are well separated, suggesting that new sites may also be colonized. New niches may be generated because excess Hh signalling causes a large increase in the number of cells in the stem cell region. It is not clear whether the extra stem cells and niches are as stable as normal SSCs in the endogenous niche, however, as they were lost relatively quickly[64].

Endodermal niches

Gut crypts

The final candidate stem cell niche that we consider here maintains a steady flow of cells from crypts (Fig. 5b) in the vertebrate gut to outwardly projecting villi. Crypts are small inpocketings of the gut surface lined by a single layer of columnar cells that lie against a basement membrane. Lineage-tracing experiments identify perhaps 4–5 stem cells that reside near the bottom of each crypt[68]. Stem cell progeny differentiate into four main cell types — Paneth, enteroendocrine, goblet and columnar — as they migrate up the walls of the crypt. Lineage labelling, including the use of a gut-directed promoter that drives *cre* recombinase and the use of a floxed marker gene[69], has still not pinpointed the exact number and location of stem cells in the

basal region of the crypt. Mosaic crypts generated in such experiments, or by somatic mutation *in vivo*, progress with time to become either all labelled or all unlabelled[69,70]. Such rapid equilibration is the expected result when a niche is maintained by a population mechanism rather than by asymmetric stem cell divisions.

Several specific cell types have been ablated to determine whether they contribute to the maintenance of stem cells. Between 30 and 50 Paneth cells reside near the stem cells in the basal region of the crypt, where they secrete antimicrobial 'cryptidin' peptides. When Paneth cells are ablated using a cryptidin2–diptheria toxin construct there is no change in cell proliferation, despite the fact that these cells also produce potential growth modulators[71]. Targeted ablation of the secretin-producing cells in the crypt[72] also has no effect on proliferation. Immature cells in these lineages are probably not removed in such experiments and cannot be ruled out as potential growth regulators.

Signals emanating from mesenchymal cells underlying the basal region of the crypt are thought to maintain the activity of crypt stem cells. One of the most important signals is mediated by the Wnt pathway acting through the Tcf-4 transcription factor. Stem cells are missing from the intestinal epithelium in mice lacking Tcf-4, indicating that Tcf-4-mediated signals are needed to form and maintain crypts[73]. Conversely, mutations in Apc or β-catenin can cause constitutive nuclear Tcf-4/β-catenin complexes, and accelerate proliferation. Disrupting the *Fkh6* gene, which encodes a transcription factor that is expressed in mesenchyme, causes intestinal cells to overproliferate[74]. It is important to note, however, that because gut stem cells cannot be identified individually, it has not be possible to prove that these genes act on stem cells rather than on their proliferating progeny.

Regulation in development

Niches modulate their numbers during development and in response to environmental factors. Normally, the number of hair follicles does not increase after a mouse is born[75]. In many tissues, however, niches multiply to keep pace with growth from juvenile to adult stages: there are many more crypts in adult guts than in newborns. Crypts usually duplicate by branching[76] — possibly when the number of stem cells or key stromal cells surpasses a critical threshold[77]. Experimental manipulations show that certain signals also have the capacity to induce *de novo* production of niches. For example, new hair follicles form in adult skin when Wnt signalling is upregulated[60]. In the *Drosophila* ovary, excess Hh signalling expands the size of the SSC niche and may create new niches[64]. Perhaps the regulated expression of these signals controls niche growth during development.

Niches also modify their regulatory properties in response to changing conditions to ensure that stem cell activity parallels the organism's needs for particular differentiated cell types. Bulge stem cells proliferate in response to local wounding[47]. After irradiation of the testis, the remaining stem cells are induced to divide equally with high probability to regenerate the population of stem cells and spermatogonia[78]. Hormonal cycles regulate the proliferation of stem cells in breast and uterine epithelium, whereas ovariolar stem cells respond to nutritional levels[63].

Developmental mechanisms that activate or silence niches are just beginning to be studied at the molecular level. The hair cycle provides one of the best-studied systems. Outer root sheath cells produce FGF-5, which signals to terminate anagen[79]. Consequently, mutants in the *angora* locus encoding FGF-5 have unusually long hair. Signalling through the EGF receptor seems to be required for entry into catagen. Expression of a dominant-negative EGF receptor in epidermal cells leads to long hairs[80], whereas loss of EGFR[81] or TGF-α[82] produces wavy hair and disorientated hair follicles. The *hairless* gene encodes a transcription factor that is required to maintain follicle integrity during catagen[83].

Common properties

Although niches seem to be structurally and functionally diverse, there may be a few common, organizing principles. Many niches use one or more specialized cell groups, such as the hub, the dermal papilla or the terminal filament/cap cells. A principal signal received by the stem cells in the niche often controls their behaviour, and may originate from within the specialized cells. Unpaired or Dpp fulfils this role for *Drosophila* male or female germ cells. Hedgehog may have this function in mouse and *Drosophila* epithelial cells. Overproduction of this signal can cause stem cell hyperplasia. In all cases, the actual signalling milieu in the niche is complex and involves additional components. A basement membrane may be part of most of the niches discussed. Extracellular matrices may help to structure niches spatially and modulate the concentration of adhesive and signalling molecules locally. In contrast, internally programmed mechanisms of generating asymmetry have not been found to play a significant role as yet.

Underlying similarities in niche regulation may ultimately minimize the distinction between lineage- and population-based regulation. It is becoming clear that the same niche may use either mechanism under particular circumstances. *Drosophila* GSCs normally use a lineage mechanism, but when replacing a missing stem cell a *Drosophila* female GSC divides equally and shifts its division plane so that both daughters contact cap cells. Similarly, stem cells in the mammalian testis are likely to alternate between the two mechanisms, although it is not known whether shifts in divisional orientation are involved. Clearly, identical stem cells might give rise to either mechanism depending on whether extra maintenance sites in the niche were available at the time and place that a stem cell divides. But not all niches may be this flexible. So far, there is no indication that the nematode gonad or the gut crypt operates other than by the population mode.

Implications

Stem cells may often be regulated in niches because of their developmental history. Like early embryonic cells, stem cells must have the capacity to grow and differentiate along several pathways in response to external signals. Rather than evolve complex, specialized internal mechanisms and signals to confer these characteristics, stem cells might have first appeared when niches acquired an ability to sequester, preserve and control undifferentiated embryonic cells that already had the necessary cellular properties. Such an origin would explain why most of the characterized niche regulatory mechanisms and signals also function in developing embryos. Although there are probably small differences among stem cells, this model predicts that the expression profiles of embryonic stem cells and those of stem cells from diverse tissues will be highly similar, encouraging attempts to reprogramme and interconvert them. Understanding niches and their signals will suggest excellent starting points for such efforts. □

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