The stem cell niche: theme and variations
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Stem cells in animal tissues are often located and controlled by special tissue microenvironments known as niches. Studies of stem cell niches in model systems such as Drosophila have revealed adhesive interactions, cell cycle modifications and intercellular signals that operate to control stem cell behavior. Candidate niches and regulatory molecules have also been identified in many mammalian tissues, including bone marrow, skin, gut and brain. While niches are an ancient evolutionary device with conserved features across diverse organisms, we suggest that certain niches display important differences in their organization and function.

Introduction
The notion that tissue stem cells reside within specific anatomical locations termed ‘niches’ arose nearly four decades ago from studies of transplanted hematopoetic progenitors [1]. The likely existence of microenvironmental factors produced by niche stromal cells has long cautioned that some aspects of stem cell biology may be difficult to deduce from purified stem cells. When the first niche to be defined at the cellular and functional level was described in the Drosophila ovary [2], stem-cell-extrinsic factors were indeed found to play a paramount role. Subsequently, stromal microenvironments likely to act as niches have been associated with an increasingly wide and diverse set of stem cells (Table 1). Hematopoetic stem cells (HSCs) reside in niches located in trabecular bone where they contact osteoblasts. Stem cells maintaining the multiple cell types of the mammalian digestive tract map to precise locations within discrete substructures (e.g. gastric units or intestinal crypts). Epidermal stem cells with the potential to replenish basal keratinocytes, hair and sebaceous glands are found in the hair follicle bulge. Ongoing cell production in the adult mammalian brain depends on astrocytes that reside in special niches within the subventricular zone of the cerebellum and the subgranular zone of the hypothalamus. These findings have already expanded the focus of stem cell research and deepened our understanding of how cell production is regulated in vivo.

The structure and properties of the best-characterized niches have been reviewed recently [3–5]. Despite this, our knowledge of niches remains limited. Variations in niche anatomy and regulatory mechanisms are just beginning to emerge. Here we consider recent studies of stem cell niches and suggest that multiple subtypes of niche may exist. Discerning such differences is likely to help us better understand how these remarkably small, simple units influence tissue growth, repair and aging.

Simple niches
We define a stem cell niche as ‘a specific location in a tissue where stem cells can reside for an indefinite period of time and produce progeny cells while self-renewing’. Many recently characterized niches, especially those in gonadal, epithelial and digestive tissue, appear to be surprisingly simple in structure and to operate using common mechanisms (Figure 1a). Often, specific junctions anchor a small number of stem cells adjacent to particular stromal partner cells (Table 1). In several tested cases, adherens junctions are involved [6**,7], while interactions with extracellular matrices are suspected to play a role [8]. Tight association with a permanent cell probably locks the stem cell in place and positions it to receive one or more critical intercellular signals. Transduction of certain intercellular signals in stem cells, for example bone morphogenetic protein (BMP) reception in Drosophila germ-line stem cells (GSCs), directly controls germ-line stem cells (GSCs), directly controls

Abbreviations
Bam bag of marbles
BMP bone morphogenetic protein
Dpp decapentaplegic
Gbb glass bottom boat
GEP gastric epithelial precursor
GSC germ line stem cell
HSC hematopoetic stem cell
IGF insulin-like growth factor
SSC somatic stem cell
SVZ subventricular zone
VEGF vascular endothelial growth factor
whose loss affects stem cell function will necessarily qualify as a stem cell regulator. Intercellular signals are a ubiquitous feature of tissues and in some cases may simply play general roles maintaining tissue physiology and/or cellular differentiation. Finally, niches must ensure that daughter cells differentiate appropriately as they leave the niche. The Drosophila testis recently joined those systems known to employ oriented divisions to direct stem cell daughters away from the stem cell microenvironment [10].

A true stem cell niche constitutes a stable aspect of tissue anatomy whether or not stem cells are present. Indeed, the ability of ‘empty’ niches to re-acquire and maintain introduced stem cells remains the definitive proof of their existence. Such assays have been carried out in the bone marrow, spleen and testis of the mouse and in the Drosophila ovary [1,11,12**]. In the ovary it has been possible to show that ectopic cells return to the exact location of the original stem cells at the cellular level.

Stem cell loss and replacement within simple, anatomically defined niches is not confined to experimental assays, but appears to be a major mechanism ensuring a stable and long-lasting supply of progenitors. Replacement of lost stem cells in the Drosophila ovary by rare symmetric divisions of an adjacent stem cell maintains stem cell number in vivo [2]. Similar behavior is likely to occur in mammalian testes, as stem cell niches are filled first within a region of the seminiferous tubule following introduction of germ cells [11]. Such a system not only ensures that lost stem cells will be replaced, but keeps stem cell numbers near maximum by quickly eliminating empty niches. Some tissues appear to regularly construct new niches to maintain an adequate stem cell supply. For example, bone is continually undergoing remodeling, and it is precisely in these areas that HSCs are enriched [13**,14**]. HSC number responds to changes in the number of osteoblasts [14**,15]. New intestinal crypts [16] and new hair follicles [17] can be generated de novo. Any stem-cell-based tissue that is capable of growth is likely to have the capacity to produce new niches.

### Complex niches

Some niches appear to be more complex than the relatively simple examples described above. For example, subventricular zone (SVZ) neural stem cells closely associate with and sometimes specifically contact other astrocytes, neuroblasts, ependymal cells, endothelial cells and a factor-rich basal lamina [18–20,21**,4]. The great cellular complexity of the nervous system may require that stem cell activity be subject to more controls than are required for other tissues. Indeed, the complex shape and junctional specializations of neural stem cells may facilitate diverse, wide-ranging interactions. While most other stem cells seem simpler in structure, suspicion is growing that they may be able to communicate with muscle, nerve and connective tissue cells surrounding the niche proper, and via humoral factors. For example, HSCs, gastric epithelial precursors (GEPs), somatic stem cells (SSCs) and probably other stem cells respond to insulin-like growth factors (IGFs) [22–24].

Complexity may also arise when multiple stem cells reside within a niche (Figure 1b). Several different stem

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**Table 1**

<table>
<thead>
<tr>
<th>Niche</th>
<th>Stem cell</th>
<th>Partner cell</th>
<th>Stem cells per niche</th>
<th>Important proteins</th>
<th>Junction</th>
<th>Gene profile</th>
<th>Other references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila ovariole</td>
<td>GSC</td>
<td>Cap cell</td>
<td>2–3</td>
<td>BMP, Nanos</td>
<td>DE-Cad, β-Cat</td>
<td>[12*,20,41]</td>
<td></td>
</tr>
<tr>
<td>Drosophila testis</td>
<td>GSC</td>
<td>Hub cell</td>
<td>7–15</td>
<td>JAK-STAT, BMP</td>
<td>DE-Cad, β-Cat</td>
<td>[37,38]</td>
<td></td>
</tr>
<tr>
<td>Mouse testis</td>
<td>GSC</td>
<td>HSC</td>
<td></td>
<td>BMP, Nanos, Plzf</td>
<td>E-Cad, β-Cat, Rac1, Rac2</td>
<td>[11,43–45]</td>
<td></td>
</tr>
<tr>
<td>Mouse bone marrow</td>
<td>HSC</td>
<td>Osteoblast</td>
<td></td>
<td>Sca-1, Bmi-1</td>
<td>[46–48]</td>
<td>[13**,14**,15]</td>
<td>[30,33,49]</td>
</tr>
<tr>
<td>Mouse intestinal crypt</td>
<td>IEP</td>
<td>GEP</td>
<td>1–2</td>
<td>Wnt, Shh</td>
<td>[50]</td>
<td>[9**]</td>
<td></td>
</tr>
<tr>
<td>Mouse gastric ismus</td>
<td>GEP</td>
<td></td>
<td></td>
<td>Shh, IGF</td>
<td>[22]</td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>Drosophila ovariole</td>
<td>SSC</td>
<td>Inner sheath cell</td>
<td>1</td>
<td>Hh</td>
<td>DE-Cad, β-Cat</td>
<td>[7,24]</td>
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<tr>
<td>Mouse skin</td>
<td>IESC</td>
<td>Dermal papilae</td>
<td>&gt;50</td>
<td>c-myc, p63, Wnt, BMP</td>
<td>Integrin, β-Cat</td>
<td>[8,17,25**,53,54]</td>
<td></td>
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<tr>
<td>Mouse hair follicle bulge</td>
<td>ESC</td>
<td></td>
<td></td>
<td></td>
<td>[51]</td>
<td>[51]</td>
<td></td>
</tr>
<tr>
<td>Mouse hair follicle</td>
<td>MPC</td>
<td></td>
<td></td>
<td></td>
<td>[51,55,56]</td>
<td>[51,55,56]</td>
<td></td>
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<tr>
<td>Mouse lateral ventricle</td>
<td>Melanoblast</td>
<td></td>
<td></td>
<td></td>
<td>[26*,57]</td>
<td>[26*,57]</td>
<td></td>
</tr>
<tr>
<td>Mouse hippocampus</td>
<td>SGZ astrocyte</td>
<td>Vascular cells; astrocytes</td>
<td></td>
<td>Shh, VEGF, Bmi-1, TLX</td>
<td>N-Cad, β-Cat</td>
<td>[6**,21**,29,31,58,59]</td>
<td>[8,19,19,29,31,59–62]</td>
</tr>
</tbody>
</table>
cells have been localized in the hair follicle bulge, but it is not known if they interact [25*,26*]. Stem cell–stem cell communication is likely in the Drosophila testis, where separate stem cells for germ cells and for somatic cyst cells lie in contact [27]. While coordinated activity of these stem cells has yet to be demonstrated at the single cell level, both stem cell types must divide to generate a new spermatogonial cyst containing one germ cell and two cyst cells. It may be that niches should not be thought of as units of stem cell maintenance, but rather as units of production of specific cellular outputs — spermatogonial cysts, ovarian follicles, intestinal villi, etc. If so niches might be expected to contain whatever stem cells and coordination mechanisms are adequate for the job.

Seemingly simple niches may exhibit complex temporal behavior. For example, it may be possible to support new stem cells without maintaining any special, pre-existing stromal architecture (‘empty niches’). Local structures to anchor and maintain new stem cells might simply be induced following stem cell arrival. Finally, some tissues may have the capacity to support stem cells without any anatomical specializations beyond a large expanse of basement membrane. The basement membrane of mammalian epidermis or seminiferous tubule may fall in this category. If stem cells can be supported by spatially uniform signals and non-specific stromal contacts alone, it would follow that niches are sometimes unnecessary for stem cell maintenance or else that they can be extraordinarily large.

**Storage niches**

A potentially different type of niche, the ‘storage niche’, may contain quiescent stem cells (Figure 1c). The bulge region of the mouse hair follicle currently represents the canonical example of such a niche. During most of the hair cycle and in the absence of wounding, transient epithelial stem cells and melanoblasts in the basal keratinocyte layer and the hair follicle matrix support ongoing skin and hair production. Reserve stem cells located in the bulge do not divide during this period and hence can preferentially retain labeled DNA, a trait often associated with stem cells. Following wounding or hair cycle completion, however, sub-populations of bulge stem cells activate, exit the niche, and migrate to the site of damage or stem cell loss [25*]. Melanoblast progenitors are also stored in or just below the bulge [26*]. It is not known whether bulge stem cells comprise distinct subtypes or interact with each other and/or partner cells, or even whether they migrate directly out of the niche or only send their daughters to serve as new transient stem cells. Likewise, the adhesive contacts and molecular signals that mediate their responses have not been characterized. Storage niches may simply be normal niches that are located in favorable, damage-resistant regions or they may contain unique mechanisms to facilitate the safe maintenance of quiescent cells.

**Programming daughter cells**

Niches with active stem cells must contain routes for progeny cells to exit lest they burgeon into tumorous nodules. For example, HSC daughters move away from the osteoblasts of the trabecular bone and toward the center of the marrow, while spermatogonia leave the basal layer and migrate toward the lumen of the seminiferous tubule. We consider a cell to have left the niche when it reaches a location that cannot itself support a stem cell because one or more critical adhesive or signaling factors is no longer present. Even before it has done so, the daughter cell may begin to differentiate. Thus, niches are likely to contain specific structural features and
mechanisms designed to ensure appropriate daughter cell movement and to initiate differentiation.

Daughter cells continue to divide and specialize long after leaving the niche; hence it is technically very difficult to sort out the relatively rare niche-specific differentiation mechanisms. Gene profiling studies are currently being used to identify candidate genes expressed differentially in stem cells or their progeny (see Table 1). The difficulty of obtaining highly pure cell populations remains the major obstacle to this approach. Although no common molecular processes (‘stem cell genes’) have yet been identified, some candidate molecules likely to control the differentiation of particular daughter cells are beginning to emerge. For example, Bmi-1 and TLX encode putative transcriptional regulators that may play a key role regulating HSCs and possibly neural stem cells [28–31]. HSCs also require Wnt signals [32] and are modulated by Sca-1 [33].

Our knowledge of the mechanisms by which stem cell daughters are programmed has progressed furthest in studies of the Drosophila ovarian cystoblast (Figure 2). The bag-of-marbles (bam) gene is necessary and sufficient for the cystoblast fate; this gene is normally off in stem cells and young daughters (pre-cystoblasts). Consequently, daughter programming involves the differential activation of bam expression only in the stem cell daughter that will become a cystoblast. Stem cells lie adjacent to cap cells, a site that for unknown reasons strongly favors BMP signal reception [12]. BMP receptor activation in stem cells directly represses bam transcription [34,35]. Consequently, the stem cell daughter that loses cap cell contact will downregulate BMP signaling, upregulate Bam expression and acquire a cystoblast fate.

Prolonged cytokinesis, a cell cycle modification characteristic of all early germ cells [36], appears to provide a
final, sophisticated level of control (Figure 2). There is a strong temporal correlation between furrow closure, loss of daughter cell BMP signal reception and the onset of daughter cell bam expression. This suggests that bam transcription can continue to be repressed through the open cytokinesis furrow, possibly by the movement of active Smad complexes. Using furrow closure as the final switch to shut off the BMP signal and to initiate cystoblast differentiation may ensure that daughter cells have fully separated before transcribing a gene (bam) that could cause their stem cell mothers to differentiate.

Gonialblasts, the GSC daughters in the Drosophila testis analogous to cystoblasts in the ovary, have recently been shown to utilize this pathway as well. Male GSCs, like female GSCs, do not express Bam, and recent studies demonstrate that high Bam levels cause male GSCs to differentiate [37,38]. Furthermore, BMP signaling is activated in male GSCs by Dpp (decapentaplegic) and Gbb (glass bottom boat) ligands and acts to represses bam expression, as in female GSCs [37,38]. Unlike in the ovary, however, Bam is not immediately required to initiate gonialblast divisions, and evidently other genes contribute to gonialblast specification and development. Once formed, reciprocal signals between gonialblasts and somatic cyst cells promote continued differentiation [39].

Stabilizing daughter cell fates

A stem cell daughter that encounters an empty niche can sometimes enter and become a stem cell again. Most cells, by contrast, once they have begun to differentiate, appear to be precluded from reverting, at least under normal conditions. Recently, Kai and Spradling [36] showed that interconnected germ cells in four- and eight-cell cysts can detach from their neighbors and revert to fully functional stem cells with very high efficiency when placed in the context of a larval ovary, or an adult germ cell tumor. Brawley and Matunis [40] induced stem cell differentiation by shifting STATs flies to the non-permissive temperature, and then looked for reversion of the remaining cysts following a return to normal temperature. They observed that male cystocytes (spermatogonia), like female cystocytes, could revert into apparently functional GSCs. Interestingly, somatic stem cells were always restored as well, suggesting that they might play some role in the reversion process. These studies clearly show that stem cell daughters do not immediately lose the capacity to function as stem cells. In each stem cell lineage, specific mechanisms are probably required to stabilize the early steps of differentiation, steps that can sometimes be reversed when these mechanisms are absent or are overridden. Transit amplifying cells often greatly outnumber stem cells in vivo; thus, these cells are likely to represent a valuable source of replacement stem cells for normal or therapeutic tissue repair.

Summary

Niches have emerged as a major mechanism of stem cell regulation. Our knowledge of niches remains very limited, but is starting to grow and solidify. Discerning differences such as those proposed in this review may help reveal how these remarkably small, simple units influence tissue development, growth, repair and aging.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


HSCs are shown to reside near osteoblasts in regions of trabecular bone and may adhere via adherens junctions. Expanding the number of osteoblasts by disrupting BMP receptor type IA function increases the number of functional long-term HSCs. In conjunction with the paper by Calvi et al. [14], this study begins to define the structure and regulation of an HSC niche in mammalian bone marrow.


The authors show that the number of HSCs depends on the number of osteoblasts, and implicate the activation of the Notch receptor in HSCs by the osteoblast-expressed Jag1 ligand. In conjunction with the paper by Zhang et al. [13], this study begins to define the structure and regulation of an HSC niche in mammalian bone marrow.


Mice expressing the BMP signal inhibitor noggin under the control of the villin promoter display intestinal defects similar to human patients with juvenile polyposis, a disease associated with BMP pathway mutations. The authors propose that BMP signals from mesenchymal cells act in intestinal epithelial cells to repress de novo crypt formation.


Coculture of neural stem cells with endothelial cells, but not vascular smooth muscle or NIH3T3 cells, inhibits stem cell differentiation and results in increased neuron production following their removal from coculture. This inhibition is probably mediated by activation of the Notch and Hes1 genes. The results of this paper therefore suggest an in vivo role for maintenance of neural stem cells by adjacent endothelial cells.


The authors show using a histone H2B-GFP reporter that slow-cycling mouse epidermal cells (stem cells) are found primarily in the hair follicle bulge. Subsets of these cells can be activated and migrate to the site of an epithelial wound, or to a receding hair matrix. In addition to illuminating epithelial stem cell biology, this scheme can be used to enrich the stem cells for molecular studies.


By following the behavior of cells of the melanocyte lineage through multiple hair cycles and experimental conditions using a transgenic marker, melanocyte stem cells were localized to the vicinity of the hair follicle bulge. Melanocyte stem cell progeny are able to exit the niche, proliferate and then re-populate vacant niches in other hair follicles.


BMP signaling is shown to directly repress the expression of the cysto-blots differentiation gene bam in Drosophila ovarian GSCs. This suggests a very simple model for how the different daughter cell fates of an asymmetric stem cell division are programmed.


The authors use a brief pulse of the bam gene product to induce formation of four germ cell types (which are normally fully stable) in young larval ovaries. After turnover of the Bam protein, cyst cells break up and revert into fully functional stem cells. These findings, and those of Brawley and Matunis [40], document that differentiation away from the stem cell fate is not irreversible.


Loss of JAK-STAT signaling pathway function from Drosophila males results in the differentiation of all germine stem cells into spermatocytes. Restoring JAK-STAT signal reception within these spermatocytes results in their reversion to functional stem cells. These findings, and those of Kai and Spradling [36], document that differentiation away from the stem cell fate is not irreversible.
42. Schultz N, Hamra FK, Garbers DL: A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. Proc Natl Acad Sci USA 2003, 100:12201-12206.