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Socializing with the Neighbors: Stem Cells and Their Niche

Review

Elaine Fuchs,* Tudorita Tumbar, and Geraldine Guasch
Howard Hughes Medical Institute
The Rockefeller University
New York, New York 10021

The potential of stem cells in regenerative medicine relies upon removing them from their natural habitat, propagating them in culture, and placing them into a foreign tissue environment. To do so, it is essential to understand how stem cells interact with their microenvironment, the so-called stem cell niche, to establish and maintain their properties. In this review, we examine adult stem cell niches and their impact on stem cell biology.

The Existence and Importance of Adult Stem Cells and Their Niches

The magnificent ability to generate an embryo from a single fertilized oocyte or to regenerate certain tissues, upon injury or natural physiological turnover, is a direct result of stem cells, nature's gift to multicellular organisms. The gold standard of stem cells is the fertilized egg, which produces an organism replete with a myriad of specialized cell types, including reproductive germ stem cells (GSCs). As the embryo first develops, an outer protective shell of support cells encases an undifferentiated mass of pluripotent embryonic stem cells (ESCs) that will make the animal. As development proceeds, pluripotent embryonic stem cells disappear as more restricted somatic stem cells (SSCs) give rise to the tissues and organs.

Although cell diversification is largely complete at or shortly after birth, organs must possess a mechanism to replenish cells as they die, either by natural wear and tear (homeostasis), or by injury. To accomplish this feat in the adult world, many developing tissues set aside life-long reservoirs of somatic stem cells, which retain some of the versatile characteristics of their early ESC counterparts, including the capacity to seemingly endlessly self-renew, i.e., divide and create additional stem cells (Schofield, 1978).

Even though the elite privilege of differentiating into most if not all cell types appears to be reserved for the more versatile ESCs, adult SSCs residing within an organ or tissue are nevertheless able to progress to differentiate along at least one (unipotent) and typically multiple (multipotent) lineages. Given the life-long importance of stem cells, they must be tucked safely from harm's way. The protective niches are composed not only of stem cells but also a diverse gathering of neighboring differentiated cell types which secrete and organize a rich milieu of extracellular matrix and other factors that allow stem cells to manifest their unique intrinsic properties, including the ability to self renew, while keeping their repertoire of differentiation programs on hold.

The importance of the niche is perhaps best exemplified by experiments in which the fate of ESCs is monitored following their subcutaneous injection into nude mice. ESCs isolated from a blastocyst-stage mouse embryo can be propagated indefinitely in tissue culture without losing their pluripotent potential (Thomson et al., 1998; Shamblott et al., 1998 and references therein). However, when faced with a foreign environment of surrounding in vivo tissue, ESCs unleash a Pandora's box of differentiation programs, forming ugly multicellular tumor masses, known as teratomas, which contain a multiplicity of cell types. Without the appropriate microenvironment of specific intercellular interactions and cellular organization, the ESC can become an undesirable beast. By contrast, as shown by Beatrice Mintz and Martin Evans in the 1970's, when injected instead into the center of a recipient mouse blastocyst, analogous to their native niche, ESCs resume normal behavior and contribute to generating all of the tissues of a healthy normal chimeric offspring. Taken together, these findings imply that it is the combination of the intrinsic characteristics of stem cells and their microenvironment that shapes their properties and defines their potential.

Exactly when and how most somatic stem cell niches develop is still a mystery. And in the world of stem cell niches, there is considerable variation in niche design. Some adult stem cells exist in relative isolation, making it seem as though they lack a specified niche within their respective tissue. Muscle stem cells, known as satellite cells, for instance, normally remain quiescently attached to the basal lamina that ensheathes each muscle fiber bundle, and only become reactivated to proliferate and fuse into differentiated myotubes when damaged fibers from injury need repairing. By contrast, tissues that undergo continual turnover are typically subdivided into units, each of which is supported by a small reservoir of stem cells responsible for replenishing and rejuvenating the tissue as needed. Figure 1 illustrates some examples of organized stem cell compartments that reside within adult tissue units.

Taking a Hold on Stem Cells

Various lines of evidence suggest that once a stem cell compartment is formed in a tissue, stem cells take up long-term residence there. What keeps stem cells in their niche? Much of what we have learned about this process comes from studies on *Drosophila* germ stem cells (GSCs). These studies show that direct physical interactions between stem cells and their nonstem cell neighbors in the niche are critical in keeping stem cells in this specialized compartment and in maintaining stem cell character.

In the adult female fly, clusters of 2-3 GSCs can be found in the germarium, located at the anterior end of each ovariole (Figure 1). At the very tip of each germarium are cap cells, which make direct physical contact with surrounding GSCs. When female GSCs divide, the cell directly contacting the cap remains a GSC; the daughter that loses cap contact differentiates and initiates oogenesis. Similar niche architecture also sets the stage for GSC retention in *Drosophila* testis, where GSCs maintain male stem cell character through direct

*Correspondence: fuchslb@rockefeller.edu

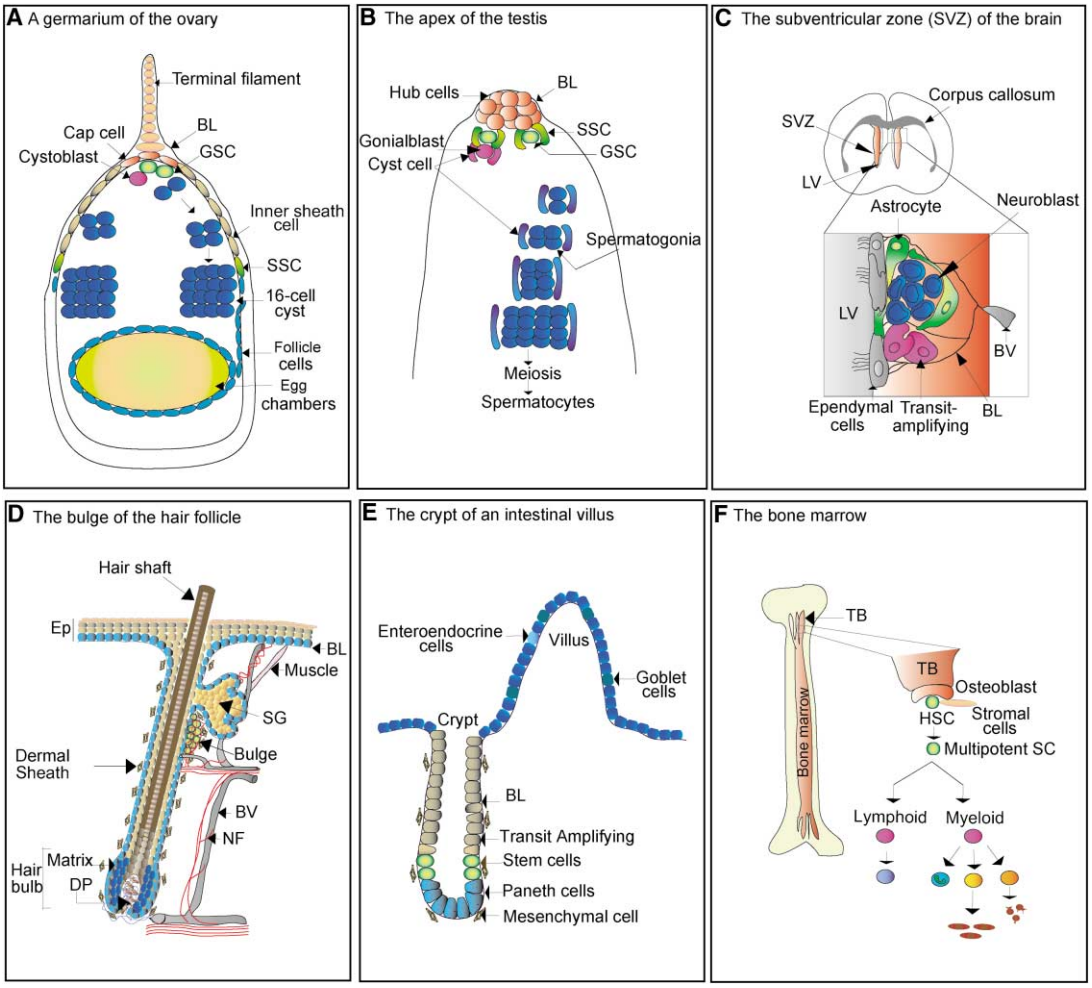

Figure 1. Stem Cell Niches

Shown are six different stem cell niches, along with their stem cells, committed progeny, and various associated nonstem cell types. In each frame, stem cells are represented in light green. Transit amplifying and/or differentiated progeny are in blue and the nonstem niche cells are highlighted in pink/red.

(A) Sagittal view of a *Drosophila* germarium, showing the location of germline (GSCs) and somatic (SSCs) stem cells. Cap and terminal filament cells contact the basal lamina (BL) and constitute the stem cell niche. GSCs are maintained by cap cell contact, but if their division yields a cell that loses cap cell contact, a cytoblast is produced. These transit-amplifying cells divide and then differentiate to produce the cysts (in blue). At the posterior zone, 2-3 SSCs produce prefollicle cells and form an epithelium surrounding the 16 cell cyst to produce an egg chamber.

(B) Sagittal view of the apical tip of the *Drosophila* testis. Germline stem cells (GSCs) attach to a cluster of hub cells. Loss of hub contact results in a commitment of the GSC to differentiate into a gonialblast. These transit-amplifying cells undergo 4 synchronous divisions, followed by differentiation to form spermatogonia. Later, spermatogonia enter meiosis and become spermatocytes.

(C) Frontal schematic of the adult mouse brain showing the location of the subventricular zone (SVZ) between the lateral ventricle (LV) and the striatum. A single layer of ciliated ependymal cells separates the SVZ from the LV. SVZ astrocytes are thought to be the stem cells, which divide to generate transit-amplifying cells, which in turn generate the neuroblasts that migrate to the olfactory bulb. An ECM-rich basal lamina makes contact with all SVZ cell types and constitutes an essential component of the SVZ stem cell niche. BV, blood vessel.

(D) In the skin, multipotent stem cells reside in the hair follicle, in a region known as the bulge. The bulge is located below the sebaceous gland (SG) and at the juncture of the arector pili muscle. The entire follicle is surrounded by a basement lamina (BL), which is surrounded in turn by a dermal sheath. The bulge also likely receives inputs from sensory nerve endings and blood vessels, which encase this region. Bulge stem cells give rise to transit-amplifying matrix cells, which proliferate and then differentiate to produce the hair shaft and the channel that surrounds it. Matrix cells are surrounded by a pocket of specialized mesenchymal cells, the derma papilla (DP), with potential hair growth inductive properties. Nerve fibers (NF, red) innervate the epidermis (Ep) and the bulge, and blood vessels (BV, gray) provide nutrients.

(E) The intestine is compartmentalized into crypts. Stem cells (green) reside near the base of each crypt, depicted here as a tube of cells. Intestinal stem cells give rise to four different progeny: paneth cells, enteroendocrine cells, goblet cells, and adsorptive villus cells. The crypt epithelium is separated by a basal lamina (BL) that is surrounded externally by gut mesenchyme. The mesenchymal cells emit signals that participate in regulating stem cell activity.

(F) The hematopoietic stem cell (HSC) resides in the bone marrow. This complex microenvironment comprises many different cell types (e.g., macrophages, adipocytes, fibroblasts), which together secrete a specialized extracellular matrix. Critical in HSC maintenance, the osteoblasts line the inner surface of the trabecular bone (TB). When osteoblast-HSC contact is lost, the HSC progresses to form myeloid and lymphoid progenitors. The lymphoid lineage produces B, T, and NK cells whereas the myeloid lineage produces granulocytes, erythrocytes, and platelets.

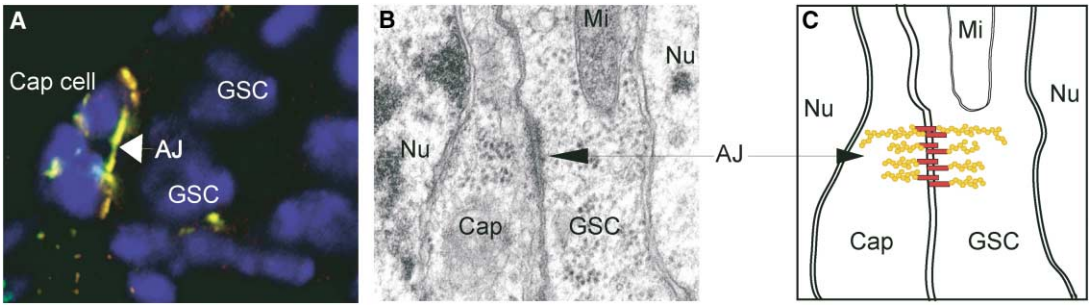

Figure 2. A Role for Adherens Junctions in Sequestering and Maintaining Stem Cells in their Niche

(A) Apical tip of a wild-type germarium that is immunofluorescently labeled for DE-cadherin (red), Armadillo/β-catenin (green), and nuclei (blue). The yellow band indicates colocalization of DE-cadherin and Arm, prominent at the border between cap cells and GSCs. AJ, adherens junction.
(B) Electron micrograph illustrating an adherens junction (arrow) between a cap cell (left) and a GSC (right). Nu, nuclei. Mi, mitochondria.
(C) Schematic of the adherens junction in (B), depicting a homotypic interaction of the extracellular domains of the transmembrane DE-cadherins (red) that are linked via catenins to the actin cytoskeleton (orange). [photos in A, B, are reprinted with permission from T. Xie and *Science*, as previously published in Figures 1F, 1G in Song et al., Science 296, 1855-1857, 2002; copyright 2002 AAAS].

association with the hub, composed of somatic cells at the apical tip of the testis. Daughters that detach from the hub initiate a differentiation program to become spermatogonia.

The molecular glue that anchors GSCs to their niches is at least in part DE-cadherin, which along with its partner, Armadillo (β-catenin in vertebrates), concentrates at GSC-niche borders (Figure 2A). Cadherins and catenins participate in the formation of specialized intercellular junctions, called adherens junctions, which can be remodeled by virtue of their association with the actin cytoskeleton (Figures 2B and 2C). The importance of adherens junctions in GSC retention by the cap cells has been revealed through genetic studies, which have shown that mutations in either DE-cadherin or in *armadillo* result in a failure of the cap cell niche to recruit and maintain GSCs (Song et al., 2002).

The ability of stem cells to reside within niches is an evolutionarily conserved phenomenon, and hence it is not surprising to see that the basic molecular features of stem cell retention are broadly utilized across the eukaryotic kingdom. Although the genetic details are still unfolding even in the best-studied *Drosophila* systems, there are already hints of such conservation and usage in vertebrate somatic stem cells. One such example is the bone marrow, where HSCs traverse along the inner surface of the bone, lined with spatially oriented osteoblasts. As HSCs progressively mature, they lose contact with these neighboring stromal cells, become more proliferative, head toward the central bone marrow cavity and traverse into the blood vessels. When mice are genetically altered to increase osteoblast numbers, HSC numbers concomitantly rise and intriguingly, the ability of these new HSCs to retain their slow-cycling characteristics appears to rely upon their ability to adhere directly to the osteoblasts through N-cadherin-mediated adherens junctions (Zhang et al., 2003).

Other putative players in establishing stem cell retention are the integrins, which mediate adhesion of cells to a basal lamina composed of extracellular matrix. Elevated levels of integrins are often characteristic of stem cells, and loss of function studies in mice reveal that both integrins and adherens junctions play critical roles in maintaining the location, adhesiveness and proliferative status of epithelial cells within tissues (reviewed by Watt and Hogan, 2000). By providing a unique milieu of ECM ligands for the integrin receptors on the surface of stem cells, the niche can further strengthen its ability to retain its precious residents. Thus, for example, HSCs express the integrins $\alpha 4\beta 1$ and $\alpha 5\beta 1$, which bind to fibronectin and promote adhesion to the bone marrow stroma; antibodies against these integrins block hematopoiesis in long-term bone marrow cultures (reviewed by Whetton and Graham, 1999).

The ability of the niche to retain its stem cells is also likely to play a role in recruiting stem cells, a process referred to as "homing" (Whetton and Graham, 1999). Although the molecular details of this process are still unknown, it seems likely that niches develop concomitantly with input from both the stem cells and the surrounding tissue. Once niches are established, however, they seem to be able to survive at least transiently as signaling centers to attract stem cells.

Several examples illustrate this point. In vertebrates, transplanted HSCs from one animal can find their way into the irradiated bone marrow of a host animal. Studies in *Drosophila* further indicate that when GSC niches are experimentally "emptied" of their GSCs, the niches still persist and can even signal incoming somatic stem cells to take up foreign residence and at least transiently maintain some of their stem cell features (Kai and Spradling, 2003). Although properly tailored ECM and cell-cell adhesive molecules in the niche are no doubt important parameters in helping stem cells to find their way to niches, the process may also be actively regulated through longer range extracellular signaling molecules. In vertebrates, this might be particularly important during injury when inflammatory- and wound-response stimuli are generated within the tissue (Whetton and Graham, 1999).

Governing Stem Cell Quiescence and Activation in the Niche

Although somatic stem cells can take advantage of an emptied GSC niche, the niche microenvironment does not appear to convert these masquerading aliens into

bona fide GSCs (Kai and Spradling, 2003). Similarly, when outside the niche, mammalian somatic stem cells that are injected into the backs of immunocompromised mice seem only able to generate cellular masses largely composed of the stem cell's developmentally established, differentiated lineages; for SSCs, these are considerably more restricted than for ESCs. Despite intensive investigation, evidence is lacking for an external environment(s) that can induce comprehensive, stable reprogramming of a somatic stem cell's normal repertoire of lineages (reviewed by Wagers and Weissman, 2004). On the other hand, the niche is critical in maintaining the intrinsic self-renewing, undifferentiated character of the resident stem cells, and a priori, this may even extend to alien stem cells. What is so special about niches that endow them with this wonderful talent?

Some insights are gained by examining the properties of stem cells in and out of their niches. Inside the niche, stem cells are often quiescent. Since proliferation can often be induced, e.g., in tissue culture, this feature suggests that the niche's microenvironment is both proliferation- and differentiation-inhibitory. How then do stem cells get activated in their niche?

A priori, one might envision a constant flux of slowly dividing stem cells, such that as the niche becomes occupied, excess stem cells are displaced as or shortly after they divide, thereby physically loosening connections with the niche. In this scenario, when faced with a new environment, the expelled stem cells or their recently divided progeny progress to differentiate. Alternatively, stem cells might simply remain dormant within the niche until they have to become functional, e.g., in response to injury. In this case, an environmental change from the tissue might actively signal to the niche to mobilize their residents.

Based upon the evidence at hand, the first mechanism appears to be more frequently utilized during development, while the second model may be more prominent in adult tissues that do not undergo dramatic tissue turnover. The stable quiescence of adult stem cells in their niche is best demonstrated by measuring the frequency at which they undergo DNA synthesis. Typically, after a sufficiently long pulse of bromodeoxyuridine or tritiated uridine, cells within the self-renewing target tissue become labeled. After a chase period, rapidly dividing cells dilute out the label and terminally differentiating cells are lost in the homeostatic flux of tissue turnover (Taylor et al., 2000; Oshima et al., 2001 and references therein). By adapting this method to employ tetracycline-controlled expression of a fluorescently tagged histone in mice, label-retaining cells (LRCs) can be visualized in stem cell niches (Tumbar et al., 2004). In adult haired skin, the brightest LRCs reside in each bulge of the hair follicle (Figure 3A).

The hair follicle represents an interesting case where the niche receives a periodic stimulus from specialized mesenchymal cells, known as dermal papilla (DP; Figure 1D). During the hair cycle, a growth period is followed by a destructive phase, in which the region below the bulge degenerates. The receding follicle carries the DP from the base of the hair bulb up to the bulge. This cyclic alteration in microenvironment appears to stimulate one or two LRCs to exit the niche, divide rapidly and terminally differentiate to regrow the follicle and produce a new hair (Figure 3B; Tumbar et al., 2004). By contrast, in response to injury, bulge LRCs exit the niche and move upward to repair the skin epidermis (Taylor et al., 2000; Figure 3B). In this case, many LRCs appear to be mobilized, and some may even migrate prior to dividing, perhaps reflecting the increased urgency for the reaction (Tumbar et al., 2004). The precise factors and cues that directionally rally skin stem cells to execute one of these two separate lineage programs are not yet known, although in both cases, a distinct change in microenvironment is clearly involved. The normal microenvironment, established by signals from the various other cells that normally surround the niche (Figure 1D) seem to be important in maintaining the slow-cycling properties of LRCs and keeping them in reserve.

The Role of the Niche in Regulating Symmetric Versus Asymmetric Stem Cell Divisions

Regulating stem cell self-renewal is an essential feature of the niche, and outside the niche, stem cells must either possess sufficient intrinsic factors to overcome differentiation or succumb to such a fate. The ability to divide symmetrically to generate identical twins is a feature of most cells, including stem cells. However, it is becoming increasingly clear that a number of multipotent stem cells possess an added ability to undergo asymmetric cell divisions, yielding one committed progenitor daughter and one stem cell daughter (Figure 4A). In these niches, regulating the balance between symmetric and asymmetric stem cell divisions becomes critical in maintaining proper stem cell number within the niche and in meeting the demand for differentiated cells within its surrounding tissue. Additionally, such niches could participate in precisely orienting asymmetric divisions in order to orchestrate the flow and directionality of the committed progeny.

For a daughter to be a stem cell, it must retain self-renewal and differentiation inhibitory factors. For a daughter destined to proliferate and differentiate along a particular lineage, this progeny cell must either receive too few stemness factors to maintain this state, and/or inherit proliferation and/or differentiation factors that can overcome this state. Based upon the studies so far, both features are likely to occur as a consequence of asymmetric stem cell divisions. How is this remarkable imbalance achieved and how is it spatially oriented?

The overall problem can be conceptualized by linking three distinct mechanisms: (1) the generation of cell polarity; (2) the orientation of the mitotic spindle; and (3) the segregation of differentiation and/or stem cell determinants, either equally into each of the two daughter cells or asymmetrically into one or the other daughter. In the hypothetical model illustrated in Figure 4A, contact of stem cells with a basal lamina participates in polarizing stem cell and differentiation-specific cell determinants. In this scenario, if the strongest spindle-polarizing signal emanates from the basal lamina pole, the cell determinants will be asymmetrically partitioned following mitosis (example at left). By contrast, if the strongest spindle polarizing signal localizes at sites of cell-cell contact, the determinants are partitioned equivalently, leaving two identical daughters (example at right). Thus, the overriding key in assessing whether a

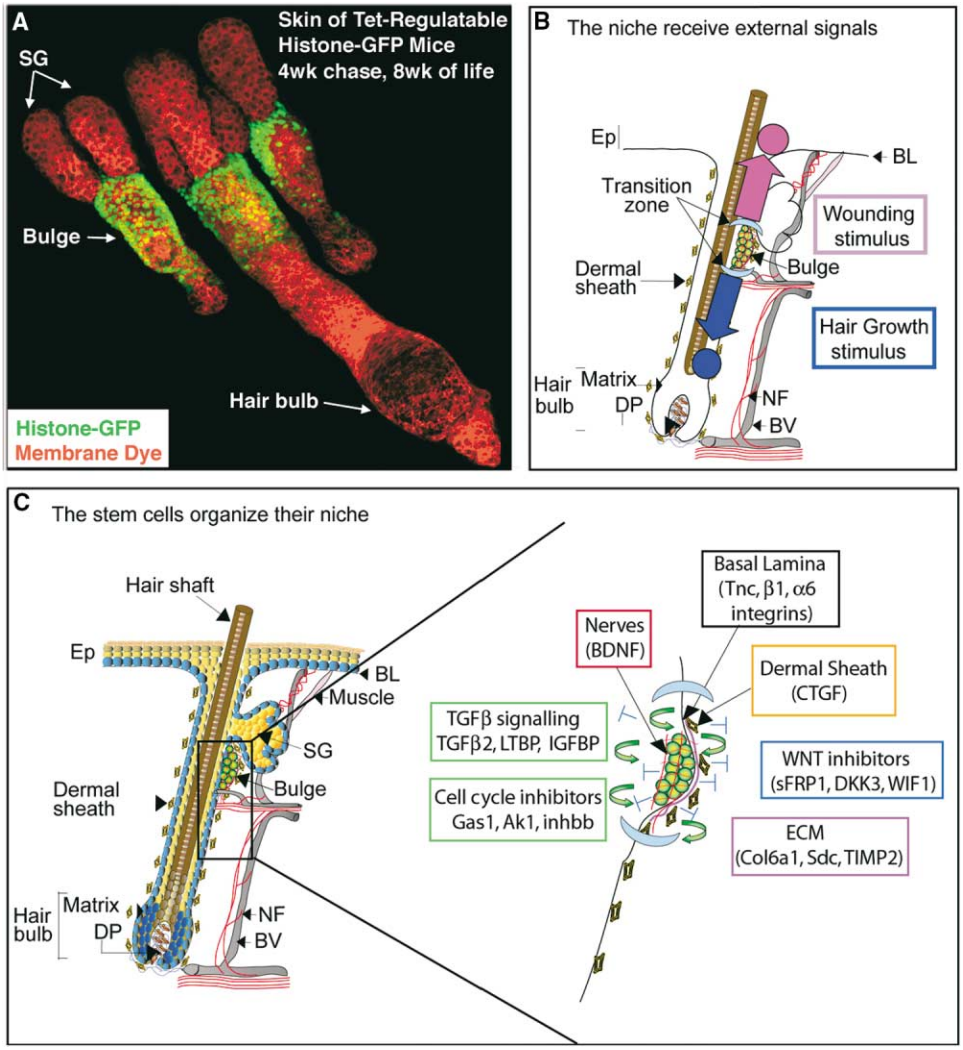

Figure 3. Signaling between Stem Cells and Their Niche

Several key concepts about the stem cells and their niches are illustrated here, using the follicle bulge niche as a model.

(A) The niche is a quiescent and differentiation-inhibited environment. Shown are three follicles from the skin of a transgenic mouse engineered to regulate histone H2B-GFP (green) under the control of a tetracycline responsive transcription factor (Tumbar et al., 2004). After four weeks of postnatal H2B-GFP expression, the gene was shut off, and four weeks later, the skin epithelium was removed, treated with a red fluorescent dye that intercalates into cellular membranes, and visualized by fluorescence microscopy. [courtesy of Valentina Greco].

(B) The niche microenvironment undergoes changes in response to various external cues. At the start of each hair cycle, signaling between the bulge and the dermal papilla (DP) (blue arrow) stimulates the stem cells to differentiate downward and regrow a new hair follicle. In response to wounding (pink arrow), the stem cells are stimulated to differentiate upward to reepithelialize the skin epidermis.

(C) Model for how stem cells and niche cells may develop and be maintained in a tissue. Transcriptional profiling of the label-retaining cells (LRCs) in the bulge suggests that stem cells not only receive signals from their niche, but may also signal to the surrounding niche cells (Tumbar et al., 2004). In parentheses some of the genes are upregulated by 2X in LRCs versus their closely related progeny. Some secreted factors (e.g., BDNF and CTGF) are potentially stimulatory for the niche rather than the stem cells. The ability of stem cells to signal to prospective tissue niche cells and vice versa provides insights into how niches may develop within a tissue.

division will be asymmetric or symmetric is whether the orientation of the mitotic spindle is parallel to or perpendicular to the major cell polarity axis that aligns the cell fate determinants.

The mechanisms underlying the establishment of cell polarity and spindle orientation are complex and in some cases, appear to involve overlapping subsets of factors, including APC, dynein-dynactin, PKCξ, actin, and myosins (reviewed by Perez-Moreno et al., 2003; Petritsch et al., 2003; Etienne-Manneville and Hall, 2003). The ability of many cell types to attach to an underlying basement membrane and to make cell-cell connections with their neighbors links tissue architecture to intrinsic polarity cues. This polarity is initiated through stimuli that prompt reorganization of the actin cytoskeleton, often making integrins and cadherins central to the process.

Intriguingly, male GSCs divide with their mitotic spindle oriented perpendicular to the interface of the hub cell and the GSC, with one of the spindle poles nestled into this crescent of heterotypic contact (Figure 4B). As at the GSC-cap interface, the GSC-hub interface is a zone rich in adherens junctions, suggesting a possible

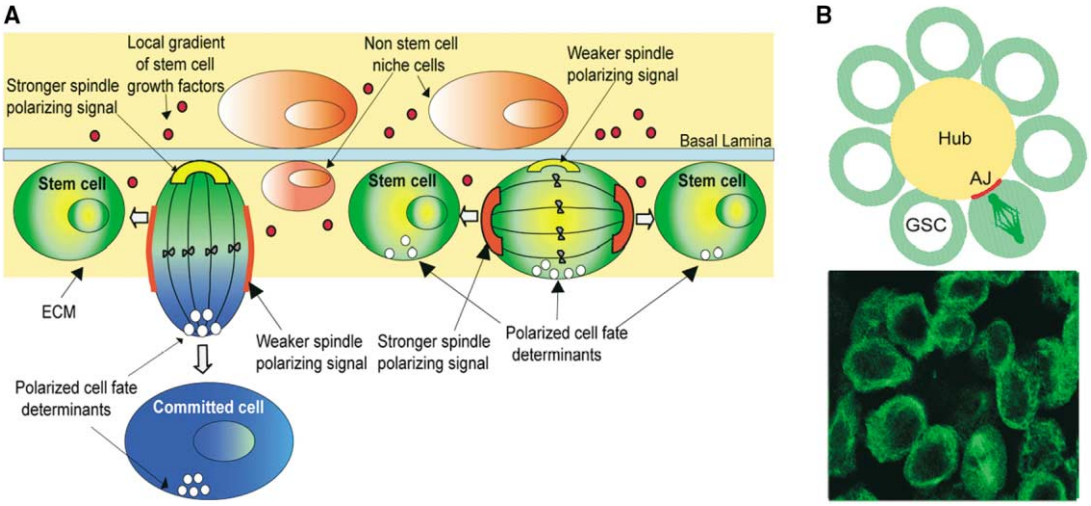

Figure 4. Potential Role of the Niche in Orienting and Defining the Nature of Stem Cell Divisions

(A) Schematic depicting the dual importance of cell polarity and spindle orientation in determining whether a stem cell division will be symmetric or asymmetric. In this hypothetical example, interactions between the basal lamina (basal surface) and neighboring stem cells (lateral surfaces) establish the polarity of the stem cells that in turn leads to the concentration of cell differentiation factors (white dots) at the apical surface. Two putative spindle-polarizing signals are depicted as aligned perpendicularly to one another. In the example shown, the spindle polarizing and cell fate determinants could, but need not necessarily be, the Par complex (yellow) and adherens junctions (red). Depending upon the relative strength of these spindle-polarizing signals, the ensuing stem cell divisions will either asymmetrically or symmetrically partition the cell differentiation determinants and/or stem cell factors.

(B) Top, schematic of GSCs (green) surrounding the hub (tan). One spindle pole in GSCs is always associated with the hub-GSC interface (red). Bottom, visualization of male GSCs with *nos-gal4-VP16/UAS-GFP-β-tubulin*. GSCs form a rosette of GFP-positive cells around and touching the GFP-negative hub. Note that the GSC in the same relative position as shown in the diagram undergoes mitosis with its spindle oriented perpendicular to the adherens junction contact (red) between hub and GSC. This orientation is dependent upon the microtubule-associated protein APC, which in mammalian cells sometimes localizes to adherens junctions. [photos in (B) are reprinted with permission from Y. Yamashita, M. Fuller, and *Science*, as previously published in Figures 1A, 1B in Yamashita et al., Science *301*, 1547-1550, 2003; copyright 2003 AAAS].

role for these junctions in orienting the spindle poles to permit asymmetric cell divisions (Yamashita et al., 2003 and references therein). Furthermore, in the absence of APC, GSC spindle pole orientation is compromised, resulting in excess GSCs.

By contrast, if adherens junctions are genetically disrupted during fly neurogenesis, the neuroepithelial cells, which normally divide symmetrically, can now also divide asymmetrically (Lu et al., 2001). The underlying difference appears to be that in the testis, adherens junctions reinforce heterotypic connections between the hub niche and GSCs, whereas neuroepithelial cells utilize adherens junctions for homotypic cell-cell associations within the monolayer.

An additional spindle-governing factor in neurogenesis is the Par-containing protein complex, which provides a potent orientation signal perpendicular to the monolayer and along the apical-basal axis of what will become the asymmetric cell division of the developing neuroblast. At the apical pole, atypical protein kinase C (PKCξ) is recruited to the Par complex, triggering the phosphorylation of Lgl, which in turn dissociates from the apical cortical actin cytoskeleton (Betschinger et al., 2003). The loss of apical Lgl results in the concentration of a Notch signaling inhibitory protein, numb, toward the basal pole (Zhong, 2003 and references therein). When Notch signaling regulators and other cell fate determinants are aligned with the spindle, they partition asymmetrically during the ensuing cell division, and the fates of the two daughter cells become distinct (Figure 4A).

The extent to which adherens junctions and Par protein complexes preside over spindle alignment in somatic stem cells remains largely undetermined. However, the ability to divide asymmetrically as well as symmetrically appears to be a property of at least some somatic stem cells, as judged from studies such as those on the mammary gland where both types of cell divisions have been detected at the terminal end bud, where stem cells are localized (reviewed by Clarke et al., 2003). Such dual talents can set the scene for controlling the number of stem cells in a niche as well as their ability to generate and orient differentiating progeny. Consequently, it seems likely that the basic molecular mechanisms underlying this process will be utilized across the eukaryotic kingdom by a spectrum of somatic as well as germ stem cells. Future research in this direction will be exciting to follow.

To Be or Not to Be? The Role of Niche Signaling in Stem Cell Self-Renewal Versus Differentiation

The examples outlined thus far underscore the powerful effects of the niche on their resident stem cells in maintaining a balance of quiescence, self-renewal, and cell fate commitment. How do these effects translate into specific external signals, and can we distinguish those involved in maintaining stemness from those promoting cell fate commitments?

As outlined above, neurogenesis has revealed the importance of Notch, a transmembrane receptor that upon ligand engagement cleaves a cytoplasmic segment, which translocates into the nucleus, where it converts the transcriptional repressor, "Suppressor of Hairless," into a transcriptional activator driving target gene expression. In development, the ability of one neighbor to activate and the other to repress Notch signaling sets up a boundary that allows the cells to choose between alternative fates. This concept is critical in stem cell lineage determination, a process where a stem cell division results in one daughter cell becoming a stem cell and the other a committed cell. Evidence continues to point to Notch as a critical player in stem cell plasticity, and in the bone marrow, osteoblasts may maintain HSC self-renewal and suppress differentiation by expressing Notch ligands to stimulate receptors on the HSC surface (Calvi et al. 2003 and references therein).

Another key family of niche signaling molecules is the bone morphogenetic protein (BMP)/transforming growth factor β (TGFβ) superfamily. In the fly ovary, one of these members, Dpp, is secreted by Cap cells to activate receptors on the GSC surface. BMP/TGFβ signaling results in the phosphorylation and activation of transcriptional corepressors (I-Smads), which in this case, silence the *bag-of-marbles* gene, encoding the differentiation factor Bam (Chen and McKearin, 2003). The Dpp signal appears to be short-range, as direct contact between GSCs and Dpp-emitting cap cells is required to suppress *bam* and maintain GSC stemness. When GSC density increases and daughter cells are displaced from the cap niche, the Dpp signal is diminished, inducing Bam and the concomitant commitment to embark upon a differentiation program.

The TGFβ/Bmp superfamily is also involved in HSC stem cell maintenance. However, in contrast to the ovary, where the stem cells receive the Dpp signal from the niche, it is the osteoblasts in the bone marrow that appear to receive the Bmp signal. Moreover, they appear to respond negatively to this signal, as the number of osteoblasts increases when their surface Bmp receptor IA is genetically mutated (Zhang et al., 2003). This change in the stroma in turn indirectly affects the HSCs, enabling an increased number of them to adhere to osteoblasts and thereby prolong their stemness. The extent to which cap cell number might be negatively controlled by Dpp signaling is as yet unknown.

In the mammalian testis, the Dpp homolog Bmp8b is essential for the initiation and maintenance of GSCs (Zhao et al., 1996), and in fly spermatogenesis, a Dpp cousin "glass bottom boat" (Gbb) maintains Bmp/TGFβ receptor signaling. Like Dpp, the outcome of Gbb signaling is *Bam* suppression (Shivdasani and Ingham, 2003), and it is possible that both Gbb and Dpp, perhaps as a heterodimer, may signal to male GSCs in the testis to prevent Bam from promoting GSC differentiation. Taken together, these studies suggest a universal role for Bmp signaling in GSC maintenance.

Interestingly, Gbb is also found in the cyst cells of the testis, where attenuation of Gbb correlates with the promotion of transit amplifying to differentiating cells. These findings underscore the importance of precisely when and where the signal comes from in determining the outcome of the response. They also illustrate how the Bmp/TGFβ signaling pathway can act on either stem cells or their committed progeny, a theme observed for most signal transduction pathways that impact on stem cells.

While Bmp/TGFβ signaling seems to be necessary for maintaining stemness, it may not be sufficient. Studies in *Drosophila* suggest that in the testis, this task is also assigned to the "Janus kinase signal transducer and activator of transcription," or JAK-STAT, pathway (Kiger et al., 2001). Hub cells express Unpaired (Upd), the signaling ligand that activates the JAK receptor. Curiously, the JAK-STAT pathway is also required during oogenesis, but there it functions in follicle cell differentiation (McGregor et al., 2002; Baksa et al., 2002). Recent studies suggest that the JAK-STAT pathway may function in the maintenance and/or differentiation of vertebrate stem cells as well. Thus, STAT3 activation appears to maintain cultured mouse embryonic stem cells in an undifferentiated state (Matsuda et al., 1999), and JAK2, complemented by a second signal activating either c-Kit or Flt-3 tyrosine kinase receptors, has been implicated in the self-renewal process of HSCs (Zhao et al., 2002). Interestingly, when activated, stromal-induced matrix metalloproteinase-9 (MMP9) in the bone marrow can enzymatically release the Kit ligand, stimulating the proliferation of normally quiescent HSCs (Heissig et al., 2002).

The picture emerging is that balance between whether stem cells maintain their stemness or commit to transiently proliferate and differentiate along a particular pathway is complex and involves many additional signal transduction pathways. While a comprehensive coverage is beyond the scope of the current review, a few additional players bear mentioning. Of particular interest are Wnts, which play global roles in cell fate specification during embryogenesis, and have recently been implicated in governing both the proliferation and the cell fate lineage specification of somatic stem cells in the adult (reviewed by Alonso and Fuchs, 2003; Sancho et al., 2003). Genetic studies implicate the pathway in regulating SSCs in the skin epithelium (Gat et al., 1998; Huelsken et al., 2001), the fly ovary (Song and Xie, 2003), the intestinal crypt (Korinek et al., 1998; van de Wetering et al., 2002), and the brain (Chenn and Walsh, 2002). Additionally, purified Wnts stimulate isolated HSCs to proliferate in culture (Willert et al., 2003; Reya et al., 2003), and following skeletal muscle injury, Wnts appear to mobilize resident stem cells during the regeneration process (Polesskaya et al., 2003). Wnts can also promote stem cells to adopt a particular cell fate at the expense of others. Thus, for example, high levels of Wnt signaling promote bulge stem cells to adopt the hair shaft lineage (Gat et al., 1998) and coax neural crest cells to form nearly exclusively sensory neural cells (Lee et al., 2004). Conversely, inhibition of Wnt signaling can lead to sebaceous gland differentiation (Merrill et al., 2001) and epidermal differentiation (Huelsken et al., 2001; Niemann and Watt, 2002) at the expense of hair differentiation in skin. Blocking Wnts causes villus differentiation in the intestine (van de Wetering et al., 2002). In summary, how specific stem cells will respond to Wnts is likely to depend not only upon their specialized microenvironment and who delivers the signal, but also on their intrinsic genetic program. These lessons are

strikingly similar to the lessons gained from studying Bmp/TGFβ signaling in germ cell niches.

Irrespective of these complexities, the Wnt pathway, like the TGFβ and STAT pathways, seems poised at a critical crossroads in balancing stem cell self-renewal versus differentiation. In this regard, it has not gone unnoticed that β-catenin functions prominently not only in Wnt signaling, but also in cadherin-mediated cell-cell adhesion. In studies in *Drosophila*, GSC maintenance was not affected by a β-catenin (*armadillo*) mutant allele that appeared to block Wingless signaling but not cell adhesion. Other mutations in the Wingless pathway behaved similarly, failing to reveal any obvious effects on maintaining GSCs within their niche. That said, in embryonic skin development, Wnt pathway stimulators, and Bmp inhibitors seem to collaborate to activate Lef1/Tcf transcription factors, where surprisingly, *E-cadherin* is a negatively regulated target gene (Jamora et al., 2003). If this pathway is operative in some adult stem cells, it could have a significant impact on stem cell self-renewal and cell fate determination.

Transcriptional Profiling of Stem Cells in Their Niches: Insights into Stem Cell to Niche Signaling

With the advent of microarray technology, scientists have begun to search for features common to all stem cells. The prominent impact of the environment on stem cell behavior clearly poses a challenge in defining such a molecular "signature," underscoring the need to isolate stem cells from their native niches. As important in meeting this challenge is to isolate and compare the stem cells' immediate progeny to reveal the true differences between the stem cells before and after commitment to a particular fate. When these conditions are met, microarray data can be highly reliable and informative.

To date, only a few attempts have been made to obtain transcriptional profiles of stem cells isolated from their native adult niches (Ramalho-Santos et al.., 2002; Ivanova et al., 2002; Stappenbeck et al., 2003; Tumbar et al., 2004), and only HSCs, intestinal crypt cells, and skin LRCs have been compared against specific progeny (Ivanova et al., 2002; Stappenbeck et al., 2003; Tumbar et al., 2004). Some intriguing parallels have already surfaced. At least 10% of the mRNAs upregulated in adult skin LRCs relative to their closest progeny are shared by the mRNAs upregulated in adult bone marrow HSCs relative to their closest progeny (Ivanova et al., 2002; Tumbar et al., 2004). Additionally, members of the TGFβ pathway, so prominent in self renewal of both male and female fly GSCs, are also enriched in all of the populations of stem cells isolated thus far, including those such as ESCs and NSCs that have been subjected to culture. Members of the JAK/STAT and Notch signaling pathways are also upregulated in many of the mammalian stem cell populations, consistent with their predicted roles in stem cell maintenance.

While previously established parallels between stem cells have been strengthened by array analyses, the differences may be equally important, and reflective of the tailoring of adult stem cells to their unique niches. Consistent with the marked quiescent state of the hair follicle LRCs, these cells express a number of inhibitors of the cell cycle, including inhibitors of the Wnt pathway (Tumbar et al., 2004 and references therein). Intriguingly, the LRC progeny outside the niche express cell cycle activators and Wnt stimulatory factors, suggesting that the niche microenvironment changes from Wnt-restrained to Wnt-promoting as stem cells transit from a quiescent to an activated state. Additional differences in gene expression that bear closer attention in the future are chromatin-remodeling factors, integrins and their extracellular matrix ligands, cell-cell adhesion and polarity proteins and their cytoskeletal associates, and inhibitors of cell type-specific differentiation programs.

In addition to examining the transcriptional profile of stem cells in their niches, researchers are just beginning to conduct microarray analyses on nonstem cell types within niches. At present, these studies have been limited to cultured niche cells of a single type (Hackney et al., 2002). The number of potential stem cell modifiers expressed by even this fraction of the niche cellular repertoire is daunting, and underscores the likelihood that no one single factor or signal transduction pathway will be able to support stem cell behavior in the absence of others.

One final note is that the crosstalk between nonstem and stem niche cells is not likely to be a one-way communication line. In fact, transcriptional profiling of stem cells reveals that stem cells produce a number of growth factors which appear to be tailored not for the stem cells per se, but rather for their neighboring niche cells (for example, see Tumbar et al., 2004). Although the cellular communication pathways remain sketchy, the profiles provide rich avenues for future studies in this area. An intriguing twist to this notion is the concept that during development, stem cells participate with nonstem cell residents of a tissue to organize and create stem cell niches. These niches might then be maintained in adult tissues by virtue of these symbiotic communications. Together, adult stem cells may thus be able to preserve certain traits of their more versatile embryonic counterparts and utilize these features to organize and maintain their specialized niche, which in turn impacts on their stem cell characteristics. Such a mechanism would explain why nonstem niche residents differ according to tissue type, and how they are nevertheless able to impact on the overall microenvironment and architecture of the niche. Figure 3C illustrates this model for the hair follicle niche.

Conclusions

Research over the past decade has made it increasingly clear that the stem cell niches provide a microenvironment that is important in protecting and perpetuating the self-renewing, undifferentiated state of their precious residents. The niche's adhesive milieu allows it to retain stem cell daughters, but expel terminally differentiating daughters. At least in some cases, this seems to be accomplished by utilizing nonstem niche cells to provide a polarizing foundation for spatially oriented, asymmetric cell divisions.

The architectural design of a niche appears to be specifically tailored to suit the particular needs of its resident stem cells, and conversely, stem cells may play an important role in organizing and specifying the niche. Thus, although niches share similarities in activating

common signal transduction pathways to achieve the slow-cycling, self-renewing, undifferentiated state of their residents, each niche is composed of different nonstem cell as well as stem cell types that establish this microenvironment. When coupled with multigene redundancies and pathway intricacies, the critical genes involved are likely to differ across stem cell types even if the mechanisms and basic principles are the same.

Overall, it is a combination of the intrinsic behavior of the stem cell and the extrinsic cues provided by the niches' unique microenvironment and architecture that protect the stem cells, maintain their stemness, determine how fast they will divide and specify whether to divide asymmetrically or symmetrically. Microarray and protein array analyses on purified populations of stem cells, their closely related progeny and their nonstem cell niche neighbors will allow researchers to assess the extent to which stem cells share gene expression patterns in order to achieve common properties such as self-renewal or multipotency. Additionally, these avenues will be critical in addressing the extent to which a stem cell's transcriptional profile and/or "stemness" changes when it exits the niche and is either mobilized for tissue homeostasis, wound repair, or clinical applications of cell culture and regenerative medicine. Finally, the approach will be important in ascertaining the extent to which cancers may be generated from malignant stem cells, and if so, the extent to which such mutant stem cells maintain the pattern of gene expression analogous to their wild-type counterparts.

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