Stem Cells and Their Niches

Kateri A. Moore* and Ihor R. Lemischka

A constellation of intrinsic and extrinsic cellular mechanisms regulates the balance of self-renewal and differentiation in all stem cells. Stem cells, their progeny, and elements of their microenvironment make up an anatomical structure that coordinates normal homeostatic production of functional mature cells. Here we discuss the stem cell niche concept, highlight recent progress, and identify important unanswered questions. We focus on three mammalian stem cell systems where large numbers of mature cells must be continuously produced throughout adult life: intestinal epithelium, epidermal structures, and bone marrow.

What Is a Stem Cell Niche?

Stem cell niches are composed of microenvironmental cells that nurture stem cells and enable them to maintain tissue homeostasis. An appropriate spatiotemporal dialog occurs between stem and niche cells in order to fulfill lifelong demands for differentiated cells. The niche concept was introduced in 1978 (*1*); however, it was largely neglected until *Drosophila* studies provided a stimulus for its resurgence (*2*). Niche cells provide a sheltering environment that sequesters stem cells from differentiation stimuli, apoptotic stimuli, and other stimuli that would challenge stem cell reserves. The niche also safeguards against excessive stem cell production that could lead to cancer. Stem cells must periodically activate to produce progenitor or transit amplifying (TA) cells that are committed to produce mature cell lineages. Thus, maintaining a balance of stem cell quiescence and activity is a hallmark of a functional niche.

The Intestinal Stem Cell Niche

The epithelial villus/crypt structure and its surrounding pericryptal fibroblasts and mesenchyme in the small intestine make up an anatomical unit that generates four cell lineages: absorptive enterocytes and the goblet, enteroendocrine, and Paneth cells of the secretory lineage (Fig. 1A). The crypt is a contiguous pocket of epithelial cells at the base of the villus. Intestinal stem cells (ISCs) and TA cells within the crypt regenerate the entire villus every 3 to 5 days (*3*). Genetic marking shows that crypts are derived from individual or few ISCs and that each villus is the product of cells from several adjacent crypts (*4*). There are four to six ISCs per crypt that are located in a ring about four cell diameters from the crypt bottom. Progeny of activated ISCs migrate upwards to become TA cells. When they reach the top of the crypt, TA cells stop proliferating, differentiate, and assume their appropriate positions within the villus structure. As such, proper cell-fate decisions are organized within the microanatomy of the crypt structure. Asymmetric cell division mediated by oriented mitotic planes, together with defined migratory activities within the overall crypt structure, could produce the correct localization of distinct differentiated cell types. Although asymmetric cell division along the vertical crypt axis is an attractive mechanism, this process has yet to be rigorously demonstrated in the ISC system.

DNA label-retention studies suggest that ISCs are normally quiescent relative to their surrounding cells (*5*). This interpretation assumes symmetric partitioning of the label into both daughter cells after cell division. In contrast, an "immortal DNA strand" model proposes that a stem cell retains an initially labeled strand with each division (*6*). Such a mechanism would result in ISC label retention that is independent of proliferation. This issue needs to be clearly resolved and awaits the development of methods including those that allow the prospective isolation of ISCs.

Mesenchymal cells surround the crypt. It is likely that the mesenchymal signals that mediate different cell fates along the vertical crypt axis are spatially organized into distinct domains. The canonical Wnt pathway regulates ISCs (Fig. 1B). This pathway triggers cell-type–specific gene expression programs due to the stabilization and nuclear localization of β-catenin. Mutations in Tcf-4, a transcriptional regulator and partner of nuclear β-catenin, allow essentially normal intestinal development; however, continued proliferation and maintenance of this tissue are severely compromised. Additional studies implicate Wnt signaling in ISC and TA cell proliferation, as well as in intestinal tumorigenesis; however, as is the case in most stem cell systems, it is difficult to say with certainty that a given signaling pathway functions directly in stem cells (*7*).

Genetic experiments have shown that Wnt signals pattern the physical structure of the ISC niche by generating opposing and complementary gradients of Ephrins and their tyrosine kinase receptors, the Eph proteins (*8*). Ephrin/Eph interactions within the crypt control cell migration patterns. A Wnt gradient is predicted by the distribution of nuclear versus cytoplasmic β-catenin along the crypt axis (*9*). A comprehensive study has now shown that Wnt signaling components are expressed by both crypt epithelial cells and surrounding mesenchymal cells, predicting an even broader role for this pathway in normal homeostasis than is indicated by genetic studies (*10*). There is also evidence that Wnt inhibitors such as Dkk3 may be expressed in a graded manner in this tissue, suggesting an intricate quantitative balance between positive and negative regulators of this pathway (*11*).

The bone morphogenetic protein (Bmp) signaling pathway functions as a negative regulator of ISC proliferation, completing a Yin-Yang axis with Wnt. Bmp-4 is expressed in mesenchymal cells adjacent to the ISCs. Conditional deletion of the Bmp receptor 1A (Bmpr1a) in crypt cells results in hyperproliferation and duplication of ISCs, as shown by staining with an ISC-specific marker (*12*). Analysis of adjacent wild-type and mutant crypts shows that Bmp signals repress nuclear β-catenin accumulation. Pten/PI3k/Akt signaling is implicated in the cross talk between Wnt and Bmp. Inhibition of Bmp signaling also results in the generation of new ISCs, ectopic crypts, and precancerous polyps (*13*). Therefore, it appears that an ISC can organize an intact and normal crypt. When crypt structures are first established during development, Hedgehog signals from the intervillar epithelium regulate the underlying mesenchyme in a paracrine manner (*14*). A role for this signaling pathway in the formation of ectopic crypts in adults has not been established.

Periodic activation of ISCs appears to depend on the transient expression of Noggin, an inhibitor of Bmp signaling. Noggin is expressed by ISCs and adjacent mesenchymal cells (*12*). The in vivo dynamics and regulation of Noggin expression need to be defined. Transient Noggin expression may be triggered by an oscillator mechanism within the niche. The Notch pathway can set up oscillating gene-expression patterns during somitogenesis (*15*). Many components of this pathway are expressed in the ISC niche (*7*). Genetic analyses also implicate Notch signaling in the maintenance of undifferentiated crypt cells and in ensuring proper cell-fate outcomes (*16*, *17*). It has also been difficult to ascertain if this pathway is active in the ISC itself, in more committed TA progenitors, or in both cell populations.

Laser capture technology has been used to isolate ISCs for genomic analyses. Various regulatory molecules were identified, including

Department of Molecular Biology, Princeton University, Lewis Thomas Laboratory, Princeton, NJ 08544, USA.

*To whom correspondence should be addressed. E-mail: kamoore@princeton.edu

components of the above signaling pathways (*18*). This type of technology provides an extremely useful tool for capturing other crypt cells for profiling. An analysis of surrounding mesenchyme and pericryptal fibroblasts is lacking in this system and would provide much-needed information.

The Hair Follicle Epidermal Stem Cell Niche

Skin epidermis and its associated structures arise from two stem cell populations within the hair follicle and interfollicular regions. One, in the basal layer of skin, normally gives rise to stratified skin layers. A second, the hair follicle stem cell (HFSC), resides in a region of the outer root sheath called the bulge, and it is responsible for the regeneration of hair and sebaceous glands (*19*). It had been suggested that bulge stem cells are also responsible for the long-term replenishment of the interfollicular epidermis. It is now clear that bulge stem cells are not required for normal epidermal homeostasis, although they can contribute transiently to this tissue in wound healing (*20*, *21*).

The hair follicle structure is complex and multilayered (Fig. 2A). Dermal cells surround and underlie the epidermal cells and are the likely source of many HFSC regulatory signals. Hair follicles possess unique spatial and temporal features. During each hair cycle, follicles undergo temporal structural alterations that bring the HFSCs closer to the dermal papilla. This proximity is necessary for transient HFSC activation, migration to the lower follicle, and the generation of a new hair structure (*22*).

There are differences between hair follicles identified in the pelage and vibrissae; however, the overall principles that govern their function appear to be similar. Vibrissal hair follicles in rodents are large and can be microdissected into segments at different stages of the hair cycle. Whereas HFSC activity was found in the bulge at all hair cycle stages, identical activity was detected in other segments in a stage-dependent manner. Transplantation of microdissected segments under the kidney capsule of hairless mice provided the first demonstration that cells from the bulge could generate a morphologically intact hair follicle (*23*). Lineage tracking experiments in the pelage hair follicles have also demonstrated that the bulge is the origin of cells in the lower follicle (*24*).

The last 2 years have seen an explosion of papers that provide rigorous measures of self-renewal and multipotent differentiation potentials of HFSCs. Experiments from the early 1990s showed that quiescent, label-retaining cells are located preferentially in the bulge region and that they can form large clones in vitro (*22*, *25*). A major advance was the development of two transgenic strategies for the prospective isolation of viable HFSC populations (*24*, *26*). One of these is a variant of label retention as a means to identify quiescent cells. A fluorescent protein marker is introduced into chromatin at a time when all epidermal cells are dividing. The transgene encoding the marker is then turned off, and the fluorescent label is chased out of cells that continue to divide. Nondividing or slowly dividing cells retain the label, and as expected, these cells are in the bulge. These results confirmed that HFSCs are generally quiescent, and they also permit direct functional analysis of HFSCs. This strategy can be combined with other markers such as α6-integrin and CD34 to further subdivide the HFSC-containing population (*26*). A second strategy used the keratin-15 promoter to express fluorescent marker protein in bulge cells. This, together with differential levels of α6-integrin expression, provided substantial enrichment for HFSCs (*24*). Enriched cells were transplanted and robustly produced hair and, to a lesser extent, sebaceous glands and skin epidermis. Genomic profiling was performed with sorted populations in both of these studies and provided the first molecular profiles of HFSCs.

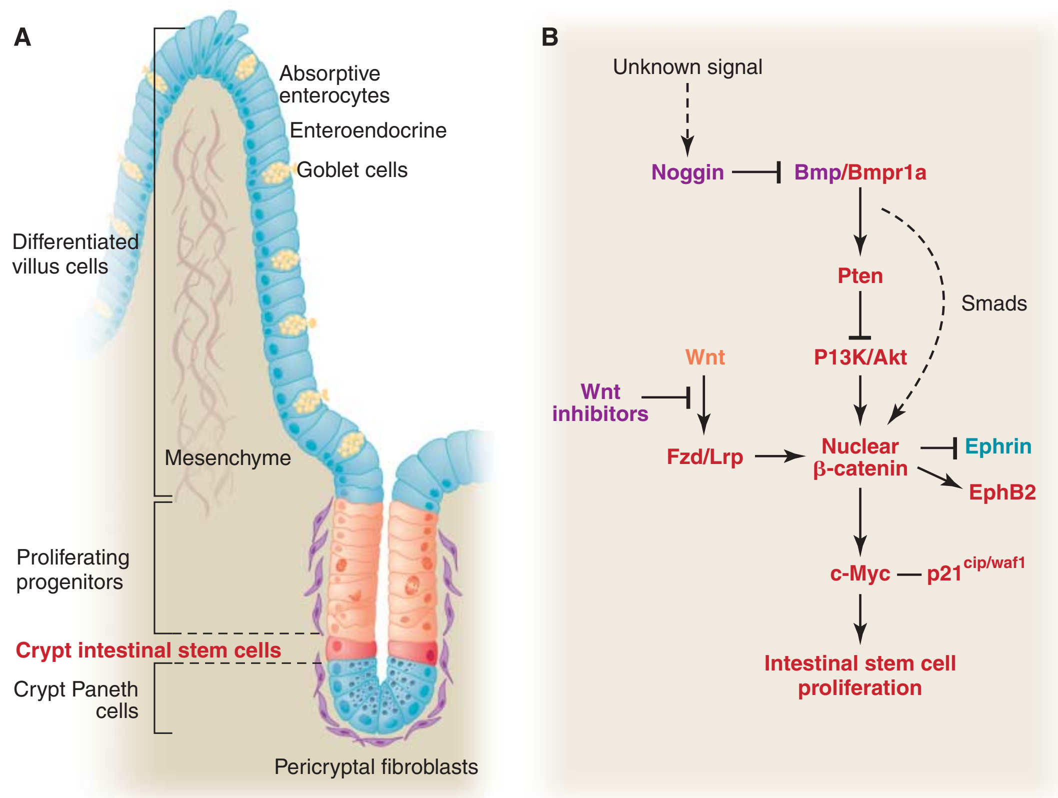

Fig. 1. Stem cells within their niche in the small intestine. (**A**) Schematic diagram of the major types and spatial orientations of cells found within the crypt niche and the villus. (**B**) Interactive signaling pathways that mediate ISC proliferation. Colors represent the cell types sending and receiving the signals as displayed in (A).

Although the aforementioned studies provided valuable prospective definitions for HFSCs, in no case was it directly shown that individual cells can be multipotent, nor was it possible to rigorously measure their self-renewal capacity. Two important studies have addressed these issues (*27*, *28*). In the first study, single bulge HFSCs were purified and shown to self-renew in vitro to produce long-term proliferating clones. Transplantation of clonally expanded cells yielded new morphologically intact hair follicles. Molecular analyses were also performed. Collectively, the molecular analyses of enriched HFSC populations provide suggestions

as to relevant regulatory pathways. Among the identified genes are components of several signaling pathways, adhesion and extra-cellular matrix proteins, as well as molecules involved in cell-cycle control. These genes provide a rich source for future functional analyses. The second study combined single bulge cell isolation, expansion, genetic marking, and transplantation to demonstrate the multipotentiality of rat vibrissal follicle HFSCs. The expanded cells were transplanted into mouse skin at a time when endogenous pelage follicles were first forming, and they contributed to normal intact follicle structures. The transplanted HFSCs could function for at least six to seven hair cycles for over 300 days. Moreover, reisolation and serial transplantation conclusively demonstrated self-renewal abilities (*28*). Serial transplantation first established in the hematopoietic system is the "gold standard" proof of self-renewal.

To date, multipotentiality of single HFSCs has been shown by using cells expanded in vitro; therefore, it may be an acquired property. It remains possible that in situ, individual bulge cells are destined to produce distinct subsets of lineages. Even if in situ, single bulge cells have distinct fates, these may be incompletely "locked in," and thus, the overall differentiation potential may be "expanded" during in vitro culture. An analogous situation can be found in neural development and highlights the complexities inherent in defining stem cells and their immediate progeny as completely deterministic fixed entities (*29*). Freshly isolated bulge cell populations have been used in transplantations (*24*), but not yet as single cells. Such techniques, or an ability to track the progeny of single HFSCs in situ, will be required to accurately assess the multipotential activity of these cells in normal homeostasis. An in situ tracking method has shown that progenitors in the hair follicle contribute to single lineages and possess limited self-renewal potential, suggesting that it may be possible to rigorously measure when and how various lineage potentials are segregated after HFSC activation (*30*).

As mentioned previously, there is little evidence for asymmetric cell division in mammalian stem cell systems. An important study has provided such evidence for basally located cells that generate the skin epidermis during development (*31*). It will be interesting to see if this can be demonstrated in the bulge HFSCs during homeostatic function.

As in the ISC niche, the Wnt pathway is important in the hair follicle system (Fig. 2B). Numerous components of this pathway were identified in molecular profiling studies, and a number of Wnt inhibitors were found. Several transgenic studies demonstrate the role of β-catenin in the skin. A transgenic stabilized form of β-catenin causes de novo follicle morphogenesis and, eventually, skin tumors (*32*). Transient increases in β-catenin levels also accelerate the transition from the resting to the growth phase of the hair cycle (*33*). Additional studies suggest that transient activation of β-catenin in adult epidermis leads to new follicles derived from existing follicles, interfollicular epidermis, and sebaceous glands, but not from HFSCs within the bulge region (*34*, *35*). These authors had previously suggested that distinct stem cell pools exist in interfollicular epidermis, sebaceous glands, and hair follicles (*36*).

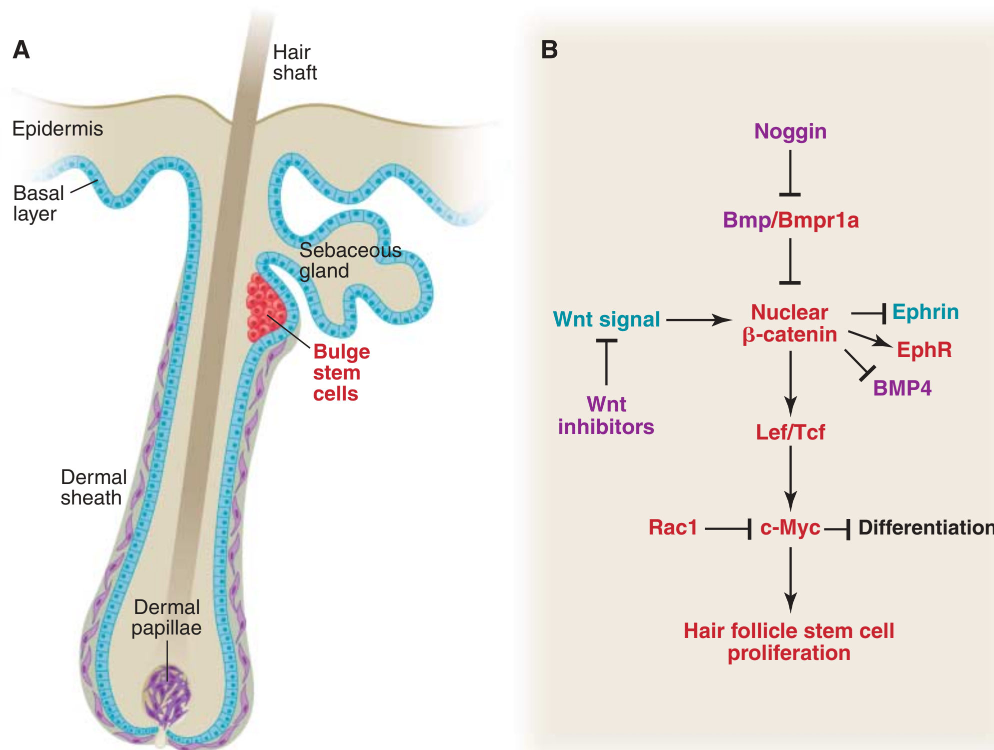

Fig. 2. Stem cells within their niche in the hair follicle. (**A**) Schematic diagram of the major types and spatial orientations of cells that make up the hair follicle. (**B**) Interactive signaling pathways that mediate HFSC proliferation. Colors correspond to the cell types that mediate the interactive signaling leading to the proliferation and differentiation of the hair follicle cell types as displayed in (A).

Bmp signaling is also crucial in the HFSC system. Conditional ablation of Bmpr1a results in hair follicle defects (*37*, *38*). Moreover, mice lacking the Bmp inhibitor Noggin show defects in the function of the canonical Wnt pathway (*39*). Mesenchymal cells produce Noggin in this system (*40*), potentially establishing one way in which these cells can activate the HFSCs. Both activation of the Wnt pathway and inhibition of the Bmp pathway appear to be necessary for functional β-catenin/Lef1 transcriptional complexes. The collective evidence suggests that integration of the Bmp and Wnt signaling pathways occurs in a manner similar to the ISC system.

An emerging theme is the implementation of the same signaling pathways in distinct stem cell systems. This is perhaps not surprising given the limited number of such pathways in all of biology. Nonetheless, it is critically important to identify precisely the actual cells affected by a given signaling pathway. In the hair follicle, Wnt signaling has been shown to affect all phases of stem cell regulation, from quiescence and identity to proliferation and terminal differentiation (*41*). Subtly elevated levels of transgenic stabilized β-catenin cause precocious activation of HFSCs without an increase in their overall numbers. Activated HFSCs return to quiescence in the in vivo niche. Moreover, conditional ablation of β-catenin results in the failure to maintain intact follicles with quiescent HFSCs (*42*). Taken together with other data documenting roles for this pathway in regulating differentiation, a model is proposed where a gradient of Wnt signaling acts on different developmental

stages of the hair follicle system. How quantitative differences in the levels of signaling are interpreted to yield distinct cell-fate outcomes is an unanswered question of fundamental importance.

Other intriguing insights about HFSC regulation are emerging. For example, overexpression of the catalytic component of telomerase promotes HFSC-activating transitions resulting in robust hair growth (*43*, *44*). This occurs through a mechanism that does not involve the synthesis of telomeres. In addition, the deletion of Rac1, which normally negatively regulates c-Myc, stimulates proliferation and terminal differentiation of HFSC and interfollicular stem cells (*45*). Clearly, there will be a need to integrate these observations with the more traditionally studied signaling pathways discussed above.

The Hematopoietic Stem Cell Niche in the Bone Marrow

Bone marrow (BM) hematopoietic stem cells (HSCs) are the best characterized stem cell population. Single HSCs are multipotent, highly self-renewing, and cycle with slow kinetics. Ironically, little in situ information is available to define the anatomical and structural relationships of stem cells, their progeny, and microenvironmental cells. In the ISC and HFSC systems, such information provides the framework for understanding the patterning of fate decisions and the flow of regulatory information. Bone and marrow are intrinsically linked with HSCs, and their primitive progeny are located proximal to the endosteal surface of trabecular bone (Fig. 3A) (*46*). Studies have shown that osteoblast (OB) cells are required for this localization. Genetically engineered increases in OB numbers lead to elevated HSC numbers without changes in committed progenitor populations. In one case, OB numbers were increased after conditional ablation of Bmpr1a (*47*). Bmpr1a is not expressed in HSCs, and Bmp signaling was shown to act cell-autonomously in OB cells. This may contrast with the ISC and HFSC systems and needs further investigation. In a second transgenic study, OB numbers were increased via an activated parathyroid hormone–related protein receptor (PPR) expressed specifically in these cells (*48*). Similar increases in HSC numbers were also observed.

The Bmpr1a and PPR studies provide mechanistic insights into OB-mediated HSC expansion. The Bmpr1a studies identified a specific subset of N-cadherin–expressing OBs that form an N-cadherin/β-catenin adherens complex with HSCs, perhaps mediating the attachment or adhesion of HSCs within their niche. N-cadherin is negatively regulated by c-Myc in differentiating HSCs, perhaps promoting displacement from the endosteum (*49*). In the PPR studies, Notch signaling was implicated, because the Notch ligand Jagged 1 was highly expressed in OBs and Notch activated in HSCs. Wnt protein was previously shown to promote HSC proliferation (*50*, *51*), and now, an additional study has shown that Notch and Wnt inputs are integrated by HSCs. Specifically, Notch signaling appears to inhibit differentiation programs that accompany Wnt-induced proliferation (*52*). However, genetic ablation studies suggest that at least some aspects of these pathways may be dispensable for in vivo HSC function (*53*, *54*). Unfortunately, none of the above studies addressed HSC self-renewal rigorously by long-term reconstitution and serial transplantation. Therefore, the exact roles of Wnt and Notch signaling will require further analysis. A recent study demonstrated that the inhibition of glycogen synthase kinase–3 (GSK-3) activity enhances HSC progenitor activity and maintains but does not expand the stem cell pool (*55*). The GSK-3 inhibitor was shown to modulate Wnt, Notch, and Hedgehog signaling specifically in primitive HSCs. Direct roles for these pathways in self-renewal were not demonstrated; however, roles in stem cell maintenance with an expansion of progenitors are supported by the data. A plausible explanation for maintenance of HSC levels by these signaling pathways could lie in controlling asymmetric cell division. Other mediators of HSC self-renewal have been identified; such as p21 (*56*), p18 (*57*), and bmi-1 (*58*); but how these are controlled by extrinsic signals from the niche has not been determined. Nevertheless, and although different in details, the overall integration of positive and negative stimuli by HSCs is similar to that of ISCs and HFSCs (Fig. 3B).

In transgenic mice where OB cells have been ablated, the marrow is aplastic and extensive extra-medullary hematopoiesis occurs (*59*). This raises questions about the existence of HSC niches in other tissues. HSCs can, in fact, be found in tissues that have no OBs (*60*). Thus, although BM HSC niches are at least in part composed of OBs, other cell types may also provide this function. The contribution of other cellular elements, such as stromal cells or perivascular cells, is yet to be defined. It is has been shown that HSCs can be recruited to a "vascular niche" in the BM (*61*). Such vascular structures could serve as components of extramedullary niches. One intriguing study has demonstrated that HSCs express a calcium-sensing receptor. Stem cells lacking this receptor fail to localize to the endosteal niche and do not function normally after transplantation (*62*). This study highlights the importance of the ionic mineral content of the bone itself and of the bone-derived matrix in the lodgment and retention of HSCs within the endosteal niche.

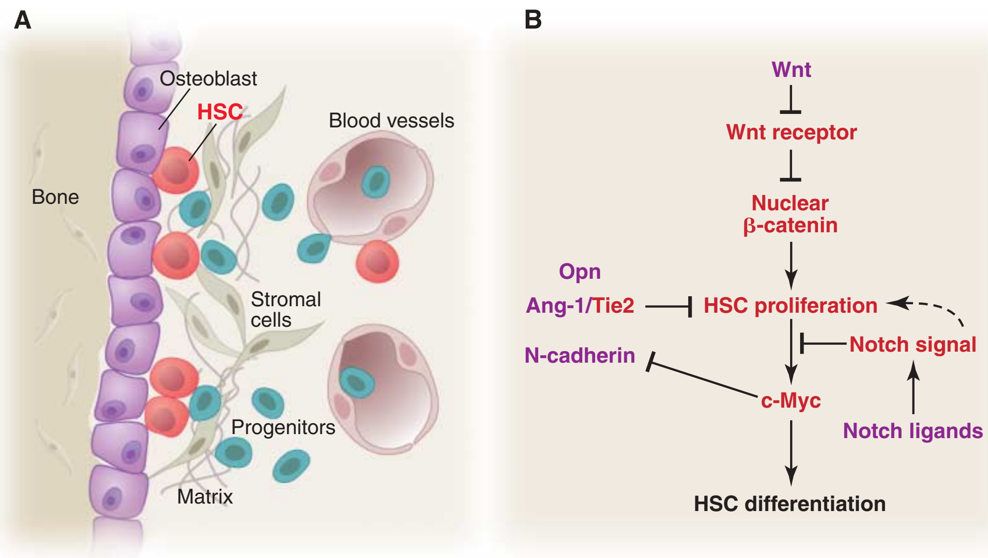

Fig. 3. Stem cells within their niche in the bone marrow. (**A**) Schematic diagram of hematopoietic and niche cellular components in the bone marrow. The exact spatial relationships are not well defined. (**B**) Candidate extrinsic signaling pathways that regulate proliferation and differentiation of HSCs. The colors represent the potential cellular elements that send and receive signals as in (A).

Differential expression of three members of the signaling lymphocyte activation molecule (SLAM) cell-surface receptor family have been used to distinguish HSCs from more committed progenitors in situ (*63*). Vascular and endosteal HSC locations were observed. The existence of multiple types of HSC niches begs the question of potential niche-dependent differences in cell fate. Do niches away from the endosteum contain activated HSCs fated to differentiate? Can HSCs traverse among different niche environments? Indeed, parabiotic experiments suggest that HSCs circulate and return to marrow (*64*).

A major challenge is to define accurately the precise cellular components and anatomical structure of the HSC niche. There are only 10,000 to 20,000 HSCs per mouse, suggesting a limiting number of true niches that can support these cells. In addition, transduction of homeobox genes into HSCs can result in dramatic in vitro expansion (*65*). Yet after transplantation of the cultured cells, normal HSC numbers are restored in vivo. Proper HSC localization within a true niche may impose quiescence and thus limit supra-physiological expansion. Alternatively, the available ''space'' for HSCs within a niche may be limited.

In the ISC and HFSC systems, more committed TA progenitor cells have been localized within the niche. In the hematopoietic system, such populations have been prospectively identified using cell-surface markers (*66*); however, very little is known about their anatomical relationships to the HSCs. If the utility of the SLAM markers is confirmed by other investigators, perhaps these and other markers from numerous genomic profiling efforts will determine if quiescent and activated HSCs, as well as distinct progenitor cells, occupy specific locations within a niche. If so, then a correlated distribution of microenvironmental signals might be expected. In the HFSC system, one study has indeed shown that progenitors committed to different lineages occupy unique positions adjacent to the dermal papilla microenvironment (*30*). Given the circulatory activity of HSCs, a similar analysis will be more difficult. In contrast to the geographical confines of the ISC and HFSC systems, the emerging picture of the HSC niche must allow for the mobile and fluid nature of this tissue.

Relating mechanisms to functional roles in HSC niches is a key area of investigation. Tie2 (receptor)/angiopoietin-1 (Ang-1, ligand) signaling regulates HSC anchorage and quiescence (*67*). Ang-1–expressing OBs and Tie2-expressing, label-retaining HSCs colocalize. The matrix glycoprotein osteopontin (Opn) expressed by endosteal OBs is a negative regulator of HSC proliferation (*68*, *69*). These and other studies (*61*) provide direct evidence for the involvement of matrix components in HSC regulation and further emphasize the importance of regulating anchorage and quiescence as essential features of niche function.

A mechanism for HSC protection within the niche has been identified. Mice with a truncation mutation in the ataxia telangiectasia mutated (ATM) gene have progressive marrow failure due to an HSC defect (*70*). ATM activates a cell-cycle checkpoint that senses DNA damage, telomeric instability, and oxidative stress. Reactive oxygen species (ROS) are elevated in mutant HSCs, and antioxidant treatment rescues their defects. Overexpression studies of candidate mediators implicate the p16^{INK4a} Rb pathway in HSC dysfunction. ATM mutant mice are likely to be intolerant to radiation, precluding their use as recipients of wild-type HSCs. Nevertheless, because the mice are hematologically deficient, transplantation without conditioning may show if the HSC-depleted niches in these mice can support wild-type stem cells. This may provide insights into possible roles for ATM within the microenvironment. Bone is a very low–oxygen tension environment, and mesenchymal progenitors generate OBs more efficiently in such conditions (*71*). Perhaps a normal function of the marrow HSC niche is to provide an environment of low oxygen tension that would inhibit exposure to ROS. Other reports have indicated the importance of low oxygen tension in the maintenance of hematopoietic and neural crest stem cell populations (*72*). The ATM pathway has also been implicated in radioprotective mechanisms that are directed to the ISCs (*73*). It is therefore possible that this pathway may play an essential protective role in all stem cell niches.

Global gene-expression profiles of quiescent and activated HSCs, as well as more committed progenitor populations, have been described (*74*). Numerous members of signaling and other regulatory pathways are present in these molecular signatures. In addition, a comprehensive genomic analysis of an HSC-supportive microenvironmental cell type has been performed (*75*). It is likely that valuable further insights into HSC regulation will emerge.

If Niches Were Wishes

A stem cell niche is an interactive structural unit, organized to facilitate cell-fate decisions in a proper spatiotemporal manner. Key signaling and molecular cross-talk events are patterned to occur in the right place at the right time. Among these three mammalian systems, certain themes emerge: (i) Anatomical organization, best defined for ISC and HFSC niches, coordinates stem cell function in space and time. (ii) Both positive and negative signaling are integrated. The Bmp/Wnt axis represents one such example. (iii) Intercellular signaling pathways are shared.

Challenges for the future include the following: (i) The development of equivalent definitions and assay systems for all three stem cell systems. For example, in the HFSC and HSC systems, prospective isolation and transplantation assays are available, whereas these do not yet exist for ISCs. The distinction between true stem cells and TA cells needs to be clarified and made uniform in all three systems. In addition, it will be necessary to ask if this and other commitment decisions are ''hard and fast'' or reversible. The reversibility of early commitment events mediated by the niche has been shown in the *Drosophila* germ line (*76*, *77*). A recent study in the hematopoietic system showed that constitutively active β-catenin, expressed in committed progenitors, results in a ''reacquisition'' of some stem cell properties (*78*). (ii) A more comprehensive analysis of niche signaling pathways. There are suggestions that other pathways, such as Hedgehog signaling, are important. Comprehensive molecular analyses of directly isolated microenvironmental cells would provide their signaling repertoire. This type of study has been performed with hair follicle dermal papillae and surrounding cell types to investigate the mesenchymal-epithelial cross talk (*79*). Molecules such as Noggin and other components of the Bmp signaling pathway are preferentially expressed in the papillae, further supporting its role as a key signaling center for the HFSC. However, numerous other molecules, such as Wnt proteins and their inhibitors, are also expressed, precluding a coherent picture of orchestrated biological functions. The development and application of more quantitative techniques to analyze the dynamics of signaling pathways at the single cell level may provide further insights. (iii) The development of in vitro systems that accurately recapitulate the in vivo functions of niches. Ultimately, it will be necessary to reconstruct these from defined cellular and molecular components. This will allow a definition of asymmetric division, as well as the intricate macromolecular aspects of multicellular interactions within niches. (iv) The development of effective real-time imaging technologies to analyze stem cell behavior in vitro and niche function in vivo. (v) The description of macromolecular assemblies at the interfaces of cells within the niche. In immunology, such assemblies are called ''immunological synapses,'' and they integrate intercellular signaling. (vi) Elucidation of how signals in the niche are coupled to processes such as cell-cycle regulation and distinct transcriptional programs in a cell-type specific manner. (vii) Elucidation of how niches are altered in situations of stress or pathology. Finally, we suggest that a proper understanding of dysregulated stem cells in cancer requires not just a description of intrinsic processes but also a functional analysis of intact cancer stem cell niches. Indeed, the ability of a tumor cell to orchestrate the establishment of a favorable niche for metastasis has now emerged (*80*).

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