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Visions & Reflections

Stem cells and their niche: a matter of fate

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Abstract. Embryonic stem cells provide an *in vitro* model for developmental biologists to study cell fate decisions during ontogenesis, while somatic stem cells allow physiologists to understand tissue homeostasis in the adult. The behavior of stem cells is dependent on an intimate relationship with a supportive niche. This brief review highlights some of the most important recent trends in stem cell biology, focusing in particular on the supportive microenvironments for both embryonic and adult stem cells. Known intrinsic and extrinsic molecular players from the best-characterized stem cell types are summarized, illuminating a number of shared environmental cues among tissues originating from all three embryonic germ layers.

Keywords. Embryonic stem cell, somatic stem cell, niche, asymmetric division, self-renewal, differentiation, regenerative medicine.

During the past decade, stem cells have gained prominence as invaluable tools for research and as a promising resource for cell replacement therapies. The history of somatic stem cell biology traces back over 50 years, and mouse embryonic stem cells have been exploited for half that time. However, it was the report by Thomson and colleagues in 1998 on the isolation of human embryonic stem cells (hESCs) together with striking but controversial reports on somatic stem cell plasticity that energized the field and started scientists imagining broader clinical applications for a novel discipline called regenerative medicine. Ultimately realizing the significant promise of stem cells for cell replacement therapies will require a deeper understanding of the molecular mechanisms that regulate stem cell fates. The pace of discovery is accelerating, and during the recent past progress has been made on several key questions relating to the instructive influences of the stem cell niche, as well as cell-intrinsic factors governing the decision of stem cells to either self-renew or differentiate. In this brief review, we highlight several of the most interesting recent trends regarding stem cells and their microenvironment.

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Stem cell fate: replication versus differentiation

The most robust and regenerative stem cells are defined by their ability to permanently reconstitute full tissues from a single cell. The ability to replenish a tissue requires the stem cell to undergo one or more of three types of mitotic divisions: (i) replicating division, where both daughters retain stem cell properties, (ii) differentiating division, where replication causes both daughter cells to commit further down the lineage, and (iii) self-renewal or asymmetric division, where one daughter cell retains stem cell properties and the other one differentiates. It is the intrinsic ability to perform asymmetric divisions that is unique to stem cells in the adult.

According to their origins and developmental plasticity, stem cells can be classified into two major categories with contrasting properties: embryonic stem cells (ESCs) and somatic stem cells (SSCs). ESCs are isolated from the inner cell mass of the blastocyst and are pluripotent, meaning that they have the developmental versatility to regenerate all tissues in the adult body, as can be demonstrated when labeled ESCs are returned to the developing blastocyst and shown to contribute to formation of all tissues, including the germ line. Many of the cell types constituting such tissues have been generated in small quantities from mouse and human ESCs through specific differentiation protocols *in vitro*, and these efforts represent much of the current research focus of scientists working in the stem cell field. In contrast to ESCs, SSCs can produce a limited but diverse set of cell types, typically those contained only in the tissue they generate. SSCs in the adult are responsible for the lifelong homeostatic maintenance of tissue mass, and tissue repair after injury. SSCs have been identified in numerous tissues in the adult mammal, namely the central nervous system, epidermis, mammary gland, muscle, bone marrow, intestinal epithelium and gonads. Stem cells have also been postulated for tissues like the heart [1], lung [2], and prostate [3]. However, the extent of cell turnover and the rather limited regenerative response to injury of these tissues has hindered the ability to confirm the existence of their tissue-specific stem cells, highlighting the dramatic difference in proliferative potential of SSCs from different organs. Furthermore, not all somatic tissues appear to harbor SSCs, and not all critical cell types in the adult may regenerate effectively from stem cell pools *in vivo*. The insulin-producing beta cells of the pancreatic islets, which are destroyed in the course of type I diabetes mellitus, have recently been shown to regenerate only through mitosis of pre-existing beta cells in the mouse, and as such, do not appear to be replenished from adult SSCs [4]. An alternative view has been provided by Gershengorn et al. [5], who propose that pancreatic islets may be replenished in the adult through epithelial to mesenchymal transitions, a hypothesis that needs to be addressed through careful clonal studies.

In addition to ESCs and SSCs, there are multipotent adult stem cells which lie in between the two. In terms of origin, like SSCs, they are derived from the adult. In terms of plasticity, they are more plastic than SSCs because they can generate tissues from all three embryonic layers. However, they are not as plastic as ESCs because they don't go as far as being pluripotent. It is accepted in developmental biology that three embryonic germ layers are irreversibly specified in the early embryo. The fact that multipotent adult stem cells might by definition generate tissues from all three embryonic germ layers after embryonic development has finished in the adult opens new uncertainty. The extent to which their plasticity in the adult is physiological or a secondary effect of extended *in vitro* stem cell culture is still an open question (reviewed in [6]). The hypothesis that multipotent adult stem cells exist in the normal adult came from observations that freshly isolated bone-marrow-derived adult stem cell transplants could produce multiorgan seeding [7]. However, there are at least two possible explanations for this finding. Either it is a consequence of true trans-embryonic-layer differentiation in the adult or a false positive due to fusion events. Donor-derived hematopoietic cells of the monocytic lineage can fuse to recipient cells in non-hematopoietic tissues [8]. Such fusion creates the illusion that the fused cell, now containing donor markers, is a true trans-embryonic-layer differentiation in the adult (reviewed in [9]). Fusion events are still to be carefully explored in some of these multipotent adult stem cell transplants. Four variants of multipotent adult stem cells have been recently described. Multipotent adult progenitor cells (MAPCs) were isolated by extensive passaging of non-phenotyped adherent cells from human, mouse and rat mesenchymal tissues such as bone marrow and muscle [10]. Unrestricted somatic stem cells (USSCs) were isolated from human cord blood [11]. Finally, marrow-isolated adult multipotent inducible (MIAMI) [12] cells and human bone marrow-derived multipotent progenitors (hBMSC) were isolated from adherent bone marrow cultures [13]. If MAPCs are indeed found *in vivo*, they would indicate the existence of a heretofore unappreciated stem cell hierarchy in the adult, or a continuum of stem cell transitory and reversible states which could explain such inexplicable recent findings as the identification of germ cell precursors in the bone marrow [14]. If MAPCs are an artifact of *in vitro* culture, their de-differentiation process caused by extended *in vitro* passaging may provide important clues to the process of nuclear reprogramming that is ultimately mastered by the oocyte's cytoplasm during natural reprogramming of the zygotic nucleus or during somatic cell nuclear transfer. Unfortunately, for clinical applications, MAPCs are significantly more difficult to culture than ESCs, and their extremely low proliferative rate compromises their usefulness. Enhanced culture conditions must be developed before determining whether they could substitute for ESCs as a source of cell replacement therapies.

The potential use of ESCs, SSCs or MAPCs for regenerative cell therapy requires that these cells be successfully (i) obtained, (ii) expanded and (iii) differentiated *in vitro* prior to transplant. An additional challenge to cell therapy is the need for histocompatibility between donor cells and the recipient patient. Patient-matched stem cells originate either from the patient directly, through isolation and culture of autologous SSCs, or from a cloned human ESC line, whose generation by somatic cell nuclear transfer is still to be demonstrated for human cells. Acceptance of research aimed at deriving human ESCs through

nuclear transfer (ntESCs) has been hindered by ethical debate in many countries. However, wider application of SSCs in regenerative medicine faces its own unique set of challenges. While ESCs enjoy unlimited expansion potential, SSCs from most tissues have been extremely difficult to expand or even to maintain *in vitro*. Thanks to cytokine cocktails, stromal support cell lines and genetic engineering strategies, ESCs (and MAPCs to a very limited extent) have been successfully differentiated *in vitro* into very diverse murine and human SSC types from all three embryonic germ layers. *In vivo* differentiation presents some remarkable differences between ESCs and MAPCs. When transferred into the bloodstream of an adult, ESCs seed teratomas, whereas mouse MAPCs contribute to the hematopoietic system, liver, lung and intestinal epithelium, without apparent tumorigenicity [15], just as SSCs would do. Once differentiated, however, ESC-derived tissues have not been proven tumorogenic, a qualitative difference whose study could give insight into the mechanisms of benign versus malignant cell proliferation. The use of both SSC and ESC-derived tissues in regenerative medicine can be thus envisioned, whereas MAPCs require reproducible protocols for their isolation and expansion. In this scenario, ESCs offer the advantage that, with today's technology, (i) they can be more readily expanded *in vitro* than SSCs, (ii) they can differentiate into tissues for which SSCs are inaccessible (i.e. the central nervous system) and (iii) they most efficiently undergo homologous recombination as a means for gene repair.

Cell fate: intrinsic versus environmental influences

Replication and differentiation constitute the key fate decision for stem cells. Should replication predominate (in an ESC-like fashion), cell transplants would carry the risk of tumor formation *in vivo*. In contrast, a tendency towards differentiation, as appears to predominate when SSCs are cultured *in vitro*, causes exhaustion of the tissue stem cells over time. A population balance of replicative and differentiative divisions, or alternatively sustained asymmetric division is required to maintain the homeostatic balance of stem cell and tissue populations. However, the mechanisms that control replicative, asymmetric and differentiative stem cell divisions are still ill-defined for most stem cell contexts. Are these properties determined in a cell-autonomous manner, in response to instructive influences of the stem cell niche, or a combination of both? What are the molecular mediators that determine such decisions? Does tissue replenishment from stem cells in the adult recapitulate tissue specification in the embryo? How do the lessons learnt from *in vitro* culture nurture our understanding about stem cell physiology?

In vitro stem cell self-renewal: ESC culture

Mammalian ESCs derive from a very transient population that cannot be readily accessed in its native context, the inner cell mass of the blastocyst. Therefore, our understanding of the molecular mechanisms of embryonic stem cell fate is restricted to the *in vitro* culture system. In this context, ESCs appear to differentiate spontaneously, in the absence of added cytokines, simply as a consequence of removal of anti-differentiation factors. It is thus suggested that differentiation, not replication, is the default pathway of the ESC. SSCs share this tendency to differentiate in current culture conditions, and to date, efforts to identify potent anti-differentiation factors for native human or murine SSCs harvested from the adult have been largely unsuccessful, even after decades of effort in the hematopoietic system.

Contrary to SSCs, ESCs can be successfully blocked in their natural tendency to differentiate. Replicative cell divisions in murine ESCs can be maintained robustly by the cytokine leukemia inhibiting factor (LIF). Human ESCs (hESCs) are not dependent on LIF [16] but can be maintained in a replicative state by activation of the Wnt signaling pathway through pharmacologic inhibition of GSK3 [17], or through sustained exposure to high levels of basic fibroblast growth factor fibroblast growth factor (FGF) [18, 19] as summarized in Figure 1. These recent discoveries constitute significant advances that should facilitate culture of hESCs, and might lead to derivation of novel hESC lines in completely serum-free conditions, free of contamination by animal products and therefore preferable for use in human clinical trials. One recent success is the isolation of an hESC line in the presence of mouse sterilized fibroblast extracellular matrix as the only animal-derived product [20]. LIF, Wnt and bone morphogenic protein-4 (BMP4) are known to contribute to ESC self-renewal, ensuring through their signaling transduction pathways (STAT3, β-catenin and Smad-Id, respectively) the transcription of downstream genes required for sustained pluripotency, e.g. Oct4 and Nanog. Mammalian target of rapamycin (mTOR) seems to be another factor required for ESC proliferation [21]. In addition, c-Myc has recently been found to play complex roles in ESC replication both as a mitotic activator and as a key response element downstream of Wnt signaling [22].

The manner in which these molecular players interact to sustain replicative cell division (and hence pluripotency) during prolonged cell culture remains unknown, as is the manner in which these signals are downregulated during the transition to differentiative cell divisions. These mechanisms are bound to have important parallels to the signals received as the developing epiblast relinquishes pluripotency and segregates somatic and germ lineage fates within the developing embryo. Also largely unexplored are the cell cycle molecular dynamics of stem cell

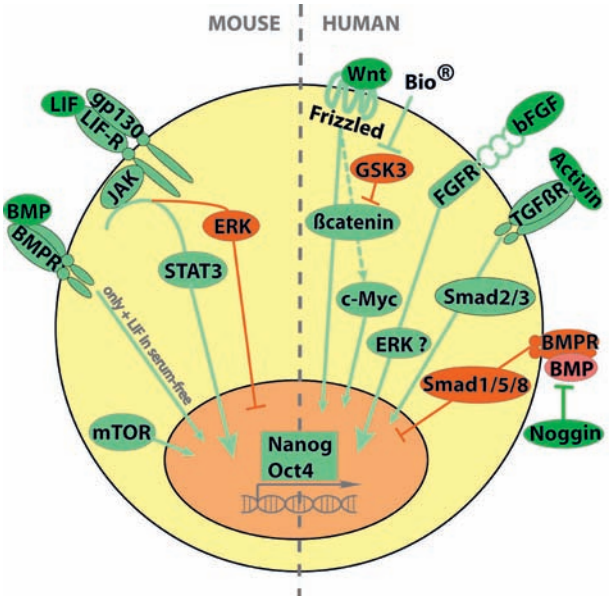

Figure 1. Molecular features of ESC self-renewal. Ligands, receptors and signal transduction pathways involved in ESC self-renewal are indicated, mouse on the left and human on the right. Green indicates factors or pathways that drive self-renewal; red indicates factors or pathways that direct differentiation. LIF and serum, or LIF and BMP4 in serum-free conditions can support mouse ESC self renewal. Human ESCs self-renew in serum-free conditions in the presence of GSK3 inhibitor Bio, Wnt3a, and high doses of FGF2 as individual factors or in combinations such as FGF2 plus the BMP inhibitor Noggin. To substitute routinely used fibroblast feeder layers, both human and mouse ESCs require basal lamina-like extracellular matrix during culture.

self-renewal. Because tumors consist of self-renewing cells that have lost their ability to differentiate, discovering disparities in cell cycle control between stem cells and malignant cells is likely to shed light on mechanisms of tumorogenic transformation.

In vivo stem cell self-renewal: the stem cell niche

In spite of the great difficulties in obtaining robust SSC proliferation *in vitro*, self-renewal of SSCs is clearly observed within their normal physiologic context. The quest for the missing cytokine or the perfect SSC expansion cocktail has taken an unexpected new direction following recent studies of the adult SSC microenvironment, or stem cell niche. We now realize that stem cells exist in specific histological sites within the structure of the tissue where they reside. It is the integration of cell-autonomous properties from the stem cell together with extrinsic signals from the adjacent stroma that ultimately determines self-renewal and differentiation potential. In this context, a niche is histologically defined as the immediate interaction between the stem cell, the surrounding supporting mesenchymal cells and the basement membrane that separates them. Functionally, the niche is defined as the supporting structure necessary and sufficient for stem cell asymmetric divisions to occur, forming with the stem cell an independent unit of stem cell function. Niches are thought to persist in the absence of stem cells [23]. Currently, the best-characterized stem cell niche at the histological and molecular level is the germinal stem cell niche in the ovary and testes of *Drosophila* and *Caenorhabditis elegans*. Additionally, four mammalian stem cell niches have been described for the bone marrow, central nervous system, intestinal crypt and skin. The molecular players of the four best-characterized niches are summarized in Figure 2, their roles further explained below.

The germinal stem cell (GSC) niche is formed by a tight cluster of stromal cells sitting on the apex of *Drosophila* gonads. In the testis they are termed hub cells, in the ovary cap cells. They both intimately contact GSCs, providing the critical membrane-membrane interactions required for self-renewal [24]. The molecular cascade initiates with homologous DE-cadherin interactions at the adherens junction connecting stromal and germinal cells, which triggers activation of the Armadillo pathway on the GSCs. The homologue of Armadillo in vertebrates is β-catenin, a transcription factor whose nuclear translocation has proven sufficient but not essential to drive mammalian stem cell self-renewal. Additionally, soluble decapentaplegic (Dpp), the homologue of BMP2 and BMP4 in mammals, is required for maintaining the GSC population in the ovary through inhibition of Bam [25], while hedgehog (Hh) signaling appears to regulate the commitment to differentiation of germ cell progeny. Therefore, a gradient of decreasing Dpp and increasing Bam marks GSC differentiation when moving away from the cap cells along the axis of the ovary. Similar mechanisms have been unraveled in the *C. elegans* gonad, where a gradient of the pro-differentiative factor GLD-1 distal to the tip cells and its inhibitor GLP-1/Notch proximal to the tip are necessary for homeostatic GSC maintenance [26]. In the context of the *Drosophila* and *C. elegans* gonads, mechanisms that regulate cell polarity may also contribute to the control of stem cell asymmetric division. To date, several gene products have been shown to split asymmetrically in daughter cells, and thereby establish distinct cell fates. These factors include the products of the partition genes PAR-1 and Bazooka/PAR-3 [27], which act as intrinsic regulators, and APC/Wnt [28], which acts as an extrinsic regulator in the *Drosophila* ovary.

Regarding the mammalian supportive SSC niches, significant progress has been made on their histological description, although the molecular picture is not yet as detailed as for their invertebrate counterparts [29]. After numerous studies had pointed to the intimate relationship between blood production and bone [30], a central role for the osteoblast in sustaining the hematopoietic stem cell (HSC) niche was recently confirmed for the mouse by two different groups [31, 32]. Notch1/Jagged2, N-cadherin, Wnt/Frizzled, Tie2/Angiopoietin 1, osteopontin [33, 34]

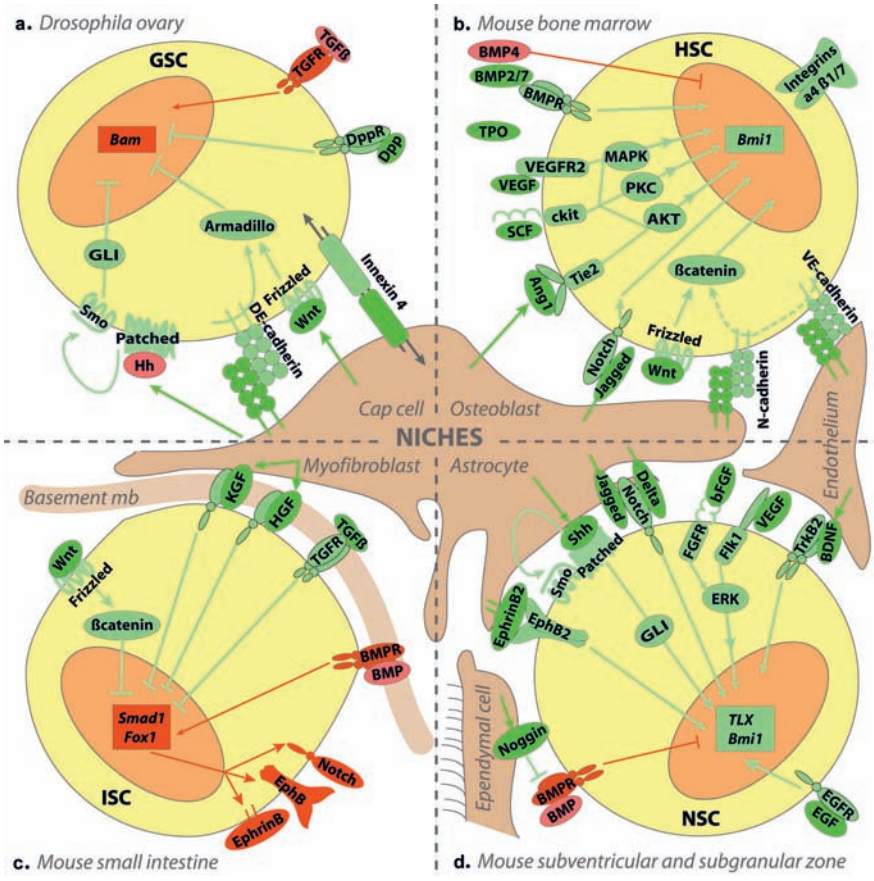

Figure 2. Molecular players of adult stem cell self-renewal in the context of their niches. Ligands, receptors and signal transduction pathways involved in the self-renewal of four well-characterized adult stem cell niches are indicated. (*a*) Germ line stem cell in the *Drosophila* ovary; (*b*) hematopoietic stem cell (HSC) in the mouse bone marrow – mesodermic origin; (*c*) intestinal stem cell (ISC) in the mouse intestinal crypt – ectodermic origin; (*d*) mouse neural stem cell (NSC) – ectodermic origin; NSC contact endothelium in the subgranular zone and ependimal cells in the subventricular zone. Green indicates factors or pathways that drive self-renewal; red indicates factors or pathways that direct differentiation. The stromal cell producing each of the extrinsic factors is indicated when known. Dpp, decapentaplegic; Smo, smoothed; Ang1, angiopoietin 1; Hh, hedgehog; Shh, sonic hedgehog. β-Catenin is the mammalian homologue for Armadillo; BMP is the mammalian homologue of Dpp.

and BMP/BMP receptor were identified as important extrinsic interactions in this context [35, 36], while Bmi1 was established as an intrinsic self-renewal factor in HSCs. Analysis of the downstream factors for these microenvironment-responsive elements has suggested redundancy, as shown by the viability of β-catenin-deficient hematopoietic stem cells, in which the Wnt/Frizzled pathway has been impaired [37]. Through the analysis of conditional tissue-specific knockouts, c-Myc was shown to play a central role in regulating the transition between replicative and differentiative divisions for the HSCs. Contrary to c-Myc expression patterns in ESCs, c-Myc deficiency in HSCs led to accumulation of long-term HSCs with impaired differentiation potential, whereas c-Myc overexpression caused increased differentiation and exhaustion of the stem cell pool [38]. Recent data showing that antibody mediated blockade of VE-cadherin can disrupt hematopoietic engraftment of irradiated mice has implicated the endothelium as a component of a distinct 'vascular niche' for the HSC [39, 40]. Similarly, a tight association with endothelial cells was shown to double the proliferation rate of neural stem cells (NSCs) and to prevent their *in vitro* differentiation in the presence of fibroblast growth factor 2 (FGF2) [41]. The molecular mediator provided by endothelial cells in this context is unknown, but brain-derived neurotrophic factor (BDNF) is hypothesized to play a major role. These complementary data raise the provocative hypothesis that the endothelium plays a central role as a niche element for a variety of SSCs, especially during tissue formation in the embryo and tissue regeneration in the adult. The intestinal crypt offers the best histologically characterized mammalian SSC niche both in the mouse and human models [42]. In the small bowel, the five cell types that form the intestinal epithelium of each villus are clonally derived from a single population of stem cells that sit at cell position 4–5 from the base of the villus. Laterally, stem cells are surrounded by differentiating cells that move upwards and are ultimately shed into the intestinal lumen. Basally, the stem cells rest on a fenestrated basement membrane at

close proximity with myofibroblasts and other mesenchymal cells that produce hepatocyte growth factor (HGF), keratinocyte growth factor (KGF) and transforming growth factor beta 2 (TGF-β2), which are essential cytokines for the regulation of epithelial proliferation and differentiation. The Wnt/Frizzled/β-catenin pathway is responsible for intestinal stem cell proliferation. The BMP-activated Smad4 and Fox 1 transcription factors seem to offer the counterbalance responsible for stem cell differentiation [43]. Notch levels fine-tune the fate of the stem cell progeny, thereby determining the lineage of differentiating cells. Even if these molecular cues have been well studied in the context of intestinal adenocarcinoma, the precise cells providing these extrinsic signals *in vivo* have not been clearly established. Finally, an elegant strategy has been developed to probe the mouse skin SSC niche and isolate the skin SSC, based on retention of a histone H2B green fluorescent protein (GFP) reporter in slow-cycling epidermal stem cells [44]. Skin SSCs were confirmed to reside in the bulge of the hair follicle and migrate to the basal epidermis upon injury. Microarray data suggested an important role for Wnt/Frizzled and Eph/Efs ligand-receptor pairs on this epidermal stem cell niche [45]. This strategy will surely prove valuable for the identification of other SSCs and their niches when the histone H2B-GFP reporter is used under the control of other tissue-specific promoters.

Conclusions and future directions

Now that key mammalian stem cell niches have been identified, the description of molecular mechanisms that determine stem cell fate both during homeostasis and injury will inevitably follow. Although some cytokines and morphogens have already been implicated, the role of adhesion molecules with outside-in and inside-out signaling properties such as integrins is still to be fully explored. Furthermore, the downstream signaling pathways governing the stromal-stem cell interaction are still being unraveled, and the specific stromal cell subsets responsible for niche support remain to be isolated. A compelling hypothesis to explain the transition from replicative to differentiative cell divisions is the unequal distribution of key transcription factors or even mammalian homologues of the partition proteins known to regulate asymmetric division during *C. elegans* and *Drosophila* embryonic development [46], as recently suggested for mammalian skin epithelium [47]. To this end, an important report by Takano et al. [48] has explored asymmetric division in mammalian HSCs on a single-cell basis, instead of a population-based approach, a protocol that could be applied to reveal the segregation of transcription factors and partitioning determinants during asymmetric or differentiating SSC divisions through the use of gain-of-function/loss-of-function mutants.

The identification of the cellular and molecular components of the niche for several adult stem cell types provides an opportunity to test the relative importance of cell-extrinsic and cell-intrinsic signals on stem cell fate as well as the stability of the fate they induce. In the *Drosophila* ovary, an 'empty niche' can be filled by ectopic tissue [49], and revert the fate of already differentiating cells [50]. This phenomenon of partial dedifferentiation could result from reversion of a transient and reversible differentiated phenotype, or a niche-induced reprogramming event that could shed light on the potential for SSC plasticity, and might illuminate mechanisms of stem cell 'alchemy' – the reprogramming of somatic cells into alternative fates through cell-extrinsic means alone.

A more thorough understanding of the instructive signals emanating from the SSC niche, together with a deeper analysis of the cell-intrinsic mechanisms governing replicative versus differentiative cell divisions, is needed to reliably expand and differentiate both ESCs and SSCs for either investigational or therapeutic ends. Given the enormous challenge that this represents, realizing the promise of stem cells for regenerative medicine seems as much a matter of faith as a matter of fate.

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1 Laugwitz K. L., Moretti A., Lam J., Gruber P., Chen Y., Woodard S. et al. (2005) Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature **433:** 647–653
2 Kotton DN, Summer R, Fine A. (2004) Lung stem cells: new paradigms. Exp. Hematol. **32:** 340–343
3 Xin L., Ide H., Kim Y., Dubey P. and Witte O. N. (2003) *In vivo* regeneration of murine prostate from dissociated cell populations of postnatal epithelia and urogenital sinus mesenchyme. Proc. Natl. Acad. Sci. USA **100 (suppl 1):** 11896–11903
4 Dor Y., Brown J., Martinez O. I. and Melton D. A. (2004) Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature **429:** 41–46
5 Gershengorn M. C., Hardikar A. A., Wei C., Geras-Raaka E., Marcus-Samuels B. and Raaka B. M. (2004) Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. Science **306:** 2261–2264
6 Quesenberry P. J., Dooner G., Colvin G. and Abedi M. (2005) Stem cell biology and the plasticity polemic. Exp. Hematol. **33:** 389–394
7 Krause D. S., Theise N. D., Collector M. I., Henegariu O., Hwang S., Gardner R. et al. (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell **105:** 369–377
8 Willenbring H., Bailey A. S., Foster M., Akkari Y., Dorrell C., Olson S. et al. (2004) Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. Nat. Med. **10:** 744–748
9 Vogel G. (2004). Developmental biology. More data but no answers on powers of adult stem cells. Science **305:** 27
10 Jiang Y., Vaessen B., Lenvik T., Blackstad M., Reyes M. and Verfaillie C. M. (2002) Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle and brain. Exp. Hematol. **30:** 896–904
11 Kogler G., Sensken S., Airey J. A., Trapp T., Muschen M. and Feldhahn N. (2004). A new human somatic stem cell from pla-

cental cord blood with intrinsic pluripotent differentiation potential. J. Exp. Med. **200:** 123–35
12 D'Ippolito G., Diabira S., Howard G. A., Menei P., Roos B. A. and Schiller P. C. (2004) Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. J. Cell Sci. **15:** 2971–2981
13 Yoon Y. S., Wecker A., Heyd L., Park J. S., Tkebuchava T., Kusano K. et al. (2005) Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. J. Clin. Invest. **115:** 326–338
14 Johnson J., Bagley J., Skaznik-Wikiel M., Lee H. J., Adams G. B., Niikura Y. et al. (2005) Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell **122:** 303–315
15 Jiang Y., Jahagirdar B. N., Reinhardt R. L., Schwartz R. E., Keene C. D., Ortiz-Gonzalez X. R. et al. (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature **418:** 41–49
16 Daheron L., Opitz S. L., Zaehres H., Lensch W. M., Andrews P. W., Itskovitz-Eldor J. et al. (2004) LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. Stem Cells **22:** 770–778
17 Sato N., Meijer L., Skaltsounis L., Greengard P. and Brivanlou A. H. (2004) Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat. Med. **10:** 55–63
18 Xu C., Rosler E., Jiang J., Lebkowski J. S., Gold J. D., O'Sullivan C. et al. (2005) Basic fibroblast growth factor supports undifferentiated human embryonic stem cell growth without conditioned medium. Stem Cells **23:** 315–323
19 Xu R. H., Peck R. M., Li D. S., Feng X., Ludwig T. and Thomson J. A. (2005) Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. Nat. Methods **1:** 185 – 190
20 Klimanskaya I., Chung Y., Meisner L., Johnson J., West M. D. and Lanza R. (2005) Human embryonic stem cells derived without feeder cells. Lancet **365:** 1636–1641
21 Murakami M., Ichisaka T., Maeda M., Oshiro N., Hara K., Edenhofer F. et al. (2004) mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Mol. Cell. Biol. **24:** 6710–6718
22 Cartwright P., McLean C., Sheppard A., Rivett D., Jones K. and Dalton S. (2005) LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent Mechanism. Development **132:** 885–896
23 Spradling A., Drummond-Barbosa D., Kai T. 2001. Stem cells find their niche. Nature **414:** 98–104
24 Lin H. (2002) The stem-cell niche theory: lessons from flies. Nat. Rev. Genet. **3:** 931–940
25 Chen D. and McKearin D. (2003) Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. Curr. Biol. **13:** 1786–1791
26 Hansen D., Wilson-Berry L., Dang T. and Schedl T. (2004) Control of the proliferation versus meiotic development decision in the C. elegans germline through regulation of GLD-1 protein accumulation. Development **131:** 93–104
27 Benton R., St Johnston D. (2003) Drosophila PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells. Cell **115:** 691–704
28 Yamashita Y. M., Jones D. L. and Fuller M. T. (2003) Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. Science **301:** 1547–1550
29 Fuchs E., Tumbar T. and Guasch G. (2004) Socializing with the neighbors: stem cells and their niche. Cell **116:** 769–778
30 Taichman RS (2005). Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. Blood **105:** 2631–2639
31 Calvi L. M., Adams G. B., Weibrecht K. W., Weber J. M., Olson D. P., Knight M. C. et al. (2003) Osteoblastic cells regulate the haematopoietic stem cell niche. Nature **425:** 841–846
32 Zhang J., Niu C., Ye L., Huang H., He X., Tong W. G. et al. (2003) Identification of the haematopoietic stem cell niche and control of the niche size. Nature **425:** 836–841
33 Nilsson S. K., Johnston H. M., Whitty G. A., Williams B., Webb R. J., Denhardt D. T. et al. (2005) Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. Blood **106:** 1232–1239
34 Stier S., Ko Y., Forkert R., Lutz C., Neuhaus T. and Grunewald E. (2005) Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. J. Exp. Med. **201:** 1781–1791
35 Arai F., Hirao A., Ohmura M., Sato H., Matsuoka S., Takubo K. et al. (2004) Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell **118:** 149–161
36 Murdoch B., Chadwick K., Martin M., Shojaei F., Shah K. V., Gallacher L. et al. (2003) Wnt-5A augments repopulating capacity and primitive hematopoietic development of human blood stem cells in vivo. Proc. Natl. Acad. Sci. USA **100:** 3422–3427
37 Cobas M., Wilson A., Ernst B., Mancini S. J., MacDonald H. R., Kemler R. et al. (2004) Beta-catenin is dispensable for hematopoiesis and lymphopoiesis. J. Exp. Med. **199:** 221–229
38 Wilson A., Murphy M. J., Oskarsson T., Kaloulis K., Bettess M. D., Oser G. M. et al. (2004) c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes Dev. **18:** 2747–2763
39 Kopp H. G., Avecilla S. T., Hooper A. T., Rafii S. (2005) The bone marrow vascular niche: home of HSC differentiation and mobilization. Physiology (Bethesda) **20:** 349–356
40 Kiel M. J., Yilmaz O. H., Iwashita T., Yilmaz O. H., Terhorst C. and Morrison S. J. (2005) SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell **121:** 1109–1121
41 Shen Q., Goderie S. K., Jin L., Karanth N., Sun Y., Abramova N. et al. (2004) Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science **304:** 1338–1340
42 Leedham S. J., Brittan M., McDonald S. A. and Wright N. A. (2005) Intestinal stem cells. J. Cell. Mol. Med. **9:** 11–24
43 He X. C., Zhang J., Tong W. G., Tawfik O., Ross J., Scoville D. H. et al. (2004) BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. Nat. Genet. **36:** 1117–1121
44 Tumbar T., Guasch G., Greco V., Blanpain C., Lowry W. E., Rendl M. et al. (2004). Defining the epithelial stem cell niche in skin. Science **303:** 359–363
45 Alonso L. and Fuchs E. (2003) Stem cells in the skin: waste not, Wnt not. Genes Dev. **17:** 1189–1200
46 Faubert A., Lessard J. and Sauvageau G. (2004) Are genetic determinants of asymmetric stem cell division active in hematopoietic stem cells? Oncogene **23:** 7247–7255
47 Lechler T. and Fuchs E. (2005). Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature **437:** 275–280
48 Takano H., Ema H., Sudo K. and Nakauchi H. (2004) Asymmetric division and lineage commitment at the level of hematopoietic stem cells: inference from differentiation in daughter cell and granddaughter cell pairs. J. Exp. Med. **199:** 295–302
49 Kai T. and Spradling A. (2003) An empty Drosophila stem cell niche reactivates the proliferation of ectopic cells. Proc. Natl. Acad. Sci. USA **100:** 4633–4638
50 Kai T. and Spradling A. (2004) Differentiating germ cells can revert into functional stem cells in Drosophila melanogaster ovaries. Nature **428:** 564–569