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Leading Edge
Review

# Stem Cell Trafficking in Tissue Development, Growth, and Disease

Diana J. Laird,[1,6] Ulrich H. von Andrian,[2] and Amy J. Wagers[3,4,5,*]
[1]Developmental Biology Program, Sloan Kettering Institute, 1275 York Avenue, New York, NY 10058, USA
[2]Department of Pathology, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA
[3]Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, USA
[4]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
[5]Harvard Stem Cell Institute, 42 Church Street, Cambridge, MA 02138, USA
[6]Present address: Institute for Regeneration Medicine and Department of Obstetrics, Gynecology and Reproductive Medicine, University of California, San Francisco, 505 Parnassus Avenue, San Francisco, CA 94143, USA.
*Correspondence: amy.wagers@joslin.harvard.edu
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**Regulated movement of stem cells is critical for organogenesis during development and for homeostasis and repair in adulthood. Here we analyze the biological significance and molecular mechanisms underlying stem cell trafficking in the generation of the germline, and the generation and regeneration of blood and muscle. Comparison across organisms and lineages reveals remarkable conservation as well as specialization in homing and migration mechanisms used by mature leukocytes, adult and fetal stem cells, and cancer stem cells. In vivo trafficking underpins the successful therapeutic application of hematopoietic stem cells for bone-marrow transplant, and further elucidation of homing and migration pathways in other systems will enable broader application of stem cells for targeted cell therapy and drug delivery.**

Stem cells are unspecialized precursor cells that are uniquely capable of both differentiation, to produce mature daughter cells that carry out particular tissue functions, and self-renewal, to sustain and replenish the stem cell pool. Stem cells play a critical role in the establishment of embryonic tissues during development and in some cases are retained into adulthood, where they support ongoing replacement of short-lived mature effector cells as well as injury-induced regeneration of diseased or damaged daughter cells. Regarding hematopoietic stem cells (HSCs), recent evidence suggests that these cells may also participate directly in immune surveillance and defense against invading pathogens (Massberg et al., 2007).

Implicit in the generative, regenerative, and immunological functions of tissue-specific stem cells is the proper localization of these precursors, which is essential to build organs and tissues during development and to foster localized tissue defense and repair after damage. New studies provide increasing support for the notion that stem cells in vivo require inputs from particular defined microenvironments, or "niches," which support their unique stem cell functions (see Review by S.J. Morrison and A.C. Spradling, page 598 of this issue). Long-term maintenance of stem cells, therefore, requires their migration to and engraftment within supportive stem cell niches.

Beyond the essential role of micro- and macroanatomical positioning in normal stem cell activity, it is becoming increasingly clear that achieving targeted trafficking of stem cells will be critical for effective tissue regeneration from transplanted cells in the clinic. In addition, with the ability to manipulate stem cell homing and migration, these cells become potential vectors for in vivo delivery of therapeutic genes or drugs. Finally, with our new view of many cancers as stem cell-maintained diseases of dysregulated organogenesis (reviewed in Dalerba et al., 2007), understanding the similarities and differences in the homing and migration of malignant cancer stem cells, as compared to their normal tissue counterparts, takes on new import for clarifying the molecular events supporting tumor progression and metastasis.

Here, we take a broad view of stem cell migration and homing in normal development, tissue regeneration, and disease, to identify conserved and distinct molecular mechanisms and biological themes governing the movement of stem cells in the body. We focus on three different stem cell types—primordial germ cells (PGCs), skeletal muscle satellite cells, and hematopoietic stem cells (HSCs)—and find new insights by comparing their trafficking in the embryo and adult. This analysis is informed by ground-breaking work in the cell-adhesion and -trafficking fields, which over several decades have elucidated discrete steps that support the homing and migration of immune and stromal cell types in the body. Strikingly similar mechanisms appear to govern the in vivo migration of stem cells, arguing that application of analogous principles to the analysis of stem cell homing will uncover the key steps in this process and provide new information that can be used therapeutically to target these cells for regenerative medicine and anticancer therapy.

## Migration and Homing

Stem cells in vivo participate in organogenesis, normal cell turnover, and repair from catastrophic injury. In each of these settings, appropriate stem cell function often requires stem cell trafficking, defined as the oriented or directed movement of a cell

towards a particular anatomic destination. For clarity, we distinguish in this review two principal modes of stem cell trafficking—homing and interstitial migration.

We define homing as a process whereby stem cells are disseminated throughout the body by the flowing blood until they recognize and interact with microvascular endothelial cells in a particular target organ. Homing is best understood for HSCs, but this process may apply also to some other stem cell types—for example, therapeutically infused mesenchymal stem cells (Pittenger and Martin, 2004) and metastasizing cancer stem cells (reviewed in Balkwill, 2004; Burger and Kipps, 2006). The intravascular dissemination of homing stem cells is essentially passive, although homing is always preceded and followed by an active migratory phase during which cells must navigate the extravascular compartment to access the blood from their point of origin and to reach their final destination in a distant target organ. A special requirement of blood-borne stem cells is that they must have the means to recognize tissue-specific microvascular features in target organs, and once this recognition has occurred they must adhere to the vessel wall with sufficient strength to overcome the considerable shear stress exerted by the flowing blood. The extravasation of blood-circulating stem cells into extravascular tissues appears to invoke a multistep adhesion cascade similar to that initially described in the homing of mature blood leukocytes.

The second mode of trafficking, interstitial migration, requires that stem cells recognize and obey extravascular guidance cues. In contrast to homing, this mode of trafficking requires active ameboid movement and can occur independent of blood flow. The developmentally timed, *trans*-tissue migration of primordial germ cells (PGCs) and of somite-derived skeletal muscle precursor cells are classic examples of interstitially migrating stem cells.

### *Multistep Adhesion Cascades*

Trafficking (migration) via homing appears to involve three (or more) consecutive steps that rely on distinct receptor-ligand pathways: (1) tethering and rolling, mediated by primary adhesion molecules (selectins or $\alpha$4-integrins) with fast binding kinetics and high tensile strength but short bond lifetime; (2) a chemotactic/activating stimulus provided by soluble or surface-bound chemoattractants, which signal mostly through G$\alpha$i-coupled (i.e., pertussis toxin-sensitive) seven transmembrane domain receptors; and (3) sticking mediated by secondary adhesion molecules, mostly integrins (ß2 or $\alpha$4) that interact with endothelial ligands of the immunoglobulin superfamily (IgSF). These sequential and molecularly distinct steps were originally defined in early studies of the recruitment of circulating leukocytes from blood to tissues, where each stage is characterized by distinct biophysical requirements and the ordered involvement of discrete molecular entities (Springer, 1994). For example, neutrophil extravasation (movement out of the blood and into the tissues) in postcapillary venules of inflamed tissues requires first a selectin-mediated step that allows flowing cells to marginate and roll along the vessel wall. This rolling must be followed by a chemoattractant stimulus that induces rapid activation of ß2-integrins, which mediate firm arrest (von Andrian et al., 1991). Genetic defects in either selectin- or integrin-mediated adhesion steps results in leukocyte-adhesion deficiency (LAD) syndrome and severe recurrent bacterial infections. Multistep adhesion cascades also operate during lymphocyte homing to lymph nodes (LNs), Peyer's patches, and other organs (von Andrian and Mackay, 2000) and during seeding of the thymus by bone marrow-derived lymphoid progenitors (Scimone et al., 2006). A similar multistep adhesion cascade also mediates hematopoietic stem and progenitor cell homing to mouse bone marrow (BM), and the molecular effectors of this process are beginning to be identified (Mazo et al., 1998, 2002).

## Stem Cell Trafficking during Development

Stem cells comprise the building blocks of many tissues and organs formed during embryogenesis. The specification and morphogenesis of these tissues hinges upon proper localization of stem cells or their precursors, which in many cases mandates long-distance homing or interstitial migration in the embryo. Here we compare the developmentally timed trafficking of three distinct itinerant stem cells across multiple model organisms. Stem cells of the germline and skeletal muscle, and a subset of early HSCs, migrate interstitially through many different embryonic tissues. In contrast, late embryonic and fetal HSCs rely on homing as a primary mode of dispersion throughout the body. These comparisons reveal a striking conservation of stem cell movement throughout evolution and a remarkable overlap in the molecular effectors of interstitial migration and homing.

### *Germ Cells*

The male and female gametes that carry genetic material to the next generation arise from a transient stem cell population in the embryo called primordial germ cells (PGCs). A small number of these founders originate early in development, before distinct germ layers or organs exist, and then traverse many different tissues in the growing embryo while proliferating, presumably by symmetric divisions (Anderson et al., 2000; Molyneaux et al., 2001). After reaching the gonad primordia, bipotential PGCs undergo genome-wide imprint erasure and commence sex-specfic differentiation, which terminates in the production of oocytes or spermatozoa during adult life (reviewed in McLaren, 2003). PGC migration is essential to development of the germ lineage, as evidenced by its ubiquity across multicellular organisms (see below).

In the fruit fly *Drosophila melanogaster*, germ cells (referred to as pole cells) are the first cells to be formed and are established at the posterior pole of the embryo. The morphogenetic movements of gastrulation carry the PGCs from the extreme periphery into the hindgut. PGCs subsequently migrate through the hindgut epithelium, and once in the surrounding mesoderm, resolve into two bilateral clusters that move toward each developing gonad. After arriving at the gonad, they differentiate according to the male or female program, which is specified by the soma (reviewed in Kunwar et al., 2006). Mouse PGCs travel a surprisingly similar route, though their origins differ (see Figure 1A). Anointed from the pluripotent epiblast by a series of signals around embryonic day 7 (E7), murine PGCs traverse the epicenter of gastrulation, the primitive steak, to the extraembryonic region (Anderson et al., 2000). This peripheral positioning of PGCs is reminiscent of fly pole cells. The exiled PGCs return to the embryo following gastrulation and at the nascent endoderm become incorporated into the hindgut (Molyneaux et al., 2001). Similar to *Drosophila*,

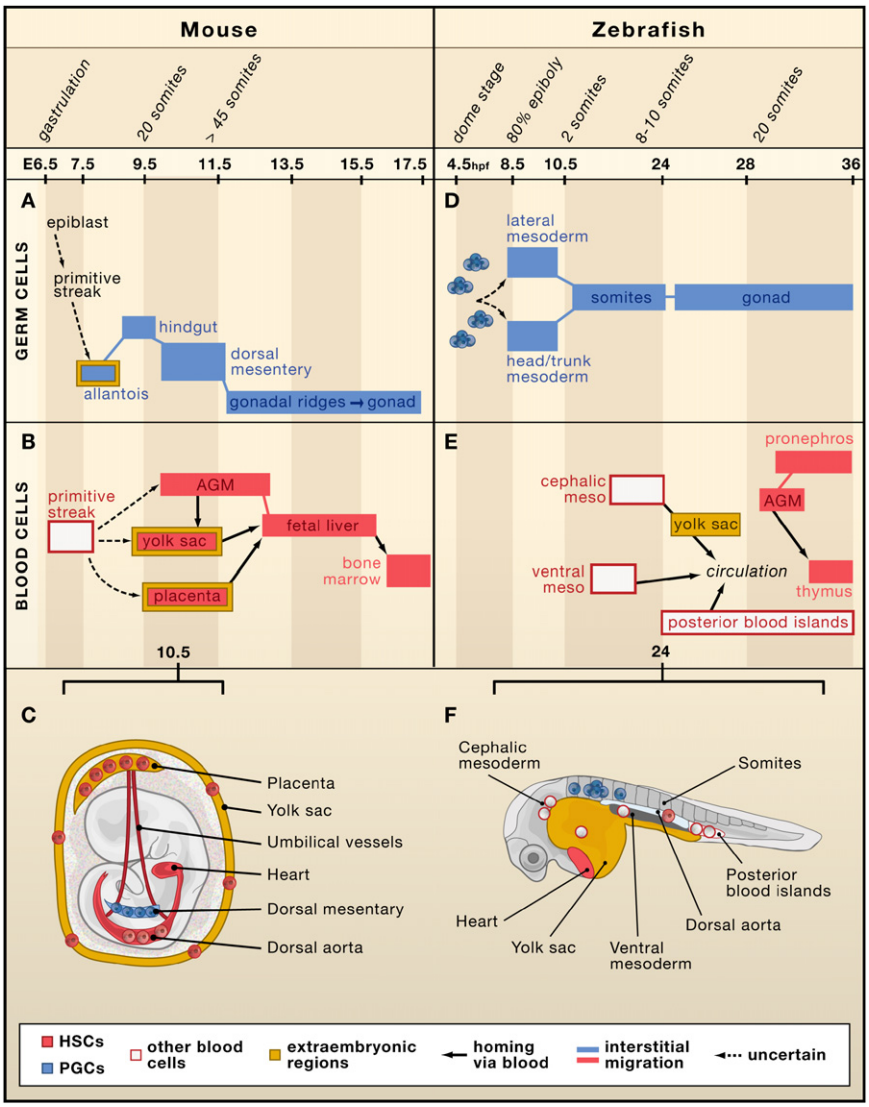


**Figure 1. Migration of Germ Cells and Blood Cells during Development**

(A) Primordial germ cell (PGC) precursors in the mouse epiblast move through the primitive streak, become committed PGCs in the allantois (yellow denotes extraembryonic tissues), then migrate into the developing hindgut. PGCs then emerge into the dorsal mesentery and colonize the gonadal ridges, which develop into the gonad.

(B) Mouse HSC progenitors and primitive blood cells also migrate through the primitive streak to the yolk sac; HSCs arise later in the placenta and aorta-gonad-mesonephros region (AGM) and then home through the embryonic and extraembryonic vessels to the fetal liver before colonizing the bone marrow.

(C) Relative positions of PGCs (blue) and HSCs (red) in the mouse embryo at E10.5.

(D) Zebrafish PGCs are specified as four clusters in the deep cell layer of the pregastrula. They migrate into two regions of mesoderm before separating into clusters flanking the somites, finally moving into the gonads.

(E) Hematopoiesis in zebrafish begins later as three transient populations: macrophages in the cephalic mesoderm (which enter circulation via the yolk sac), erythrocytes (which arise in the ventral mesoderm), and erythromyeloid progenitors (which arise in the posterior blood islands). HSCs are specified in the AGM and migrate to the thymus and the pronephros, which, like the bone marrow in mammals, is the main site of adult hematopoiesis.

(F) PGCs, HSCs, and blood progenitors in the zebrafish at ~24–28 hours postfertilization.

mouse PGCs emerge from the gut, migrate into the dorsal mesentery, and bifurcate as they approach the gonads, finally arriving around day 11.5 of gestation. By contrast, zebrafish PGCs travel a less linear route to the gonad (Figure 1D). They begin not as a single cohort, but as four randomly oriented clusters that converge on two regions of mesoderm during early gastrulation, then aggregate on either side of the first somites (Yoon et al., 1997). Zebrafish PGC migration concludes similarly to fly and mouse, with directed movements through mesoderm to colonize the gonads (Raz, 2003).

Although the migration routes differ between these three phylogenetically distant model organisms, the interstitial migration of PGCs in all cases proceeds in discrete steps and targets successive anatomical landmarks. Each step in PGC migration involves a specific kind of movement—from squeezing between epithelia, to drifting through mesentery, to passive transit within the hindgut—and PGCs in various organisms progress through each migration step vacillating from polarized ameboid cells, to rounded individual cells, to aggregated cells (Blaser et al., 2005; Godin et al., 1990; Molyneaux et al., 2001). Is this morphological progression simply a perfunctory response to the obstacle course that lies between the birthplace of PGCs and their arrival at the gonad, or does their meandering provide factors necessary for PGC development and function? The answer still is not clear: mouse PGCs progress through these morphological changes in vitro, suggesting that at least some of their behavior is autonomous (Godin et al., 1990), but heterochronic (interstage) transplants in fish and flies suggest that the niche plays an instructive role in their development (Blaser et al., 2005; Jaglarz and Howard, 1994). What is perhaps even more puzzling than the remarkable length and complexity of the PGC pilgrimage to the gonad is their disparate origin from the gonad. Would it not be more efficient to generate the germ lineage where (and when) it is needed, rather than conveying PGCs throughout the embryo and risking losing them along the way? A surprising realization upon comparing PGC development across different species is that the route from PGC inception to arrival in the gonad seems to be perpetually changing; yet, perhaps such variation in the specific paths of PGC migration belies a conserved requirement for migration itself. Furthermore, when comparing PGC migration to that of other developing stem cells, a remarkable theme emerges in

which a small number of founder cells expands while transiting through successive anatomic sites. These observations suggest that for many stem cells migration is more than just practical and represents an essential aspect of their development and perhaps even maintenance.

### Blood

In contrast to the simple bipotentiality of PGCs, which generate either oocytes or sperm, hematopoietic stem cells (HSCs) give rise to many different types of mature blood cells through a branching series of progenitors with increasingly restricted potential (see Review by S.H. Orkin and L.I. Zon, page 631 of this issue). Furthermore, unlike the leisurely germ cell whose services will not be required until adulthood, HSCs must from the start balance the immediate physiological demands of the growing embryo with the need to produce sufficient reserves for sustaining hematopoiesis throughout life. Moreover, whereas PGCs navigate predominantly along interstitial routes, HSCs often home to distant sites by making use of passive transport via the circulating blood. Nonetheless, the development and movement of blood and germ stem cells in the embryo share surprising similarities, beginning with their early specification, to their sojourn to extraembryonic tissues in mammals, to their serial stopovers in distinct embryonic sites. In addition, the cellular phenotype of HSCs, like PGCs, is not static during development, but changes to reflect different kinds of migration and different hematopoietic requirements.

Although the earliest studies on blood development were carried out in birds and amphibians, the mouse ultimately became the main model organism in this field. Surprisingly, it remains unclear even today precisely when and where the very first HSCs are established. Mouse hematopoiesis first becomes evident in the yolk sac just after gastrulation at E7.5 as a band of specialized nucleated erythrocytes called blood islands (Figure 1B) (Moore and Metcalf, 1970). Grafting studies into newborn recipients detect the first HSCs at E9.0 in the yolk sac and para-aortic splanchnopleurae, a mesoderm-derived tissue that becomes the aorta-gonad-mesonephros (AGM) region around E10.5 (Yoder et al., 1997). However, earlier commitment to the HSC lineage has been implied by genetic marking of the first Runx1$^+$ cells in the yolk sac blood islands (Samokhvalov et al., 2007) and by successful adult engraftment of E8.5 splanchnopleural HSCs after a culture period (Medvinsky and Dzierzak, 1996).

In addition to the AGM and yolk sac, the placenta is the most recently appreciated and prolific source of HSCs in the developing mouse embryo (Gekas et al., 2005; Ottersbach and Dzierzak, 2005). It is possible that developing hematopoietic precursors emerging from the primitive streak remain in the allantois, which fuses with the chorion and contributes to the umbilical cord and placenta; alternatively, HSCs might arise de novo in the placenta (Corbel et al., 2007). This second possibility has now been supported by a new study documenting the emergence of HSCs in the placenta of embryos that lack blood circulation due to failure to initiate a heartbeat (Rhodes et al., 2008).

At E11.5–12.5 of mouse development, a hematopoietic diaspora ensues, as HSCs abandon the yolk sac, AGM, and placenta and home to the fetal liver (Johnson and Moore, 1975), which for the next 5–6 days promotes both rapid HSC expansion and differentiation to pools of various blood progenitors. Just 1–2 days before birth, stem and progenitor cells begin to seed the bone marrow (Christensen et al., 2004). Soon after their arrival at this site of continued adult hematopoiesis, fetal mouse HSCs curb their proliferative activities and enter a state of relative quiescence (Bowie et al., 2006). Yet, HSC migration is hardly finished. Indeed, HSC migration persists throughout adulthood with a continuous recirculation throughout the blood, tissues, and lymphatic system (Abkowitz et al., 2003; Massberg et al., 2007; Wright et al., 2001).

The zebrafish has only recently become a standard model for studying hematopoiesis and HSC development. Due in part to differing requirements associated with external development, the sites of early blood formation in zebrafish are quite distinct from those in the mouse. Multipotent HSCs are preceded by three separate populations of precursors with limited potential and self-renewal capacity (Figure 1E): a population of primitive macrophages arises from the cephalic mesoderm (Herbomel et al., 1999), erythrocytes develop in migrating strips of ventral mesoderm that converge into the cardinal vein (Al-Adhami and Kunz, 1977), and progenitors with erythromyeloid potential originate in the posterior blood islands (PBI) (Bertrand et al., 2007). A relevant evolutionary question is whether these evanescent blood lineages existed prior to HSCs, or if their evolution followed that of HSCs to satisfy increased metabolic or immune requirements of the growing embryo. The first HSCs were recently prospectively isolated from the zebrafish AGM equivalent. These cells home through the vasculature to the thymus and via the pronephric tubules to the head kidney, which is the site of adult hematopoiesis equivalent to the bone marrow in mice (D. Traver, personal communication). Thus, in spite of their evolutionary distance, the origination and migration of blood-forming stem cells are remarkably similar between fish and mammals.

Considered in both rodent and teleost model systems, HSCs migrate during development for comparatively less abstract reasons than PGCs; practically speaking, blood cells are required by the embryo before the formation of adult stem cell niches (located predominantly in the bone marrow in mammals). As we begin to attribute distinctive niche functions to particular HSC stopovers throughout embryogenesis, such as rapid expansion in the mammalian fetal liver and controlled quiescence in the bone marrow in mammals or head kidney in fish, the question remains whether the earliest HSCs are specified more than once and in several locations. Does this multicentered approach reflect phylogeny or a demand for production volume? Or, is there an important functional heterogeneity between HSCs derived from different sites of de novo hematopoiesis? Comparative studies of blood development in multiple organisms will help to answer this question, and such studies will certainly benefit from recently acquired capabilities for prolonged live imaging of zebrafish embryos, which marry lineage tracing and genetics to directly monitor HSC origins and migration.

### Skeletal Muscle

Skeletal muscle is a highly specialized tissue comprised of nondividing multinucleated myofibers that contract in concert to generate force (Figure 3). The ontogeny and migration of skeletal muscle precursors is highly conserved in vertebrates, and our current understanding is a synthesis of studies in amphibian, chick, mouse, and zebrafish. During development, cells that

ultimately give rise to skeletal muscle originate from somites, segmented parcels of paraxial mesoderm that flank the neural tube (Buckingham et al., 2003). These precursors to adult skeletal muscle migrate great distances to multiple sites of embryonic myogenesis, including the limb buds and brachial arches, where differentiation ensues in late fetal stages. Myogenic differentiation in these locations is accompanied by expression of the myogenic transcription factors Myf5 and MyoD and the fusion of cell bodies (reviewed in Buckingham et al., 2003; Hawke and Garry, 2001). After this initial establishment of the muscle, the primary myogenic requirement shifts from morphogenesis during development, to growth and repair in postnatal life.

To meet the demands of postnatal life, fully developed muscle retains a reservoir of cells committed to muscle regeneration. First identified ultrastructurally, these "satellite cells" were named for their peripheral location beneath the basal lamina of the myofiber (Mauro, 1961). Satellite cells persist from late embryogenesis, to neonatal stages and through adulthood, although their numbers decline after birth (Hawke and Garry, 2001). In the adult, transplantation studies demonstrate both differentiation and self-renewal capacities within the satellite cell pool, supporting their designation as a tissue-specific stem cell population (Collins et al., 2005; Montarras et al., 2005).

Recently, the developmental predecessors to muscle satellite cells were identified in the late fetus based on their lack of differentiation markers and continued expression of the paired box transcription factors Pax3 and Pax7. Grafting experiments in chick and genetic marking in mice suggest a common origin in the dermomyotome for satellite cells and skeletal muscle (Gros et al., 2005; Relaix et al., 2005). Satellite cell precursors, which seed embryonic myogenesis and give rise to the postnatal satellite cell pool, delaminate (split off) from the somites early in embryogenesis, and migrate large distances to multiple sites of myogenesis within the developing limbs and trunk (reviewed in Buckingham et al., 2003). But precisely when is the fetal satellite cell lineage established, and how is the specialized subset of this pool set aside to give rise to adult self-renewing satellite cells? Although the answers to these questions remain uncertain, it is clear that targeted migration of both satellite cells and their precursors during development is critical for the morphogenesis of muscle and that the mechanisms involved in this process overlap in many ways with stem cell trafficking events in the adult.

## Trafficking Mechanisms in Development

### *Cell-Cell Adhesion and Deadhesion*

The regulation of adhesion between cells is critical for the transition of stem cells between different tissues during development. In most cases, the breaking of existing junctions between cells, or deadhesion, represents the earliest step in migration. For muscle precursor cells in the somites, the onset of migration requires dissociation of Pax3-expressing precursors from the dorsal epithelial layer of the somite, or dermomyotome (Buckingham et al., 2003). Pax3 transcriptionally regulates the tyrosine kinase receptor c-Met (Epstein et al., 1996), which binds to secreted hepatocyte growth factor. Regional concentrations of hepatocyte growth factor in the developing limbs and brachial arches engage c-Met and induce the dispersion of satellite cell precursors along routes of myogenesis (Bladt et al., 1995).

Such epithelial-to-mesenchymal transitions (EMT) are a widespread developmental strategy for generating new cell lineages; the associated breakdown of apical junctions permits free movement of defecting cells relative to the uniform epithelial sheet (reviewed in Shook and Keller, 2003). Zebrafish PGC migration similarly begins with EMT, sparked by the expression of dead end, a germline-specific RNA-binding protein. In dome stage embryos, dead end downregulates the adhesion molecule E-cadherin in the 4 PGC clusters in the deep epithelial layer and enables their migration (Shimizu et al., 2005).

Migrating PGCs passing through the hindgut epithelium avoid adhesive interactions similar to mesenchymal cells undergoing EMT. PGCs in mice maintain low levels of E-cadherin compared to the surrounding gut epithelial cells, which may enhance their motility. Curiously, these cells upregulate E-cadherin upon egress from the gut to the surrounding dorsal mesentery (see Figure 1C), perhaps to facilitate adhesion to one another (Bendel-Stenzel et al., 2000). The role for cadherins in transepithelial migration of fly PGCs through the midgut is comparatively less clear, as it is expressed but not absolutely required (Kunwar et al., 2003).

Mouse HSCs and their precursors in the yolk sac, AGM, and placenta robustly express CD144, also known as vascular endothelial (VE) cadherin, and CD144 levels fall as they transit to the fetal liver (Fraser et al., 2002; Taoudi et al., 2005). Homotypic association through VE-cadherins on endothelial cells is essential for adherens junctions and regulates vascular permeability (Corada et al., 1999). The function of VE-cadherins in embryonic and fetal HSCs is not clear, although cadherins may be biologically important for both trafficking and niche interactions unique to prenatal HSCs (Taoudi et al., 2005). These shared and unique mechanisms of homotypic adhesion and deadhesion collectively permit stem cells to migrate individually through sheets of cells. Thus, a balance between engagement and aloofness from their neighbors maintains stem cell dispersion, promotes movement, and possibly helps to orient migrating stem cells in three-dimensional space.

### *Movement*

Integrin-mediated adhesion is important for the movement of HSCs throughout mouse embryogenesis and in adulthood. Distinct profiles of integrin heterodimer expression by HSCs in different locations and at different developmental time points suggests that integrins help to determine homing specificity. In the mouse, CD41/integrin $\alpha$2b is strongly expressed by prospective HSCs in the para-aortic splanchnopleure, placenta, and yolk sac from E8.5 onward. Expression gradually decreases during maturation and becomes undetectable in fetal liver HSCs by E11.5–12.5 (reviewed in Mikkola and Orkin, 2006). The function of CD41 is not known, although its expression in the earliest zebrafish HSCs suggests a conserved role in hematopoietic commitment or retention in the early niche (D. Traver, personal communication). Nonetheless, blood-island, embryonic, and adult hematopoiesis appear normal in mouse embryos lacking CD41, perhaps belying its functional redundancy in mammals (Francis et al., 2002). PGCs in the mouse express several integrin subunits ($\alpha$5, $\alpha$6, $\beta$1, and $\beta$3), although only $\beta$1 appears to be essential for migration. In mouse chimeras, $\beta$1-integrin-deficient

PGCs accumulate along their migration route, in the hindgut and mesentery, and very few successfully populate the gonad (Anderson et al., 1999). Taken together, these data indicate that the combinatorial display of integrins on stem cell membranes changes dynamically during development and has functional consequences in the migration of multiple types of stem cells. With the increasing feasibility of large-scale analyses, such as intravital and molecular imaging and transcriptional profiling on small populations of cells, it will be possible to directly correlate integrin expression on stem cells with their location and behaviors in various niches. Such approaches will define markers for the isolation of stage-specific stem cells in development and will clarify the functional role of particular integrin heterodimers in migration.

### Guidance Factors

Stem cells in the embryo rely on multiple navigation systems for choreographing their discrete, successive movements. Genetic screens in *Drosophila* suggest that phylogenetically ancient guidance systems depend on lipids and factors requiring lipid modification. Three independent mutants in the mevalonate pathway disrupt PGC migration in fly embryos (Table 1) (reviewed in Kunwar et al., 2006). Mevalonate is involved in the synthesis of cholesterol and isoprenoids, but the absence of genes regulating cholesterol synthesis in *Drosophila* argues that the protein prenylation pathway is required for PGC guidance.

Lipids may function more directly in fly PGC navigation by activating the receptors Wunen1 and Wunen2, resulting in deflection of PGCs from midline tissues (Starz-Gaiano et al., 2001; Zhang et al., 1997). Vertebrate Wunen homologs respond to phospholipid substrates including sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA) (Renault et al., 2004 and references therein). Both phospholipids and their receptors, the S1P or Edg family, also regulate migration of mammalian lymphocytes, endothelial cells, and cardiac progenitors (reviewed in Saba, 2004), although a role in vertebrate PGC guidance has not been reported. These observations collectively imply that lipid-mediated guidance has been reused by a variety of migrating cell types in multiple organisms, although evolution has not conserved these mechanisms in specific cell lineages.

Strategies for cellular repulsion and avoidance also appear to have evolved multiple times and are invoked by many migrating cells, including stem cells. For example, members of the ephrin family of receptor tyrosine kinases and their membrane-bound Eph ligands mediate axon guidance in the developing nervous system, as well as neural crest, endothelial cell, and satellite cell migration (reviewed in Davy and Soriano, 2005). In the chick embryo, EphA4 enables satellite cell precursors delaminating from the dermomyotome to avoid regions of EphA5 expression in the limb mesoderm (Swartz et al., 2001). Ephrin signaling between engaged ligand and receptor-bound cells produces attractive as well as repulsive guidance cues, depending on the family members (Davy and Soriano, 2005).

Similar attraction-repulsion responses arise from cellular contact between early mouse PGCs; however, in this case the molecular machinery involves a family of cell surface receptors called IFITM/Mil/Fragilis. In the allantois of the mouse embryo, PGC contenders may seal their commitment to the germline via IFITM3 homotypic interactions (Saitou et al., 2002; Yoshimizu et al., 2001). Interestingly, IFITM1 in neighboring somatic cells furnishes a repulsive signal that drives PGCs from the allantois back into the embryo, whereas IFITM3 expressed by PGCs is sufficient for their localization in the hindgut endoderm (Tanaka et al., 2005). IFITMs are not exclusive to the germ lineage, as other family members are found on lymphocytes (i.e. Leu-13) and play a role in lymphocyte homing by modulating L-selectin levels (Frey et al., 1997). Further studies may reveal common signaling pathways downstream of various IFITM receptors in PGCs and lymphocytes.

An important and pleiotropic guidance factor for several stem cells in the embryo is Kit ligand (KitL). KitL and its receptor, c-kit, were first studied 60 years ago in spontaneous mouse mutants called *Steel* (Sl) and *White spotting* (W), which had nearly identical dominant coat-color phenotypes. Homozygous Sl and W mouse embryos exhibit a profound PGC deficit, severe anemia, and failure of neural crest-derived melanocyte migration (Fleischman, 1993; Mintz and Russell, 1957). In PGCs, the c-kit tyrosine kinase receptor promotes both survival and chemotaxis in response to KitL, which is expressed by somatic cells along the PGC migration route in the hindgut and dorsal mesentery (Matsui et al., 1990; Runyan et al., 2006).

Embryonic and adult mouse HSCs also maintain high levels of c-kit on their surface from E9.0 onward (Yoder et al., 1997). In the fetal liver, KitL exerts chemoattractive effects on HSCs, suggesting that it helps to retain them there during the last days of gestation (Christensen et al., 2004). Given the distribution of KitL expression during mid-development in relation to PGC and embryonic HSC migration routes (Matsui et al., 1990), it is surprising that the respective stem cells do not confuse one another's signals. How do PGCs moving through $KitL^+$ territory in the dorsal mesentery avoid the dorsal aorta endothelium, which also expresses high levels of KitL? Conversely, do HSCs lining the dorsal aorta escape along PGC conduits in the surrounding mesentery?

Similar signal crossing also could confound the responses of different stem cells to the growth factor chemokine stromal-derived factor 1 (SDF-1$\alpha$/CXCL12). SDF-1$\alpha$ induces chemotaxis of mouse fetal liver HSCs in synergy with KitL (Christensen et al., 2004); mouse embryos deficient in SDF-1$\alpha$ or its G protein-coupled receptor CXCR4 (also known as fusin) develop severe hematopoietic defects (Ma et al., 1998; Nagasawa et al., 1996; Zou et al., 1998). SDF-1$\alpha$ , like KitL, also provides a survival as well as a guidance signal to mouse PGCs in their final movement toward the gonadal ridges (Ara et al., 2003; Molyneaux et al., 2003). PGCs apparently co-opted chemokine signaling early in vertebrate history and maintained it, as evidenced by its importance in mammals, fish, and birds. Zebrafish PGCs, unlike those in chicks and mice, thoroughly depend upon SDF-1$\alpha$/CXCR4-mediated guidance throughout all steps of migration (Knaut et al., 2003; Stebler et al., 2004).

Muscle satellite cells have also exploited CXCR4 and SDF-1$\alpha$ for long-distance migration during development. Recent work in the chick embryo demonstrated that SDF-1$\alpha$ in the limb mesenchyme and brachial arches suffices as a target for satellite cell precursors following their delamination from the dermomyotome. In satellite cells, *CXCR4* interacts genetically with *Gab1*, an adaptor molecule involved in signal transduction via c-Met

**Table 1. Molecular Mechanisms Involved in Stem Cell Trafficking during Development**

| Mechanism | PGCs | HSCs | Satellite cells | Involved in homing (blood to tissue)? | Involved in interstitial navigation? | References |
|---|---|---|---|---|---|---|
| Chemoattraction | | | | | | |
| c-Kit/KitL | survival, migration (mouse, not fish) | chemotaxis, fetal liver retention (mouse not fish) | ? | maybe | yes | (Christensen et al., 2004; Matsui et al., 1990; Mintz and Russell, 1957; Parichy et al., 1999; Runyan et al., 2006; Yoder et al., 1997) |
| CXCR4/ SDF-1α | survival (mouse), migration (mouse, fish) | homing to fetal liver, BM (mouse) | chemotaxis of precursors to limb, inhibits differentiation | yes | yes | (Ara et al., 2003; Christensen et al., 2004; Knaut et al., 2003; Ma et al., 1998; Molyneaux et al., 2003; Nagasawa et al., 1996; Vasyutina et al., 2005; Zou et al., 1998) |
| Repulsion/Attraction | | | | | | |
| Ephrins | ? | ? | EphA4-EphA5 mediate avoidance | no | yes | (Swartz et al., 2001) |
| IFITM/Mil/ Fragilis (Leu-13 in lymphocytes) | IFITMs drive PGCs into endoderm; PGC-PGC interaction (mouse) | ? | ? | yes | yes | (Frey et al., 1997; Tanaka et al., 2005; Yoshimizu et al., 2001) |
| Lipids | Wunens: guidance and survival (fly); mevalonate pathway (fly) | S1P/Edg receptors bind SIP, LPA to regulate lymphocyte migration | ? | maybe | yes | (Kunwar et al., 2006; Saba, 2004) |
| Adhesion/Movement | | | | | | |
| Cadherins | E-cadherin downregulation with onset of migration through epithelia (fish, mouse) | VE-cadherin expressed by committing HSCs (mouse) | N-cadherin retains satellite progenitor daughters in dermomyotome | no | yes | (Bendel-Stenzel et al., 2000; Fraser et al., 2002; Shimizu et al., 2005; Taoudi et al., 2005; Weidinger et al., 1999) |
| c-Met/HGF | ? | ? | delamination from dermomyotome | no | yes | (Dietrich et al., 1999; Epstein et al., 1996) |
| Integrins | β1-integrin, PGC homing (mice) | CD41 expressed on early HSCs (mouse, fish); β1-integrin essential for fetal liver and BM colonization | α6β1-integrin involved in migration to the myotome | yes | yes | (Anderson et al., 1999; Bajanca et al., 2006; Bertrand et al., 2005; Potocnik et al., 2000) |

Abbreviations: BM, bone marrow; HGF, hepatocyte growth factor; HSC, hematopoietic stem cell; IFITM, Interferon-inducible transmembrane receptor, PGC, primordial germ cell; S1P, sphingosine-1-phosphate; SDF-1α, stromal derived factor-1α; question mark indicates lack of evidence of involvement.

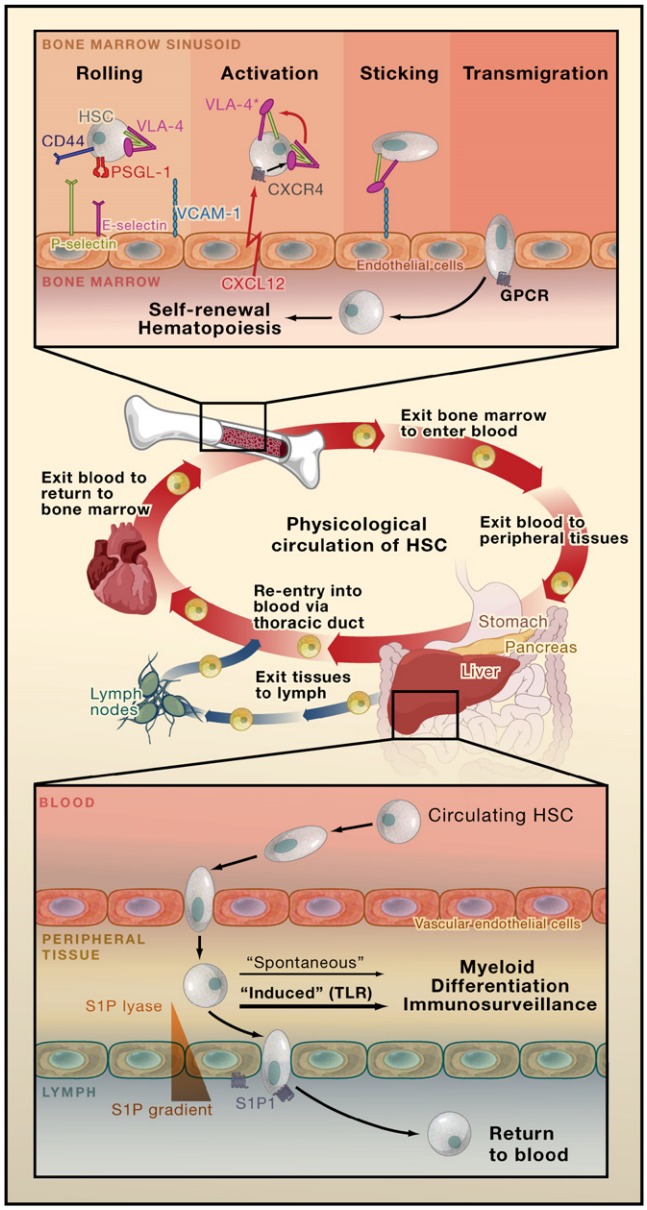


**Figure 2. Migratory Routes of Adult HSCs**

The majority of HSCs reside in the bone marrow where they undergo self-renewal and give rise to differentiated hematopoietic cells; however, some HSCs continuously leave the marrow and enter the blood. At the top, circulating HSCs can re-enter the marrow through sinusoids, which constitutively express trafficking molecules that support a unique multistep adhesion cascade for HSC homing. Initially, free-flowing HSCs are tethered to the vessel by the vascular selectins, E- and P-selectin, which bind to sialyl-Lewis$^x$-like carboydrate ligands that are associated with PSGL-1 and CD44 on HSCs. Selectin binding, together with engagement of endothelial VCAM-1 with the integrin VLA-4 ($\alpha4\beta1$), mediates HSC rolling in marrow sinusoids. The rolling HSCs are then activated by the chemokine CXCL12, which binds to the G protein-coupled receptor, CXCR4. The chemokine signal is thought to induce a rapid conformational change in the VLA-4 heterodimer (VLA-4*) that results in increased affinity for VCAM-1 and permits the rolling cells to arrest. Adherent HSCs then emigrate into the extravascular bone marrow compartment, presumably following extracellular chemoattractant signals transduced via G protein-coupled receptors expressed by HSCs. At the bottom, some blood-borne HSCs exit the blood in various peripheral organs where they spend ~36 hr before entering the draining lymphatics in a manner that depends on sphingosine-1-phosphate (S1P) and the S1P receptor. S1P level are high in the lymph but very low in tissues due to degradation by S1P lyase. While in peripheral tissues, HSCs can divide and differentiate, presumably to replenish tissue-resident myeloid cells. Exposure of tissue-resident HSCs to agonists for Toll-like receptor (TLR) 2 or TLR4 markedly amplifies HSC differentiation along the myeloid lineage. Through this mechanism, migratory HSCs contribute to immune surveillance by the innate immune system.

(Vasyutina et al., 2005), which regulates the survival and dispersal of satellite cell precursors (Sachs et al., 2000). Downstream integration of CXCR4 and c-Met signaling pathways controls both migration and survival of satellite cell precursors. In light of these data, it will be interesting to dissect the molecular crosstalk between GPCR and receptor tyrosine kinase signal transduction in adult satellite cells and to ask whether these pathways also converge in embryonic PGCs or HSCs. Curiously, SDF-1$\alpha$ appears to be a relatively common stem cell guidance cue and is used among multiple vertebrate classes and by all three stem cells considered here. As we continue to compare the development and migration of stem cells in additional organisms, it may become clearer whether SDF-1$\alpha$/CXCR4 represents a primordial navigation system of vertebrates, upon which additional migratory pathways are layered, or whether among many early migration mechanisms, SDF-1$\alpha$/CXCR4 became a refined evolutionary survivor.

## Stem Cell Trafficking in Adult Tissues

As in development, stem cell homing and migration are critical for the ongoing replacement of mature cells and regeneration of damaged cells in many adult tissues. Stem cell function in adult tissue repair and replacement often recapitulates the processes that gave rise to these cells and enabled their dissemination during development. Thus, comparative analysis of developmental and regenerative stem cell function can help to inform studies of the crucial signaling pathways that mediate stem cell movement in the body.

### *Blood*

In the adult hematopoietic system, multipotent clonogenic HSCs give rise to billions of new mature blood cells each day (see Review by S.H. Orkin and L.I. Zon). These cells replenish circulating pools of red and white blood cells whose effector functions limit their lifespan and necessitate their replacement from self-renewing precursors. Migration and homing are thus key components of normal adult hematopoiesis and are required for the dissemination and function of mature blood cells throughout the body. As discussed below, controlled migration appears to be a key feature of normal HSC activity as well (Figure 2).

In adult mice and humans, the majority of HSCs are found in the bone marrow, but HSCs are also constitutively present at low levels in the circulation. Circulating HSCs have been detected in the blood phenotypically and functionally, both by direct transplantation of peripheral blood cells and by analysis of parabiotic mice—animals surgically joined so that they share a common blood circulation (Abkowitz et al., 2003; Massberg et al., 2007; Wright et al., 2001). Significantly, parabiosis experiments directly demonstrate that circulating HSCs rapidly

re-engraft the bone marrow at distinct locations and functionally contribute to ongoing hematopoiesis (Abkowitz et al., 2003; Wright et al., 2001). Moreover, HSC crossengraftment in parabiotic mice occurs in the absence of hematopoietic ablation and does not involve surgery-induced inflammation (Wright et al., 2001). These data suggest that blood circulation is a normal physiological activity of HSCs.

Recently, the in vivo circuit of HSC recirculation has been further explored in mice, revealing the remarkable journey of these cells from the marrow into the blood, from the blood into multiple tissues (including liver, kidneys, and lung), from the tissues into the lymph, and from the lymph back into the blood, where they may return to the marrow or enter another cycle of transit (Figure 2) (Massberg et al., 2007). Although the biological rationale for this nomadic behavior is not entirely clear, one strong possibility is that the constitutive circulation of HSCs through peripheral tissues provides a rapidly recruitable source for local production of immune and inflammatory effector cells (Massberg et al., 2007). Such effectors generated "on the spot" enable fast and effective eradication of subthreshold infections, clean up circumscribed regions of cell death, and replenish rare tissue-resident leukocytes such as dendritic cells that are lost in the course of infection. In addition, analysis of mutant mice lacking the transcription factor early growth response 1 (Egr1) show enhanced HSC proliferation in the bone marrow, coupled with normal numbers of marrow HSCs and a constitutive increase in peripheral blood HSCs (I.M. Min and A.J.W., unpublished data). These findings suggest that regulated release of HSCs into the circulation also may help to limit overaccumulation of these cells in the marrow environment.

The efficiency with which HSCs re-engraft the marrow in unmanipulated animals appears to depend directly on the availability of open niches, which constantly turn over at a low rate (Bhattacharya et al., 2006). In parabiotic mice, HSC chimerism can be enhanced by hematoablative drugs and cytokines that induce the movement of endogenous cells out of the marrow niche (Abkowitz et al., 2003). Likewise, hematopoietic engraftment following transplantation in unirradiated animals can be significantly enhanced by antibody-mediated depletion of the recipient's endogenous pool of HSCs (Czechowicz et al., 2007). Ultimately, understanding how constitutive recirculation affects HSC function must await strategies that specifically block this migration. Nonetheless, the importance of HSC homing pathways is made clear by the reliance of clinical bone-marrow transplantation on the innate ability of transplanted HSCs to traffic efficiently to the bone-marrow niche.

Transplantation of human bone-marrow cells or peripheral blood progenitor cells (PBPCs) is a common treatment option for patients with hematopoietic and nonhematopoietic cancers, bone-marrow failure, or certain metabolic disorders. During hematopoietic cell transplant, donor HSCs contained within marrow or peripheral blood grafts are introduced intravenously into recipients whose own blood-forming capacity has been partially or completely abrogated by irradiation or chemotherapy. To succeed in regenerating the recipient's blood system, these HSCs must accurately and efficiently home to appropriate marrow locations and engraft within available niches that support HSC survival, expansion, and differentiation to regenerate mature blood cells. This clinically important process of transplantation is likely to use pre-existing pathways that normally support the physiological recirculation of HSCs during steady-state hematopoiesis. Intravital microscopy studies in mouse bone marrow have enabled the dissection of the multistep adhesion cascade regulating hematopoietic stem and progenitor cell homing to normal and irradiated bone marrow (Mazo et al., 1998, 2002) and have implicated particular adhesion and chemotactic receptors in this process (Table 2 and Figure 2).

In addition to blood-to-marrow homing, common clinical practice also exploits the reverse process of marrow-to-blood migration, a phenomenon known as "mobilization." In fact, because mobilization of HSCs into the circulation greatly facilitates their collection for transplantation and appears to shorten the time required for recovery of normal levels of circulating blood cells in some transplant recipients (Jansen et al., 2005), mobilized peripheral blood cells are increasingly preferred when compared to other stem cell sources for adult transplantation (http://www.imbtr.org).

Like transplantation, HSC mobilization may use pre-existing physiological migratory pathways. Mobilization can be induced by a wide variety of "mobilizing" agents, including antagonists of adhesion and chemotaxis, cytotoxic drugs, and certain cytokines. Interestingly, these agents often drive both HSC proliferation and movement from the marrow to the bloodstream (Morrison et al., 1997; Papayannopoulou, 1999), suggesting a mechanistic link between HSC cell-cycle progression and migration. In this regard, it is interesting to note that the homing efficiency of transplanted HSCs (from blood to marrow) is directly impacted by the position of HSCs in the cell cycle. In several studies, the ability of HSCs to functionally engraft irradiated recipients dramatically decreases as soon as they exit quiescence (Bowie et al., 2006; Passegue et al., 2005), although the explanation for this reduced engraftment potential remains unclear.

### *Skeletal Muscle*

Adult skeletal muscle possesses remarkable regenerative capacity, with large numbers of new muscle fibers forming only a few days after acute muscle damage (Hawke and Garry, 2001). This rapid repair is believed to occur through the action of myogenically specified precursor cells contained within the population of satellite cells located immediately adjacent to and beneath the basal lamina of muscle fibers (Mauro, 1961; Figure 3). In response to muscle growth and regenerative cues, normally quiescent satellite cells become activated and divide, migrate, and differentiate to form myoblasts that fuse with each other and with existing muscle fibers to regenerate the muscle (reviewed in Hawke and Garry, 2001).

Satellite cell activation, migration, and fusion all contribute to the maintenance and regeneration of muscle mass in normal and pathological conditions. The migratory capacity of myogenic satellite cells appears to be modulated by the integrity of the basal lamina. After rupture of the basal lamina due to injury or disease, satellite cells migrate to adjacent myofibers via tissue bridges (Watt et al., 1987). With more limited muscle injury where no rupture of the basal lamina occurs, satellite cells may traverse the myofiber underneath the basal lamina to the injury site. The signals that initiate satellite cell movement along and between damaged myofibers, as well as the molecular mediators of their migration, are not well understood. However, some myofiber-associated

**Table 2. Molecular Mechanisms Involved in the Trafficking of Stem Cells in the Adult**

| Mechanism | HSCs | Satellite cells | Involved in homing (blood to tissue)? | Involved in interstitial navigation? | Pathway also utilized during development? | References |
|---|---|---|---|---|---|---|
| Deadhesion | | | | | | |
| MMPs | cleaves KitL to generate soluble KitL; important for mobilization and hematopoietic recovery | involved in myoblast migration | indirectly | yes | yes (in flies) | (Carmeli et al., 2004; Heissig et al., 2002; Kollet et al., 2006) |
| Cathepsin K | Produced by osteoclasts; cleaves KitL and SDF-1α | ? | yes | yes | ? | (Kollet et al., 2006) |
| CD26 | Cleaves SDF-1α; involved in mobilization and homing to BM | ? | yes | no | no | (Christopherson et al., 2003) |
| Chemoattraction | | | | | | |
| c-Kit/KitL | BM homing and retention | ? | yes | yes | yes | (Bernstein et al., 1991; Fleming et al., 1993) |
| CXCR4/ SDF-1α | BM homing and retention | myoblast chemotaxis | yes | yes | yes | (De Paepe et al., 2004; Ma et al., 1998; Nagasawa et al., 1996; Petit et al., 2002; Ratajczak et al., 2003; Zou et al., 1998) |
| Rho GTPases | Rac1, Rac2, cdc42: HSC retention in BM; homing to BM | regulates M-cadherin expression | yes | yes | yes | (Cancelas et al., 2005; Charrasse et al., 2006; Gu et al., 2003) |
| Lipids (S1P) | HSC recirculation from tissues into lymph | S1P signaling induces proliferation and cell contraction | no | yes | yes | (Formigli et al., 2004; Massberg et al., 2007; Nagata et al., 2006) |
| Adhesion/Movement | | | | | | |
| Cadherins | N-cadherin adhesion implicated in some but not all studies | M-cadherin binds satellite cells to the myofiber | no | yes | yes | (Irintchev et al., 1994; Kiel et al., 2007; Zhang et al., 2003) |
| c-Met/HGF | | activation of satellite cells | no | yes | yes | (Tatsumi et al., 1998) |
| Integrins | β1-integrin essential for BM homing; BM retention | involved in myoblast migration, adhesion and fusion | yes | yes | yes | (Papayannopoulou, 2000; Potocnik et al., 2000; Schwander et al., 2003; Taverna et al., 1998) |

Abbreviations: BM, bone marrow; HGF, hepatocyte growth factor; HSC, hematopoietic stem cell; MMP, matrix metalloproteinase; S1P, sphingosine-1-phosphate; SDF-1α, stromal derived factor-1α; question mark indicates lack of evidence of involvement.

muscle stem cells contained within the satellite cell population may possess unique migratory properties that allow their re-engraftment into the satellite cell niche upon intramuscular transplant (Collins et al., 2005; Montarras et al., 2005; Sherwood et al., 2004). Re-entry into this niche after muscle damage allows myogenic stem cells to repopulate and renew the stem cell compartment in the muscle, such that muscle regenerative activity is maintained for subsequent rounds of injury repair.

Unlike HSCs, highly myogenic muscle satellite cells do not appear to traffic naturally in the circulation, as no chimerism develops in this population in parabiotic mice, even after many months of shared circulation (Sherwood et al., 2004). Recruitment of myogenic satellite cells from the circulation is also not induced following muscle injury (Sherwood et al., 2004), suggesting that these cells do not possess appropriate adhesion and signaling receptors to support their movement into or out of the bloodstream. Even within the muscle, myogenic precursors exhibit relatively limited mobility, although they do appear capable of contributing to myofibers at some distance from their initial site of activation within the muscle bed (Hughes and Blau, 1990).

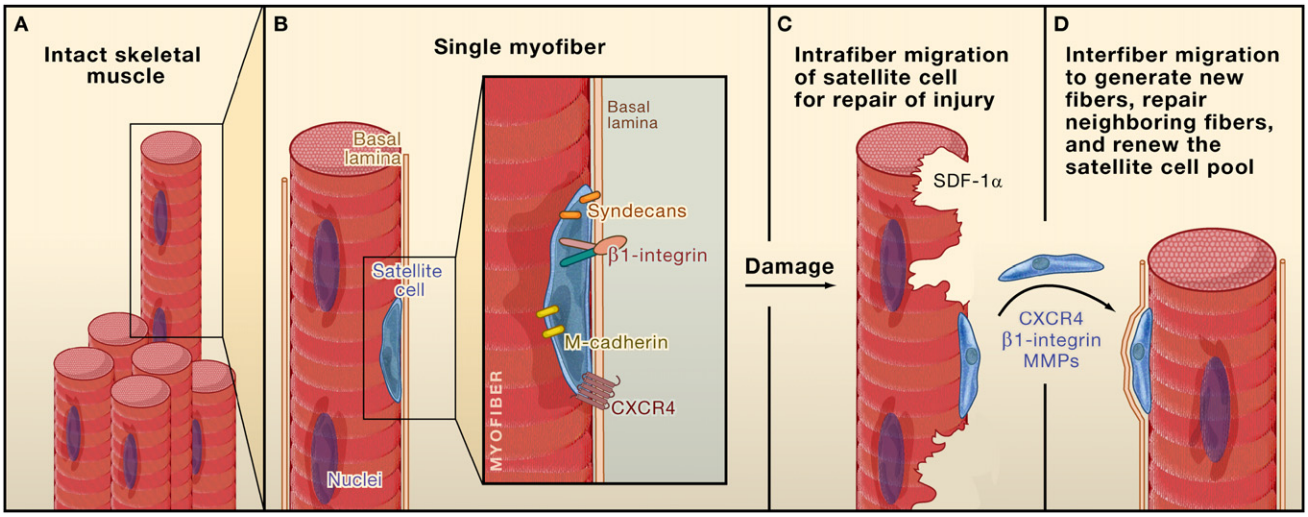


**Figure 3. Adhesion and Migration in Adult Skeletal Muscle**

(A–D) Skeletal muscle is composed of bundles of multinucleated myofibers (A). Each fiber carries a rare population of primitive muscle satellite cells. Satellite cells reside between the myofiber plasma membrane and the surrounding basal lamina, composed of collagen, laminin and other extracellular matrix-associated proteins (B). When the muscle is injured, damaged muscle fibers and infiltrating blood cells (not shown) elaborate soluble mediators, such as SDF-1α, and satellite cells become activated (C). Activated satellite cells proliferate and migrate along the myofiber (C) and through the muscle intersitium to adjacent myofibers (D) to repair damage by fusion with surviving myofibers and by de novo myogenesis. Shown in (B)'s inset is the muscle satellite cell niche. Satellite cells adhere tightly to myofibers, and M-cadherin concentrates in the region of cell-cell contact. Satellite cells also adhere to the laminin-containing extracellular matrix (black line) via β1-integrin heterodimers, and express the chemokine receptor CXCR4 and Syndecan coreceptors. These trafficking molecules regulate satellite cell proliferation and migration during muscle regeneration.

Interestingly, other populations of cells that reportedly exhibit myogenic activity in vivo—including blood vessel-associated pericytes, mesangioblasts, and muscle side-population (SP) cells—are able to engraft the muscle from the bloodstream and to contribute to donor-engrafted muscle fibers following intra-arterial (but not intravenous) delivery (reviewed in Peault et al., 2007). The homing capacity of these alternative muscle regenerative cells may in some instances be induced or enhanced by cell culture (Galvez et al., 2006; Sampaolesi et al., 2003). Such induced homing capacity may be an advantage for systemic dissemination, but given the relatively poor myogenic activity of these cells in comparison to canonical muscle satellite cells (Collins et al., 2005; Montarras et al., 2005), and the potential dangers (such as thrombosis) associated with intravascular cell delivery, it is still unclear what constitutes the "best" population for muscle cell therapy.

## Trafficking Mechanisms of Adult Stem Cells

Taking blood and skeletal muscle as prototypical examples of homeostatic (blood) and facultative (skeletal muscle) stem cell populations, one can divide the events of physiological adult stem cell movement into two distinct multistep processes. First, because long-term maintenance of tissue-specific adult stem cells typically requires association with a supportive stem cell niche (see Review by S.J. Morrison and A.C. Spradling, page 598 of this issue), movement of these cells during the course of steady-state or injury-induced replacement of mature daughter cells first requires stem cell mobilization from the niche. Yet, to enable long-term stem cell maintenance, these mobilized cells also must eventually return to the niche, in a process complementary to stem cell homing. Mobilization and return of stem cells to the niche invoke similar, although not identical, molecular processes involving adhesion/deadhesion, chemoattraction/chemoretention, and ultimately stem cell movement. In addition, they often use the same molecular mediators, illustrating the functional complementarity of these processes. Interestingly, while stem cell mobilization often evokes stem cell proliferation, return to the niche is typically accompanied by a return to mitotic quiescence (Hawke and Garry, 2001; Morrison et al., 1997; Passegue et al., 2005; Zammit et al., 2004).

### *Adhesion and Deadhesion*

In both blood and skeletal muscle, the molecular mechanisms underlying stem cell movement have been most studied in settings of induced responses to tissue injury (that is, chemotoxic drug treatments, irradiation, and transplantation). However, it is likely that factors involved in injury-induced stem cell mobilization and homing likewise mediate steady-state physiological movements, suggesting that these same pathways will almost certainly have relevance for homeostatic stem cell functions as well. In any event, stem cell movement in vivo begins with release of the stem cell from its protective environment, or niche. In the hematopoietic system, this appears to occur constitutively (Massberg et al., 2007; Wright et al., 2001), whereas in skeletal muscle, the liberation of myogenic satellite cells seems to occur almost exclusively in response to muscle damage (Hawke and Garry, 2001).

For HSCs, deadhesion from the niche involves the elaboration of proteolytic enzymes by both bone marrow hematopoietic and stromal elements; such enzymes include matrix metalloproteinase MMP-9 and the cysteine protease cathepsin K (Heissig

et al., 2002; Kollet et al., 2006). These enzymes act on extracellular matrix proteins and secreted cytokines, including SDF-1α and KitL (Kollet et al., 2006), both of which can modulate the in vivo localization of HSCs and hematopoietic progenitors (Fleming et al., 1993; Levesque et al., 2003). Recent data implicate blood-lineage osteoclasts, bone-remodeling cells found often in the endosteal region of the marrow, in the release of HSCs from their niche; direct cytokine stimulation of osteoclasts specifically increases circulating levels of hematopoietic precursors, whereas in vivo inhibition of osteoclast activity reduces both the physiological release and induced mobilization of hematopoietic precursor cells (Kollet et al., 2006). The activity of proteases produced by HSCs themselves, such as the cell surface-expressed dipeptidase CD26, also contributes to the silencing of HSC retention signals in the marrow in part by cleavage-mediated inactivation of SDF-1α (Christopherson et al., 2003). Activation of protease activity likewise appears to regulate muscle satellite cell function, and in vivo studies argue that MMP activity (likely MMP-2, MT-MMP1, or MMP-9) is essential for the migration of myogenic precursors during muscle regeneration (Carmeli et al., 2004).

Based on expression studies, cadherin-mediated cell adhesion has been suggested to facilitate HSC retention in the niche (via N-cadherin) (Zhang et al., 2003) and to correctly position muscle satellite cells along the muscle fiber (via M-cadherin) (Irintchev et al., 1994). However, mice lacking M-cadherin show no defects in skeletal muscle development or regeneration (Hollnagel et al., 2002), and recent studies argue against the involvement of N-cadherin in regulating HSCs (Kiel et al., 2007). Thus, the precise role of cadherin-mediated binding in adhesion/deadhesion of adult stem cells remains opaque, and it is possible that other adhesion molecules, such as integrins, support cadherin-like functions in adult tissues.

Satellite cells also express a number of other cell surface receptors implicated in cell-cell and cell-ECM adhesion, including CD34, VCAM-1, NCAM, c-Met, syndecan-3, and syndecan-4 (reviewed in Peault et al., 2007 and Hawke and Garry, 2001). Although in many cases the functional importance of these adhesion receptors for satellite cell activation and myogenic function remains obscure, analysis of relevant knockout mice suggests that syndecans-3 and -4 are important for satellite cell-mediated muscle regeneration. Syndecans are a family of cell surface-expressed heparin sulfate proteoglycans that act as coreceptors for tyrosine kinases and play a role in cell adhesion. Mice lacking syndecan-3 exhibit a progressive muscular dystrophy and aberrant in vitro differentiation of muscle satellite cells, whereas mice lacking syndecan-4 show defects in muscle regeneration in vivo and syndecan-4 null myocytes fail to proliferate properly when cultured ex vivo (Cornelison et al., 2004). Nonetheless, normal muscle morphogenesis in syndecan-knockout mice suggests that these molecules are not critical for specification or migration of myogenic precursors during development.

### *Chemoattraction/Chemoretention*

Receipt of appropriate stem cell retention signals appears to be essential for maintaining HSCs within the bone-marrow niche. Like their fetal counterparts, adult HSCs express the chemokine receptor CXCR4 and selectively respond to SDF-1α in chemotaxis assays in vitro (Wright et al., 2002). Administration of the CXCR4 antagonist AMD3100 effectively mobilizes HSCs in both mice and humans (Broxmeyer et al., 2005), and proteolytic degradation of SDF-1α in the bone marrow has been associated with induced mobilization of hematopoietic progenitor cells (Levesque et al., 2003; Petit et al., 2002), although the necessity of this degradation for mobilization has been questioned (Levesque et al., 2004). Likewise, conditional ablation of CXCR4 in hematopoietic lineage cells results in loss of HSCs from the marrow environment and reduced resistance to hematopoietic injury (Sugiyama et al., 2006). Signaling downstream of CXCR4, via the Rho family GTPases Rac1 and Rac2, also appears to be essential for retention of HSCs in the marrow, as ubiquitous deletion of both Rac1 and Rac2 or administration of small molecule inhibitors of Rac proteins induces spontaneous mobilization of HSCs and progenitors into the blood (Cancelas et al., 2005; Gu et al., 2003). Interestingly, different chemotactic signals appear to regulate the movement of HSCs out of tissues other than the bone marrow. HSCs arrive in these peripheral organs via the blood, but exit via the draining lymphatics in a manner that depends on sphingosine-1-phosphate (S1P) and the S1P receptor (Massberg et al., 2007). S1P levels are high in the lymph but very low in tissues due to degradation by a S1P lyase (Figure 2C). Thus, HSCs appear to respond to a gradient of S1P to exit peripheral tissues and enter the lymph, which enables their passive transport and return to the blood via the thoracic duct.

Many of the same molecules that play a critical role in HSC mobilization from the bone marrow have also been implicated in the reverse process of HSC homing to the marrow from the peripheral blood. For example, hematopoietic engraftment of human HSCs in immunodeficient mice is blocked by inhibitory antibodies to CXCR4 (Peled et al., 1999). Likewise, Rac1-deficient mouse HSCs exhibit an impaired ability to migrate to the marrow after transplant and, thus, are unable to effectively repopulate recipient hematopoietic systems (Cancelas et al., 2005; Gu et al., 2003). After arriving in the marrow, HSC engraftment in the appropriate marrow location appears to depend additionally on retention signals provided by divalent cations in the microenvironment. In particular, the $Ca^{2+}$ sensing receptor is highly expressed by HSCs, and transplanted HSCs that lack this receptor home to marrow but show impaired lodgement in endosteal niches (adjacent to the bone) (Adams et al., 2006). As a result of their defective in vivo localization, HSCs lacking the $Ca^{2+}$ sensing receptor ultimately fail to effectively engraft irradiated recipients in competitive transplant assays (Adams et al., 2006).

Signaling via the CXCR4/SDF-1α axis also appears to be involved in the action of myogenic precursor cells of adult skeletal muscle. First, analysis of cell surface marker expression by skeletal muscle satellite cells (Sherwood et al., 2004) indicates that a highly myogenic subset of these, which exhibit muscle stem cell properties (M. Cerletti and A.J.W., unpublished data), can be specifically enriched by expression of CXCR4. Therefore, these cells likely respond directly to SDF-1α, consistent with reports of SDF-1α upregulation in injured and regenerating skeletal muscle (De Paepe et al., 2004) and chemotaxis of muscle satellite cell lines in response to SDF-1α (Ratajczak et al., 2003). SDF-1α is also involved in the migration of rat neural precursor cells and may be important for attracting these cells to proliferate in the external granule cell layer of the cerebellum in the brain (Reiss et al., 2002).

### Movement

As for HSC chemotaxis, HSC mobilization from the marrow and homing to the marrow also appear to invoke an overlapping set of cell surface-expressed adhesion receptors (Figure 2C). For example, antibody-based inhibition of the integrin VLA-4 ($\alpha4\beta1$) or proteolytic degradation of its ligand VCAM-1 induces mobilization of stem and progenitor cells in both mice and primates (Levesque et al., 2001; Papayannopoulou, 2000). Conversely, functional inhibition of VLA-4 during HSC transplant prevents efficient hematopoietic reconstitution by blocking HSC homing to the bone marrow and engraftment within the marrow niche (Wagers et al., 2002). HSC homing from blood to marrow additionally invokes the activity of the endothelial adhesion molecules, E- and/or P-selectin (Frenette et al., 1998; Mazo et al., 1998).

As in the movement of HSCs, integrin-mediated cell adhesion and migration is also essential for proper muscle repair by myogenic satellite cells. During the later stages of muscle regeneration, interaction between the remodeled extracellular matrix and integrins expressed by satellite cells facilitates the adhesion and spreading of muscle precursors and thus establishes the organization of the regenerated muscle fibers (Disatnik and Rando, 1999; Zaidel-Bar et al., 2004). In addition, conditional inactivation of the $\beta1$-integrin chain, a marker of highly myogenic stem cells within the satellite cell population (Kuang et al., 2007; Sherwood et al., 2004), in developing skeletal muscle leads to an accumulation of unfused cells and a decrease in muscle fibers in mutant muscles (Schwander et al., 2003).

In summary, drawing from examples in both the blood and skeletal muscle, it is clear that a complex cascade of adhesive, chemotactic, and signaling pathways acts cooperatively and in concert to bring about the relocation of stem cells in the adult organism. Moreover, many of the molecules and mediators supporting stem cell migration and homing in adult tissues are shared with migration systems utilized during embryogenesis, suggesting a close conservation of the regenerative responses of adult tissue stem cells and the organogenic activities of embryonic and fetal precursors (Tables 1 and 2). Thus, the regulated mobilization and homing of tissue-specific stem cells is crucial for proper function of these cells in tissue homeostasis and repair.

## Stem Cell Trafficking and Disease

As a final parallel, we consider the role of stem cell homing in the pathological activities of tumor-propagating cancer stem cells, which like their normal counterparts, may rely on regulated adhesion and migration to disseminate malignant clones exhibiting a hierarchy of (dysregulated) cell differentiation. We also discuss potential new strategies to exploit stem cell homing as a vehicle for drug or gene delivery.

### Cancer, Cancer Stem Cells, and Metastasis

An abnormal increase in progenitor cell frequency in the bloodstream often correlates with neoplastic transformation. Like normal hematopoietic cell migration, the trafficking of cancer cells into and through the bloodstream relies on the expression of specific cell adhesion and chemotactic factors. Migration through the bloodstream allows dissemination of metastatic cells in both hematopoietic and solid tumors, and in both cases, specific adhesion/deadhesion pathways appear to determine the efficiency of egress from the primary tumor site and the tropism of the metastatic cells. For example, in a mouse model of melanoma, ectopic expression of integrin $\alpha4\beta1$ is sufficient to retain tumors in situ (Qian et al., 1994), whereas in a distinct mouse insulinoma model, transgenic expression of the homing receptor L-selectin (CD62L) is sufficient to direct metastasis to peripheral lymph nodes (Qian et al., 2001). Finally, expression of chemokine receptors, particularly CXCR4, has been associated with trafficking and enhanced metastasis in several blood and solid tumors including leukemia, lymphoma, multiple myeloma, breast cancer, ovarian cancer, prostate cancer, renal cell carcinoma, melanoma, and non-small-cell lung cancer (reviewed in Balkwill, 2004 and Burger and Kipps, 2006).

These data support the notion that neoplastic progenitor cells take advantage of the same or similar mechanisms of migration as those normally used by their nonmalignant counterparts and by differentiated leukocytes. But do these mechanisms truly reflect the in vivo movement of tumor propagating cancer stem cells, and can they be targeted effectively to delay or impair reseeding of malignancy at distant sites? In fact, current data from a number of model systems lend increasing support to the notion that many, although perhaps not all, malignancies have at their root a rare population of cancer stem cells, which maintains production of more differentiated malignant blasts and is capable of transferring disease to otherwise normal secondary recipients (reviewed in Dalerba et al., 2007). Recent work further suggests that marrow-derived non-neoplastic cells may be necessary to form a "premetastatic niche" that directs the organ-specific homing patterns of malignant carcinoma cells (Kaplan et al., 2005). This premetastatic niche possesses unique extracellular matrix properties and secretes chemoattractants, such as SDF-1$\alpha$, to recruit metastatic cancer stem cells to establish secondary tumors at distal sites (Kaplan et al., 2005). Importantly, interventions that prevent the establishment of this niche appear also to block in vivo metastasis, suggesting that targeting the homing mechanisms of malignant cells may be an effective strategy for limiting cancer spread.

Consistent with this notion that mobilization of malignant cancer stem cells is important in the establishment of metastatic tumors, studies in a xenograft model of acute myelogenous leukemia (AML) indicate that inhibition of leukemia stem cell (LSC) migration to a putative leukemic niche by blockade of CD44-dependent adhesion prevents leukemic engraftment (Jin et al., 2006). Thus, interference with tumor cell migration could be used to prevent or delay leukemic progression and cancer spread. Conversely, because acquisition by cancer stem cells of new adhesion receptor expression or function may confer metastatic potential upon some tumors (Balkwill, 2004; Qian et al., 1994, 2001), migration receptor expression profiling could prove useful as a diagnostic and prognostic tool. These data argue in favor of conserved mechanisms of cell deadhesion, chemotaxis, migration, and homing in the dissemination of normal stem cells and of their malignant counterparts.

### Stem Cells as Delivery Vehicles

The innate homing capacity of HSCs has been exploited clinically in the repopulation of blood cells via bone-marrow transplant. Intravascular transplant methods have also been explored

for cell-replacement therapy involving skeletal muscle precursors and mesenchymal stem cells, although in many cases these cell populations have exhibited limited efficiency for engraftment in target organs (reviewed in Peault et al., 2007). The study of in vivo stem cell homing and migration has taught us that trafficking patterns differ between stem cell lineages, and are strongly influenced by the normal interactions between these cells and their niches during development and in the adult. Thus, stem cells should not be considered as broadly acting "heat-seeking missiles" that are capable of specifically searching out and targeting diseased or dysfunctional tissues. Nevertheless, a deeper knowledge of the natural migratory properties of stem cells and of the ways in which stem cell trafficking patterns can be manipulated may reveal new ways to exploit their unique properties. For example, because stem cells possess extensive self-renewal capacity, they represent particularly attractive delivery vehicles for drug or gene therapy because they would allow long-term production of disease modulators and ongoing replacement of missing or defective gene products. In fact, recent studies in a mouse xenotransplant model of human glioma provide some support for this notion. In these studies, intracranially injected mouse neural precursor cells showed the surprising ability to migrate from the contralateral hemisphere of the brain to primary and secondary glioma foci. The targeted migration of these cells was exploited to deliver cytotoxic tumor therapy, thereby reducing tumor growth (Shah et al., 2005). The analogous capacity of hematopoietic and muscle stem cells to home through the circulation or to navigate within the tissue interstitium to target distinct niches within the body raises the intriguing possibility that these cells may likewise be useful as drug or gene-delivery vectors.

## Conclusions and Perspective

Just how critical is interstitial migration and blood-to-tissue homing for the specification of stem cells in the embryo, for the replacement and repair of adult tissues, and for the maintenance and spread of tumor-propagating cancer stem cells? On a practical level, the establishment of new cell lineages and coordination of organogenesis during development requires mechanical separation of precursors from surrounding cells. For example, to seed muscle formation and satellite cell pools at distant sites throughout the body, embryonic myogenic precursors must first delaminate from their original site of specification in the dermomyotome (Buckingham et al., 2003) and migrate to new locations. Relocation to a new environment also may permit nascent or maturing stem cells to receive appropriate induction signals or may shield them from inappropriate signals. For example, mouse PGC precursors make a brief detour to extraembryonic regions, and embryological studies demonstrate that this sojourn is required for establishment of the PGC lineage (Snow, 1981). Recent work proposes a molecular mechanism for this shielding, demonstrating that induction of the transcriptional repressor Blimp1 in these extraembryonic mouse PGCs prevents the expression of genes that would otherwise activate a somatic cell differentiation program (Ohinata et al., 2005). The next important question to answer will be how the extraembryonic niche regulates Blimp1 in PGCs to prevent the activation of default somatic differentiation programs. In any event, such observations argue that the development of some stem cells cannot be completed in a single niche and that signals gleaned from multiple microenvironments must be integrated over time. Recent advances in stem cell isolation and visualization in situ and in forward genetics will facilitate the identification and study of mutant flies, fish, and mice with defects in anatomically discrete aspects of stem cell migration. These tools will enable formal testing in multiple tissue systems of the relationship between stem cell function and stem cell migration and homing during development.

Is there something unique about the migration and homing of stem cells? Extensive migration during development and in adult life is a property of many cells, including stem cells, progenitors, and differentiated cells. In fact, not one of the molecular mechanisms discussed here operates exclusively in stem cells; on the contrary, stem cells appear to home and migrate by mechanisms common to many itinerant cells, including mature leukocytes and neural crest and endothelial cells. If in fact there are no particular molecules that function exclusively in the adhesion, movement, and navigation of stem cells, then perhaps the specific routes traveled by stem cells are patterned and timed more subtly, through the expression of particular combinations of trafficking molecules or by integration of migratory cues with other stem cell signaling pathways. Nonetheless, while stem cell trafficking may not invoke molecular mechanisms unique to stem cells, our consideration of the movement of HSCs, PGCs, and satellite cells does reveal intriguing commonalities, including utilization of the SDF-1$\alpha$/CXCR4 chemotaxis pathway and linkage of stem cell proliferation and movement, which appear to be shared in developing and adult tissues by all of these migrating stem cell populations.

Among the examples we have considered, chemotactic signaling via the SDF-1$\alpha$/CXCR4 axis stands alone as a broadly conserved migration mechanism that acts in stem cell movements in multiple tissues in both the embryo and adult. During development, SDF-1$\alpha$/CXCR4 signals direct the homing of fetal mouse HSCs to the liver and marrow (Ma et al., 1998; Nagasawa et al., 1996; Zou et al., 1998), guide fish and mouse PGCs toward the gonadal ridge (Ara et al., 2003; Molyneaux et al., 2003), and help to target mouse myogenic precursor cells as they migrate from the dermomyotome (Vasyutina et al., 2005). In the adult, SDF-1$\alpha$ and CXCR4 regulate the mobilization of mouse and human HSCs into the peripheral blood as well as their re-entry into the marrow (Broxmeyer et al., 2005; Peled et al., 1999; Sugiyama et al., 2006), and facilitate skeletal muscle regeneration (De Paepe et al., 2004; Ratajczak et al., 2003). This chemotactic pathway also functions in the dissemination of tumor-forming cells in a large number of metastatic cancers (Balkwill, 2004). The remarkable ubiquity of SDF-1$\alpha$/CXCR4 signaling in regulating a diverse array of stem cells in a diversity of contexts certainly begs the question of whether this pathway may have some unique or stem cell-specific functions. Yet SDF-1$\alpha$/CXCR4 signals also regulate several processes apparently unrelated to stem cell activity, including the normal trafficking of lymphocyte precursors and mature hematopoietic cells, migration of cerebellar neurons, and cardiogenesis (reviewed in Burger and Kipps, 2006). In any event, our understanding of this pathway in stem cell regulation is as yet incomplete; for example, it remains to be determined whether the recently identified second

receptor for SDF-1α, CXCR7 likewise regulates multiple stem cell types (although a lack of overt hematopoietic phenotypes in mice conditionally lacking this receptor argues against a crucial role for CXCR7 in HSCs (Sierro et al., 2007). Further characterization of both the upstream and downstream components of the SDF-1α/CXCR4 pathway may highlight important differences between cell lineages or identify aspects unique to stem cells. A broader understanding of SDF-1α/CXCR4 molecular regulation and response circuitry may shed new light on how this particular chemokine pathway may have become so important for the trafficking of multiple types of stem cells in development, disease, and regeneration.

In addition to frequent use of SDF-1α/CXCR4 signaling, a second common aspect of the movement of stem cells in the embryo and adult appears to be coordination of migration with regulation of stem cell numbers (Hawke and Garry, 2001; Morrison et al., 1997; Zammit et al., 2004). For example, during development, HSCs switch from a state of rapid proliferation to a state of relative quiescence soon after homing to the bone marrow (Bowie et al., 2006). For PGCs, the connection between proliferation and migration is implied by the growing list of genes that act in both processes: c-Kit/KitL (Runyan et al., 2006), SDF-1α/CXCR4 (Ara et al., 2003; Molyneaux et al., 2003), and zebrafish Dead end (Kunwar et al., 2006). Misregulation of proliferation or migration of any of these stem cell types may have dire biological consequences, such as anemia, myopathy, infertility, and cancer. For example, in the germline, aberrant PGC regulation can give rise to extragonadal tumors derived from vagrant PGCs (Runyan et al., 2006), and the prevention of germline cancers necessitates additional control of cell survival during the migration of PGCs. In mice, PGCs that fail to migrate not only fail to proliferate but are actively deleted by apoptosis pathways involving the proapoptosis protein Bax (Runyan et al., 2006) or other mechanisms such as the Wunen pathway in flies (reviewed in Kunwar et al., 2006). In the adult, direct analysis of HSC movement in vivo corroborates a mechanistic link between HSC cell-cycle progression and migration. HSC-mobilizing agents often simultaneously enhance stem cell proliferation and migration into the blood (Morrison et al., 1997). Furthermore, recent analysis of mice lacking the transcription factor Egr1 demonstrates a direct molecular link between HSC proliferation and in vivo localization, both of which are perturbed in Egr1-deficient HSCs (I.M. Min and A.J.W., unpublished data). The reverse processes of homing and bone-marrow engraftment of transplanted HSCs also appear to be linked to cell-cycle status, as dividing HSCs exhibit compromised bone-marrow homing and long-term hematopoietic reconstituting capacities (Bowie et al., 2006; Passegue et al., 2005). Finally, in skeletal muscle, activation of satellite cells following muscle injury induces both their proliferation and their migration to damaged regions of the same or adjacent muscle fibers, whereas, conversely, re-entry into the satellite cell niche is associated with a return to mitotic quiescence (Hawke and Garry, 2001; Zammit et al., 2004). These examples from several different systems reveal a coordination of stem cell proliferation and migration, which may enable "system-wide" maintenance of appropriate stem cell numbers and limit stem cell expansion to only the appropriate locations and contexts.

Further perspective on how and why stem cells migrate will likely come from studies in a diversity of model organisms. As discussed here, many insights have been gleaned from both vertebrate and nonvertebrate systems. In addition, stem cell movement both in development and in adult tissues during maintenance and repair appears to be evolutionarily conserved. Indeed, ascidians—a phylogenetic intermediate between invertebrates and vertebrates—offer a unique perspective on stem cell homing. Colonial ascidians such as *B. schlosseri* are comprised of successively regenerating clonal individuals and maintain lineages of stem cells to build bodies and to make gametes. Although their origins during the organism's adult life remain opaque, these somatic stem cells and germline stem cells migrate from old to new bodies to carry out normal colony regeneration. Like other homing stem cells, they transit through the vasculature and may reside in multiple niches; however, *B. schlosseri* stem cells also invade and colonize the bodies and gonads of other genetically distinct colonies (Laird et al., 2005). Thus, in this example, stem cell homing provides a mechanism not only for homeostatic regeneration but also for evolutionary competitiveness by enabling parasitization of the gonads of others to expand reproductive capacity. Although little is currently known regarding the molecular mechanisms that control stem cell homing and engraftment in *B. schlosseri*, ongoing genomic analyses have identified homologs of many key mammalian adhesion proteins including selectins, integrins, ICAMs, and NCAMs (A. De Tomaso, personal communication), suggesting evolutionary conservation of the mediators of stem cell migration and engraftment in this organism. Recent studies suggest that stem cell competition also occurs in a more commonly studied model organism, the fruit fly *Drosophila melanogaster*, where it appears that a major determinant of the "competitiveness" of germline stem cells (GSCs)—that is, their success in being maintained in the ovary—relates to their expression of cadherin receptors, which allow them to maintain contact with their niche. Fly GSCs that express higher levels of cadherin adhere better to supportive niche cells (called cap cells), as indicated by an expanded area of contact between cadherin$^{hi}$ GSCs and cap cells. Indeed, cadherin$^{hi}$ GSCs can actually expel less competitive cadherin$^{lo}$ GSCs from the niche and replace them via symmetric self-renewing division (Jin et al., 2008). Whether similar adhesion-based competition helps to determine "winners" and "losers" in the *B. schlosseri* gonad will be an important question to address. Elucidating the molecular basis of what appears to be a high-stakes stem cell homing competition in colonial ascidians as well as fly ovaries will likely provide unique insights into how and why stem cells home.

What do we gain from understanding stem cell migration? The migration and homing of adult HSCs has been tremendously useful in the clinical application of these cells in bone-marrow transplantation. Revealing the importance of HSC movement and the role of distinct niches in specifying their function during development and in adulthood will likely enable their improved therapeutic application. The study of stem cell migration may also catalyze ongoing efforts to derive specialized precursor cells from pluripotent embryonic stem cells or induced pluripotent stem (iPS) cells, particularly as sequential exposure to multiple distinct microenvironments during stem cell homing or

interstitial migration appears to be commonly required for appropriate stem cell specification and maturation. A deeper understanding of the migratory activity of PGCs, HSCs, satellite cells, and other stem and progenitor cell populations will likely accelerate progress towards exploiting these cells for regenerative medicine. Finally, an improved capacity to control stem cell migration will have important implications for drug delivery and anticancer therapies, perhaps enabling highly specific interventions to promote endogenous function or to ablate cancer stem cells. Through continuing comparative analysis of stem cell movement in a variety of model systems and organs, we undoubtedly will uncover additional critical mechanisms governing the decisions of these dynamic cells to migrate or to home to distant sites in the body, and these insights will be translated into new tools for regenerative medicine and anticancer therapy.

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