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The Evolving Concept of a Stem Cell: Entity or Function?

Review

H.M. Blau,[1] T.R. Brazelton, and J.M. Weimann
Department of Molecular Pharmacology
Stanford University School of Medicine
Stanford, California 94305

Introduction

Stem cells have long been regarded as undifferentiated cells capable of proliferation, self-renewal, production of a large number of differentiated progeny, and regeneration of tissues. Generally, it has been thought that only embryonic stem cells (ES) are pluripotent, since during early development such plasticity is critical. Indeed, extensive data support this supposition and differentiation of ES cells into a range of cell types has been documented both in vitro and in vivo. By contrast, stem cells in the adult have traditionally been thought to be restricted in their differentiative and regenerative potential to the tissues in which they reside. Examples include liver cells that proliferate following partial hepatectomy, hematopoietic stem cells (HSC) that reconstitute the blood following lethal irradiation, satellite cells that repair damaged skeletal muscle, and keratinocyte precursors that participate in wound healing. In addition to repairing damage, stem cells play a key role in ongoing tissue homeostasis, for example in maintaining the blood and skin throughout life. Invariably the diagrams of the differentiation of adult stem cell progeny have been linear and irreversible, depicting an orderly progression along a well-defined path concluding in a terminally differentiated cell type.

However, this view of adult stem cell potential has been challenged of late. Bone marrow (BM)-derived cells have been shown not only to replenish the blood, but also to contribute to muscle, brain, liver, heart, and the vascular endothelium. Some reports document stem cell movement in the reverse direction, suggesting that muscle or CNS-derived cells can give rise to the blood. Stromal cells in the bone marrow, which are distinct from HSC, have also been shown to yield a multitude of cell types. Although many of these cell fate transitions have been observed following tissue injury, in some cases such transitions between distinct tissue compartments have been documented in the absence of overt tissue damage.

These recent findings suggest that stem cell biology may be more complex than originally anticipated. The discovery that stem cells in adults can first reside in one tissue and then contribute to another suggests a previously unrecognized degree of plasticity in stem cell function. Indeed, it now appears that cell fate changes are a natural property of stem cells and may be involved in ongoing physiological repair of tissue damage throughout life. Although the frequency in each case is still relatively low, the numerous recent unexpected findings suggest that the concept of stem cells is in a state of flux and that the commonly held view of a tissue-specific adult stem cell may need to be expanded. Accordingly, adult stem cells may not only act locally in the tissues in which they reside, but may also be recruited out of the circulation and enlisted in regeneration of diverse tissues at distal sites. Taken to an extreme, even highly specialized cell types in tissues may be capable of reversing their differentiated state and contributing to the stem cell pool, as recent studies with multinucleated muscle and differentiated CNS cells suggest. Thus, according to this novel view, at least some stem cells in adult tissues are highly plastic and amenable to change given the appropriate microenvironment. An attractive hypothesis, given the current state of knowledge, is that the concept of a stem cell is evolving. We propose that rather than referring to a discrete cellular entity, a stem cell most accurately refers to a biological function that can be induced in many distinct types of cells, even differentiated cells.

Evolving Stem Cell Concept

The results mentioned above suggest that an expansion of the traditional view of stem cells is needed. If cells from diverse organs can migrate systemically, enter other organs and assume morphologies and functions typical of their new environment, then these changes in cell fate may not always be linear. In other words, there may be multiple sources of stem cells and routes whereby an organism can generate specific types of mature differentiated cells. A schematic of this concept is shown in Figure 1. Some stem cells can transit through the circulation, which can be envisioned as a "stem cell highway," with access to all organs of the body. BM-derived stem cells enter different organs such as those which have been documented experimentally: heart, brain, skeletal muscle, or liver (Bittner et al., 1999; Brazelton et al., 2000; Gussoni et al., 1999; Jackson et al., 1999; Krause et al., 2001; Lagasse et al., 2000; Mezey et al., 2000). Homing signals, depicted on "billboards" near on-ramps, may result from local damage and influence the migration of stem cells to specific sites, in a manner reminiscent of white blood cell homing (Butcher, 1991). Growth factors, depicted on neighboring "billboards," induce stem cells to participate in the function of the organ they enter. Thus, the microenvironment, including contact with surrounding cells, the extracellular matrix (Hay, 1991), the local milieu (Studer et al., 2000) as well as growth and differentiation factors, is likely to play a key role in determining a stem cell's function. Within organs such as brain, liver, and muscle, it is well known that there is a resident pool of stem cells, long thought to be dedicated exclusively to the repair of the tissue in which they reside. The diagram indicates that stem cells can enter an organ via the circulation, where they can either contribute to the existing pool of stem cells within that organ, or directly generate differentiated cells. The emerging knowledge that there are cells capable of both movement between tissues and cell fate changes suggests that at least a subset of stem cells may alter their function in a manner that is more plastic and dynamic than previously thought.

[1] Correspondence: hblau@stanford.edu

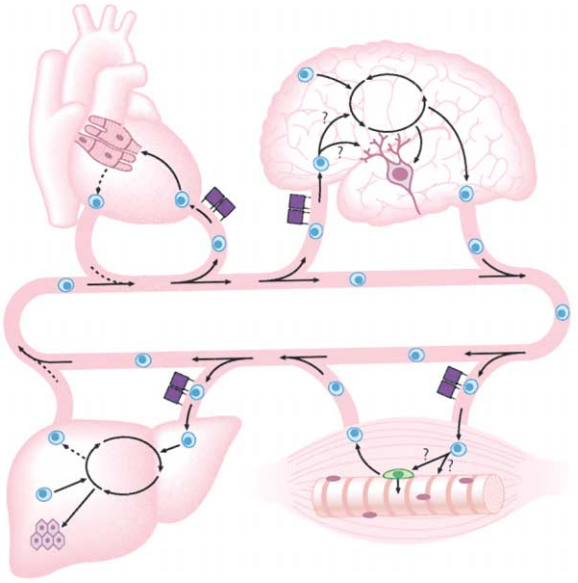

Figure 1. Evolving Concepts of Stem Cell Plasticity

Documented (solid arrows) and hypothesized (dashed arrows) transitions in stem cell identity and differentiation are illustrated. In addition to tissue-specific stem cells, some stem cells may travel throughout the body via the circulation. The scheme also suggests that cell fate decisions may not be irreversible. Flexibility is the hallmark of this depiction allowing for regeneration and changes in cell fate in response to need.

Topics Addressed in This Review

The evolving view of stem cells in adults raises a number of issues that are elaborated upon in this review. To provide a perspective for thinking about the recent stem cell findings, a historical account is given of how plasticity in adult differentiated cells was gradually recognized and its validity accepted. In addition, examples are reviewed of some of the different types of tissue-specific stem cells that are well documented to respond to local tissue damage and contribute to tissue regeneration in adults. New evidence is then described that stem cells in adults are capable of transit through the circulation and possess plasticity that allows them to alter their function according to their microenvironment. The need for specific markers is highlighted, as their paucity has stymied stem cell purification resulting in enrichment protocols that preclude an in-depth analysis of shared and distinct stem cell properties. Criteria are proposed for documenting a cell fate change. To increase the frequency of such changes, a better understanding of homing and growth signals is required. In this review the neuron is used as an example, since it represents a particularly remarkable and challenging cell fate transition. In the last section we discuss questions raised by the new body of stem cell research in adults. What is an adult stem cell? Why is there such a lack of stem cell markers? Is there a universal stem cell? Are adult stem cells not only tissue specific, but can they also transit between tissues via the circulation? Are stem cells in tissues a subset of cells that are specialized for stem cell function or can differentiated cells in these tissues also assume the function of a stem cell? If stem cells are a distinct population, does that imply that the stem cell state is actively maintained? If so, how are these cells prevented from differentiating? If differentiated, how might that specialized state be reversed? Finally, what is the physiological significance and what are the potential clinical consequences of the recently demonstrated plasticity of adult-derived stem cells?

Evidence for Plasticity of the Differentiated State in Adult Cells

That differentiated adult cells can change their fate has been known for decades. However, experimental manipulations, such as tissue damage, nuclear transplantation, or cell fusion are typically required to reveal this otherwise concealed potential for cellular plasticity. For example, transdifferentiation occurs in adult vertebrates when melanin-producing iris cells become crystallin-producing lens cells after lentectomy (Eguchi, 1988). In the case of transdifferentiation from pancreas to liver, this change can be induced by exogenous expression of a single transcription factor (Shen et al., 2000). Cloning experiments in amphibia first demonstrated that cells from differentiated adult tissues can yield nuclei that upon transplantation into enucleated eggs give rise to entire organisms (Gurdon, 1962). More than three decades later, cloning was achieved in mammals leading to "Dolly" the sheep (Schnieke et al., 1997). These studies provided the first evidence that in many types of cells DNA is generally not lost, even when only a subset of genes is expressed upon differentiation. Nonetheless, questions remained from such cloning experiments as to whether progression through the egg was required for the reawakening of genes that had been shut off.

Experimentally induced fusion of two different types of differentiated cells to yield nondividing stable heterokaryons showed that this was not the case: previously inactive genes in "terminally" differentiated cells were induced to express protein products through exposure to a novel cytoplasmic environment. When differentiated muscle cells were fused with mature cells isolated from all three embryonic lineages (hepatocytes, keratinocytes, and fibroblasts), muscle gene expression in the nonmuscle nuclei was induced within days (Blau et al., 1983, 1985; Wright, 1984). Moreover, this expression of previously silent genes occurred in the absence of changes in chromatin requiring cell division or DNA replication (Chiu and Blau, 1984). Such heterokaryon results proved to be generally true of diverse cell types other than muscle (Baron and Maniatis, 1986; Spear and Tilghman, 1990). Although the fusion of disparate cell types is experimentally forced in heterokaryons, a unifying principle has emerged. These experiments demonstrate that the differentiated state in adult mammalian cells is generally not fixed and irreversible, but instead is regulated by a dynamic active process that requires continuous regulation (Blau, 1992; Blau and Baltimore, 1991). At any given time, the differentiated state is dictated by the balance of regulators present in the cell.

The Pluripotent Embryonic Stem (ES) Cell

Pluripotency was long thought to be a property specific to embryonic cells. The existence of pluripotent stem cells in embryos became evident from studies of terato-

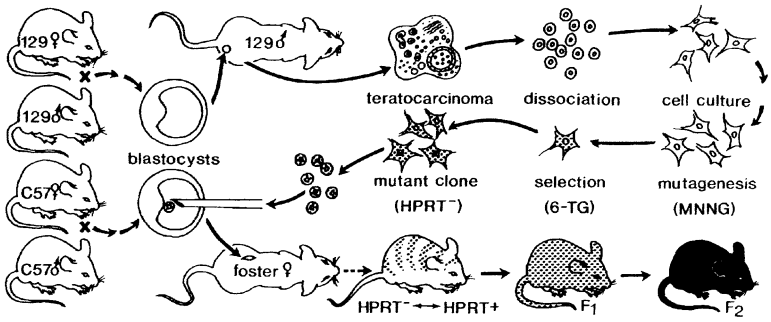

Figure 2. Evidence for Embryonic Stem Cell Pluripotency

The plan of the experiment, spanning almost a decade, is diagrammed, starting at the upper left. Grafted blastocysts form malignant teratocarcinomas in ectopic sites in the host mouse. After dissociation, teratocarcinoma cells microinjected into genetically marked wild-type blastocysts gave rise to offspring that were mosaics with chimeric tissues composed of cells derived from a mixture of both the teratocarcinoma and blastocyst-derived cells (Dewey et al., 1977).

carcinomas produced by ectopic injection of blastocysts into adult mice. In these cases, embryonic tissues exhibited a wide range of differentiated phenotypes including teeth and hair but in an aberrant disorganized manner. The remarkable pluripotency of the cells was shown in experiments by Mintz (Figure 2) (Dewey et al., 1977) in which the teratocarcinoma cells were isolated, genetically marked and implanted into the blastocyst of a foster mother. The resulting mice were normal, yet had chimeric mixtures of teratocarcinoma and wild-type cells in virtually every tissue of their bodies. That the ES cells could be isolated and cultured under conditions that either allowed extensive proliferation as undifferentiated cells, accumulation in floating embryoid bodies with features similar to early embryos, or differentiation as specialized cell types, led to a breakthrough in ES cell research (Evans and Kaufman, 1981; Martin, 1975). Adherent and nonadherent substrates, feeder layers of irradiated or mitomycin-treated cells, and specific growth factor cocktails influenced the cells to assume predominantly cardiac, skeletal, neuronal, or other highly differentiated fates.

Tissue-Specific Adult Stem Cells

That tissue-specific stem cells reside in certain adult tissues has been clearly documented, yet their specific properties often continue to elude us because of their paucity in parent tissues, heterogeneity, and technical difficulties in identifying them and tracing their progeny. Such adult tissue stem cells are responsible for regenerating damaged tissue and maintaining tissue homeostasis, for example physiological replenishment of skin and blood cells. In some cases such as hematopoiesis, the stem cells can be highly enriched and markers that distinguish these cells have been well characterized for that purpose. In other cases in which the extensive regenerative capacity of stem cells has been documented, such as in the liver, stem cell markers still remain to be identified. Below we briefly summarize pertinent data regarding a selected sample of these tissue-specific stem cells with the aim of highlighting areas that warrant further research and placing them in the context of the new findings on non-tissue-specific stem cells described in the next section.

Hematopoietic Stem Cells

The best characterized tissue-specific stem cells in adults are undoubtedly the pluripotent hematopoietic stem cells (HSC), which have the ability to reconstitute all cells of the blood (Lagasse et al., 2001; Morrison and Weissman, 1994; Weissman, 2000). Two classes of HSC have been identified: short-term (ST-HSC) and long-term (LT-HSC) that are capable of reconstituting the blood of mice for two and greater than six months, respectively (Jones et al., 1989; Lagasse et al., 2001). The majority of HSC enrichment protocols rely on fluorescence-activated cell sorting (FACS), which allows cells to be positively selected based on the expression of a set of cell surface proteins. Most protocols also use lineage depletion panels to exclude cells expressing proteins characteristic of a mature hematopoietic cell. This enrichment protocol permits the isolation of stem cell populations in which greater than 80% of the cells have the potential to reconstitute the blood (Lagasse et al., 2001). It has been suggested, based on statistical arguments, that individual stem cells can give rise to clones with full hematopoietic capacity (Lagasse et al., 2001; Weissman, 2000).

There are, of course, some caveats. The difference between negative and low expression of many of the marker proteins used in such isolation protocols can be subtle and as a result the cells obtained by lineage depletion are not always consistent among studies. Even the most rigorous isolation protocols currently available result in heterogeneous populations that are enriched for HSC but in which some of the cells fail to demonstrate pluripotency and/or long-term reconstituting ability (Morrison and Weissman, 1994). Moreover, since most of the protein markers used to identify HSC are not known to be essential to stem cell function, the expression of these proteins may not directly correlate with stem cell potential. For example, CD34 expression on LT-HSC has been found to be reversible and dependent not only on the activation state of the cells but also the developmental age of the donor (Matsuoka et al., 2001; Sato et al., 1999). Nonetheless, the hematopoietic stem cell clearly can be highly enriched up to 10,000-fold and delivered to marrow ablated recipients to fully reconstitute the blood (Morrison and Weissman, 1994).

Neural Stem Cells

The discovery that stem cells exist in the adult brain was quite unexpected and required years of investigation to become widely accepted. It was long thought that damage to the brain could not be repaired, as adult neurons could not be replaced. A series of studies in rats and in songbirds first revealed that neurons from adult brains could be formed anew (Altman, 1969; Goldman and Nottebohm, 1983). Additional studies by a number of investigators have now confirmed that mammalian adult neuronal progenitors exist and are capable

of extensive cell division and self renewal (Gage, 2000; Palmer et al., 1997; Reynolds and Weiss, 1992; van der Kooy and Weiss, 2000). Moreover, neural progenitors can migrate and home to specific sites of damage or regeneration, for instance to the olfactory bulb of rodents (Goldman and Luskin, 1998), the hippocampus of humans (Eriksson et al., 1998), and sites of tumors such as gliomas (Aboody et al., 2000).

Thus, stem cells from the CNS provide a second source of well-characterized tissue-specific stem cells. In tissue culture as well as following direct injection into brains, clones from the NSC population give rise to all three major cell types of the CNS: neurons, astrocytes, and oligodendrocytes (Gage, 2000; Qian et al., 2000; van der Kooy and Weiss, 2000). Following proteolytic dissociation of adult brain tissue, populations enriched for neural stem cells (NSC) can be obtained based on differential density in a sedimentation gradient (Palmer et al., 1999). These NSC progeny have typical morphologies, characteristic patterns of protein expression, and exhibit physiological evidence of function (Auerbach et al., 2000). Despite the extensive characterization of these cells, currently available cell surface markers allow for only a 45-fold enrichment of neural stem cells from embryonic brain (Uchida et al., 2000).

Hepatic Stem Cells

Despite the fact that the liver is an organ capable of extensive regeneration, the precise source of the tissue-specific stem cells responsible for this regeneration remains unclear. In contrast to bone marrow or skin in which a relatively small population of cells undergoes massive expansion to support regeneration, liver regeneration following partial hepatectomy, in which two-thirds of the liver of a rat is removed, involves a modest proliferation by a variety of differentiated liver cells including hepatocytes, biliary epithelial cells, and endothelial cells. However, following some types of injury which compromise hepatocytes, a smaller portion of stem-like cells near the bile ducts give rise to a proliferation of oval cells that subsequently generate hepatocytes and ductular cells (Kubota and Reid, 2000; Sell, 2001; Thorgeirsson et al., 1993). These data, taken together with morphological studies of liver regeneration following injury, have raised the possibility that the true liver stem cell with multilineage potential resides in or near the terminal bile ductules (reviewed in Theise et al., 1999; Vessey and de la Hall, 2001).

Skeletal Muscle Stem Cells

The satellite cell has been defined as a quiescent mononucleated cell ensheathed under the basal lamina that surrounds multinucleated muscle fibers (Mauro, 1961). Such cells are widely thought to constitute a reserve of stem cells for muscle regeneration. Numerous studies have shown that satellite cells can be activated, induced to proliferate and contribute to intact skeletal muscle fibers even after extensive tissue doublings (for reviews see Ozawa et al., 2000; Smythe et al., 2000). Moreover, these cells can be separated from single fibers, plated in culture and induced to divide and differentiate into myotubes (Bischoff, 1986). The Pax7 knockout mouse apparently lacks satellite cells, but whether it is capable of regenerating muscle after damage in adulthood remains to be determined (Seale et al., 2000). Evidence is also accumulating that satellite cells are not all alike but instead are heterogeneous in the genes they express; consequently the markers of such cells are not consistent (Beauchamp et al., 2000). Thus, it is clear that tissue-specific stem cells exist in skeletal muscle that can contribute to muscle growth and repair, exhibit reduced proliferative capacity with increasing age of the donor, and are rapidly depleted in chronic muscle degenerative diseases such as Duchenne muscular dystrophy. Markers for the prospective isolation of human and rodent proliferative myoblasts are well documented (Blanco-Bose et al., 2001; Webster et al., 1988) but whether they are present on satellite cells remains to be determined.

Stem Cells for Skin

Both the epidermis and hair follicle require stem cells to support high rates of epithelial turnover. Many consider the epidermis and hair follicle to be distinct tissue compartments, each with their own stem cell, while others argue that a single stem cell can give rise to both (Rochat et al., 1994). Severe epidermal injuries revealed that keratinocytes can migrate from the hair follicles to regenerate the epidermis (Taylor et al., 2000). Furthermore, several recent papers contend that keratinocyte stem cells that give rise to both epidermis and hair follicles reside in a specific region of the follicular epithelium, the bulge zone, where they cycle slowly, express keratin K5 and K14, and generate progeny to replenish the epidermal basal layer (Fuchs and Segre, 2000; Lavker and Sun, 2000; Taylor et al., 2000). Given the enormous proliferative potential of both epidermally and follicularly derived keratinocyte stem cells (Rochat et al., 1994), it is not surprising that it is difficult to determine the relationship of the origin of these cells.

The Search for a Universal Method for Isolating Tissue-Specific Stem Cells

Most stem cell enrichment protocols rely on the fluorescence-activated cell sorter (FACS) and use sets of antibodies to cell surface proteins. An alternative method for identifying both murine and human hematopoietic stem cells (HSC) was recently developed and is based on the efflux of the DNA binding dye, Hoechst 33342. This small cell population, termed the side population (SP), is identified by a characteristic pattern of fluorescence after staining with Hoechst 33342 detected in both far red ($>$675 nm) and blue (450 nm) emission channels. The SP has been isolated from both mouse and human bone marrow and contains LT-HSC (see HSC section above) expressing low or negative levels of CD34 (Goodell et al., 1997). In addition to enriching for HSC, the SP protocol can be used to identify stem cells in other tissues including skeletal muscle and epidermis (Dunnwald et al., 2001; Gussoni et al., 1999; Jackson et al., 1999). Although the SP protocol represents a significant advance, specific cell surface markers would be useful to identify stem cells definitively, compare them across tissues, and distinguish them from other cells, as currently only enrichment, rather than purification protocols exist for most tissues.

Plasticity: Adult Stem Cells that Are Not Tissue Specific

While the evidence for lineages of tissue-specific stem cells remains strong, recent studies indicate that a classification of stem cells solely by tissue compartment of

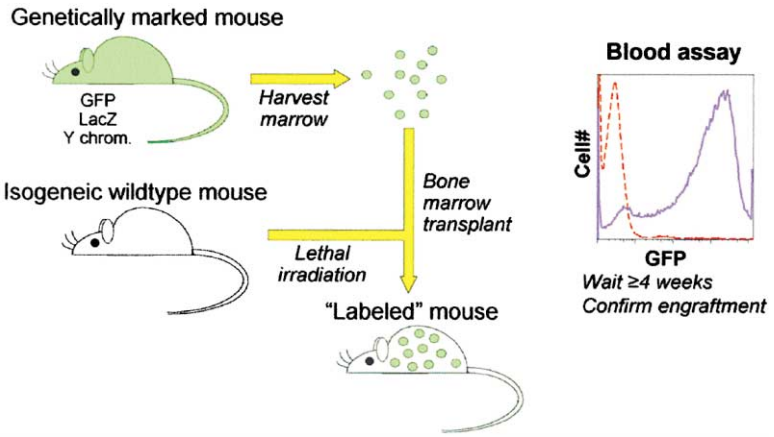

Figure 3. General Strategy for Identifying Cell Fate Transitions using Bone Marrow-Derived Cells

Bone marrow cells from a genetically marked adult mouse are delivered intravascularly into isogeneic, lethally irradiated, normal adult hosts. The bone marrow derives either from transgenic donor mice that constitutively express green fluorescent protein or β-galactosidase in all of their cells. Alternatively, cells from a male mouse can be used which, following transplantation into female mice, can be detected based on their Y-chromosome. Following irradiation at high doses, mice will die unless bone marrow is administered leading to reconstitution of all the lineages of the blood. The success of a bone marrow transplant can be ascertained by survival of the animal and the degree of chimerism in the blood, i.e., the proportion of the cells in the circulation of the recipient that express the genetic marker of the donor, determined by microscopy or FACS. Four to eight weeks are usually required to reconstitute the blood in adult mice (8–10 weeks of age) and detection in the tissue of interest requires another 2–4 weeks.

origin may greatly underestimate the potential of these cells. For example, murine and human NSC have been shown to give rise to skeletal muscle after injection into that tissue (Galli et al., 2000). The results documented below focus on recent evidence that cells derived from BM can give rise to cells typical of other tissues. These stem cells can be derived either from the hematopoietic (HSC) or stromal compartments of the bone marrow. There are also examples of tissue-specific stem cells reconstituting the blood after intravascular injection into irradiated mice (Bjornson et al., 1999; Gussoni et al., 1999; Jackson et al., 1999). Given that the blood has access to all tissues of the body, these findings may not be as surprising as they first appeared. Indeed, cell fate transitions may be an ongoing physiological property in adults, since many of the studies cited involved direct transplantation of adult cells that had not been exposed to tissue culture. The recently reported discoveries that demonstrate bone marrow giving rise to cell types typical of other tissues such as muscle, brain, heart, and liver have generally used a similar protocol (Figure 3). In all cases, the results suggest that BM-derived stem cells can undergo a multistep process entailing migration, conversion to a new phenotype, and expression of functions characteristic of the tissue in which they now reside.

Skeletal Muscle

The demonstration that BM-derived cells could home to muscle and give rise to differentiated myocytes was first shown using bone marrow cells from a transgenic donor mouse line in which lacZ was expressed under the control of the muscle-specific myosin light chain-3F promoter (Ferrari et al., 1998). β-gal was detected histochemically in muscle fibers following chemical damage. Subsequent reports showed that genetic damage characterized by chronic muscle degeneration also resulted in BM-derived cells that contributed to host muscle fibers (Figure 4A). Following transplantation of male marrow into irradiated female *mdx* mice, the mouse model of Duchenne muscular dystrophy, male muscle cells produced muscle-specific transcription factors, myf5 and myogenin, and the missing dystrophin protein as shown by fluorescence or laser scanning confocal microscopy (Bittner et al., 1999; Gussoni et al., 1999). Thus, the BM-derived cells were capable of migrating, converting to the myogenic lineage, and fusing with host myofibers in response either to chemically or genetically induced damage.

CNS

Two studies showed that intravascular delivery of BM-derived cells could give rise to cells with neuronal char-

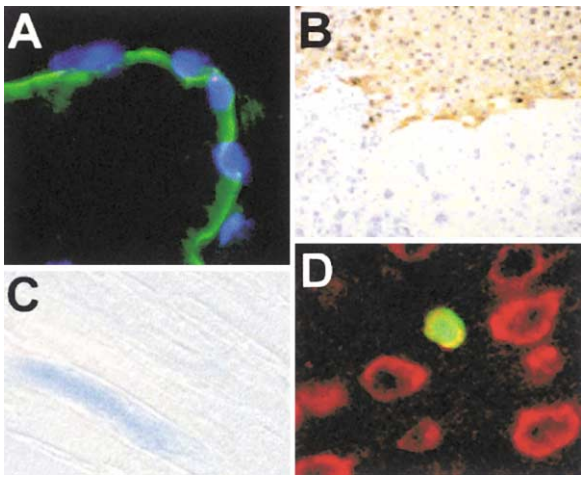

Figure 4. Derivation of Diverse Tissue-Specific Cell Types from Bone Marrow-Derived Stem Cells

(A) Dystrophin expression (green) and detection of Y-chromosome (red) in nuclei (blue) in the tibialis anterior muscle following bone marrow (BM) transplant from male into female *mdx* mice, a mouse model of Duchenne muscular dystrophy (Gussoni et al., 1999).

(B) Fumarylacetoacetate hydrolase (FAH) staining hepatocytes (donor cells, dark red) of a liver nodule 7 months after transplantation into an $FAH^{-/-}$ mouse (Lagasse et al., 2000).

(C) Bone marrow-derived β-gal expressing fibers (blue) derived from transplanted BM in the myocardium of a murine model of myocardial infarction (Jackson et al., 2001).

(D) Layer 2/3 neurons (red, NeuN), one of which is from the GFP^{+} marrow donor (green) in the cortex of mouse brain (Brazelton et al., 2000).

acteristics in the CNS of mice (Figure 4D) (Brazelton et al., 2000; Mezey et al., 2000). In one case, BM-derived cells expressing neuronal proteins were detected in the olfactory bulb of adult mice, a site of extensive regeneration, suggesting that homing to this site was physiological. These cells integrated into the tissue of the CNS and coexpressed GFP as well as one or more neural-specific markers characteristic of the brain (NeuN, class III β-tubulin, and 200 kDa neurofilament) as determined by laser scanning confocal microscopy using sub-micron thick optical sections. The second study used a genetic model, the PU.1 mouse that is unable to survive beyond birth without a bone marrow transplant. Male-derived BM introduced intravascularly into neonatal female mice rescued these animals and resulted in Y-chromosome labeled cells expressing NeuN and neuron-specific enolase in the hippocampus of the brain. The generation of BM-derived neuronal phenotypes in the adult brain in these two reports demonstrates remarkable plasticity either following minimal damage (irradiation) or no damage at all.

Cardiac Muscle

Recent reports suggest that conversion from BM to cardiac myocytes may also occur in the heart. GFP-labeled BM-derived cells were injected directly into the peri-infarct region of the left ventricle of mice resulting in replacement of dead myocardial tissue by cells that later expressed a number of cardiac muscle-specific proteins (Orlic et al., 2001). More recently, after intravascular injection into lethally irradiated rodents, the SP fraction of BM-derived cells reconstituted the blood, was incorporated into ischemically damaged myocardium, and expressed proteins typical of cardiomyoctyes such as α-actinin (Figure 4C) (Jackson et al., 2001). These results are particularly exciting in that no tissue-specific myocardial stem cells have been identified to date. The new results suggest that if the efficiency of BM-derived stem cell conversion increases, these cells may be able to contribute to repair following cardiac disease.

Liver

Perhaps the most robust and well-defined demonstration of bone marrow regeneration of nonhematopoietic tissue thus far are studies in which HSC not only regenerated large portions of liver but functioned in their new site by rescuing mice from death due to a genetic liver disease. After mice that had received a sex- or strain-mismatched BM transplant were treated both with drugs to block hepatocyte proliferation and induce hepatic injury, bone marrow-derived putative oval cells first appeared and subsequently matured into hepatocytes (Petersen et al., 1999; Theise et al., 2000a). Lagasse and coworkers, using a genetic model that resulted in sustained selective pressure for normal hepatocytes, transplanted wild-type HSC into lethally irradiated mice deficient in fumarylacetoacetate hydrolase ($FAH^{-/-}$), a model of fatal-hereditary tyrosinemia type I liver disease. The HSC-derived hepatocytes accounted for not only 30%–50% of the liver mass by 7 months post-transplant, but also restored multiple liver functions to near wild-type levels including expression of the missing liver hydrolase, leading to long-term survival (Figure 4B) (Lagasse et al., 2000). Although injury to the tissue probably played a role in the robustness of this response and generation of large numbers of marrow-derived hepatocytes, liver repopulation by marrow-derived cells can occur even in the absence of injury, albeit at a lower frequency (Theise et al., 2000a). In humans, marrow-derived cells also gave rise to substantial numbers of hepatocytes and ductular cells, both in livers from patients who received sex-mismatched bone marrow or liver transplants (Alison et al., 2000; Theise et al., 2000b). Given the lack of treatments for severe liver disease and the difficulties in growing these cells in culture, it is of particular interest that liver regeneration by adult marrow-derived cells can occur with minimal injury or even during normal physiological maintenance in both mice and humans.

Mesenchymal Stem Cells

Although it is beyond the scope of this review, which is primarily focused on evidence of circulating multipotent stem cells, it is of great interest that in addition to HSC, there is a noncirculating bone marrow-derived cell population with remarkable plasticity: the mesenchymal stem cells (MSC). MSC reside within the bone marrow cavity and can be isolated based on their adhesive properties. MSC have recently been shown, both in culture and following injection into particular tissues in mammals, to give rise to a range of cell types including chondrocytes, osteoblasts, adipocytes, and cardiac and skeletal muscle cells (Liechty et al., 2000; Pittenger et al., 1999), as well as cells typical of the CNS, such as neurons and astrocytes (Kopen et al., 1999; Sanchez-Ramos et al., 2000; Woodbury et al., 2000).

Criteria for Establishing the Occurrence of Cell Fate Changes

The surge of reports of diverse cell fate transitions has highlighted the need to standardize the criteria required to establish that a cell fate transition has occurred. Increasingly stringent criteria are proposed below. The first criterion is the demonstration that a previously silent gene specific for the new cell type becomes expressed in the cell of interest. Clearly, the expression of multiple proteins characteristic of a distinct cell type is more convincing. Documentation usually entails using one or more antibodies in conjunction with microscopy or the fluorescence activated cell sorter (FACS). Demonstrating these features in tissue culture is a first step, but more definitive is a demonstration of de novo gene expression in intact tissues in vivo.

To document clearly that proteins are expressed in the same cell in tissue sections and cell layers in culture necessitates the use of laser scanning confocal or deconvolution microscopic methods which allow analyses of optical sections of less than 1 μm. Since cell fate transitions tend to be rare events, it is essential that the occurrence of cells that overlay or wrap around one another not be mistaken as the same cell and taken as evidence for protein colocalization. Tracking of cells typically involves use of certain markers such as the Y-chromosome when male cells are introduced into female animals, green fluorescent protein (GFP), or β-galactosidase (β-gal). But even genetic markers require caution. For example, when ROSA mice are used as BM transplant donors, the ubiquitously but weakly expressed bacterial β-gal signal can be enhanced by pH changes which also increase the signal from endogenous mam-

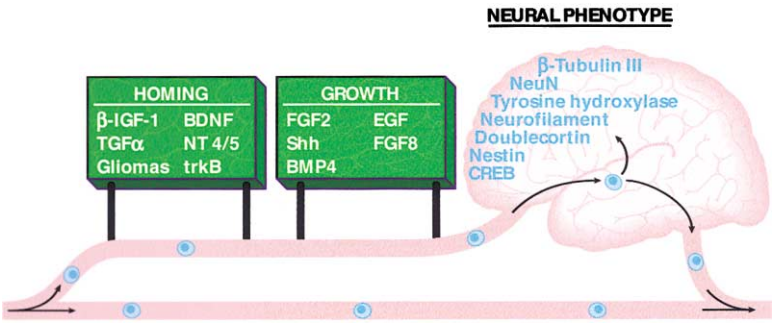

Figure 5. Enhancement of Tissue-Specific Cell Fate Transitions

Summaries of factors involved in neuronal development include homing and growth factors that lead to expression of characteristic neuronal genes. Examples derive from studies in intact brains or tissue culture experiments.

malian β-gal. Thus, it is preferable to use markers that have no endogenous counterparts, such as Y-chromosome probes and GFP.

A second criterion is that the cells are well integrated into the tissue structure and are morphologically indistinguishable from their host-derived neighbors. That a cell has arrived at its destination, was incorporated into the tissue structure, is morphologically indistinguishable from its neighbors, and expresses new proteins typical of those in its microenvironment provides quite strong evidence of cell type conversion.

The third and most stringent criterion for demonstrating a change in cell fate is a functional assay. An example is the production of a missing enzyme or other molecule specific to a particular organ in a genetically deficient animal that rescues it from lethality or ameliorates a deficit that is disease related. A demonstration of function in the heart would constitute showing that the cells derived from bone marrow not only express cardiac-specific proteins, but also contract in synchrony with the cardiac syncytia. For neurons of the CNS, the ability of a stem cell to participate actively in a neuronal circuit by generating action potentials in response to appropriate signals and producing synaptic potentials indicative of connectivity with other neurons would provide strong evidence that these cells can functionally repopulate the adult brain.

Regulation of Cell Fate Transitions: Migration and Growth Factors

In order to change its fate, a stem cell presumably responds to key migration factors and growth factors. Factors released by damage may induce stem cells to home to a particular tissue. Growth and differentiation factors within the tissue determine which genes will be activated. These factors in turn alter the pattern of genes expressed by a stem cell when it resides in a new tissue. The molecular players involved in migration and differentiation of stem cells are likely to differ depending on the tissue, degree of injury, and stem cells involved. The factors that induce stem cells to migrate may be tissue-specific or generic to injury.

Here we use the neuronal cell as the prototype, as it is perhaps the most challenging cell fate transition to achieve, and because the number of specific factors that are known to be involved in homing, growth, and differentiation is quite extensive. In accordance with the "stem cell highway" paradigm (Figure 1), we have designated some of the currently known factors as two billboards on the off-ramps to the brain (Figure 5). In the diagram of the brain, gene products characteristic of the differentiated neuron are listed. Most of the accumulated knowledge derives either from studies of patterns of gene expression during normal brain development or from studies of ES cells and adult neural stem cells.

Evidence for Neuronal Migration Factors

Strategies for inducing BM-derived cells to migrate to particular tissues of interest are critical to increasing the frequency of this cell fate transition. Several lines of evidence suggest that the NSC involved in adult neural regeneration can migrate to sites of damage in the adult brain, a feature that holds promise for future therapeutic applications. Both transplanted dissociated cortex from embryonic (E17) rat brain and endogenous adult NSC from the subventricular zone (SVZ) can migrate to lesioned areas in adult rat brains, presumably in response to local cues. Indeed, following the controlled apoptotic death of a subset of cortical neurons, transplanted E17 cortical cells migrated to the induced lesion and differentiated into multiple types of cortical neurons. Cells adjacent to the lesion (including interneurons) increased their expression of BDNF, NT-4/5, NT-3, and trkB receptors, implicating these factors and receptors as inducers of migration to damage (Wang et al., 1998). In other studies, both transplanted and endogenous NSC generated cells that migrated efficiently to the lesion, differentiated into neurons with axons that projected to appropriate targets and expressed appropriate neurotransmitters and receptors (Magavi et al., 2000; Shin et al., 2000). One factor involved in the proliferation and the migration of NSC within the brain was shown to be transforming growth factor α (TGFα). When infused into the forebrain of rats with a lesion to the *substantia nigra*, TGFα not only induced proliferation of endogenous NSC, but also diverted their migration toward the infusion site. Interestingly, these expanded and diverted neurons and glia were capable of improving a behavioral deficit in this model of Parkinson's disease (Fallon et al., 2000).

An additional example of tropism of NSC is provided by Snyder and colleagues who demonstrated that cells of a murine NSC line immortalized with v-myc homed to and infiltrated human gliomas implanted in adult rat brains (Aboody et al., 2000). Infiltrating NSC progeny were detected in the gliomas even when the NSC line was injected into the brain hemisphere opposite to the glioma or delivered into the circulatory system of the rat. These experiments suggest that factors are induced and released upon perturbation of the CNS that may be involved in the appropriate homing of newly generated

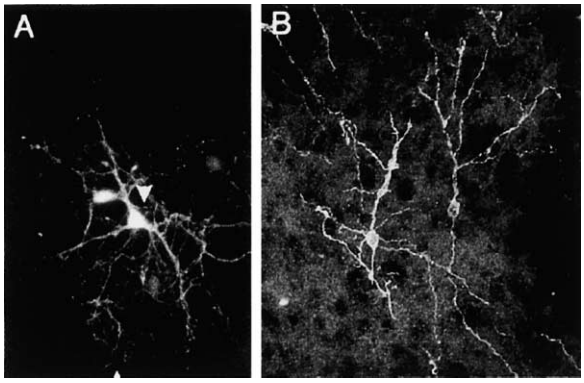

Figure 6. Neuronal Morphology of ES-Derived Cells in Tissue Culture and in Brain

(A) ES cell-derived neuron-like cells in vitro visualized with an anti-GABA antibody (Okabe et al., 1996).

(B) ES cell derived neurons injected into the brain of fetal rats show bipolar morphology and typical neuronal extensions (Brustle et al., 1997).

neurons in the adult. Although only a subset of the molecules responsible for NSC homing have been identified, it may be possible to achieve adequate homing of BM-derived cells using gene delivery methods to express known homing factors in conjunction with damage.

Strategies for Enhancing Differentiation of Stem Cells into Neurons

In order to ensure that BM-derived cells give rise to functional neurons in the CNS, a better understanding of the factors that enhance growth and differentiation of this cell type is required. One strategy for identifying such factors is based on the temporal replication of the combination of factors found in developmental or damage-induced pathways. Despite the attraction of such a rational strategy, random screening of factors is yielding unexpected but highly useful candidate molecules with increasing frequency. Here we describe examples of both.

During mammalian brain development, studies of gene expression have led to the identification of factors that preferentially induce neuronal or glial differentiation in tissue culture including basic fibroblast growth factor (FGF2), epidermal growth factor (EGF), Sonic hedgehog (Shh), fibroblast growth factor-8 (FGF8), and bone morphogenetic protein-4 (BMP4) (Lillien and Raphael, 2000; Powell et al., 1991; Ye et al., 1998). For example, the temporal interplay between FGF2, BMP4 and EGF receptors can regulate the differentiation of neurons and glia (Anderson, 2001; Lillien and Raphael, 2000). Indeed, studies in tissue culture have used these factors to expand and differentiate NCS (Reynolds and Weiss, 1992; Vescovi et al., 1993) or to transform ES cells into neurons.

Studies of ES cells in culture have also contributed to our knowledge of differentiation factors. ES cells have been shown to express a multiplicity of neuron-specific proteins and develop axons that generate action potentials and receive synaptic potentials typical of functioning neurons, providing evidence of connectivity and communication among neurons developed from non-CNS tissue in culture (Figure 6A) (Lee et al., 2000; Okabe et al., 1996). Arborized axonal and dendritic morphologies have been observed not only in tissue culture but also upon injection of ES cells into the rodent brain (Figure 6B) (Brustle et al., 1997). Indeed, ES cells can give rise to CNS-like cells with an efficiency as high as 70% under optimal conditions (Hynes and Rosenthal, 2000). Unfortunately, to date the outcome is rarely a pure population of a given cell type, irrespective of the clonality of the ES stem cell.

Of particular interest are protocols that favor the generation of dopaminergic neurons that are lost in Parkinson's disease. The ability to differentiate ES cells into specific types of neurons has also benefited from knowledge of the factors present during CNS development. For example, the interaction of FGF4, FGF8, and Shh creates inductive centers for serotonergic and dopaminergic neurons at the mid/hindbrain border and in the rostral forebrain (Ye et al., 1998). Subsequently, studies of ES cells in tissue culture demonstrated that the number of dopaminergic neurons could be increased when Shh, FGF8, and ascorbic acid were added to the culture medium (Lee et al., 2000). On the other hand, an alternate approach which was not based on in vivo findings led to the discovery that stromal cell-derived inducing activity (SDIA) also caused 30% of the neurons produced by ES cells to differentiate as dopaminergic cells (Kawasaki et al., 2000). Similarly, by using available antibodies, an adhesion molecule critical to neuronal migration was identified (Heffron and Golden, 2000) suggesting that differentiation factors can also be identified by random screening without preconceived notions. Thus, diverse factors, likely acting by different mechanisms, can enhance dopaminergic and other neuronal differentiation. Analyses of the effect of homing and growth factors, whether with ES cells, NSC, or BM-derived cells should increase the efficiency of stem cell fate transitions, which is of both fundamental and clinical interest.

Physiological and Clinical Implications

Regeneration of tissue, either with endogenous or genetically modified cells, clearly holds promise as a novel class of therapies with the ability to treat a multitude of serious diseases and injuries. Although to date the cell fate conversions described here in adults are rare occurrences, this is likely to change as experimental methods and approaches improve. Now that the phenomenon is known to exist, much research will be devoted to enhancing it and exploiting its potential therapeutically. The ability of stem cells from multiple sources to regenerate diverse tissues greatly increases the flexibility and applicability of tissue regeneration strategies. Compared to neural stem cells from the CNS or satellite cells from the muscle, bone marrow is both a safe and accessible source of stem cells that is routinely harvested for clinical use. In addition, BM-derived cells avoid the immunological problems associated with allografts, as the donor is the recipient.

What Is a Stem Cell?

Is There a Universal Stem Cell?

Since cells from adult tissues can cross tissue boundaries, it is tempting to speculate that a universal type of stem cell exists in adults. As detailed above, there

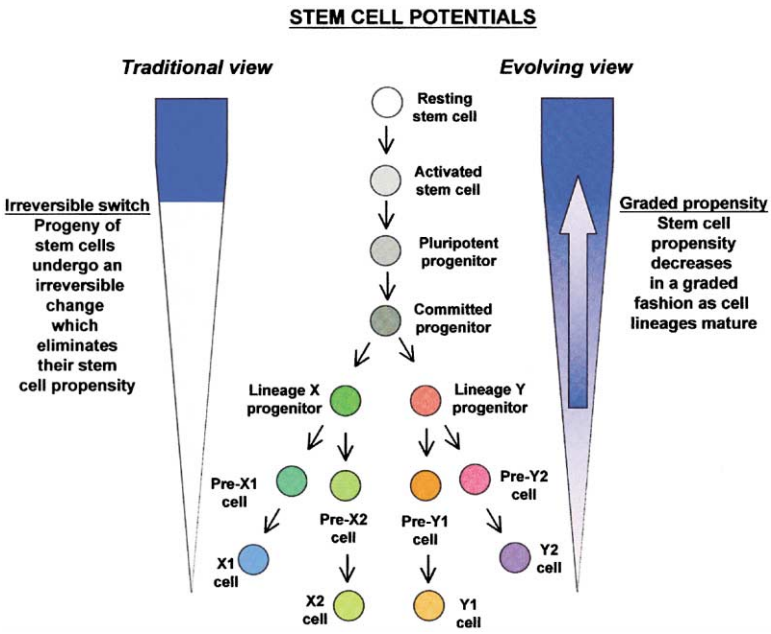

Figure 7. Comparison of Two Conceptual Views of Stem Cells

Two schemes of a cell's propensity to function as a stem cell as it progresses down differentiation pathways are shown. Stem cell potential is indicated by the intensity of color in the lateral triangular diagrams. Traditionally stem cells have been viewed as reaching a point of no return, an irreversible switch (left). The evolving view is that cells have a recruitable but decreasing propensity to act as stem cells as they differentiate.

are now reports of bone marrow giving rise to multiple tissues, multiple tissues giving rise to bone marrow, and neural stem cells contributing to skeletal muscle. Perhaps the most dramatic example of the plasticity of adult-derived cells is the demonstration that adult neural stem cells, when implanted into the amniotic cavity of stage 4 chick embryos, give rise to cells in all three germ layers (Clarke et al., 2000). At least one marker has been identified, Hoechst 33342 dye exclusion, which is a property of SP cells that has proven useful for selecting populations of stem cells including hematopoietic, skeletal muscle, and epidermal stem cells. This finding suggests the existence of shared traits among stem cells from disparate sources. However, the criterion of increased dye extrusion is not particularly definitive. Thus, the question of whether or not there is a universal stem cell remains unanswered. The general lack of specific molecular markers for stem cells may be due either to the fact that better markers have yet to be discovered or that they simply do not exist.

Heterogeneity: Support for Function Rather than an Entity

If a stem cell is a single entity, it should be possible to isolate it prospectively. Even among tissue-specific stem cells, there is heterogeneity. For example, two distinct cell types in the CNS have been reported to give rise to neurons which may reflect the existence of more than one distinct type of stem cell (Alvarez-Buylla et al., 2001; Doetsch et al., 1999; Johansson et al., 1999). Further support for this hypothesis derives from the inefficiency of purifying stem cells from the nervous system. This may be due to an absence of available markers, or alternatively, reflect the fact that a single NSC does not exist in the peripheral or central nervous systems and that heterogeneity is inherent to the stem cell pool (Morrison et al., 1999; Uchida et al., 2000). Recent evidence in skeletal muscle suggests that satellite cells, the mononucleate stem cells characteristic of that tissue, are also heterogeneous with respect to the proteins they express (Beauchamp et al., 2000). Indeed, the accumulating evidence raises the possibility that many types of cells from distinct tissues displaying different degrees of differentiation could potentially be recruited to function as stem cells. Thus, the ability to act as a stem cell may be a cellular function shared by numerous cell types expressing diverse genes. Just as most cell types in the body are able to engage in an apoptotic program in response to specific types of damage, many diverse cells in the body may be able to act as stem cells. Thus, recent evidence suggests that an expansion of the traditional view of a stem cell is warranted, i.e., that a stem cell is not necessarily a specific cellular entity, but rather a function that can be assumed by numerous diverse cell types

Maintenance of a Stem Cell State

What maintains a stem cell in a stem cell state? Perhaps like the differentiated state, the stem cell state requires active maintenance and is dependent on the composition of proteins and the balance of those proteins present in that cell at any given time (Blau and Baltimore, 1991). Given its plasticity, the stem cell state is dynamic since it can respond to a variety of signals that dictate distinct differentiated paths. For example, in the CNS, NSC that exist in diverse compartments of the brain have the potential to generate neuronal progeny, but only do so in specific locations (Palmer et al., 1999). Accordingly, stem cells may be protected or isolated from cues such as differentiation signals at most times. This precept raises the possibility that adult stem cells are maintained in a nonresponsive state by a constellation of as yet unidentified molecules until needed, for example for tissue maintenance (skin and blood) or in the repair of damage.

Could Stem Cells Exist in a Differentiated State?

It is possible that a compartment of dedicated stem cells does not exist in perpetuity, as cells capable of becoming stem cells may perform other functions in the interim. A striking example of cells that appear to be

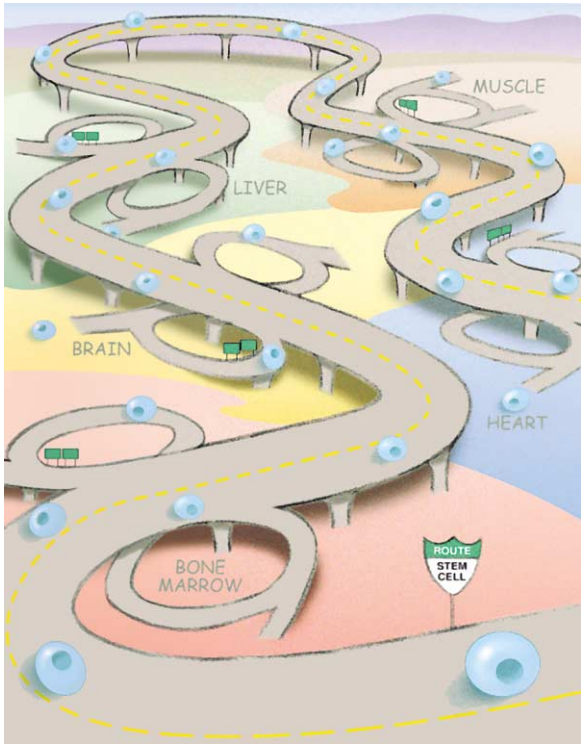

Figure 8. Route Stem Cell

The stem cell landscape depicted here illustrates the emerging characteristics of adult stem cells that include plasticity in cell fate, diversity of origin, and a multiplicity of tissue potentials. Stem cells (blue) are able to enter diverse tissue compartments from the blood stream (the stem cell highway) via "on ramps" and generate appropriate cell types in response to homing signals or growth factors depicted on "billboards." In theory, all choices are reversible. (Artwork by N. Gewertz and B. Colyear.)

well along a differentiation pathway, yet can revert to a more plastic state are oligodendrocyte precursor cells (OPC). OPCs routinely give rise to one of the three major classes of cells in the brain. Under certain culture conditions, committed OPCs can be induced to become multipotent stem cells which can differentiate once again into all three major cell types (Kondo and Raff, 2000). A second case in point is the potential for differentiated multinucleated myotubes to give rise to mononucleated proliferative myoblasts, a property first discovered in newts (Brockes, 1997). More recently, it has been shown that mouse myotubes can similarly be disrupted upon overexpression of a homeobox gene, *msx-1*, and the mononucleated "myoblasts" to which they give rise can then differentiate into adipocytes or chondrocytes in appropriate media (Odelberg et al., 2000). Such examples, which demonstrate the substantial plasticity of "committed" or "differentiated" cells and how they can be induced to give rise to cells with more stem-cell like properties, prompted the schematic described below.

Figure 7 compares the traditional and evolving conceptual views of stem cells. As shown, the traditional concept of a stem cell is of a discrete type of cell that belongs to a relatively homogeneous population of similar stem cells, that is identifiable by physical parameters, and that undergoes an irreversible loss of stem cell ability as it matures (Figure 7, left). In contrast, the evolving view proposed here, namely that stem cells reflect a functional program that can be engaged by many types of diverse cells, is shown in Figure 7 (right). We postulate that as depicted by the gradient, it is not necessary that the potential for stem cell function be equal among all cells. According to Figure 7, the propensity of a cell to initiate stem cell functions is likely to decrease as cells mature. Routine physiological processes would be expected to enlist cells with higher stem cell propensities. This may explain the ease with which bone marrow-derived cells are able to reconstitute cells not only of the blood, but also other organs. However, in the context of damage or physiological perturbation, atypical levels of environmental signals may allow cells with lower stem cell propensities to regenerate tissues. Our knowledge of stem cells and the concepts depicted here is likely to increase exponentially in coming years. As depicted in the stem cell landscape, the new data suggest the concept that an adult stem cell is subject to change and most accurately reflects a regulatable function, rather than a discrete cellular entity (Figure 8). Future research will determine the extent to which this postulate holds true.

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