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Stem Cells: Units of Development, Units of Regeneration, and Units in Evolution

Review

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Introduction

Stem cells are not only units of biological organization, responsible for the development and the regeneration of tissue and organ systems, but also are units in evolution by natural selection. Stem cells are generally defined as clonogenic cells capable of both self-renewal and multilineage differentiation (Till and McCulloch, 1961; Metcalf and Moore, 1971). Stem cells can be divided into a long-term subset, capable of indefinite self-renewal, as well as a short-term subset that self-renews for a defined interval. Stem cells give rise to non–self renewing oligolineage progenitors, which in turn give rise to progeny that are more restricted in their differentiating potential, and finally to functionally mature cells. The earliest stem cells in ontogeny are totipotent, extending from the zygote to the inner cell mass of the blastocyst; soon thereafter, totipotent stem cells give rise to somatic stem/progenitor cells and primitive germline stem cells. Very little is known of the stages somatic stem cells take between the blastocyst stage and the emergence of tissue and organ-specific stem cells at about the neurula stage. At this stage, the best studied stem cells—those that will form blood—emerge. This review begins with a detailed examination of hematopoietic (blood-forming) stem cells.

Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are probably the best-characterized stem cell population. Beginning with the groundbreaking experiments of Till, McCulloch, Wu, Becker, and Siminovitch, a population of clonogenic bone marrow (BM) cells was found to generate myeloerythroid colonies in the spleens of lethally irradiated hosts (Till and McCulloch, 1961; Becker et al., 1963; Wu et al., 1968). These clonogenic cells in some cases gave rise to cells that also could be transferred to secondary hosts and there reconstitute all blood cell lineages (Siminovitch et al., 1963). These cells could be enriched by physical or cell surface characteristics (Visser et al., 1984; Muller-Sieburg et al., 1986). With the development of clonal assays for all major hematolymphoid cell lineages (Ezine et al., 1987; Whitlock et al., 1987), cell sorter–based separation of monoclonal antibody or dye-stained bone marrow (BM) subsets led to the isolation of candidate stem cell populations in the mouse (Spangrude et al., 1988; Goodell et al., 1996; Osawa et al., 1996). In vivo limiting dilution analysis of these cells (Smith et al., 1991; Uchida, 1992; Osawa et al., 1996) allowed the isolation of at least two classes of multipotent cells—long-term (LT-HSCs) and short-term reconstitutive cells (ST-HSCs) (Morrison and Weissman, 1994). The long-term subset self-renews for the life of the host, while the short-term subset retained self-renewal capacity for approximately 8 weeks (Morrison and Weissman, 1994). The lineage of multipotent cells is LT-HSCs→ST-HSCs→multipotent progenitors (MPPs) (Morrison et al., 1997a) (Figure 1A). Each stage of differentiation of multipotent cells involves functionally irreversible maturation steps. Included in the progeny of mouse HSCs are two kinds of oligolineage-restricted cells: common lymphocyte progenitors (CLPs), which at a clonal level are restricted to give rise to T lymphocytes, B lymphocytes, and natural killer (NK) cells (Kondo et al., 1997), and CMPs, which are progenitors for the myeloerythroid lineages (Akashi et al., 1999). CMPs give rise to myelomonocytic progenitors (GMPs) and megakaryotic/erythroid progenitors (MEPs) (Akashi et al., 1999). All of these populations (LT-HSCs, ST-HSCs, MPPs, CLPs, CMPs, GMPs, and MEPs) are separable as pure populations using cell surface markers (Figure 1B) (Akashi et al., 1999).

In normal circumstances, no single step of dedifferentiation or transdifferentiation occurs between hematolymphoid progenitors (Figure 1B). The transcription profiles of each of these populations, as prospectively isolated, are quite distinct (Akashi et al., 1999). For dedifferentiation or transdifferentiation to occur, a small number of master regulators would be required.

Ontogeny of HSCs

In the mouse, hematopoiesis occurs by 8 days postconception (dpc 8) in the yolk sac blood islands (Moore and Metcalf, 1970), and the yolk sac vasculature connects via the umbilical vein to the fetal liver between dpc 8.5 and dpc 9.5. Hematopoietic progenitors can be found at dpc 8–8.5 in the yolk sac (Weissman et al., 1978) and in the embryo proper (Cumano et al., 1995; Medvinsky and Dzierzak, 1996). Yolk sac HSCs provide local hematopoiesis during development and participate in lifelong hematopoiesis in the bone marrow (BM), presumably by a natural migration of HSCs from one hematopoietic microenvironment to the other (Weissman et al., 1978; Morrison et al., 1995). At least two successive mobilizations of HSCs occur—from the embryonic loci (Weissman et al., 1978; Cumano et al., 1995; Medvinsky and Dzierzak, 1996) to fetal liver, and from fetal liver to spleen and BM (Morrison et al., 1995). These movements are genetically controlled in part via changes in expression of cell surface adhesion molecules (Hirsch et al., 1996).

In the zebrafish (Detrich et al., 1995) and frog (Turpen et al., 1997), both the ventral and the dorsal mesoderm contribute to the initiation and perpetuation of hematopoiesis. The origin of hematopoiesis and vasculogenesis has been proposed to occur through another stem cell—the hemangioblast. The isolation of definitive hemangioblasts has not yet been reported, although a number of candidates exist (Sabin, 1920; Choi et al., 1998).

Regulation of HSC Numbers

In young mice, the frequency of HSCs in hematopoietic tissues is relatively constant (Harrison, 1980; Harrison et

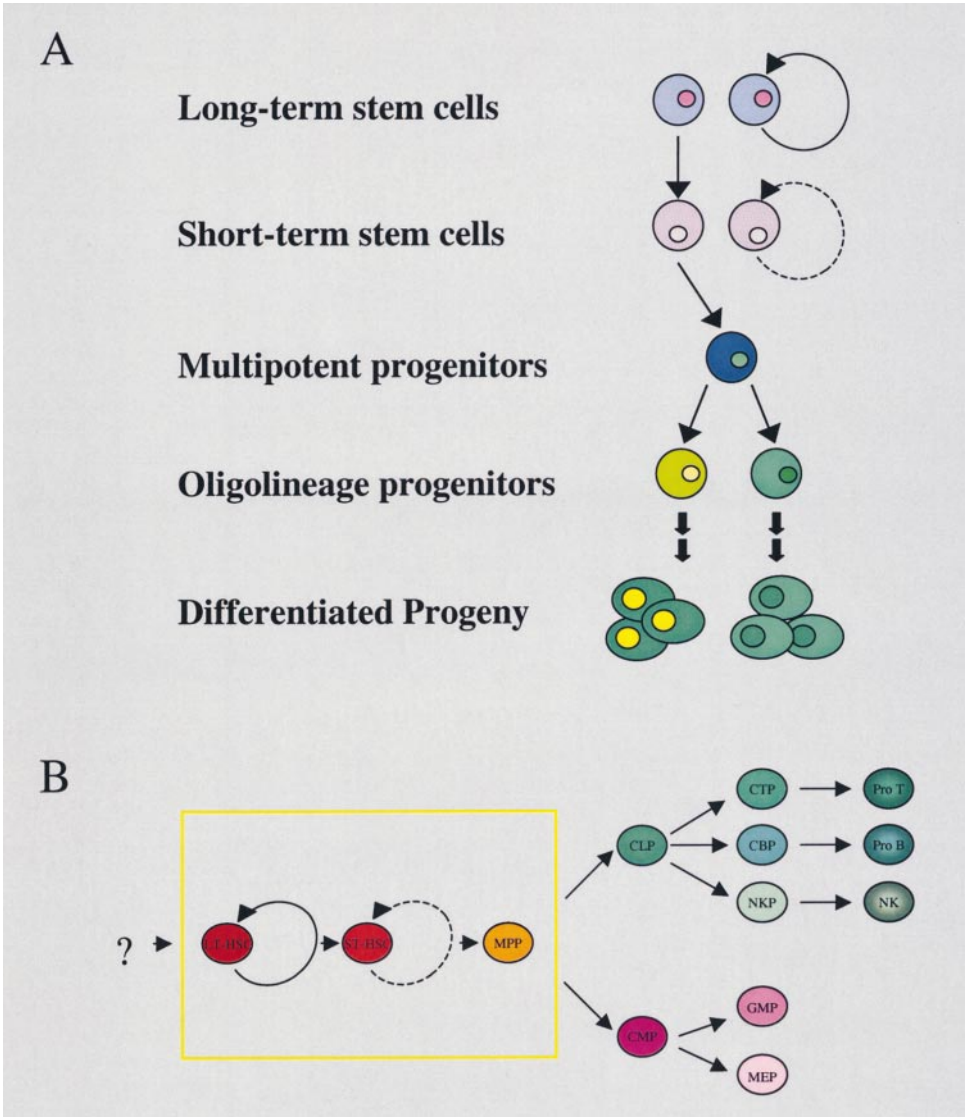

Figure 1. Hematopoietic Stem and Progenitor Cell Lineages

(A) Model of HSC self-renewal and differentiation. HSCs can either be LT-HSCs (Thy-1.1^{lo}Lin^{-}Sca1^{+}c-Kit^{+}), highly self-renewing cells that reconstitute an animal for its entire lifespan, or ST-HSCs (Thy-1^{lo}Lin^{-}Mac1^{lo}Sca1^{+}c-Kit^{+}), which reconstitute the animal for a limited period. ST-HSCs differentiate into multipotent progenitors, which have the ability to differentiate into oligolineage progenitors and ultimately give rise to differentiated progeny. ST-HSCs cannot give rise to LT-HSCs, but can self-renew for a limited time or give rise to MPPs (Thy-1.1^{lo}Lin^{-}Sca1^{+}c-Kit^{+}Mac1^{lo}CD4^{lo}). Each stage of differentiation involves functionally irreversible maturation steps.

(B) Model of HSC differentiation. Included in the progeny of mouse HSCs are two kinds of oligolineage-restricted cells: common lymphocyte progenitors (CLPs), which can be isolated with the phenotype Lin^{-}IL-7R^{+}Thy1.1^{-}Sca-1^{lo}c-Kit^{lo}, and which at the clonal level are restricted to give rise to T lymphocytes, B lymphocytes, and natural killer (NK) cells; and CMPs, which are progenitors for the myeloerythroid lineages. CMPs give rise to cells that include myelomonocytic progenitors (GMPs) and megakaryotic/erythroid progenitors (MEPs). All of these populations are separable as pure populations using cell surface markers.

al., 1988; Morrison et al., 1996b, and references therein). Early models (e.g., the clonal succession model: Kay, 1965) proposed that HSCs remain out of the cell cycle for much of the life of the animal. In that model, one or a few HSCs at a time are recruited into hematopoiesis, and when they reach the end of their productive lifespan, one or a few replace them (Kay, 1965; Lemischka et al., 1986). This would require extreme regulation and almost magically sensitive feedback mechanisms to limit the entry to hematopoiesis to only one or a few HSCs at a time. Contrary to this hypothesis, subsequent experiments showed that in young adult mice about 8%–10% of LT-HSCs randomly enter the cell cycle per day, with all HSCs entering the cell cycle in 1–3 months (Bradford et al., 1997; Cheshier et al., 1999).

Given that LT-HSC cycle many times in the life of an animal, these cells need either to be continually replenished from non-HSC precursors, or to self-renew. An apparent requirement for continuously dividing and self-renewing cells, such as HSCs and tumor cells, is the

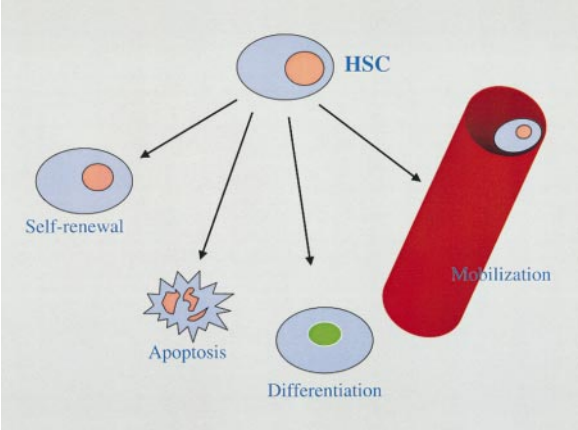

Figure 2. Model of the Alternative Fates of HSCs

The progeny of HSC cell division can self-renew, differentiate (thereby becoming a more committed progenitor), undergo apoptosis (PCD), or undergo mobilization. Under certain conditions, HSCs migrate and seed other organs. The number of HSCs is in part regulated by PCD. Adapted from Domen and Weissman (1999).

ability to avoid fatal telomere shortening through the action of the telomerase complex (Vaziri et al., 1994; Allsopp et al., 1995). In mice, LT-HSCs contain as much telomerase activity per cell as do cancer cells, while ST-HSCs/MPPs have significantly less (Morrison et al., 1996a). Whether decreased telomerase activity results in reduced telomere shortening in mouse LT-HSCs that have divided many times has not yet been demonstrated. However, enriched human stem/progenitor cell populations do show telomere shortening with age (Vaziri et al., 1994). We have proposed that high telomerase activity is a property of any cell that self-renews extensively and that any tissue-specific stem cell will be marked by high telomerase activity (Morrison et al., 1996a).

The number (or frequency) of many cell types is regulated, in part, by programmed cell death (PCD). HSCs are no exception (Figure 2 and Domen et al., 2000). Transgenic overexpression of the anti-PCD protein Bcl-2 in LT-HSCs leads to a gradual increase of LT-HSC frequency; the effect is intrinsic, occurs in the absence of LT-HSC malignant transformation, and takes place despite Bcl-2-mediated decrease of LT-HSC entry into the cell cycle (Domen et al., 2000). Ectopic expression of Bcl-2 acts to replace normal survival signals imparted to resting and proliferating LT-HSCs and allows analysis of the signals that induce cell proliferation versus those that promote cell survival.

In vivo, LT-HSCs can respond to a variety of conditions by entering the cell cycle, expanding their numbers, and mobilizing into the bloodstream (Morrison et al., 1997b). These include acute myelosuppression (Richman et al., 1976) and treatment with cytokines (Molineux et al., 1990). Following entry into the cell cycle and HSC expansion, LT-HSCs in tissues and in cycle return towards the resting "set points." During expansion, symmetric divisions of LT-HSCs must occur. However, in the steady state, it is not clear whether symmetric or asymmetric outcomes of LT-HSC divisions are the rule.

Different mouse strains maintain different LT-HSC cell cycle and numerical frequencies. Several chromosomal regions have thus far been shown to contribute to these set point polymorphisms (Muller-Sieburg and Riblet, 1996; De Haan and Van Zant, 1997). To date, none of these regulatory genes have been identified or isolated.

The regulation of stem cell frequency is a general problem in metazoan organisms. Self-renewal is the default pathway in virtually all single-cell organisms, and regulation of self-renewal versus differentiation or death is a requirement of metazoans that have tissue specialization. Expansion by self-renewal of HSCs is a hard-to-achieve goal. Perhaps that goal will be achievable not by asking which genes specify self-renewal, but by assuming that self-renewal is the default pathway. In that view, self-renewal can only occur to the extent that death and differentiation are prevented.

The Use of Histocompatible and Allogeneic HSCs in the Regeneration of the Hematolymphoid System

Understanding the biology of HSCs has led to a number of medical advances in cancer therapy, transplantation, and autoimmunity. The regeneration of the hematolymphoid system following an otherwise lethal dose of whole-body irradiation or chemotherapy became the basis for the use of bone marrow transplantation (Thomas, 1991). In the mouse, HSCs are the principal elements that are responsible for the early and sustained engraftment of myeloerythroid cells and platelets (Uchida and Weissman, 1992; Uchida et al., 1996, 1998). Isolation of human HSCs by phenotype ($CD34^+$ $Thy1^+$ Lin^-) (Baum et al., 1992) led to clinical trials wherein positive selection of $CD34^+$ Thy 1^+ cells allowed removal of all detectable tumor cells (Gazitt et al., 1995) with rapid and sustained recovery of neutrophils and platelets (Archimbaud et al., 1997; submitted).

In mouse and man, allogeneic bone marrow transplantation (BMT) leads to a graft versus host (GvH) disease caused by contaminating T lymphocytes (van Bekkum et al., 1962). In contrast, transplantation of purified allogeneic HSCs leads to dose-dependent radioprotection and donor blood engraftment without the appearance of GvH (Shizuru et al., 1996; Uchida et al., 1998). These hosts are immunologically tolerant of cells and tissues from the HSC strain donor (Gandy and Weissman, 1998; Shizuru and Weissman, 1999). Transfer of HSCs (Shizuru et al., 1996) from normal mice into allogeneic mice bearing mutations that render them highly prone to an autoimmune, T cell–mediated type 1 diabetes not only induces tolerance to donor tissues, but also blocks the progression of the autoimmune disease. Thus, HSCs are not only units of hematopoietic generation and regeneration: in a practical sense, they are also units of transplantation. The fact that allogeneic HSCs can induce tolerance of other tissue-specific stem cells from the same donor extends the practical use of these units of transplantation to the functional regeneration of the tissues and organs they generate.

Stem Cells in Other Mammalian Somatic Tissues and Organs

Nervous System Stem Cells

Stem cells are responsible for the generation of other somatic systems such as the central nervous system

(reviewed in Gage, 1998) and the neural crest–derived peripheral nervous system (Stemple and Anderson, 1992). The first direct evidence of nervous system stem cells came from the identification and isolation of rat neural crest stem cells, clonogenic precursors that give rise to all known neural crest cell types, while self-renewing the neural crest progenitors (Stemple and Anderson, 1992; Morrison et al., 1999).

The search for stem cells in the adult central nervous system was significantly delayed due to the conventional wisdom that neurogenesis is complete by puberty. This proved wrong (Altman and Das, 1966). Dividing cells in the adult mouse subventricular zone (SVZ) (of the lateral ventricles) continuously self-renew and give rise to progeny that migrate rostrally to the olfactory cortex, where they differentiate into astrocytes, oligodendrocytes, and neurons; this pathway is called the rostral migratory stream (rms) (Lois and Alvarez-Buylla, 1994). The development of methods to culture rodent neural progenitors (from either fetal ventral mesencephalon or adult sites of continuing neurogenesis) resulted in their growth as nonadherent neurospheres (Reynolds and Weiss, 1992) or adherent multilineage cultures (Ray et al., 1993). Retrovirally marked clonogenic neural precursors can self-renew, as well as produce neurons, oligodendrocytes, and astrocytes (Palmer et al., 1997). These multipotent CNS stem cells are not restricted to a particular neural fate; transplantation to the hippocampus gave progeny that included hippocampal neurons, astrocytes, and oligodendrocytes (Suhonen et al., 1996). If transferred to the SVZ, the progeny entered the rostral migratory pathway to give rise to olfactory-type neurons, astrocytes, and oligodendrocytes (Suhonen et al., 1996). If these cells were transferred into the developing rat retina, they could participate in retinal cell formation, including Muller, amacrine, bipolar, horizontal, photoreceptor, and astroglial cells (Takahashi et al., 1998).

Cell division frequency in the dentate gyrus area can be altered by more or less enriching environmental stimuli, indicating that this system might have a functional as well as a structural significance (Kempermann et al., 1997). Mouse fetal CNS cells immortalized with the viral *myc* oncogene also could be transplanted in fetal mouse brains, wherein their progeny included neurons and glia that can participate in regeneration in myelin-deficient shiverer mice (Yandava et al., 1999). Taken together, these experiments indicate that the stem cell→oligolineage progenitor→mature cell pattern of tissue and organ generation and regeneration is a property of the nervous system as well as of the hematolymphoid system.

The ability to grow multilineage neural cultures as neurospheres (Reynolds and Weiss, 1992) or adherent cultures (Gage, 1998) offered a possible assay for the identification of clonal neural-initiating cells. Recently, human fetal clonogenic CNS neurosphere-initiating cells (NS-ICs) have been isolated; their progeny are capable of self-renewal and multilineage differentiation, indicating that they are CNS stem cells (CNS-SCs) (Uchida et al., 1999). Such human neurosphere cells, when transplanted to immunocompromised rats, enter the SVZ, migrate along the RMS, and undergo multilineage differentiation (Fricker et al., 1999). The ability to use xenogeneic CNS-SCs to regenerate neural components might allow the test of whether they can regenerate regions of the brain with appropriate connections, and if so, to begin to analyze whether apparent species-specific differences in brain functions are quantitative, organizational, or qualitative.

Endodermal Stem Cells

Endodermal stem cells have not yet been isolated. Several visceral organs are derived from primitive endoderm, including gut, liver, exocrine pancreas, and endocrine pancreas. Primitive liver cells placed in culture give rise to more mature liver cells (Brill et al., 1999). Retrovirus-marked liver cells transplanted into mice undergoing genetically programmed liver failure give rise to colonies of liver cells (Overturf et al., 1997). Clonal cells can be obtained from these livers for multiple serial transplants resulting in complete liver regeneration (Overturf et al., 1997). Clonogenic liver cells can give rise to colonies in vitro, whose progeny include hepatocytes and bile duct cells. If liver stem cells exist, these clonal assays should allow their identification.

Mice expressing γ-interferon driven by the insulin promoter undergo a chronic inflammatory process, which includes the continual budding of islet cell progenitors from pancreatic ducts. Budding ductal cells give rise to insulin-producing islet cells, exocrine pancreatic cells, and hepatocytes, as well as biphenotypic progenitors (for review, see Jones and Sarvetnick, 1997). It will be important to characterize the phenotypic nature of the pancreatic and islet stem cells, when isolated, to test whether their differentiation potential is limited to the cell types found in the liver and the endocrine pancreas, respectively.

Germline Stem Cells

Early in embryogenesis, the germ plasm separates from the somatoplasm (Weissmann, 1892). Primitive germ cells (PGCs) express alkaline phosphatase (AP), which allows their passage to and from the extraembryonic mesoderm to be tracked (Chiquoine, 1954). These AP^+ cells, like hematopoietic stem cells, arrive in the extraembryonic mesoderm and yolk sac as distinguishable entities around dpc 7.2 in early mouse embryos (Mintz and Russell, 1957); AP^+ cells can be found in tracks leading to the developing genital ridges at about dpc 9–10, in the mesonephros by dpc 11.5, and in the presumptive gonads between dpc 11.5 and 12.5. Undifferentiated PGCs are germline stem cells because (1) significant numbers of PGCs are derived from smaller numbers of precursors (self-renewal), (2) PGC progeny potentially include either oogonial or spermatogonial cells (multilineage differentiation), and (3) PGCs cultured in vitro can retain multipotentiality. It is currently unclear when and where in mouse the development of the first GSCs emerged from totipotent stem cells (TSCs). A few cells in the blastocyst inner cell mass (ICM) can express AP (MacGregor et al., 1995). However, to my knowledge, pure AP^+ cells have not been isolated from these sites and tested for their differentiative potential. The central issue is the stage at which totipotent stem cells diverge into GSCs and somatic stem cells (SSCs), and also whether some TSCs are retained (for model, see Figure 3).

Unexpected Plasticities in Stem/Progenitor Cells

At the Cellular Level

Until recently, it has been reasonable to assume that developmental processes, whether they are elaborated

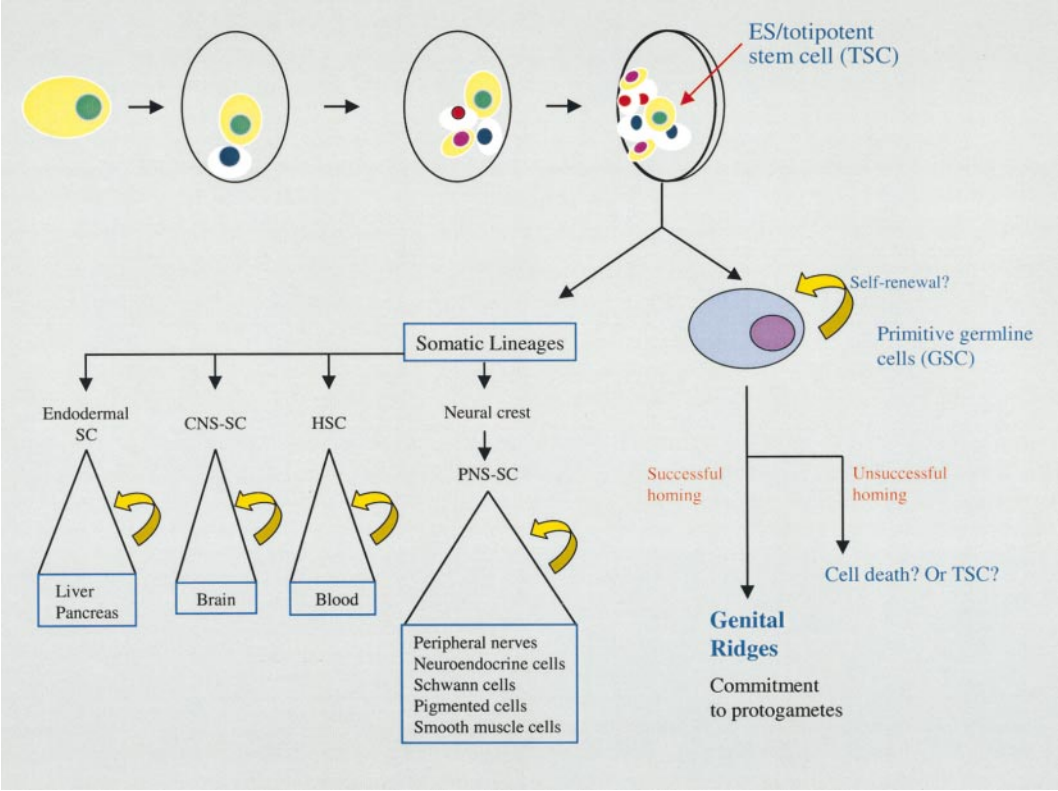

Figure 3. Model of TSC Generation of Germline and Somatic Progenitor Cells

Totipotent stem cells (TSCs) can diverge into GSCs and somatic stem cells (SSCs). A possible source of residual TSC is depicted. Although TSCs have not yet been identified or isolated either in the adult or in the embryo, it shall be important to identify the genes that regulate the outcomes of TSCs to somatic or germline stem cells.

during embryonic and fetal development or as elements of self-renewal in adult life, follow pathways of increasing lineage commitments, with little or no transdifferentiation or dedifferentiation occurring naturally (for model, see Figure 4). However, several recent findings challenge that assumption. Transplantation of clonally derived neurosphere culture cells into sublethally irradiated allogeneic hosts led to the late emergence and takeover of donor-derived hematopoietic systems (Bjornson et al., 1999). Transplantation of BM or blood cells sharing at least some markers with HSCs have been reported to participate in both angiogenesis (production of donor-derived endothelial cells) (Asahara et al., 1997) and somatic muscle development (Ferrari et al., 1998; Gussoni

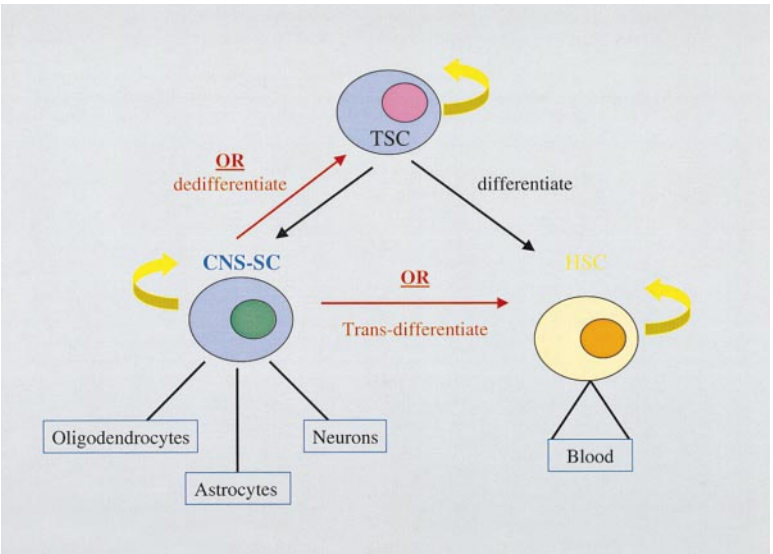

Figure 4. Possible Sources of Tissue-Specific Stem Cells from Another Tissue

In this model, the emergence of, say, HSCs from brain neurospheres could involve either transdifferentiation (brain→blood) or dedifferentiation (brain→TSCs), or by the actions of rare, but residual TSCs.

et al., 1999). BM cells have also been implicated in hepatic regeneration (Petersen et al., 1999) as well as in the formation of CNS cell types (Eglitis and Mezey, 1997). Somatic muscle satellite cells apparently can serve as progenitors for myoblasts, myocytes, and myotubes (Baroffio et al., 1996; Gussoni et al., 1999), which is not a surprise, but have recently been implicated as highly enriched HSCs as well (Jackson et al., 1999).

At the Nuclear Level

Nuclei taken from the mammary glands (Wilmut et al., 1997), intestines (Gurdon and Uehlinger, 1966), egg-lining cumulus (Wakayama et al., 1998), etc., have been inserted into enucleated eggs from frogs, sheep (Wilmut et al., 1997), cattle (Campbell et al., 1993), and mice (Wakayama et al., 1998), resulting in partial or complete development of the respective animal. Taken together, the results of these experiments have led their authors to challenge the notion that either cells or cell nuclei are committed to a particular fate. In this emerging model, cells or cell nuclei would be only temporally committed, and their placement in microenvironments that are totipotent or sites of other cell lineage outcomes would allow these cells or nuclei to change their apparent developmental fates. As diagrammed in Figures 3 and 4, these alterations in fate could involve either transdifferentiation or dedifferentiation. Alternatively, TSCs that never differentiated could seed the various tissues and be revealed in nuclear transplant assays. The above-described nuclear transplantation experiments lacked a definitive characterization of the resident cells that provide the totipotent nuclei. The frequency of nuclear transplants giving rise to embryos or adults is ~1/200–1/400. At that frequency, pluripotent cells (perhaps TSCs) could contaminate the more differentiated (e.g., epithelial) cells. Thus, the experimental verification of the cell type donating nuclei for embryo/adult development when transplanted to egg cytoplasts needs to be precise. The same applies for the transdifferentiation studies. Are the rare subsets of cells capable of hematopoiesis, or myogenesis, or neurogenesis, typical of lineage-committed stem/progenitor cells in those organs, or might they be resident TSCs? In models claiming transdifferentiation or dedifferentiation, it shall be important to identify, isolate, and characterize the cells capable of unexpected differentiation capacities. I propose that neither dedifferentiation nor transdifferentiation occurred in these instances, but rather that stem cells (whether TSCs, SSCs, or PGCs) in unexpected sites are responsible. An alternate hypothesis is presented by Fuchs and Segre in this issue of *Cell*. Current technologies will permit the elucidation of the nature of these cells with unexpected developmental potentials and the resolution of this debate.

To follow this line of speculation further, it is important to consider both the ontogeny and the phylogeny of stem cells. For instance, as described above, PGCs must emigrate from yolk sac and extraembryonic mesoderm toward their ultimate genital ridge "home." Presumably they do so via various homing receptors/chemokine receptors (Weissman, 1989; Butcher and Picker, 1996). While homing via homing receptors and chemokine receptors is reasonably specific and accurate, it is not perfect. What happens to PGCs that miss the genital ridges? Do they die, or are they distributed (and perhaps positively attracted elsewhere) to be available for residency throughout the body? Given what is currently known about markers and what can be done with transplantation of marked purified cells, this possibility could be tested.

There is also a phylogenetic perspective. The TSC→GSC or SSC paradigm is found in a wide variety of living metazoans, and perhaps all. In these solitary species, where both SSCs and GSCs are contained within individuals, it is reasonable to propose that selection for GSC traits and SSC traits, as well as their generation from TSCs, will result in inheritance of the selected GSC and SSC traits in concert by sexual reproduction. That is, the gene pool used for growth, development, and survival is representative of the gene pool collectively distributed in the gametes. However, a large part of the animal metazoan world is inhabited by colonial organisms that regularly become cellular chimeras by organismal fusions or cell lineage transfers. In these organisms, the genes that could regulate GSC and SSC behaviors might not be inherited in concert, and GSCs (and perhaps SSCs) might be relatively independent units of natural selection. That possibility will be examined in the next section.

Stem Cells as Units of Natural Selection

As described above in chordates, the blastocyst contains an ICM that includes totipotent embryonic stem cells that become the epiblast (Gardner and Rossant, 1979). Epiblast cells that travel through the posterior part of the early primitive streak give rise to PGCs and yolk sac blood islands. At a stage between blastula and gastrula development, PGCs split off from somatic stem cells, and thereafter somatic cells cannot give rise to PGCs in that individual's body (Weissmann, 1892). The expansion of PGCs that occurs between the time of their origin and their entry into the genital ridges must include, if not be entirely composed of, self-renewing divisions. The pathways of PGC development, migration to the extraembryonic mesoderm, their expansion and self-renewal, and their remigration to and commitment within the male and female genital ridges are all events that must be programmed genetically, and therefore are events in which genetic variants can arise. It is likely that such variants are targets of natural selection. In vertebrates, the genomic content of these PGCs equal the genomic content of all somatic stem cells, and therefore, with the exception of variants that might guide PGC events, the gene pool of PGCs is the same as the gene pool of the body that houses and nurtures PGCs.

For metazoans such as *Drosophila*, *C. elegans*, and all vertebrates, natural selection acts at least on individuals, which is a straight-forward process where outcomes can be predicted according to the principles of Mendel and Hardy-Weinberg. However, during metazoan evolution, sequestration of cells in one conspecific animal from another is not the rule; many species exist wherein genotypically distinct cells may intermix within a chimeric entity (reviewed in Buss, 1999; Magor et al., 1999). It is the existence of these compound or colonial metazoans—many of which are believed to be close to the phylogenetic line that leads to the vertebrates—that could provide insights into the phylogeny of stem cells,

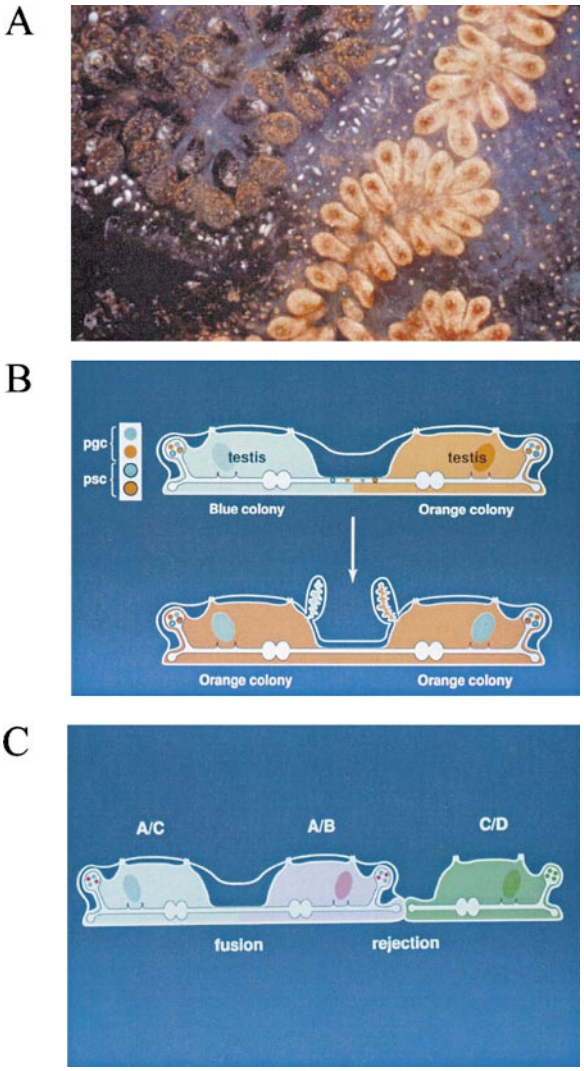

Figure 5. A Colonial Protochordate that Undergoes Natural Germline and Somatic Stem Cell Transplantation

(A) A *Botryllus schlosseri* composite colony. Each colony is composed of several systems (flower-shaped), which in themselves are composed of individual "zooids" (petal-shaped). The systems of a colony are joined by a vascular network, which can be seen at the colony margins. In this photograph, a colony bearing orange pigment cells has fused with a colony bearing purple pigment cells, which results in the interconnection of their vasculature networks.

(B) A model of somatic cell and germ cell parasitism following fusion of Fu/HC-compatible colonies. A schematic representation of a transverse section through a blue colony newly fused to an orange colony shows the common vasculature (in white), through which blood cells circulate freely. The progenitor germline cells (circulating cells without an outline) and progenitor somatic cells (cells with black outline) enter all buds (upper left and upper right, respectively, of the blue and orange zooids) of both colonies. The progenitor cells of the two colonies compete for the sites of organogenesis within the buds. In this example, following the next asexual generation, the orange progenitor somatic cells have won over the colonies' somatic progenitors, and the zooid is a genetic clone of the orange colony. Independently, the progenitor stem cells of the blue colony have won over orange for the gonadogenesis sites, and thus, the testes in both colonies are derived from the blue colony. The degenerated zooids from the first generation are shown as fernlike appendages on the proximal flanks of the zooids. The second and subsequent generation colonies retain a mixture of circulating cells from both the orange and the blue colonies.

(C) Schematic representation of fusion and rejection between *Botryllus schlosseri* colonies. Three Fu/HC-genotyped colonies (A/C, A/B, and C/D) are depicted, with fusion occurring between A/C and A/B and rejection occurring between A/B and C/D. Little or no exchange of circulating cells occurs between the rejecting pairs. Adapted from Magor et al. (1999).

and how the bodies of one genotype protect themselves against predatory allogeneic stem cells of another genotype within the chimera.

A Case Study: Chimeric Colonial Protochordates

Life History Characteristics

Protochordates share developmental history with vertebrates at least through their early stages (Millar, 1971). As in the vertebrates, the protochordates develop embryos that progress through blastula and gastrula to form tadpole larvae, each with tail, notochord, neural tube, segmented musculature, etc. (Millar, 1971). The colonial protochordate that is the object of this discussion is *Botryllus schlosseri*, a largely sessile subtidal colonial tunicate species that exists in shallow sea water in virtually all temperate zones around the world (Grosberg, 1981). *Botryllus* tadpoles upon hatching leave the mother colony, swim to another subtidal surface, attach to that surface, and undergo a metamorphosis that results in the loss, by apoptosis, of the chordate characteristics of tail, notochord, neural tube, and segmented musculature (Lauzon et al., 1993). Metamorphosed *Botryllus* offspring then commence an asexual budding process that begins with the formation of one to three small vesicles from outpouchings of the lateral body wall, apparently including all germ layers as well as blood cells (Berrill, 1941). The vesicles then undergo a series of cell divisions, invaginations, and evaginations, to develop blastozooid adults that have body plans which are essentially the same as that of the oozooids, lacking the chordate intermediate stages (Berrill, 1941). Thus, protochordates like *Botryllus* have at least two genetic pathways for development, one following sexual reproduction and the other governing asexual reproduction. *Botryllus* zooids are complex individuals with a two-chambered heart and a cardiovascular system that ramifies throughout the organism and also throughout the gelatinous tunic as an extracorporeal circulation. They have a gastrointestinal tract, an endostyle which contains epithelial cells that produce and secrete hormones, a nervous system, gills with gill slits, and at some later stage of development, both testes and ovaries. When *Botryllus* blastozooids reach their full maturation, the individual from which they budded dies and is resorbed (takeover). The newly developed blastozooids in the common tunic fuse their extracorporeal blood vessels to form a multiindividual colony. A colony of colonial tunicates arranges itself in systems of individuals arranged like the petals of flowers, with several systems existing within a clonal colony (Figure 5A). As observed by John Steinbeck,

> There were great colonies of tunicates, clusters of tiny individuals joined by a common tunic and looking so like the sponges that even a trained worker must await the specialist's determination to know whether his find is sponge or tunicate. This is annoying, for the

sponge being one step above the protozoa, at the bottom of the evolutionary ladder, and the tunicate near the top, bordering the vertebrates, your trained worker is likely to feel that a dirty trick has been played upon him by an entirely too democratic Providence (Steinbeck, 1955, p.50).

Stem Cells as Units of Natural Selection in Botryllus

At the inception of bud formation, the cells in the vesicle are morphologically undifferentiated. It is reasonable to assume that they are stem cells, either TSCs or a variety of somatic stem cells and GSCs. If these stem cells self-renew during asexual reproduction and are free to circulate, they could participate in the processes of organogenesis and gonad formation that occurs in the bodies of their clonemates in a vascularly fused colony. If a colony is clonal, the genomes of circulating GSCs and SSCs are the same. As far as natural selection is concerned, the individual that was the tadpole is a target of natural selection. However, the metamorphosed tadpole gives rise to a colony (with the same genome) that is represented by a large number of interconnected individuals, a colony that inhabits space, competes for food, and must survive the challenges of its environment. The loss of a single or several individuals of the colony does not end its survival, and therefore, it can reproduce (Chadwick-Furman and Weissman, 1995). This contrasts with the tadpole, wherein the death of the individual removes its genome from the selectable pool. The fact that the colony also may be a unit in natural selection was also noted by Steinbeck:

There are colonies of pelagic tunicates which have taken shape like the finger of a glove. Each member of the colony is an individual animal, but the colony is another individual animal, not like the sum of its individuals . . . So a man of individualistic reason, if he must ask, "Which is the animal?" must abandon his particular kind of reason and say, "Why, it's two animals and they aren't alike any more than the cells of my body are like me. I am much more than the sum of my cells, and, for all I know, they are much more than the division of me" (Steinbeck, 1955, p. 136–137).

When two *Botryllus* colonies or oozooids come into contact, the terminal vascular ampullae touch the sides of the vessel of the adjacent colony, and either fuse or reject (Bancroft, 1903). A single, highly polymorphic gene locus (called *Fu/HC*) controls fusion or rejection; colonies that share one or two *Fu/HC* alleles fuse, but those that share no alleles reject (Oka and Watanabe, 1957; Scofield et al., 1982). In 1982, Buss was the first to propose that chimeric metazoans should possess genetically polymorphic self/non self–recognition systems to prevent parasitization of germline niches by genomes that are closely related (Buss, 1982).

Colony fusion offers the opportunity for GSCs or SSCs to move from one colony to a genetically distinct colony. Because the *Fu/HC* locus is so highly polymorphic, individuals found in the wild are Fu/HC heterozygotes; only relatives share *Fu/HC* alleles (Scofield et al., 1982). Thus, the opportunity for the movement of GSCs and SSCs should be limited to relatives. To test this hypothesis, we used highly polymorphic microsatellite sequences to track somatic and germline cells in fused allogeneic colonies. Both in the laboratory and in the wild, fused, Fu/HC-compatible colonies were full blood chimeras, and in many instances the prevascularized buds were also chimeric (Pancer et al., 1995; Stoner and Weissman, 1996; Stoner et al., 1999). Surprisingly, isolated sperm from one colony were often derived wholly or in part from the fused partner, and this was reflected in sexually reproduced offspring from the chimera, whether the chimera provided eggs or sperm (Stoner et al., 1999). We interpreted these experiments to show that GSCs and SSCs circulate, take part in organogenesis during asexual development, and can enter the developing bud of another, fused individual (Stoner et al., 1999). Fu/HC-incompatible pairs do not fuse vessels and therefore are not at risk for competitive GSCs and/or SSCs (Figures 5B and 5C). The winners of the germline competitions were unlinked to the winners of the somatic competitions for the prevascular buds. The phenotype of germline winner or loser was reproducible and heritable over all asexual generations tested, as were the unlinked phenotypes of somatic winners and losers. The phenotypes of germline winners or losers were also heritable through a pedigree and therefore are probably genetically determined (Stoner et al., 1999). We propose that natural selection could be operating on the basis of the heritable traits (selection at the level of the gene), the germline or somatic stem cells that express these genes (cell lineage selection), as well as the aforementioned individual and colony (group) selections (Stoner et al., 1999). It should be pointed out that cell lineage selection is a special form of individual selection wherein the whole genome contained in, for example, a germline stem cell is the unit of selection; it differs from most individual-based selections in that the body that houses the individual germline may not have an identical genome.

The imposition of Fu/HC-determined fusibility has several interesting consequences when one considers stem cell lineage selection in the context of natural selection. First, supercompetitor GSCs are limited in their parasitic spread through the species to Fu/HC-compatible siblings, limiting the tendency to genetic homogeneity. Rare GSCs (or for that matter, SSCs) that develop unregulated self-renewal capacity would be, in effect, malignancies, and these malignancies would end the survivorship of self and Fu/HC-compatible siblings with which they fuse; Fu/HC-different colonies would be protected by rejection. Can inheritance of highly competitive GSCs lead to coselection of SSCs? It is clear that a trait that makes a GSC more competitive results within one generation in its improved chances of passing that trait on in the next round of sexual reproduction, but what about improved SSC traits? If the differentiated progeny of SSCs express genes that will help them tolerate certain environmental conditions, nonfused colonies are less adaptive to environmental changes than fused colonies. The dynamic nature of interchanges of SSC cell lineages during asexual reproduction could result in a chimera whose body parts are successively rebuilt by the favored SSC genotype in the chimera. Contained within a fused colony are GSCs from both colonies, and the ability of those GSCs to pass on their traits depends partly on the fitness of the bodies they inhabit, at least until the gonads form and sexual reproduction is complete. Fu/HC-limited fusion guarantees that the genomes of the GSCs are approximately 50% identical to the genomes of the partner sibling colony. It is reasonable to propose that over time GSCs will be selected

that also carry genes which determine more successful SSC-derived bodies. In the wild, not only sibling pairs, but also groups of three or more Fu/HC-compatible siblings can form multichimeras, enabling a wider possibility of competition and selection of circulating SSCs and GSCs.

Potential Lessons for Vertebrate Stem Cells from Protochordate Colonies

What kind of traits might one expect in successful GSC or SSC competitors? It is reasonable to search for genes regulating self-renewal, programmed cell death, homing receptors, addressins, chemokines, chemokine receptors, and their signal transduction pathways as good candidates for GSC (and SSC) competitive behaviors. The genetics that vertebrate GSCs, HSCs, and other stem cells use for self-renewal, homing, etc., are probably phylogenetically conserved. It is therefore not difficult to conceive of GSCs and SSCs in contemporaneous protochordates using genes inherited from ancestral cell lineages present over 550,000,000 years ago prior to the protochordate/chordate split. Also, it is not difficult to conceive of self/non–self recognition as a means to limit the passage of these cells from any individual to individual, even in the vertebrates. The passage of hematopoietic stem cells between vertebrate individuals can and does occur in nature. In 1945, Ray Owen documented that nonidentical bovine twins sharing a placenta were often hematopoietic chimeras for life (Owen, 1945), and Medawar et al. substantiated that these were transplant tolerant of each other for life (Billingham et al., 1954). Similar in vitro chimeras occur in a primate species—the cotton-topped marmoset (Watkins et al., 1990). Although nonidentical human twins sharing a common placental vasculature is rare, what is not rare is the cross-circulation of small numbers of cells between mothers and their fetuses. It shall be important to test in the bovine and marmoset cases whether germline chimerism can accompany hematopoietic chimerism, and in fetuses and adults whether the chimerism is strictly limited to cells of the hematolymphoid system following pregnancy. Perhaps the descendants of *Fu/HC* genes are still operative in limiting chimera formation in the vertebrates, and if so, the identification of such genes in protochordates might reveal another layer of self/non–self regulation in them. Similarly, once the genes are found that are used by protochordate GSCs and SSCs to compete, they might be used by vertebrate GSCs and other cells. Finally, neoplasms can be considered to be differentiated cells that have assumed the properties of self-renewal that stem cells possess. It is not inconceivable that these genes, too, may be descended from ancestral genes in primitive protochordates that regulate GSC, and perhaps SSC, self-renewal.

Concluding Statement

In essence, this review is about the evolution of development. Stem cells were defined as clonogenic cells capable of self-renewal as well as differentiation. The practice of medicine has led to the finding that hematopoietic stem cells are not only units in the development of the hematolymphoid system, but also its maintenance and regeneration. It is this aspect of regeneration that accounts for the expanding field of hematopoietic stem cell and tissue-specific stem cell transplantation. The themes of stem cell–based organogenesis, stem cell mobility, stem cell–based regeneration, and the coevolution of polymorphic histocompatibility that limits stem cells to self or close relatives are here proposed to be interrelated and evolutionarily coselected.

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