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The Stem State: Plasticity Is Essential, Whereas Self-Renewal and Hierarchy Are Optional

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Abstract
The prevailing stem cell concept is derived from the large body of evidence available on the structure of the blood-generating system. Hemopoiesis is organized such that a multipotent stem cell, endowed with self-renewal capacity, is viewed as being positioned at the origin of a hierarchical tree of branching specificities, increasing maturity and decreasing self-renewal ability. Data accumulated in recent years on various stem cell systems often contradict this traditional view of stem cells and are reviewed herein. It is suggested that other options should be considered and put to experimental scrutiny; it is argued that the organization of the hemopoietic system may not represent a general structure of stem cell systems. The basic trait of the stem state is proposed to be plasticity. Self-renewal is not a specific stem cell trait; rather, it is exhibited by some mature cell types, whereas other particular stem cells are endowed with relatively poor renewal ability. Hierarchical structuring is also proposed to be an optional stem cell trait and may exist only in specific tissues where it serves the need for rapid expansion. The stem state is therefore defined by the highest degree of plasticity of a cell, within the repertoire of cell types present in the organism. STEM CELLS 2005;23:719–726

The Prevailing Stem Cell Theory and Stem Cell Types
While observing the whereabouts and changes that cell populations undergo in vivo and in vitro, investigators arrived at the “stem cell” notion that basically explains a major puzzle: cells in the embryo and in the adult organism reach a state of full functional differentiation that is viewed as “terminal” in that it is irreversible. The cells may further die through apoptosis or other death mechanisms. How is this cell loss compensated? The prevailing explanation is that tissues contain a small fraction of stem cells that are endowed with unique properties inherently different from those of the mature ones. These rare cells can divide by producing more of themselves; thus, they are capable of self-renewal and also possess the potential to differentiate into many different mature cell types and therefore are multipotential. This theory was dramatically successful upon the isolation of rare cells within the bone marrow, hematopoietic stem cells (HSCs), which are multipotent in that they give rise to all types of cells in the blood and are capable of long-term repopulation of irradiated recipients. Isolated HSCs have been shown to give rise to committed progenitors that are restricted to a single lineage within the hematopoietic system (myeloid, erythroid, megakaryocytic, or lymphocytic series). However, intermediate stages wherein the cells are still multipotent but are already biased to a particular pathway have been described. The committed progenitors are followed by yet further differentiated progeny that are gradually losing their proliferation potential while maturing and acquiring differentiated functions (Fig. 1A) (reviewed by [1]). This succession of steps from the early stem cell to differentiated products is referred to as the stem cell tree. Thus, the hematopoietic system is viewed as a hierarchical organization wherein the stem cell is positioned at the origin, while branching leads to the differ-
entiation into mature cells that are the end products (Fig. 1A). The direction of flow in this system is from the stem cell down to the mature cell, a flow that is irreversible. The identification of HSCs and the hemopoietic tree is commonly regarded as the ultimate proof of the stem cell concept, pointing to the possible presence of such cells in different organs and tissue. Indeed, evidence to this effect exists for the embryo [2], adult skin [3], nervous system [4, 5], pancreas [6], heart [7], liver [8], and others. The depth of the available information about these other stem cell–based systems is not as detailed as that relating to HSCs. Nevertheless, while examining the different kinds of stem cells, the general impression that emerges is that indeed the stem cell theory has a sound basis.

The early mouse or human embryo at the blastocyst stage is a source of cells designated embryonic stem (ES) cells. The mode of derivation of these, which are in fact cell lines capable of continuously growing in culture, is isolation of the inner cell mass (ICM) from the blastocyst and propagation of this tissue fragment in tissue culture. At a later stage it is possible to clone single cells and further propagate them while maintaining their growth potential. ES cell lines are pluripotent, as judged by their capacity to differentiate in vitro into a variety of mature progeny according to the stimulus provided to them. More importantly, upon introduction into an early embryo, cultured mouse ES cells integrate into the developing animal and give rise to cells of most tissues and organs, including the germ line. This pluripotentiality is suggested to be a distinct property of embryo-derived stem cells, a notion that seems reasonable and logical in view of the fact that the cells within the embryo are indeed capable of differentiating into all eventual cellular constituents of the organism. By contrast, HSCs are multipotent in that they give rise to all the constituents of the blood system; however, they are viewed as being tissue-specific and capable of giving rise to cells of their tissue of origin only. Accordingly, stem cells derived from other tissues, such as the heart, liver, and brain, are supposed to be restricted to their respective organ of origin. Figures 2A–2C show schematically how a totipotent zygote is supposed to give rise to pluripotent ES cells that characterize the early embryo, whereas these will eventually produce tissue-specific stem cells that are first multipotent and eventually oligopotent or monopotent. At this stage they are ready to terminally differentiate. Once again, this is logical in view of our knowledge of development and differentiation. However, data accumulated in recent years seem to shatter the current model of stemness. Alternative models have been proposed [9–11], and I have recently suggested the concept of the “stem state”; cells may enter a stem state reversibly, and thus stemness is a state rather than a cellular entity [12]. The present review is aimed at further re-evaluating the stem cell notion and suggests that plasticity is the major trait of the stem state. On the other hand, properties such as ability to self-renew or give rise to a hierarchy of branching specificities are dispensable options.
Inconsistencies in the Prevailing Stem Cell Theory

Pluripotent Stem Cells Are Not Specific to the Early Embryo

The introduction referred to the generally accepted notion that the early embryo, at the blastocyst stage, is a source for ES cells and that this property is lost at later stages from the soma. The only other site in the embryo that is a source of pluripotent stem cells is the genital ridge, which gives rise to cells termed embryo germ (EG) cells. Embryonic development and the subsequent adult life are viewed as a continuum of decreasing potencies (Fig. 2C). However, the mere detection of EG cells argues against this notion. Recently, doubts have been raised as to the origin of ES cells, and it has been suggested that they are of germ cell origin [13]. Furthermore, pluripotent fetal somatic stem cells (FSSCs) recently have been demonstrated among mesenchymal fibroblasts isolated from murine and porcine embryos at 11.5 to 14.5 and 25 days postcoitum, respectively. FSSCs are relatively abundant, as evidenced by in vitro culture, and can be propagated effectively in serum-rich medium; upon introduction into the blastocysts, they participate in formation of liver, tongue, and the genital ridges [14]. Similarly, in the late human embryo, unrestricted somatic stem cells (USSSCs) were obtained from the placental cord blood at 34-42 weeks’ gestational age in approximately 40% of pregnancies examined. USSSCs were tested for their stemness in vitro and were found to be pluripotent. More importantly, they were examined in a noninjury-preimmune fetal sheep model; upon inoculation into the sheep embryo, these cells contributed to the liver parenchyma and heart muscle [15]. Cord blood collected upon delivery was also a source of pluripotent mesenchymal stem cells (MSCs) giving rise to cells of all three germ layers [16], and neonatal mouse testis was found to harbor pluripotent stem cells [17]. Thus, pluripotent stem cells are found in the late embryo proper as well as in cord blood and persist until birth (Fig. 2D).

Pluripotent Stem Cells Occur in the Adult Organism

The stem cell theory entails the notion that adult stem cells are tissue specific and thus have a restricted spectrum of differentiation options. The HSCs would thus give rise to blood cell only, and the stem cells of the epithelial skin components would be devoted to this direction of differentiation only. However, it is clear to date that adult tissues contain cells that upon in vitro culture exhibit pluripotent capacity comparable to that of ES cells. The studies of Verfaillie and colleagues [18, 19] demonstrated that the bone marrow contains a population of cells designated multipotential adult progenitor cells (MAPCs). These are adherent mesenchymal cells that, like the previously discovered bone marrow-derived MSCs [20, 21], are capable of differentiating into mesodermal derivatives such as bone, cartilage, muscle, and fat. However, MAPCs can further give rise to ectoderm cells such as neurons and to endoderm products such as hepatocytes. Upon introduction into the blastocysts, MAPCs integrate into the developing embryo and give rise to cells of the three germ layers [19]. Thus, the adult bone marrow, much like the embryo, contains cells that are pluripotent. A reservation often raised regarding this last conclusion is that MAPCs appear in culture after 20 to 40 population doublings and may therefore be an in vitro artifact. It is noteworthy that the same reservation can be made to dismiss ES cells, because these emerge after long-term culture of whole ICM tissue followed by cell line derivation. Strangely enough, criticism on this cell type as being an in vitro artifact is seldom expressed despite the fact that it has not been possible thus far to isolate a single cell from the ICM and derive from it an ES cell line. It should be emphasized therefore that the in vivo counterparts of all stem cell types that have been demonstrated by virtue of in vitro culture should be identified in situ before definitive conclusions regarding their nature can be made. However, the studies of Miller and colleagues [22] indicate that the identification of candidate stem cells using in vitro methods may indeed reflect the existence of in vivo counterparts; several years ago this group first described a cell type derived from skin, the skin progenitor cell (SKP). These cells differentiate into neuroectoderm and mesoderm derivatives, including smooth muscle, neurons, glia, and adipocytes. Recently, a seminal study by this same group showed that SKPs originate in the neural crest, migrate to the hair papillae, and remain there in adulthood [23]. SKPs can be directly cultured in vitro without the need for long-term passaging and exhibit a capacity to differentiate into neurons and Schwann cells after a single passage as well as after 20 in vitro passages. In view of the data described above, it is suggested that pluripotent stem cells prevail, and in fact are abundant, in the adult organism (Fig. 2D).

Stem Cells Are Not Tissue-Restricted and Occur Even at Old Age

The study of SKPs demonstrated unequivocally that this stem cell has potencies that exceed the restricted requirement of the skin, the tissue of residence of these cells. Other examples are MSCs and MAPCs. Apart from residing in the bone marrow, MAPCs were also derived from muscle and brain [18]. Likewise, MSCs were described in a multitude of tissues and organs, and their presence was verified even in geriatric human donors [24]. D’Ippolito et al. [25] used culture conditions reminiscent of those used by Jiang et al. [18] and isolated a class of pluripotent human mesenchymal cells (MIAMI cells) that differentiate in vitro into mesodermal derivatives as well as into neural cells and can be directed to express genes associated with pancreatic islets. MIAMI cells were recovered from human donors of ages ranging from 3–72 years. Thus, the assumption that each tissue harbors a specific stem cell subtype committed to a tissue-restricted differentiation scheme is incomplete. Clearly, tissue- or organ-specific multipotential and oligopotential stem cells exist in the adult. Along with
these, adult organs at any age may also contain pluripotent stem cells that have the potential to differentiate into mature cells that are irrelevant to the tissue of residence of the specific stem cell (Fig. 2D). Because stem cells are endowed with migratory properties (see below), their function may not be restricted to their tissue of residence. Rather, stem cell pools may serve as depositories activated at need to supply cells to distant body sites.

Self-Renewal May Not Be an Obligatory Stem Cell Trait

At the single-cell level, self-renewal is defined as the capacity of the progenitor to divide while giving rise to at least one cell out of the two daughters that remains identical to the progenitor cell. One contention of the prevailing stem cell model is that proliferation potential and self-renewal capacities are properties that are gradually lost during differentiation. However, lymphocytes, which are clearly downstream in the differentiation cascade and as distant from the HSC as any mature cell may be, can proliferate and self-renew extensively after antigenic stimuli. Thus, in the hematopoietic system, T and B lymphocytes, which are fully mature and functional, maintain renewal capability that is equivalent if not superior to that of their precursors, the HSCs. After choriomeningitis virus infection of mice, naive CD8 T cells increase in number over 1,000-fold to create a large pool of memory cells that may respond again to antigenic challenge [26]. T cells may be cultured as antigen-specific entities and can be propagated without limit [27] and are therefore not different, from this viewpoint, from ES cells. These cultured T cells have a defined T-cell receptor and corresponding specificity and are thus mature, monopotent, and committed cells despite their great self-renewal capacity. Conversely, in vitro maintenance of HSCs is difficult despite all efforts put into this issue, and attempts to expand HSCs in culture has, by and large, failed thus far. By contrast, factor-dependent hematopoietic cell lines exhibiting properties of later, more restricted differentiation stages are relatively simple to derive. Is this a technical problem or rather a feature of the HSCs? It suffices to say that despite the apparent consensus among researchers that stemness entails high capability for self-renewal, the HSC has not been unequivocally proven to have this property. Successive transplantations of HSCs result in a decline in the effectiveness of engraftment [28, 29]. Thus, HSCs show some renewal activity; however, they seem to lack infinite self-renewal capacity, such as that exhibited by ES cells in culture. Because most hematopoietic cells are quiescent (in the G0 of the cell cycle), it is possible that upon maturation, the bone marrow builds up a pool of stem cells that periodically sends out few stem cells that repopulate the blood system for months or years. Self-renewal needed to replace these lost stem cells should be minimal and a very slow process. This possibility has not been excluded by experimental evidence available to date. The fact that the W/Wv mutation, which results in a relative lack of earlier stem cells and anemia, nevertheless allows for prolonged survival indicates that intermediate progenitor populations can renew and allow expansion in the absence of self-renewing early stem cells. In fact, the in vivo population analysis indicating gradual increase in HSC numbers could be equally explained by processes of dedifferentiation rather than self-renewal, as defined above [30]. A mathematical phase-space model of hemopoiesis, suggested recently, dismisses the compartmentalization aspects of the blood-producing system [11] and is in line with the view presented in this text. Thus, the bone marrow may be designed to discharge a few stem cells at a time, and little renewal would be needed because the stem cell pool is concentrated in this single anatomical site and is well protected by the bone marrow microenvironment [31–33]. On the other hand, MSCs or MAPCs seem to be spread throughout the organism in every tissue and in small numbers at each site. These cells may not have their own protective niche. They should exist independently and differentiate readily upon call. MSCs must therefore have a high renewal capacity or else they would be extinct. This is probably the reason that mesenchymal cell types are relatively easy to maintain in vitro while exhibiting high renewal capability.

Contrary to the weak experimental support for the self-renewal of HSCs, the study of ES cells does provide evidence for an incredibly high self-renewal potential of this stem cell type. It must be remembered, however, the ES cells are cell lines selected from the cultured ICM tissue. It is thus far unclear whether single cells in the ICM can give rise to ES cells or whether this is a rare property that emerges in vitro. In fact, within the embryo the ICM exists for a few days only, and no information is available to show that descendants of cells from the ICM, being those that give rise to cultured ES cells, indeed self-renew in vivo.

The idea that self-renewal is not a characteristic property of early stem cells is supported by analysis of hierarchical organization of neural cell populations. Neural crest cells were found to be ordered in a hierarchy starting from pluripotent stem cells followed by a succession of intermediate progenitors of more restricted potencies. However, renewal capacity was a feature of the late bipotent progenitors rather than that of the earlier multipotent stem cell stage [34]. Similarly, in the intestinal crypt, transit cells may assume a stem cell behavior [35]. Thus, hierarchical organization of cells with decreasing range of differentiation directions in this stem cell system does not entail decreasing self-renewal ability. In view of these considerations, I suggest that self-renewal is not a stem cell–specific property; rather, more mature and even fully mature cells such as lymphocytes have this property and may be more potent than stem cells in their renewal ability.

Limited Evidence for Hierarchical Organization in Stem Cell Systems

The human hematopoietic system is designed to produce over $10^8$ cells per minute, starting from a small pool of multipotent stem
cells. Such a task requires a logarithmically expanding population. The hierarchical branching structure facilitates the formation of large numbers of cells belonging to the different bone marrow lineages. However, is such hierarchical organization found in other stem cell systems? It has been suggested by several investigators that such hierarchy is present in MSC populations (Fig. 1B) [36, 37]. Stromal cell lines derived from mouse bone marrow show properties of adipocytes, osteoblast, endothelial cells, and hemopoietic supportive stroma [38–40]. Although they have relatively stable phenotypes, the adipocytes, to mention one example, may return to a preadipogenic state or assume a hemopoietic supportive stroma phenotype and therefore exhibit a plastic nature. Analysis of myogenic markers in such stromal cell lines shows that they express these markers to various degrees and were therefore ordered as a myogenic differentiation cascade (Fig. 1B) [36]. Several of the very same cell lines express osteogenic properties to various degrees [41]. Mesenchymal cells therefore seem to express a variety of gene products related to the directions of differentiation that they may assume. The proposed models for MSC differentiation hierarchy are primarily deductions from the structure of the hemopoietic system, whereas solid evidence for hierarchical organization of mesenchymal populations is not available; the hemopoietic tree has been constructed using cell-surface markers and functional assays of isolated subpopulations and shows clear branching into committed progenitors followed by a continuum of differentiation steps forming a clear hierarchy of increasing maturation. By contrast, researchers have been unable thus far to demonstrate steps in mesenchymal differentiation in which clear branching is observed. Rather, an ill-characterized “mesenchymal stem cell” ends up giving rise to a whole range of cell types, demonstrating pluripotency. Where are the missing steps of maturation, a complex hierarchical tree of branching direction of differentiation assumed by an ES cell entails several different steps of maturation, a complex hierarchical tree of branching specificities involving various degrees of commitment, equivalent to those found in the hemopoietic tree, are unknown. Is this a result of insufficient experimental work or a property of ES cells? This author proposes that indeed the second option is plausible and should be examined. Another type of pluripotent stem cell is the adult MAPC. This MSC type is capable of differentiating in vitro, and upon inoculation into early embryos, it differentiates into different cell types. Again, similarly to the ES cell, MAPCs do not show hierarchical structuring. The cells can be directed readily into different lineages and cell types according to the stimulus given to them. In the hemopoietic system, methods have been designed to physically separate the bone marrow into distinct subpopulations of progenitors that represent a descending cascade of potencies, from multipotent stem cells down to mononopotent committed cells. No similar cascades are characterized for ES, MAPC, or MSC populations. In different culture conditions, mesenchymal cells with varying differentiation capacities have been isolated and were given specific designations such as MSC, MAPC, or USSC. Yet whether they represent stages in commitment is at present unclear. It is therefore suggested that
The Collapse of Hierarchical Organization of Stem Cell–Based Systems: Stem Cell Plasticity and Transdifferentiation

A final blow to the notion that the stem state entails hierarchical organization is the discovery of unexpected plasticity. HSCs have been reported to differentiate into a variety of cell types of endoderm or ectoderm origin as well as into mesoderm derivatives other than blood cells [48–51]. This discovery denotes a complete collapse of the orderly and hierarchical hematopoietic tree. However, it is important to note that controversy exists as to the degree of plasticity that HSCs possess. Some investigators argue that this plastic behavior is a rare event [52, 53] or that HSCs do not differentiate at all into cardiac myocytes [54]. Others propose that rather than being able to differentiate into nonhematopoietic progeny, HSCs or their descendants fuse with other tissue cells, such as hepatocytes [55, 56], and consequently their genome is reprogrammed. Nevertheless, clear examples of plasticity that do not entail fusion and are not rare events have been reported [57].

The ability of the HSCs to give rise to cell types that do not belong to the hematopoietic system is but one surprise. That HSCs may differentiate into muscle [50] is acceptable from the viewpoint that the differentiation resulted in a cell type belonging to the same germ layer that gives rise to blood cells, i.e., mesoderm. A reciprocal process in which muscle cells differentiate into hematopoietic cells, while retaining myogenic potential, has also been reported [58]. Thus, the ability to cross the tissue barrier and give rise to other cells of the same germ layer is referred to herein as plasticity. On the other hand, when HSCs differentiate into intestinal epithelial cells, the event represents crossing of the embryo germ layer barrier (mesoderm to endoderm) and is thus referred to herein as a transdifferentiation phenomenon, a subtype of plasticity. Stem cell types like MSC, MAPC, USSC, FSSC, MIAMI, and SKP are both plastic and capable of transdifferentiation as they give rise to various cell types belonging to different embryonic germ layers [14, 15, 18, 19, 25, 59, 60]. Figure 3 presents stem cell differentiation in a “radiating sun scheme” wherein the source is the stem cells, which send out differentiation “rays” to all germ line directions (Figs. 3A, 3B). This presentation qualitatively indicates the possibility of occurrence of such differentiation without reference to the qualitative differences between the various directions. Clearly, in the case of the HSCs, one such ray that leads to production of hematopoietic cells is quantitatively dominant within the bone marrow (Fig. 3C). The plastic phenomena are not restricted to hematopoietic and mesenchymal populations; endothelial cells differentiate into cardiac muscle [61], and hepatic stem cells turn into pancreatic cells producing endocrine hormone [8], to mention just two examples.

An Alternative Stem Cell Concept: The Stem State Entails Plasticity While Other Traits Are Optional

In view of the above considerations, I propose that cells with stem cell characteristics are highly plastic and thus pluripotential. All other properties that were associated with the stem cell phenotype are just options, or traits specific to one type of stem cell, and therefore do not define the stem cell per se. This definition goes along with the notion of the stem state; although it is commonly believed that stemness defines a cellular entity, it has been suggested that stemness is in fact a state in the life cycle of the cell [12]. Thus, cells may be proliferating or may enter a differentiation stage. Under these latter circumstances, they are highly restricted in their options. However, cells maintain a degree of potency to exit proliferation or differentiation to enter the stem state entailing high plasticity. This conclusion obviously assumes capacities of dedifferentiation that need to be verified in mammalian systems, although in Drosophila [62], amphibians [63], and plants [64], this is a well-studied and widely accepted phenomenon. A major assumption of the stem state model is that almost any cell has the potential to enter the stem state. Clearly, if a cell within the hematopoietic lineage is entering the stem state, it will nonetheless be biased to hematopoietic differentiation to the same extent that a liver stem cell would maintain some liver characteristics. This is the reason for the notion that stem cells are a
tissue-specific entity, while the model suggested here supports the idea that all stem cells are basically equal and share common properties characteristic of the stem state (Fig. 3). The molecular basis of this stem state is yet to be defined, but it seems to entail a promiscuous gene expression pattern, as evidenced by the study of gene expression of MSCs \[65\], HSCs \[66\], and transcription comparison of this stem state is yet to be defined, but it seems to entail a pro-
properties characteristic of the stem state (Fig. 3). The molecular basis of cell plasticity will enable the control of cell fate and the direction of cells into a stem state at will. Such a development will have a dramatic impact on cell therapy and medicine at large.

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