

Our Perception of Developmental Plasticity

Esse Est Percipi (to be is to be Perceived)?

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Abstract: The continuing interest in the biology of stem cells is enhanced by new discoveries surrounding developmental plasticity of both embryonic and adult stem cells. Adoptive transfer of concepts and definitions from the hematopoietic system to other tissue stem cells suggests inclusion of characteristics such as ability to self-renew and differentiate to functionally reconstitute a tissue/organ of origin. How adequate and accurate are these definitions? Within the great unknown of how these cells function, modulate their gene expression patterns and respond to extrinsic signals, it is apparent that there are numerous levels of stemness. We may envision a scale of developmental flexibility. At one end of the scale are positioned the embryonic stem cells, and at the other end are positioned partially-differentiated, differentiation restricted (committed) tissue/organ stem cells. There is evidence that some stem cells in the adult are pluripotent, thus positioned close to the embryonic end of the stem scale. It is uncertain yet to what extent stem cells can move back and forth along the stem scale.

INTRODUCTION

The abundant information that has recently become available on a potential plastic behavior of stem cells is still being submitted for critical review. Data and facts are being assessed and confronted by our pre-existing set of notions, concepts and hypotheses, the ensemble that form our opinion. When does our mode of thinking become a biased prejudice, and to what extent does it interfere with the arena of stem cell plasticity? The dictionary definition of perception is *to become aware of something via the sense, as a basic component in the formation of a concept*. We attempt to apply this definition to our current understanding of the developmental potential of stem cells assuming that *something* represents function, *become aware via the sense* represents phenotype, and the *concept* is plasticity.

Information on "peculiar" behaviors of adult cells derived from one system that develop and/or adopt the phenotype of a different tissue imposes quite severe difficulties in methodology and interpretation. If we must rely on a systematic paradigm that fits our way of interpretation of the hierarchical and linear differentiation of stem cells, we need to draw an integrative platform for this behavior. There are several converging notions that shed light on the virtual characteristics of this algorithm. First, the single cell experiments indicate that some adult stem cells can indeed differentiate into a variety of cell phenotypes, most important unrestricted by the embryonic layer partition. Eager efforts are being directed to the induction of differentiation of embryonic stem cells [1]. This pattern of behavior is, at least, preserved by some stem cells in the

adult. Second, our way of analysis of the system relies on phenotypic characterization of the cells, a possible source of bias. Great difficulties are encountered in analyzing systems with intrinsic heterogeneity, fluctuating phenotypes and non-deterministic variables. These consist primarily of markers with unknown function at the singular level, for which we have even less insight into their function in a multi-factorial system. Third, the cell fate is directed in part by signals received from the environment, in particular injury signals for regenerative biology in the transplant setting. These signals likely include both inductive and restrictive elements, tightly concerted to regulate a particular physiological process. In our search for molecular mechanisms that trigger pivotal events, in this case differentiation pathways of stem cells, we frequently forget that a single action potential in a neuron is the integrative result of multiple inputs. Lessons from the nervous system also have taught that this highly specialized and sophisticated system operates in an inhibitory mode, while the excitatory cells inherently initiate activity.

Plasticity has caught our interest and imagination, as evident from the large number of original research manuscripts and the equal number of reviews/points of opinion on this topic. Here is one more that addresses the coupling between phenotype and function, in analogy: brain and mind. It is perhaps the enthusiasm preceding the discovery of a great unknown that may shake the ground of our prior notions of cell development and differentiation. The bounding limits of the perception of stem cell plasticity are a-priori antagonism, based on the deviation from the common and prevalent wisdom (consensus), and fanatic want-to believe, based on our limited understanding of biology. In between lays the healthy skepticism and critical review.

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IN SEARCH FOR THE HEMATOPOIETIC STEM CELLS

The hematopoietic compartment is a developmental system extensively investigated over the last 4 decades [2], with significant applications to clinical bone marrow transplants. In search of the definition of the hematopoietic stem cells (HSC), a series of phenotypic characterizations have been suggested. In mice, the putative HSC that reconstitute hematopoiesis in myeloablated recipients fall largely in the $c\text{-kit}^+ \text{lin}^- \text{Sca-1}^+ (\text{K}^+\text{L}^-\text{S}^+)$ [3-5], $\text{Thy-1.1}^{\text{lo}} \text{Lin}^{-/\text{lo}} \text{Sca1}^{\text{hi}} (\text{T}^{\text{lo}}\text{L}^-\text{S}^+)$ [6-8], $\text{CD34}^+ \text{lin}^-$ [9-12] and $\text{CD34}^{-/\text{lo}} \text{lin}^-$ [13,14] populations. Essential roles of $c\text{-kit}$ and SCA-1 in the development of normal hematopoiesis have been demonstrated in knockout mice [15-17]. Other definitions have used functional markers of the putative HSC, in their ability to exclude Rhodamine 123 and Hoechst 33342 dyes [18-20], and possess high activities of aldehyde dehydrogenase [21-24]. Exclusion of the Hoechst 33342 dye was used for flow cytometric definition of a side population (SP) enriched in stem cells, which have a predominant phenotype of $\text{K}^+\text{L}^-\text{S}^+$ [25,26]. Other enrichment techniques have used counterflow centrifugal elutriation (CCE) and lineage depletion to obtain a population of small blasts [21,27,28]. All these cells were convincingly shown to possess the capacity of robust, multilineage and long-term reconstitution (LTR) of the lympho-hematopoietic system in myeloablated recipients. *The first conjecture is that multiple bone marrow-derived stem and progenitor cells (HSPC) with variable phenotypes is endowed with hematopoietic reconstituting potential.* In adopting concepts of stem cell behavior from the hematopoietic system we must consider the possibility that HSC may be not the pluripotent stem cell residing in the bone marrow, as emphasized by its expression of several fate-commitment markers [29]. So far, $\text{T}^{\text{lo}}\text{L}^{-/\text{lo}}\text{S}^+$ stem cells were found to have little capacity to generate cell types other than hematopoietic [30]. There are however populations of cells in the bone marrow that can derive various cell types [31-42]. We will refer to these bone marrow-derived stem cells as the bone marrow stem cell compartment.

HETEROGENEITY OF HEMATOPOIETIC STEM CELLS

Heterogeneity within the stem cell compartment has been a constant struggle for biologists. A recent review echoed the problem: "Investigators studying plasticity will need to assess findings of plasticity in light of the definition commonly accepted to define stem cells – that is, demonstrate that single cells derived from adult tissue differentiate into multiple lineages characterized not only based on phenotype but also on function and support robust, sustained and functional multilineage engraftment *in vivo*" [43]. The challenge was addressed in single cell transplant experiments, not only to achieve homogeneity but also used to quench the difference between plasticity and trans-differentiation. This is without ignoring the concern that single cell experiments may yield different results than population studies that may be more physiological [44].

Single cell transplant experiments demonstrating durable multilineage reconstitution of lympho-hematopoiesis in

myeloablated hosts are perhaps the most convincing source of evidence on the phenotype of putative HSC. Such experiments have shown that one $\text{K}^+\text{L}^- \text{CD34}^-$ cell [13], one $\text{K}^+\text{L}^-\text{S}^+ \text{CD34}^{\text{lo}/-}$ cell [14] and one $\text{K}^+\text{T}^{\text{lo}}\text{L}^-\text{S}^+$ cell [30] can reconstitute hematopoiesis. Failure to repopulate all the recipients has been attributed to an estimated homing efficiency to the host bone marrow of 20%, the only site that supports sustained and multilineage hematopoiesis. Cells with strongest dye efflux activity (Tip-side population) and a phenotype of $\text{K}^+\text{L}^-\text{S}^+ \text{CD34}^-$ engrafted in almost all myeloablated hosts [45]. Single $\text{K}^+\text{L}^-\text{S}^+$ and K^+L^- cells were shown to regenerate hematopoiesis and incorporate into injured muscles with appearance of muscle phenotypes [46,47]. The apparent common phenotype of single cells with hematopoietic reconstituting potential is $c\text{-kit}^+ \text{lin}^-$.

In other single cell transplants, small blasts were shown to possess a much wider differentiation capacity [41]. In this study transplantation of one low-density CCE lin^- bone marrow-homed cell was found to reconstitute hematopoiesis and generate a variety of epithelial tissues. These LTR cells expressed low-to-undetectable levels of Thy-1 , Sca-1 , $c\text{-kit}$ and CD34 , and showed high activities of aldehyde dehydrogenase [21]. Other studies showed that the low-flow CCE fraction (without proved bone marrow homing capacity) contained all the phenotypic combinations of $c\text{-kit}$ and SCA-1 positive/negative cells, and implied that the $\text{K}^+\text{L}^-\text{S}^-$ cells are more primitive than the $\text{K}^+\text{L}^-\text{S}^+$ cells [48]. Thus the only common phenotypic denominator of these successful single cell transplants is low or absent expression of lineage markers. The absence of consensus on the definitive phenotype of HSC seems irrelevant, once the hematopoietic reconstituting potential of all these phenotypes was demonstrated experimentally at the single cell level. *The second conjecture states that at the single cell level the only available common phenotype of LTR cells is low or absent expression of lineage markers.*

THE INTRINSIC NATURE OF STEM CELL HETEROGENEITY

The homogeneity achieved by single cell experiments is short lived. Stem cells may divide symmetrically and asymmetrically, to yield daughter cells with variable degrees of common and different properties. In selected cases, short (2-3 days) *in vitro* culture of single murine $\text{K}^+\text{L}^-\text{S}^+ \text{CD34}^{-/\text{lo}}$ cells with SCF and TPO resulted in hematopoietic reconstituting capacity of cells that divided once similar to that of cells that did not divide [14]. If this result was the case of a symmetric division, we would expect the daughter cells to share similar engraftment potentials and phenotypes. For a strict definition of symmetric division, as a process that yields two identical daughter cells by all means, the chances for such an occurrence are quite low, and the probability of asymmetry is rather high. As elegantly delineated, asymmetry may evolve from differential gene expression, asymmetric partition of cytosolic factors and/or unequal allocation of membrane-associated molecules [49]. Thus, even if the hematopoietic reconstituting potential of the daughter cells is equal, variable engraftment efficiencies may evolve because of uneven distribution of the cell surface molecules. For example, failure of single cell experiments to yield hematopoietic reconstitution of all the recipients has

been attributed to a limited bone marrow-homing efficiency of the cells [13,14,30,41]. In most cases, one asymmetric division of a stem cell may yield two daughter cells with distinct phenotypes, as well as different functional, self-renewal and differentiation potentials [49-51]. Single stem cells lost their hematopoietic reconstituting potential with addition of more stimulating factors and with prolongation of the culture time [14]. In most culture conditions that are currently available, *ex vivo* incubation or expansion is usually associated with loss of engraftment potential and extensive changes in phenotype [52]. *The third inference is that the heterogeneity of proliferating and differentiating stem cells is in fact a path of developmental plasticity.* This is commonly expected to occur within each differentiating system, while developmental diversity beyond a particular tissue, organ or system is evidence of pluripotency. This conjecture suggests that plasticity is one of the intrinsic properties of stem cells, and heterogeneity becomes less well dissociated from plasticity [53], in part because the promiscuous gene expression patterns in hematopoietic stem cells [54,55].

THE POTENTIAL OF PLASTICITY EXISTS

Studies of nuclear transfer have demonstrated that the nucleus of embryonic, fetal and adult somatic cells can reconstitute the genetics of an entire organism in mammals [56,57]. Nuclei of “terminally differentiated” mature B lymphocytes transferred into enucleated oocytes, and embryonic stem cells (ESC) inserted in blastocysts were shown to develop into adult monoclonal mice. The unlimited potential of the nucleus should be dissociated from the technical and biological difficulties associated with this system [56]. This line of studies may be interpreted as a demonstration of the competence of the genetic material within the nucleus of a somatic cell to sustain wide organogenesis. An example of reprogramming at the gene level is modulation of globin expression when adult HSC were placed in various environments [58,59]. Wide differentiation potential of adult cells is often considered aberrant if it deviates from the linear pattern of terminal differentiation. If terminal differentiation is not the rule for adult somatic stem cells, as demonstrated by nuclear transfer [57] and introduction of cells into blastocysts with chimeric representation of these cells [60-62], then pluripotent potential is a “normative” rather than “aberrant” mode of differentiation. *We can draw the fourth inference, that under appropriate conditions the nucleus of adult, mature, formerly termed “terminally differentiated” somatic cell is endowed with the potential to express the full genome required for development of an entire organism.*

FLUCTUATIONS IN STEM CELL PHENOTYPE

Without the ability to select cells according to their phenotype, much of the work could have been not performed. Yet, how accurate or binding are the phenotypic definitions? Skepticism on the definitive phenotype of stem cells has been already stated: “You can’t judge a stem cell by its cover: perhaps the only infallible traits of stem cells are their robust proliferative capacity and their ability to self-renew” [29]. Although the phenotypic clues are important for

description of various cell subsets, stringent adherence to phenotypic definitions may be not the optimal approach.

Within highly purified HSC populations, phenotypic and functional heterogeneity has been demonstrated at multiple levels. The expression of putative HSC markers changes widely during ontogenesis, differentiation, migration, activation and in culture. One prominent example is the debated expression of CD34 [63-65]. Determination of the kinetics of CD34 in mitotically- and functionally-quiescent versus activated cells has been hampered by the lack of understanding of the function of this molecule. Fetal hematopoietic stem cells are considered to be CD34⁺ [66,67], and murine HSC convert from the CD34⁺ to the CD34⁻ phenotype at 7-10 weeks of age [68]. There is solid evidence that CD34 demarcates engrafting cells derived from the adult bone marrow [9-11,69,70], CD34 is dispensable in the phenotypic characterization of HSC [12,21,71-73] and murine LTR cells are within the CD34^{-/lo} fraction [13,26,73]. The evidence points to the presence of murine and human LTR and short-term repopulating (STR) HSC in both CD34⁺ and CD34⁻ subsets [63,65,74,75].

Reversibility of CD34 expression in murine K⁺L⁺S⁺ HSC has been demonstrated in experiments where secondary hosts were successfully engrafted with CD34⁻ cells harvested from primary recipients of (5-fluorouracil-stimulated) CD34⁺ cells [73,76]. Similar data were shown for human cells [77,78]. These data imply that loss of CD34 expression is associated with long-term repopulating activity of HSC [13,26]. Consistently, a small subset of AC133⁺ CD34⁻ CD38⁻ lin⁻ with progenitor activity equivalent to that of CD34⁺ CD38⁻ lin⁻ cells was shown to acquire expression of CD34 during HSC development [74]. Human and rhesus bone marrow-derived SP CD34^{-/lo} cells acquired expression of CD34 in long-term stromal culture [26], and human CD34⁻ CD38⁻ lin⁻ cells upregulated CD34 in culture upon initiation of growth [79]. Furthermore, expression of mRNA encoding for CD34 has been variably detected in CD34⁻ HSC populations with proven STR and LTR activities [12,13,21,63]. Similar to CD34, another putative stem cell marker c-kit has been brought into question by studies demonstrating hematopoietic reconstitution with c-kit⁺ [13,14] and c-kit⁻ cells [80-82]. C-kit is an essential factor in the evolution of hematopoiesis [15,16], that is downregulated in the bone marrow cells by 5-FU treatment [82], and its expression is modulated at different stages of hematopoiesis [48,83].

One way to organize phenotypically-defined cells according to their repopulating activity is to assume that CD34⁻ cells are more primitive than CD34⁺ cells and thus are more “pure” stem cells [12,13,26]. LTR cells can support hematopoiesis for more than 4-6 months in myeloablated recipients, and STR cells are radioprotective and can repopulate blood for several weeks [84,85]. For example, murine T^{lo}L^{-/lo}S⁺ [7,8] and CD34⁺ [63] cells have both STR and LTR activities. Other murine lin⁻ cell populations characterized by CD38⁺ [86], K⁺S⁺ CD34^{lo} CD38⁺ [87], low density CCE [84], CD34⁻ [13,14,63] and human CD34⁻ [26,68,69] are restricted to LTR activity, while K⁺L⁺S⁺ CD34^{lo} CD38⁻ and high-density CCE cells are restricted to STR activity [13,84,87]. Thus, according to a hierarchic organization CD38⁺ cells should be more primitive and self-renew better than CD38⁻ cells within the CD34⁺ subset [87].

A similar interpretation was proposed for putative stem cell markers, such as c-kit, in the low-density CCE cell population with LTR activity, despite the extensive phenotypic variability of these cells [88]. Within the CCE K⁻L⁻ subset, SCA-1⁻ cells are considered to be more dormant than SCA-1⁺ cells, and precede them in the HSC maturation pathway [48]. Based on these functional and phenotypic definitions, it was hypothesized that LTR cells proliferate slowly and generate late and sustained engraftment, assumed to be the "true" stem cells.

These experimental approaches are of cardinal importance towards the characterization of stem cell activation, function, self-renewal and differentiation. They provide however little insight into the exact phenotype of the stem cells, which possess varying degrees of self-renewal and differentiation potentials [63,87-90]. Conclusively, functional activation of the putative hematopoietic stem cells is accompanied by reversible expression of CD34 and c-kit [14,73,76,83,86]. The function-phenotype coupling is not extinct; it may be just premature for our state knowledge to draw stringent lines of differentiation. Recent work suggests that stem cells are continuously changing their phenotype at higher frequencies than we used to think. *The fifth conjecture suggests that the bone marrow stem cell compartment includes many types of stem cells. In conditions of functional activation and growth, the phenotypes of stem cells are dynamic.*

CELL CYCLE DEPENDENCE OF THE STEM CELL PHENOTYPE

Stem cells, previously considered to be divisionally-quiescent [20,91], are continuously or intermittently cycling [92,93]. An early model of development suggested that HSC are out of cycle most of the adult life, and at times one or few cells are recruited to generate hematopoiesis, with few others replacing them to sustain steady state in the stem cell pool [94,95]. An apparent contradicting finding was the random entry into the cell cycle of ~10% of the putative HSC per day in adult mice, and virtually all cells cycling within 3 months [96,97]. Within the heterogeneous compartment of bone marrow stem cells it is possible that cell fates are determined in both stochastic and deterministic modes. In the stochastic model of development there are limited frequencies of HSC self-renewal [98]. As evident from the single cell experiments, a first self-renewal division is absolutely necessary towards generation of multiple lineages within the hematopoietic system and more tissues out of this system [13,14,30,41,45-47]. The biological clock set by the progressive shortening of telomeres upon cell proliferation would allow a replicative span of ~100 divisions [90]. High activities of telomerase may prevent telomere shortening and expand the life-span of stem cells by preventing loss of the proliferation potential [99,100]. In between the slow cell cycles, there is compelling experimental evidence that stem cells spend most of the time in a cycle-quiescent state [101-106]. Cells residing in G₀/G₁ are considered to be dormant, such as the majority of CD34⁺ CD38⁻, c-kit^{lo} CD34⁺ CD38^{-/lo} and CD34⁺ HLA-DR⁻ cells.

Different subsets in the bone marrow stem cell compartment are positioned in phases of the cell cycle at

variable rates. These analyses however were performed on bulk samples at one point in time, thus little information is available on the kinetics of stem cell cycle. It is estimated that ~8% of the HSC engage in cell division every day, and they should perform a number of symmetric and asymmetric divisions to keep the number of HSC in steady state [107,108]. Most cells enter the cycle within 1-3 months [92,97], the frequency of entry in cycle increases in progenitor cells and with age, such as the majority of HSC in aged mice are cycling [96,108]. Compared to 70% rhodamine 123-bright cells positioned in G₀/G₁, virtually all (97%) Rho123^{lo} T^{lo}L^{-/lo}S⁺ HSC were in G₀/G₁ [109]. Modulation of CD34 was found to relate to the cell cycle in culture (79). GM-CSF-mobilized human CD34⁻ cells expressed the CD34 antigen within 2 days from initiation of growth, and did not express this molecule if remained quiescent. Consistently, CD34⁺ precursors lost CD34 expression in culture if they remained in G₀, and could regain expression of CD34 antigen upon initiation of growth. These data suggested that CD34 antigen is an early indicator of proliferation [79], yet it is uncertain if CD34⁻ cells divided before or after upregulation of CD34 [77].

During transit along the cycle stem cells change in their capacities of engraftment [110,111], generation of progenitors [112], display of adhesion molecules [113] and gene expression [111]. The progenitor/stem cell inversion states that phenotypic changes occur during transit of cells in cycle, with temporary loss of engraftment potential in the progenitor phase, despite a doubling in cell number [112]. The engraftment potential is thought to recur prior to cell division. These inversions contribute to the marked heterogeneity within the stem cell compartment, with transient phenotypic changes that persist for variable periods of time, and are likely related to the stability of the proteins. On the one hand, the engraftment of murine T^{lo}L⁻S⁺ in G₀/G₁ was markedly superior to that of cells in the S/G₂/M phase, suggesting that engraftment is related to the cell cycle phase of the cells [109,114]. On the other hand, comparison of 1-3% of the Hoechst SP population of cells that were in S/G₂/M phase of the cell cycle to cells in G₀/G₁ revealed equivalent reconstitution capacities [25], suggesting that cell cycle position is not a principal determinant of engraftment and hematopoietic potential [109,115]. These views are not necessarily contradicting, since the cells may have advanced in cycle at variable rates [93].

Divisional quiescence is a relative term, with cells engaging in slow cycling presenting differential capacities to engraft [92,96,111,116]. A cell cycle-dependence is also invoked to affect the efficiency of nuclear cloning [117]. Cell cycling is in fact an open frame condition when the chromatin is exposed and offers permissive conditions for gene expression [118]. During cycle the cells may experience an exogenous stimulatory signal or removal of an inhibitory signal and progress to a differentiation mode, while undisturbed cells complete the cycle in a mode of self-renewal [49,51]. This was elegantly exemplified by equal hematopoietic reconstituting potential of cells that divided once in culture and cells that did not divide, and the progeny of both cells successfully reconstituted hematopoiesis in secondary recipients [14]. From the functional standpoint, engagement in fast cycle does not necessarily imply loss of engraftment and stem cell potential [93,116]. In the case of

human candidate HSC it has been suggested that slowly dividing cells have higher proliferative potential than do rapidly dividing cells [49,90]. In fact, cells that progress towards a commitment to differentiate enter a stage of fast proliferation, during which large numbers of transiently amplifying cells are generated [29]. From the phenotypic standpoint, we must accept the possibility that cells may undergo quite extensive changes in marker expression that do not directly affect their engraftment, differentiation and proliferation potentials. *The sixth conjecture states that if phenotype fluctuates with the cell cycle, perhaps many phenotypes attributed to different stem cells are in fact transiently displayed by one cell.*

HOW MUCH IS THE PHENOTYPE A BIAS?

The phenotype evolves as a dynamic characteristic of the cells with limited relationship to their function or developmental potential. The differentiation is viewed by some as a linear and hierarchic pattern of development, accompanied by gradual and progressive acquisition of functional and phenotypic characteristics [107,119,120]. If the restricted activities of phenotypically-defined stem cells are perceived as a developmental commitment that not only directs, but also limits the differentiation potential of these cells, one must adhere to the stringent rules of hierarchical and linear differentiation (Fig. 1). It is not a matter of semantics, but a way of thinking: are stem cells functional units, physical entities, or both. The community has experienced such a transition in thinking with demonstration of the allowed transitions between particles and waves. Now we accept that transitions between these conditions are allowed and both are best referred to as energy.

In previous sections we quoted evidence of phenotypically-defined cellular entities within the bone marrow stem cell compartment that perform the task of hematopoiesis. Characterization of these cells at one time point represents a phenotypic singularity within the differentiation, proliferation and functional status (Fig. 2). Beyond this point, the phenotype of these cells changes without obvious relation to function, as far as our experimental capacity reaches out. On the one hand there are numerous singular phenotypes of LTR-HSC, on the other hand the cues on the phenotypic changes that accompany cell development are variable and ambiguous. With further technological advances, it will hopefully be possible to characterize at precision the phenotypes of various cell functions [121]. At this stage, the only harm of the phenotype-function coupling is the confusion between functional states and phenotypic entities. While we adhere to the requirement that a stem cell is recognized by its functional ability, our perception of plasticity is blurred by the phenotype [122-124]. The foggy perception may be an intrinsic limitation even if very stringent rules are being applied to assess the phenomenon of plasticity [125]. We submit that a definitive stem cell phenotype is characteristic of a stationary state, which has little relevance to the functional potential of the cell. In between the definition of phenotypic markers and assessment of the stem cell function, there are likely numerous changes in gene expression.

Interpretation of the biological system of stem cells is in evolution. From the phenotypic perspective the potential mechanisms of stem cell plasticity may occur sequentially or concomitantly [107,126-128]. If a stem cell is endowed with pluripotent differentiation potential, it can generate multiple stem cells, which we define by our way of observation rather

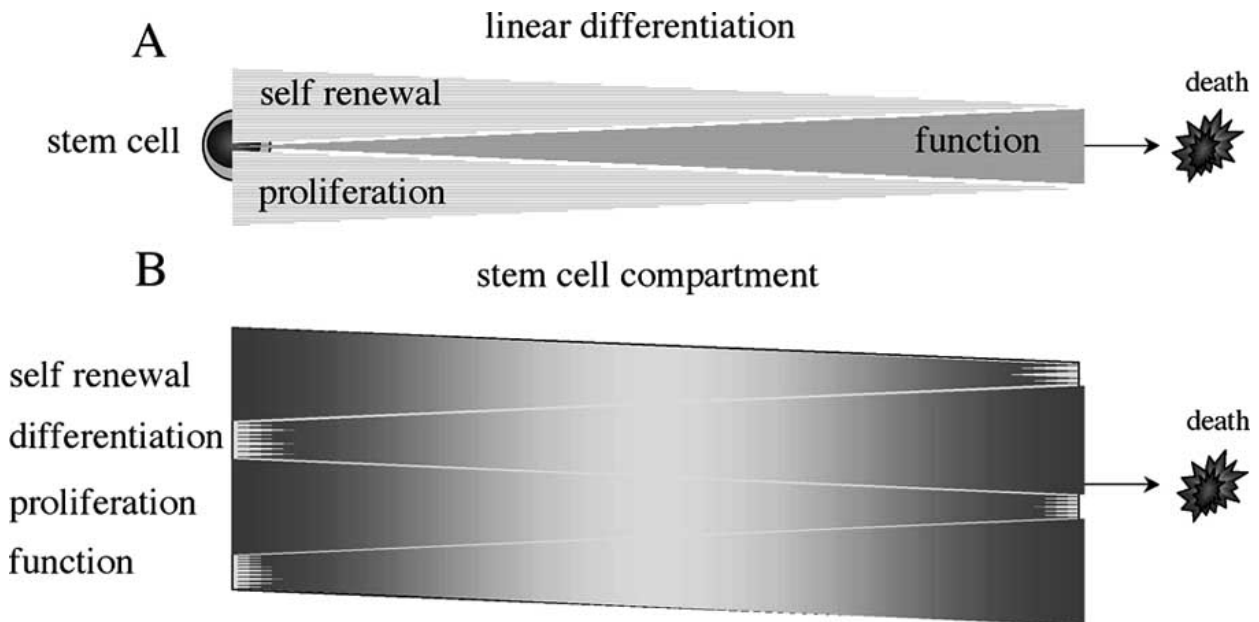


Fig. (1). A. Stem cell as a singular entity in a developmental program. In functional terms the stem cell is expected to undergo a differentiation program that is accompanied by finite capacities to self-renew and proliferate. Engagement in the differentiation program is irreversible and the only possible outcome for the mature cell is death upon extinction of its functional potential. **B. The stem cell compartment/state.** If differentiation is not a programmatic pathway, a stem cell has many options in modulation of various parameters. It is difficult, and perhaps impossible to determine phenotypic and functional singularity within the heterogeneous stem cell compartment.

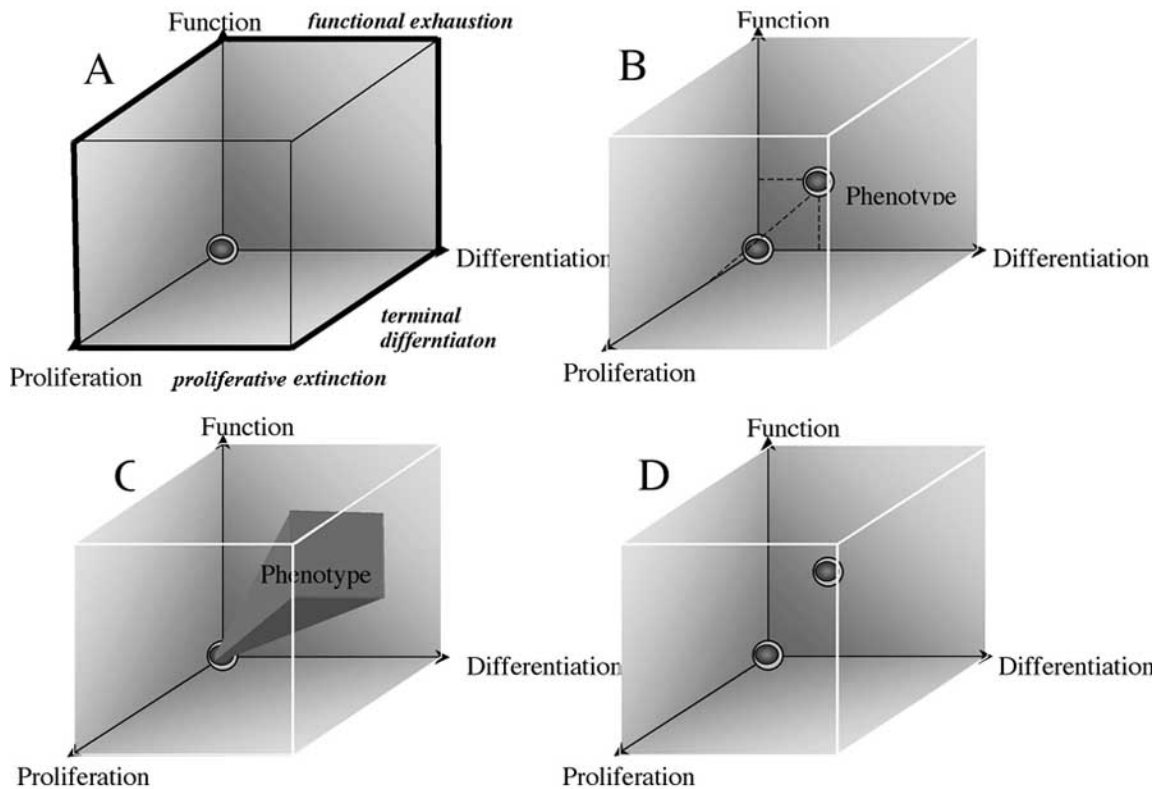


Fig. (2). Limitations of the phenotypic-functional characterization of stem cells. A stem cell in a virtual resting state is non-cycling, undifferentiated and functionally quiescent. A. In a three dimensional imaginary space, it should be located close to the origin. The limiting conditions of this space are terminal differentiation, proliferative extinction and functional exhaustion. B. A linear evolution of differentiation and proliferation should advance the cell along a definitive path that determines its function and a concrete phenotype. C. Evolution of functionally competent stem cells (for example hematopoietic repopulation) places the cell at a 3D position with multiple phenotypes and functions. This delineates a functional-phenotypic flexibility, as exemplified by the varying phenotypes of short- and long-term hematopoietic reconstituting cells. D. Unlimited flexibility should allow a cell to occupy any differentiation and proliferation position without imposing a definitive path to achieve this functional/phenotypic state. With identification of distinct developmental pathways to achieve this position, it is likely that many of them will lead to equivalent functional states.

than the pattern of stem cell behavior. If some phenotypic characteristics of the developing cell persist, we may term the switch in differentiation as trans-differentiation. Likewise, loss of phenotypic markers will be interpreted as de-differentiation. The capacity of a healthy and potent stem cell to fuse with other cells is indeed a distinct pattern to disguise in a different phenotype [129], yet there is insufficient evidence to determine whether the nucleus of the original stem cell is reprogrammed.

INTRODUCTION OF EVOLVING CONCEPTS IS DIFFICULT AND SLOW

The hierarchical model of differentiation thought to guide the cell throughout development, is perhaps a derivative of linear interpretation. At the one end we all recognize the capacity of the fertilized ovum, to initiate and sustain the formation of a viable, functional and fertile organism. At the terminal end, cells succumb by senescence, apoptosis or necrosis. That is, if a cell assumes a state of terminal differentiation, the only possible outcome is death, even if it will await the entire life span of the organism. These may be

taken as the points of agreement. Along the process of development, the stem cells become much less defined entities. The prevalent classification suggests a series of transitions that define the potency of the stem cell, from totipotent, through pluripotent and multipotent, to omnipotent (organ-specific) [119,120]. The fact that all these terms employ the term “potent” acknowledges the basic fact that all cells possess the genetic material for universal organogenesis within the particular organism. Emergence of the classical dogma of hierarchic differentiation states “the concept of stem cell plasticity, which holds that the lineage determination of a differentiating stem cell may not be rigidly defined, but is instead flexible” [107]. The alternative trails a hematopoietic stem cell may undertake, i.e. self renewal, differentiation, migration and apoptosis [120], are now recognized to occur on a single time axis, with stem cells performing all these tasks in partially reversible modes until the fatal process of apoptosis occurs (Fig. 3).

Rigidity of the developmental process is being questioned: “A classic perception of stem cells is that they are undifferentiated cells, making them pliable to adopt various different cell fates. The realization that stem cells can

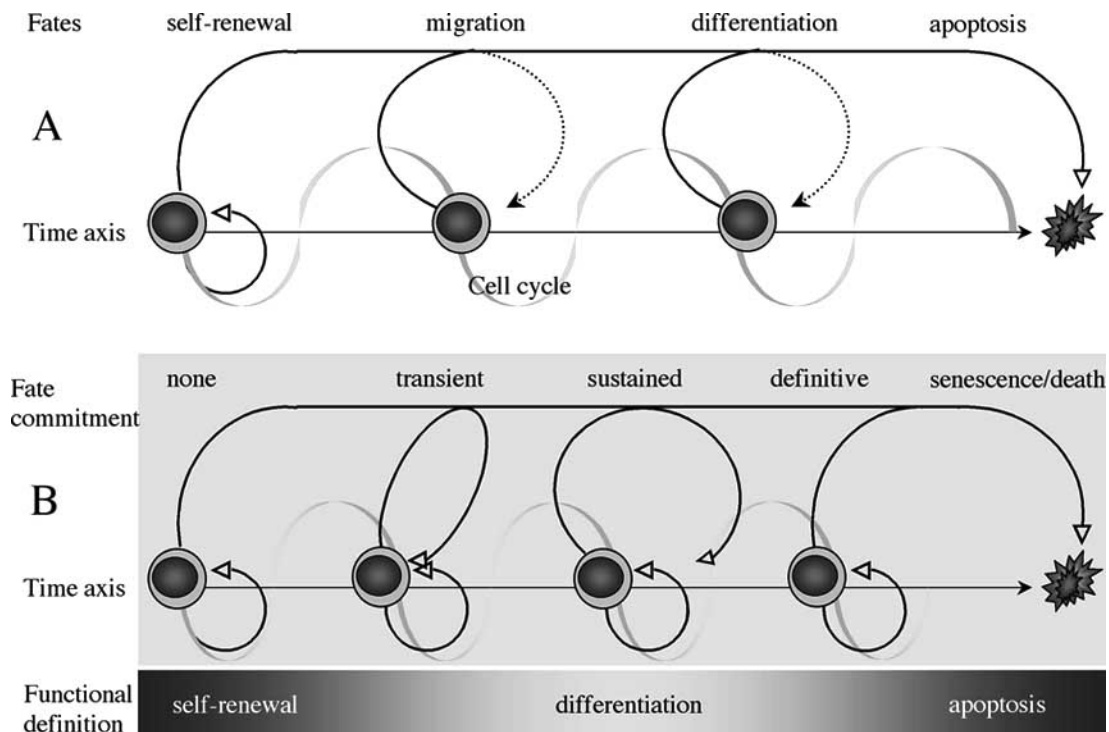


Fig. (3). A. Linear representation of cell fates along the time axis. At certain points of the cycle the cells may undergo through several alternative fates along the time axis. The first option is to perform a slow cycle of self-renewal during which few or none differentiation markers are expressed, and the cell returns to its potent position of the maternal stem cell. Some of the trails may be reversible, except death. **B. Linear representation of stem cell fate commitments.** Along the time axis of a cell’s life it performs recurrent cycles and differentiation tasks. The option of self-renewal is preserved at all times. At certain positions in cycle the cell can engage in differentiation, transient, sustained or definitive, which are accompanied by multiple changes in phenotype (gray box). Regression of the cell from a more differentiated state to an undifferentiated configuration may not completely reverse the phenotypic changes. If the cell engages in a definitive fate commitment, the only path it can follow is towards senescence and death.

masquerade behind morphological and biochemical features normally attributed to differentiated cell types has revolutionized our view of what stem cells look like and where to find them” [29]. The stem cell propensity was suggested to decrease along differentiation, rather than the cell crossing an irreversible threshold: “A stem cell is not necessarily a specific cellular entity, but rather a function that can be assumed by numerous diverse cell types” [130]. Further, the concept of the stem state proposes: “Cells may enter a stem state reversibly, and thus stemness is a state rather than a cellular entity. The molecular basis of this stem state is yet to be defined, but it seems to entail a promiscuous gene expression pattern” [55]. In this model the pluripotent potential originates from the plastic behavior of cells that may undertake various development and differentiation traits [131]. If de-differentiation and trans-differentiation are developmental options, any cell may enter the stem state at various points in its life cycle.

Function of the cells under stipulated conditions of fusion and plastic differentiation evolves as a highly dynamic process that is difficult to define without reference to the site where the cell develops, the injury it responds to and its cellular companions. Fusion of stem cells with somatic cells does indeed occur in selected systems at low frequencies [132-139]. This is not the only mechanism of plasticity [129,140-143]. An additional level of confusion is

the payoff between intrinsic cellular factors and exogenous stimuli [29,144]. In order to respond, the cells must express the appropriate receptors, and the sequence of exposure to inductive stimuli is of prime importance [145]. The nature and extent of injury have to be considered and analyzed within the multitude of signals originating from any damaged tissue [145].

CONCLUDING REMARKS

Our perception of stem cell development is confronted by stipulations grounded both in experimental findings and their interpretation. The complex process of cell differentiation includes many steps, some of which are transient, some of which are definitive. In the early stages of differentiation, it is still a stem cell, yet we do not know if commitment restricts its pluripotent capacity. The hierarchical model postulates that once engaged, commitment is above all other developmental possibilities and will advance the cell to complete the differentiation program. In other words, programmatic development is a terminal fate of the cell. Stem cells may not be required to complete the program and some cells may deviate due to incidental signals to which they are able to respond. These are the trans-differentiation, de-differentiation and reprogramming processes. In its state of stemness, the cell is

endowed with a certain pluripotent potential and procures one or several developmental options. Plastic cell behavior, irrespective of its potential to self-renew and proliferate, may be the adequate definition of the stem function.

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