Asymmetry of Stem Cell Fate and the Potential Impact of the Niche

Observations, Simulations, and Interpretations

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Abstract

Asymmetric cell division is a common concept to explain the capability of stem cells to simultaneously produce a continuous output of differentiated cells and to maintain their own population of undifferentiated cells. Whereas for some stem cell systems, an asymmetry in the division process has explicitly been demonstrated, no evidence for such a functional asymmetry has been shown for hematopoietic stem cells (HSC) so far. This raises the question regarding whether asymmetry of cell division is a prerequisite to explain obvious heterogeneity in the cellular fate of HSC.

Through the application of a mathematical model based on self-organizing principles, we demonstrate that the assumption of asymmetric stem cell division is not necessary to provide a consistent account for experimentally observed asymmetries in the development of HSC. Our simulation results show that asymmetric cell fate can alternatively be explained by a reversible expression of functional stem cell potentials, controlled by changing cell–cell and cell–microenvironment interactions. The proposed view on stem cell organization is pointing to the potential role of stem cell niches as specific signaling environments, which induce developmental asymmetries and therefore, generate cell fate heterogeneity.

The self-organizing concept is fully consistent with the functional definition of tissue stem cells. It naturally includes plasticity phenomena without contradicting a hierarchical appearance of the stem cell population. The concept implies that stem cell fate is only predictable in a probabilistic sense and that retrospective categorization of stem cell potential, based on individual cellular fates, provides an incomplete picture.

Index Entries: Stem cell fate; niche; asymmetric division; mathematical model; simulation analysis.

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Classical Concepts of Stem Cell Self-Renewal and Differentiation

Beside the production of a continuous output of terminally differentiated cells, the ability to maintain their own population of undifferentiated cells is one fundamental characteristic defining tissue stem cells. Frequently, this capability is referred to as self-renewing potential. Although it is not the only criteria to define tissue stem cells *(1–3)*, the explanation of the self-renewing potential has always played a common role in stem cell biology. A number of conceptual approaches has been proposed to explain the self-renewing/differentiation duality of stem cells *(4–8)*. A very frequently cited concept to explain stem cell self-renewal is *asymmetric cell division* (Fig. 1A). This concept suggests that a stem cell on division always generates one stem and one differentiated daughter cell. By this means, a continuous output as well as a stable stem
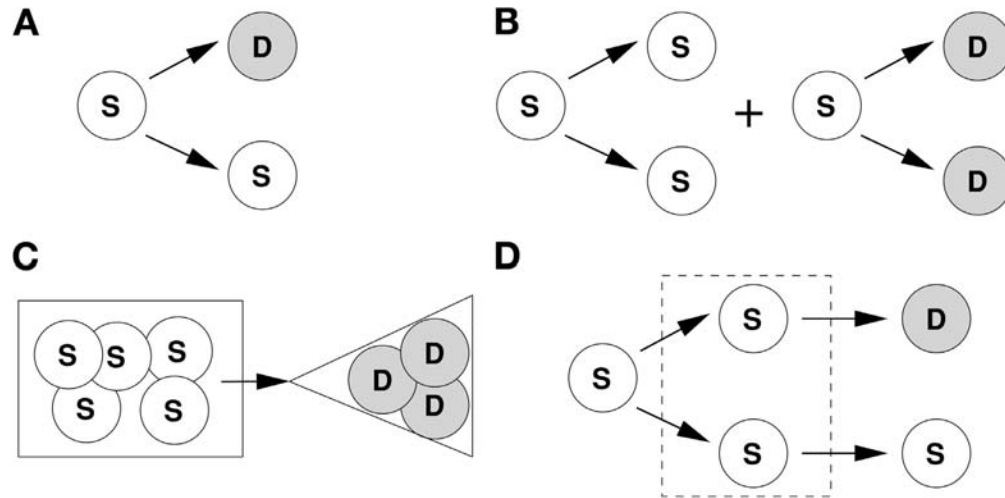
Fig. 1. Classical stem cell concepts. **(A)** Asymmetric stem cell division, generating one stem (denoted by S) and one differentiated (denoted by D) daughter cell. **(B)** Symmetric stem cell division, either into two stem or two differentiated daughter cells. **(C)** Clonal succession, with a fixed pool of stem cells, which successively releases cells to form clones of differentiated cells. **(D)** Symmetric stem cell division together with an independent process of differentiation.

cell population is guaranteed. However, this process by itself does not realize a true self-renewal potential because neither the stem cell number nor a specific stem cell property can ever be *renewed* if it has previously been lost (e.g., in case of injury or stem cell depletion). In other words, asymmetric cell division does not allow a dynamic regulation of the production rates for stem and differentiated cells and it is therefore more appropriate to denote the resulting process as self-maintenance *(8)*. To overcome the inflexibility of constant self-renewing and differentiation rates, two further possible types of stem cell division, namely *symmetric division*, either to produce two identical stem cells or two identical differentiated daughter cells, have been proposed (Fig. 1B). By changing the particular rates one can induce growth or reduction of the stem cell population. Using this type of division scheme (which might additionally include also asymmetric division) and its regulation, true self-renewal of the stem cell pool with respect to cell numbers can be achieved. However, self-renewal with respect to cellular properties, such as lineage commitment or proliferative potential is still not possible because differentiation is perceived as an irreversible one-step process. It should be noted that with respect to the average behavior on the population level, a system based on fixed equal rates of symmetric self-renewing and differentiating divisions is identical to a system based on asymmetric division only. A conceptually different option to describe a life-long production of differentiated cells is the clonal succession theory, which has been proposed by Kay (Fig. 1C; *9*). It postulates a restricted division potential of each cell. Consequently, the existence of a dormant (nonproliferating) stem cell reserve pool is required. Kay hypothesized that this reserve pool is formed during embryogenesis. On demand, individual cells from this reserve can be activated to initiate the production of a differentiated clone.

If the process of differentiation is considered to be independent of cell division (suggested e.g., in *[4,7]*), all the previously described approaches can be summarized into one common theoretical concept, which is assuming only one type of symmetric self-replicating division and a differentiation process (Fig. 1D). Because differentiation can be induced for none, one, or both daughter cells, this concept includes all possible combinations of symmetric and asymmetric cell divisions. Also the clonal succession approach is captured by this description if stem cell amplification is restricted to a specific phase of development, i.e., embryogenesis. Relaxing this restriction and allowing for self-replicating (symmetric) divisions within the stem cell reserve pool throughout the whole life, as proposed by Abkowitz et al. *(10)*, both concepts are identical.

All the concepts described so far rely on a homogeneous population of stem cells. As it became obvious that most stem cell populations are heterogeneous, it was necessary to incorporate some degree of substructure into the conceptual description. Therefore, the assumption of differentiation to be a singular event was altered into the description of differentiation as a multistep process. In other words, stem cells gradually gain a differentiated phenotype while they gradually lose their stem cell potential. The majority of these theories share the concept of a developmental hierarchy of tissue stem cells implying the irreversibility of the differentiation process (c.f. *[2,8,11]*).

Microenvironment Dependence of Stem Cells: the Niche Concept

Although the outlined concepts model several different developmental choices of stem cells, they do not explicitly describe a mechanism that controls this choice. Different possibilities, such as a pure stochastic decision, a cell intrinsically predetermined program, or a cell external control have been proposed and are still under debate. Currently, it is widely accepted that stem cell fate is at least partially dependent on environmental signals. One specific concept is that of a stem cell niche as a particular growth environment consisting of
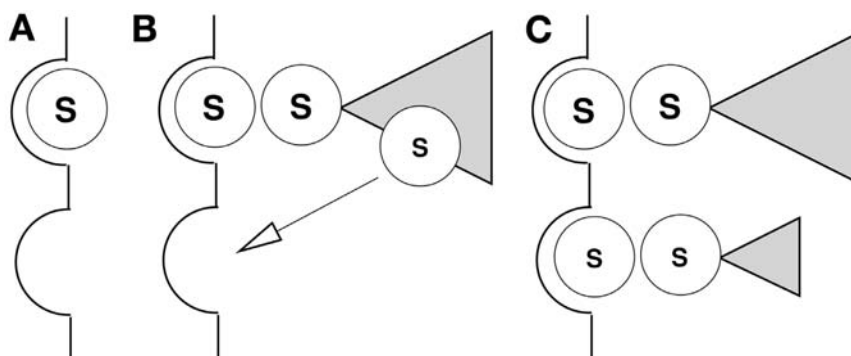
Fig. 2. Stem cell niche concept (after *[2]*). **(A)** Stem cell in the niche. **(B)** If dividing, one daughter cell leaves the niche, initiating a clone. It is still possible for cells which have already undergone few differentiating divisions to reoccupy empty niches. However, the proliferative potential (size of produced clones) of these cells is already reduced **(C)**.

different (e.g., stroma) cell types and extracellular matrix components. Schofield first proposed the niche concept for hematopoietic stem cells (HSC) in the late seventies *(see* Fig. 2; *12)*. It distinguishes different developmental fates of a stem cell, depending on the microenvironmental signals it receives. In its original form the niche concept represents a unidirectional differentiation model combined with the assumption that HSC reside in a specific microenvironmental niche. The proliferation of cells that do not receive niche signals is tied to an irreversible differentiation process. In contrast, cells that do receive niche signals divide symmetrically without differentiating. However, whereas one of the daughter stem cells remains in the niche, the other has to leave it. In this sense, the division process might also be regarded as asymmetric with respect to the spatial arrangement of the daughter cells. If there are empty niches, stem cells have the potential to re-enter these niches, which would prevent further differentiation. Still, according to the paradigm of an irreversible unidirectional differentiation process, proliferative potential lost during one or more differentiating divisions cannot be regenerated.

The assumption of an environment induced differentiation arrest has also been adopted, for example, by Muller-Sieburg and Deryugina *(13)*. These authors assume a differentiation block of HSC, explicitly induced by their direct attachment to stroma cells. The proposed mechanism is the occupation of receptor molecules by the attachment process, which otherwise, could receive and transmit differentiation signals.

Although originally suggested for the hematopoietic system, stem cell supporting microenvironments (i.e., niches) have been characterized for other tissue systems, such as epithelial (intestinal crypts, hair follicles) or neural stem cell systems. For a review on different stem cell niches and their signaling components it is referred to Li and Xie *(14)*.

A generalization of the idea of different signaling contexts is the concept of *actual* and *potential* stem cells, which was originally proposed for the intestinal crypt by Potten and Loeffler *(1,7)*. Actual stem cells are exposed to appropriate signals, inducing them to actively perform self-maintenance (or even self-renewal) and differentiation. In contrast, potential stem cells are not exposed to appropriate signals. However, they have the same potential and are able to turn into actual stem

cells if these are lacking. These authors, furthermore, suggested the possibility for stem cells to regain previously lost self-renewal potential under certain circumstances, such as regeneration after injury. In these situations, potential stem cells that have already undergone a number of differentiation steps are still able to take the job of depleted stem cells, e.g., by occupying their place within the appropriate growth environment (stem cell niche). Beside the fact that differentiation and cell division are regarded as independent processes, this concept is the first, which explicitly assumes a potential reversibility of stem cell development.

Experimental Evidence for Asymmetric Cell Division

The self-renewal/differentiation duality in stem cell function obviously requires a certain degree of asymmetry in the fate of stem cells. Although this asymmetry in the development of stem cell progeny can be demonstrated experimentally, the knowledge of the mechanisms inducing this fate asymmetry is still incomplete. One possibility to explain asymmetric stem cell fates is obviously *asymmetric cell division*. There are a number of systems where asymmetry in the division process has been observed. Prominent examples are germline stem cells in *Drosophila* ovary and testis. These cells are attached to specific cap (ovary; *15–18*) and hub (testis; *19–21*) cells. Following division, one daughter stem cell remains attached, whereas the other loses direct contact. It has also been demonstrated that this process is accompanied by an active control of spindle orientation during mitosis *(see* e.g., ref. *21)*. Other examples for an asymmetry in the cell division process are germline stem cells in *Caenorhabditis elegans* and *Drosophila* neuroblasts *(22–25)*. For detailed reviews on asymmetric cell division mechanisms in invertebrates the reader is referred to Knoblich *(26)* and Kaltschmidt and Brand *(27)*.

For vertebrates and in particular for mammalian systems, asymmetric cell division has explicitly been demonstrated only for a small number of specific cell types, such as neural and epidermal stem cells. In these systems, the different developmental fates have been shown to correlate with asymmetric protein segregation and with alteration of spindle orientation during mitosis *(28–30)*. A common feature of both systems is that asymmetric fate is closely linked to physical location and

microenvironmental structure: neural stem cells (e.g., in the subventricular zone of the lateral ventricle) are attached to ependymal cells at the ventricular surface *(31)*. If, on division, one daughter cell loses direct contact, it is free to migrate and to differentiate into a neuron. Similarly, embryonic (day E12.5) epidermis stem cells normally form a single-layered structure, allowing for direct contact with the basement membrane. In contrast to membrane-parallel cell division, this contact is lost in case of divisions perpendicular to the basement membrane (induced by altered spindle orientation). The daughter cell, which is now no longer attached to the basal membrane, then gives rise to a committed suprabasal cell *(30)*. Another example where the microenvironmental structure plays an important role in the determination of cellular fates is the intestinal crypt. In this system, the stem cells are believed to be located at the bottom of the crypt, tightly linked to so called Paneth and mesenchymal cells *(32,33)*. On division a proportion of the daughter cells move upwards, providing a supply of new functional cells in the villus, which grow out of the top of the crypt. During this process the cells change their phenotype, including the loss of proliferative activity.

In all these systems, a strong correlation of spatial arrangement and stem cell fate is visible. However, it is not clear, whether the dislocation of a daughter stem cell from niche components is a system-controlled process intended to induce differentiation. It might also be the case that the dislocations simply emerge as a result of the physical tissue structure *(34,35)* subsequently leading to differentiation. The key difference between these explanations is that in the second perspective, a stem cell does not have to "know" its developmental direction prospectively. It acts as a stem cell (i.e., taking one of two alternative developmental fates) because it is directed by its microenvironment. Clearly, the cells still need to have the general potential to react on the altered signaling context, induced for example by spatial dislocation.

Asymmetric Stem Cell Fate in the Hematopoietic System

Although the hypothesis of a hematopoietic stem cell (HSC) niche has been around for more than 20 yr *(12)*, only recently members of a well-defined subpopulation of osteoblastic cells, the spindly-shaped N-cadherin osteoblastic cells (SNO), have been explicitly proposed as potential components of the HSC niche *(36)*. Primitive HSC are able to attach to SNO cells using two specific adherence junction molecules, *N*-cadherin and β-catenin. In such an attached state, the HSC are kept dormant (i.e., noncycling) and are able to preserve their primitive phenotype. These observations, together with the fact that the binding of stem cells to specific stroma cell types is promoting the maintenance of long-term repopulating potential in vitro *(37,38)*, suggest that in the hematopoietic system it is also the microenvironment and the spatial arrangement of a stem cell niche that influences the developmental fate of HSC. On the other hand, there is no experimental evidence for an asymmetric cell division in HSC, induced by mechanisms such as alteration of spindle orientation or asymmetric protein distribution during mitosis.

Although no asymmetric division mechanism can be identified explicitly, cell division events of hematopoietic stem or progenitor cells are frequently categorized as *symmetric* or

asymmetric. This categorization is made on the basis of different criteria, such as proliferation activity or lineage contribution of the daughter cells, applying retrospective analyses of either cell division trees, e.g., by using time-lapse videos, or colony-forming assays of twin cells by separate plating of the daughter cells immediately after division *(39–42)*. For example, if the two daughter cells are both giving rise to a colony within a defined period of time, the corresponding cell division (which generated the two daughter cells) is denoted as symmetric. If, as another example, the daughter cells give rise to progeny of different lineages (e.g., one producing erythroid and the other myeloid precursor cells), the cell division is denoted as asymmetric. However, what is actually characterized by this notation is the cellular fate, rather than the type of division. The observable asymmetry in cellular fate is interpreted as the consequences of an asymmetric cell division. If this interpretation is correct, it should be possible to identify asymmetries within the cell division events. Herein, it is not sufficient to describe an arbitrary asymmetry to prove that differences in cellular fate are determined by asymmetric cell division. It is almost beyond doubt that no cell division event is absolutely symmetric. By looking long and hard enough, one would eventually discover asymmetries with respect to some transcription factor, cell surface receptor, or other molecular components. To determine a candidate molecule, characterizing an asymmetric division, inevitably requires the proof of a *functional asymmetry*. This means that the asymmetry causally determines the differences in cellular fate.

These considerations raise the question as to whether the assumption of asymmetric cell division is a prerequisite to explain the experimentally observed asymmetry in the cellular fate of HSC. In our work, we have applied a mathematical model of hematopoietic stem cell organization *(43)* to test the hypothesis of whether the experimentally described asymmetry in the fate of HSC can be explained without the assumption of asymmetric cell division. This mathematical model, which is based on self-organizing principles, has already been demonstrated to consistently describe a broad variety of experimental phenomena in the murine system *(43–45)*. A brief outline of the model is presented in the next paragraph.

Self-Organization, Phenotypic Reversibility, and Cellular Fate

As discussed, classical concepts of stem cell organization tightly link cell division with the decision on self-renewal/maintenance and differentiation. That means, on division a stem cell explicitly "knows" somehow the fate of its daughter cells. In contrast, our approach strictly avoids assumptions that require a direct or indirect predetermination of cellular fates and, therefore, does not require inherent stem cell "knowledge." Cells are characterized by functional properties, which can reversibly change within a range of given options. Induced by such a phenotypic reversibility, cells are able to react flexibly on specific cell–cell and cell–microenvironment mediated signals. As a consequence, the system behavior selects some of the cells by virtue of the properties, such that these cells actually fulfill the criteria of stem cells (i.e., the simultaneous maintenance of the stem cell populations and the production of differentiated cells). In contrast to the perspective of a predetermined stem cell identity, this self-organizing view allows to predict stem cell fates only in a
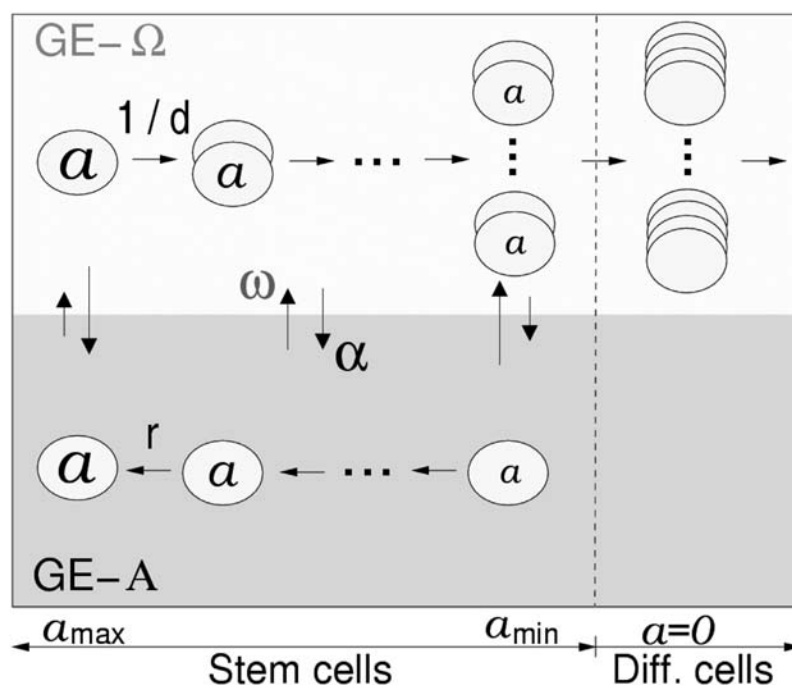
Fig. 3. Schematic representation of stem cell model (reprinted from ref. *43* with permission from International Society for Experimental Hematology). Cells are assumed to reside in one of two growth-environments GE-A or GE-Ω. Proliferation is only possible in GE-Ω. Whereas affinity *a* decreases by factor *1/d* per time step in GE-Ω, it increases by factor *r* per time step in GE-A. The actual quantity of *a* is depicted by different font sizes within each cell. If *a* has fallen below a_{min} the cell has lost its potential to switch to GE-A (represented by empty cells: *a = 0*) and is called differentiated. Transition between GE-A and GE-Ω occurs with intensities α and ω.

probabilistic sense. Applying these general principles to the hematopoietic system, we particularly consider stem cells to be able to receive and interpret signals induced by two different growth environments, denoted as GE-A and GE-Ω. The current state of a cell is characterized by its actual membership to either GE-A or GE-Ω, its position in the cell cycle, and by an affinity (*a*), which determines the propensity of a cell to reside in GE-A. Whereas cells in GE-A are assumed to be nonproliferating, cells in GE-Ω are actively dividing. All cell divisions are assumed to be symmetric, i.e., both daughter cells are identical to their mother cell. Furthermore, while exposed to the signaling context of GE-Ω, cells will gradually lose their affinity *a*. This process is characterized by the differentiation coefficient *d*. The loss of affinity *a* can be regained when cells come under the influence of GE-A. Here, affinity *a* is incrementally increased (regeneration coefficient *r*) up to a maximum value a_{max}. GE-transition, i.e., the change of a cell from GE-A to GE-Ω and vice versa, is modeled as a stochastic process. The transition intensities (probabilities of GE-change per time step) are assumed to be dependent on the individual affinity *a* of a cell and on the total number of stem cells in the target GE. If the affinity of a particular cell has fallen below a threshold a_{min}, it is set to zero. This implies that the cell cannot change to GE-A and, therefore, cannot regain *a* anymore. Whereas all cells with *a > 0* are denoted as stem cells, cells with *a = 0* are called differentiated. Figure 3 provides a schematic representation of the model. For further details it is referred to Roeder and Loeffler *(43)*.

Although this perspective of stem cell organization does explicitly preclude asymmetric cell divisions, it still accounts

for asymmetric cell fates. This asymmetry, however, is not predefined, but emerges as a result of different cell–cell and cell–microenvironment interactions. Interpreting self-renewal as the generation of cell progeny with identical functional potential as the original cell, this is achieved in the self-organizing model through the use of a two-stage process rather than by a singular event, such as asymmetric division. To illustrate this, let us have a look at an example cell with initial affinity a_1. If this cell divides under the influence of GE-Ω, it generates two identical daughter cells. However, owing to the fact that completing a cell division takes a certain time, the affinity changes to a new value a_2 (with $a_2 < a_1$) for both daughter cells. If one of the daughter cells now changes to GE-A, subsequently regaining its affinity to the starting value a_1, whereas the other daughter cell continues to decrease *a* (staying in GE-Ω), we finally arrive at cells with different properties: one with the initial affinity a_1 and one (or more) with a new affinity a_3, with $a_3 < a_1$. The asymmetric fate of the two daughter cells generates the situation of self-maintenance, i.e., maintaining a cell identical to the original cell with respect to affinity *a*. The situation of true self-renewal (i.e., amplifying the number of cells with the original functional potential) is achieved whenever both daughter cells regenerate their affinity up to the initial value a_1. Finally, symmetric differentiation is obtained in the case that none of the daughter cells changes to GE-A (*see* Fig. 4 for a graphical illustration).

Depicting cellular fates at the clonal level (Fig. 4, lower panel), clearly points to the potential for (mis-)interpreting symmetric and asymmetric cellular fate by means of different
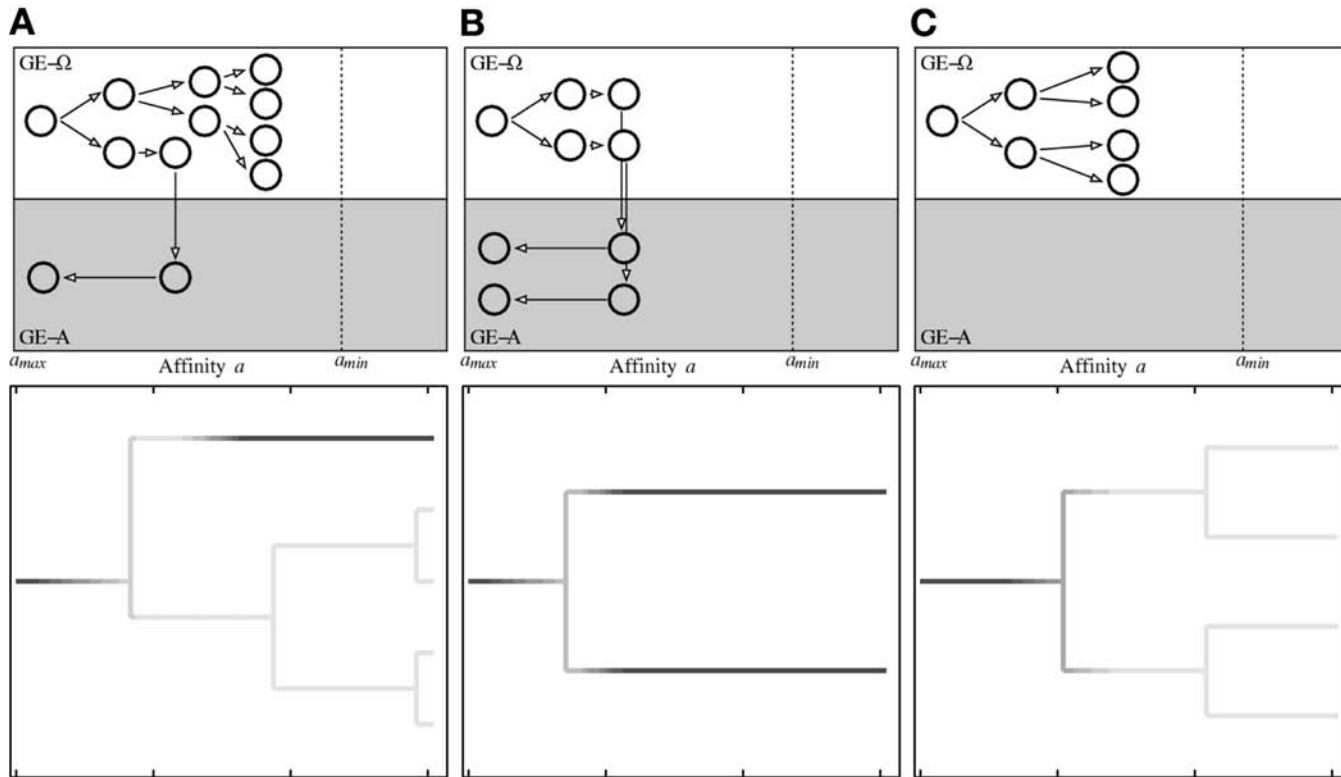
Fig. 4. Self-renewing and differentiating stem cell fates. The three panels illustrate the realization of asymmetric **(A)**, symmetric self-renewing **(B)**, and symmetric differentiating **(C)** stem cell fates in the context of the self-organizing stem cell model. Upper graphics show schematic representations of clonal development with respect to the two model growth environments and affinity a. Lower graphics give the corresponding clone tree representations (cell fate over time) with gray-scale coding of actual affinities a (dark gray: high a, light gray: low a).

division types: If a particular division event is retrospectively categorized as symmetric or asymmetric purely based on the state (e.g., the actual affinity a) of the daughter cells at a certain point postdivision, then the clone tree could easily suggest a causal relation of developmental fate and division, although no asymmetry in the division process was involved.

Quantitative Description of Cellular Fate

In the last section the possibility for a consistent explanation of asymmetric cell fate without assuming asymmetric division was described at a qualitative level. It is now demonstrated that it is also possible to quantitatively explain experimental results on asymmetric stem cell fate within the context of the self-organizing stem cell model. To do so, the earlier outlined mathematical model is applied to a particular type of stem cell asymmetry, which has been described experimentally by Punzel and colleagues *(41)*. In particular, these authors analyzed the in vitro cell cycle activity of human cord blood cells. They sorted individual CD34$^+$/CD38$^-$ cells into 96-well plates, which were previously coated with either bovine serum albumin (BSA), fibronectin (FN), or a specific stroma cell line feeder layer (AFT024). Using the membrane dye PKH-26 and time-lapse fluorescence microscopy, the authors kept track of the division history of each individual cell within a culture period of 10 d. A cell division is defined as asymmetric if one of the

first-generation daughter cells did not divide during the culture period, whereas the other daughter cell performed at least one division. To quantify the occurrence of asymmetric cell divisions, the authors used the percent of cells that show asymmetric division with respect to all cells deposited (denoted as AD index). The experiments demonstrated that about one fourth to one third of the plated cells showed asymmetric division activity (AD = 22.9 % for BSA cultures, 22.8 % for FN cultures, 31.1 % for AFT024 cultures). This data also suggest that stroma coculture (AFT024) is able to increase the asymmetric behavior.

To simulate these cell cultures within our model, several individual systems are initiated with single cells. The model parameters are based on previous simulation studies and are given in Appendix A. After model initiation with a single cell, the system is traced for 10 d according to the experimental protocol. Whenever only one of the two first-generation daughter cells is performing further cell divisions within the 10 d period, the division is denoted as asymmetric. To compare simulation and experimental results, the amount of asymmetric division is determined according to the previously described AD index. Owing to the fact that the two-stroma free culture conditions (BSA, FN) did show almost identical results in the experimental situation, we only consider one type of stroma free (BSA) and the stroma-dependent culture settings (AFT024) for comparison with model results.

Table 1
Parameter Dependence of Asymmetric Cell Fate

Initial affinity	Regeneration coefficient	Proportion of asymmetric divisions (%)
$a = 0.1$	$r = 1$	0.0
$a = 0.5$	$r = 1$	11.4
$a = 1$	$r = 1$	30.8
$a = 0.1$	$r = 1.1$	12.4
$a = 0.5$	$r = 1.1$	37.2
$a = 1$	$r = 1.1$	39

Given are the simulated proportions of "asymmetric divisions" (according to the definition specified in the text) for different choices of initial affinity a and regeneration coefficient r. The proportions represent average results from 500 simulation runs for each setting.

A sensitivity analysis of the simulation model showed that the proportion of asymmetric divisions is especially sensitive to the initial affinity a of *in silico* culture initiating cells. The higher the initial affinity values of the cells, the higher the proportion of asymmetric cell divisions (Table 1). Because the affinity directly correlates to the probability of a cell to long-term repopulate a model system, these results can be interpreted such that highly potent stem cells, i.e., cells with high long-term repopulating potential, more frequently exhibit an asymmetric cell fate compared with cells with a lower long-term repopulating ability.

The experimental results, showing that coculture of CD34$^+$/CD38$^-$ cells with AFT024 stroma cells is enhancing the number of asymmetric divisions, can be explained in terms of the model by differences in the regeneration coefficient r. Whereas $r = 1.0$ (which does not allow for a regeneration of affinity a in GE-A) produces low proportions of asymmetric cell fates, $r > 1$ leads to an increase in asymmetric cell fates (Tab. 1). This result shows that the stem cell supporting potential of particular stroma cell types in vitro can consistently be represented in the model by a growth environment (GE-A) that allows for a certain degree of a-regeneration. Moreover, these results suggest that a regeneration supporting GE-A ($r > 1$) can serve as an appropriate description for an in vivo stem cell niche. This interpretation also implies that differences in the stem cell support of various in vitro systems and of (possibly different) stem cell niche microenvironments in vivo might adequately be described by a common quantitative measure. The described effects of varying model parameters are also visible at the clonal level. Figure 5 shows a number of typical examples of *in silico* clones. Significant qualitative differences of the structure and of the heterogeneity of the clone trees are detectable. Particularly, the heterogeneity of cellular fates points to the fact that a (retrospective) classification of the stem cell potential of an individual cell, on the basis of its actually realized fate in a specific situation, can be misleading.

Based on these results, we were able to derive particular parameter configurations that consistently reproduce the published experimental results. The variation of the initial affinity range and the regeneration coefficient leads to a good quantitative fit of simulation results and observed proportion of asymmetric divisions according to the definition giving earlier (Fig. 6). Whereas both simulation scenarios shown in Figure 6

use initial affinities a, uniformly distributed on the interval (0.5; 1), the stroma free situation is described by a regeneration coefficient of $r = 1.00$, in contrast to an $r = 1.05$ for the situation of a stroma supported culture system. It should be noted that the latter r-value is still smaller than 1.1, which is the regeneration coefficient assumed for the description of in vivo situations *(45)*.

Interpretation and Implications

Classically, the experimentally observed heterogeneity of stem cell fate is explained by the assumption of asymmetric cell divisions. However, the observed asymmetries in the fate of hematopoietic progenitor cells should not be confused with a predetermined functional asymmetry in the division process. Our results clearly show that the experimental results regarding the proliferative stem cell activity in vitro can consistently be explained without assuming a specific type of asymmetric cell division. It should be noted that our results are not restricted to this specific experimental situation. It is also possible to explain asymmetries in the lineage specification of multipotent hematopoietic progenitors (as experimentally described e.g., in *[39,40]*) along the same lines of argumentation (data not shown). Although the presented model analysis does not exclude the general possibility of asymmetric cell division, it clearly suggests an explanation, why a functional asymmetry in the cell division process of primitive hematopoietic progenitors has not been demonstrated to date. Besides this alternative explanation of asymmetries in cellular fate, the proposed perspective of understanding stem cell systems as flexible, self-organizing structures rather than a collection of preprogrammed cellular entities incorporates a number of implications.

First of all, the proposed perspective does require a certain amount of reversibility in the differentiation process to generate a sustained heterogeneity of the stem cell population. This heterogeneity is necessary to allow the system to react flexibly in response to particular needs, such as regeneration following injury or enhanced production of differentiated cell output in case of infection. There are a number of experimental results, which provide evidence that stem cells are indeed able to flexibly alter their properties and functionalities. Examples of such phenotypic reversibility of stem cells encompass phenomena of tissue and lineage plasticity *(46–50)*, as well as reversibility in the expression of cell surface receptors *(51)*, cell cycle status *(52,53)*, or engraftment potential *(54–56)* of cells within one specific tissue or lineage.

The assumption of phenotypic reversibility leads directly to a second implication of the self-organization perspective. Although the experimentally described hierarchy of stem and progenitor cells (e.g., regarding their repopulation potential) is also generated by the self-organizing system, there is a marked difference in the nature of this hierarchy compared with the classical interpretation in terms of a unidirectional differentiation process. Whereas the structure of the hierarchy is generated and stabilized by the self-organizing system, the individual components, i.e., the cells, are able to reversibly change between different positions in the hierarchy. In this sense, the stem cell hierarchy is a dynamically stabilized structure with continuously changing properties of individual cells. This can be compared with the situation of the dynamical equilibrium of a chemical (forth and back) reaction. Although
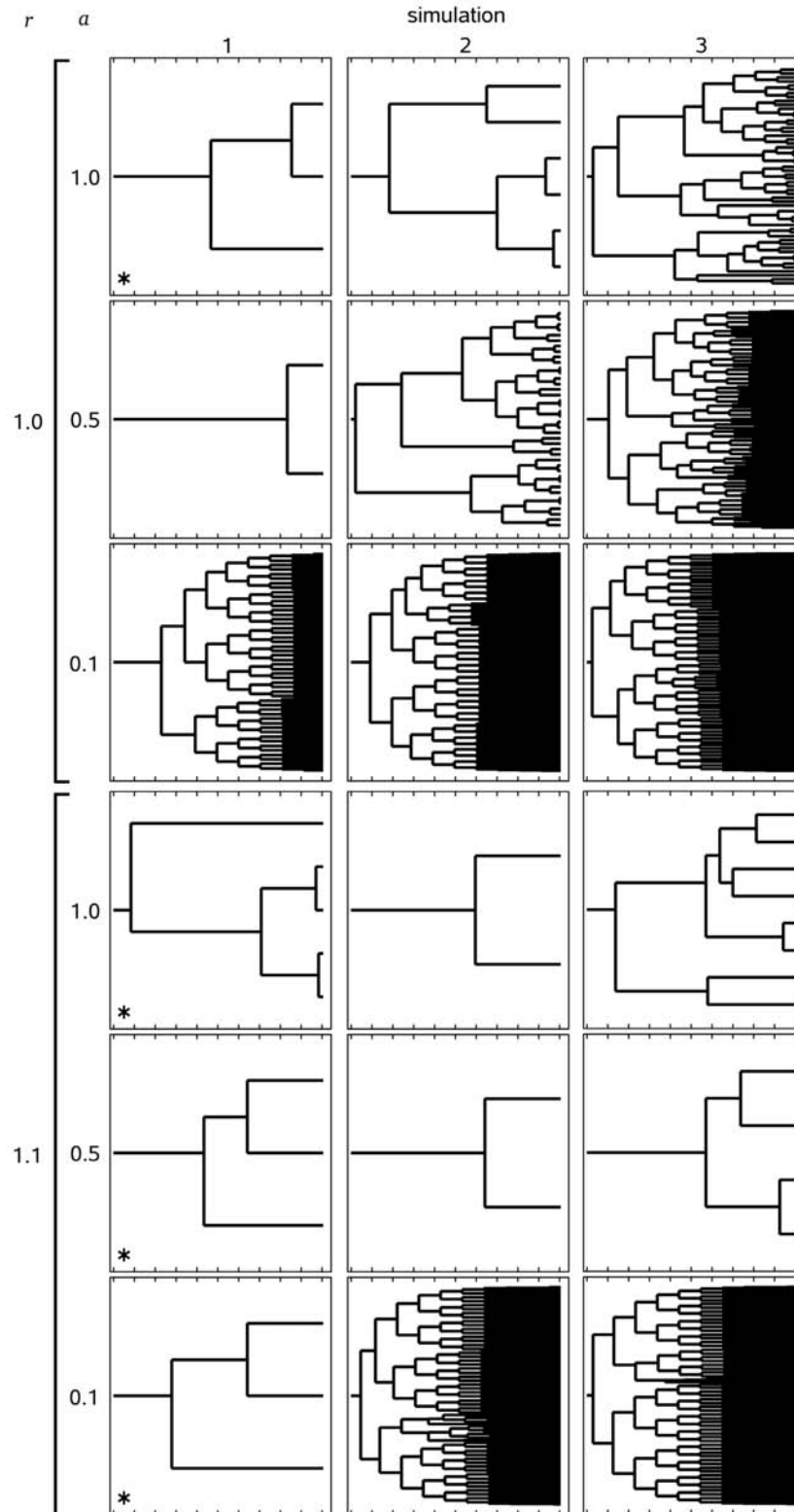
Fig. 5. Individual cell fates. Given are representative simulation examples of individual, single cell induced *in silico* cultures over a 10 day period according to the indicated parameter values (compare also Tab. 1). In contrast to Figure 4, the clone trees simply illustrate the division history, not (color) coding for different affinity values. Clone trees representing "asymmetric divisions," according to the stated definition, are marked by an*.
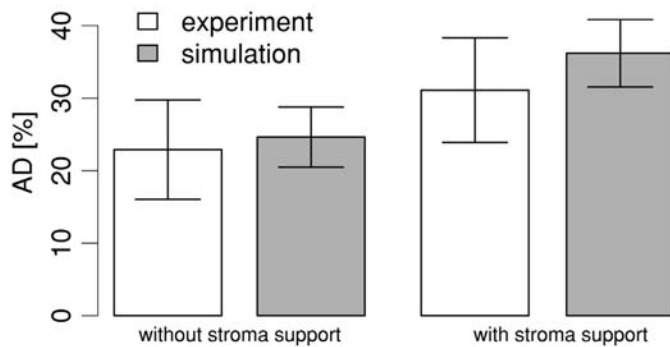
Fig. 6. Comparison of experimental and simulation data. Bars represent the mean percent and the standard deviation for the proportion of asymmetric divisions (AD score). The experimental results (taken from ref. *41*), are based on *n* = 13 independent evaluations of 96-well plates per culture condition. Corresponding simulation results have been obtained by evaluating *n* = 100 *in silico* experiments per setting, each consisting of 96 individual, single cell induced model systems. Culture conditions "without stroma support" and "with stroma support" refer to the BSA and the AFT024 culture as described in ref. *41*.

such equilibrium appear to be stable, there is still a constant flux of individual molecules reacting in one or the other direction. It should be noted that the capability of individual stem cells to fulfill specific functions is not a constant property, but is continuously changing and adapting in response to system requirements. Some cells actually have a larger potential to take over the function of cells at another point in the hierarchy than others. However, this picture might change in another situation. As an example, the loss of highly potent stem cells (at the top of the hierarchy) owing to injury might be compensated by cells, which would not act as repopulating stem cells without the injury event *(57)*. Although not identical, this situation can be compared with a management hierarchy of an enterprise with rather fixed functions and responsibilities, but with changing persons to fulfill these. Although unlikely, it is not impossible for an ordinary but qualified employee to become a leading manager. Of course, this is a rare event; however, it might be more likely under certain extraordinary circumstances. Along these lines, stem cell plasticity can be interpreted as the infrequent realization of an unused potential of cells, which can be made more likely when cells are challenged by certain external events, such as artificial assay conditions or injury.

A third implication of the self-organizing perspective is the importance of cell–cell and cell–microenvironment interactions. Generally, self-organization is characterized by an interaction of individual system components acting according to local rules. These local rules determine the generation of a global system structure without being governed by any other external instruction. Within the proposed stem cell model, the local rules characterize the individual reactions of cells in response to the signals of their actual growth environment. It is the interaction of stem cells and their local growth environment, which induces the asymmetry of cellular development and, by this means, the generation of heterogeneity. Therefore, the local stem cell growth environment has to be regarded as an integral component of the self-organizing concept. In this sense, the model growth environment GE-A can be interpreted as the description of a stem cell supporting niche.

Acknowledgments

Appendix A

Model parameters used for simulating single-cell-induced cultures

Model parameter	a_{min}	initial	τ_c	d	r (without stroma support)	$f_\alpha(0)$	$f_\omega(0)$
	a_{max}	*a* range	τ_S		r (with stroma support)	$f_\alpha(\tilde{N}_A/2)$	$f_\omega(\tilde{N}_\Omega/2)$
			$\tau_{G2/M}$			$f_\alpha(\tilde{N}_A)$	$f_\omega(\tilde{N}_\Omega)$
						$f_\alpha(\infty)$	$f_\omega(\infty)$
						$\tilde{N}_A$	$\tilde{N}_\Omega$
Value	0.01	(0.5; 1)	24	1.03	1	0.5	0.5
	1		8		r = 1.05	0.3	0.3
			4			0.01	0.1
						0.0	0.0
						400	80

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