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A novel dynamic model of hematopoietic stem cell organization based on the concept of within-tissue plasticity

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Objective. **At present, no dynamic quantitative models of stem cell organization are available that fulfill all criteria of the prevalent functional definition of hematopoietic stem cells and, at the same time, provide a consistent explanation of cell kinetic and functional stem cell heterogeneity, reversibility of cellular properties, self-organized regeneration after damage, fluctuating activity and competition of stem cell clones, and microenvironment dependency of stem cell quality. To solve this problem, we propose a new, comprehensive model concept.**

Materials and Methods. **A single cell-based stochastic model is described. It makes the novel concept of within-tissue plasticity operational. Within a range of potential options, individual cells may reversibly change their actual set of properties depending on the influence of the local growth environment. Stochastic switching between the growth environments introduces fluctuations that eventually generate heterogeneity. Extensive model simulations are compared with experimental data.**

Results. **Although stemness is not an explicit cellular model property, the system behavior is consistent with the functional definition of stem cells and explains a large set of experimental observations on stem cell function in vivo and in vitro on the level of cell populations and individual cells. Classic results such as the colony-forming unit spleen assay, as well as recent experimental observations on stem cell kinetics, individual clone tracking, and fluctuating clonal contribution, are discussed.**

Conclusions. **This concept introduces a fundamentally new perspective on stem cell organization treating stemness not as an explicit cellular property but as the result of a dynamic process of self-organization. The model needs to be extended to incorporate lineage specification and tissue plasticity.** **© 2002 International Society for Experimental Hematology. Published by Elsevier Science Inc.**

Hematopoietic as well as all other tissue stem cells are defined by their functional properties. They need to fulfill a set of criteria, including the capability to proliferate, differentiate, self-renew, to reconstitute the entire tissue after damage, and to have flexibility in the use of these options [1–3]. We recently reexamined the definition and concluded that the list should be amended by an additional criterion considering an appropriate growth environment to which the cells may home and by an extension of the flexibility criterion encompassing reversibility and plasticity of the properties adopted by individual cells [4].

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It has been documented that the stem cell population is heterogeneous with respect to cycling activity [5,6], colony-forming ability [7], and phenotypic markers [8]. Correlations of specific phenotypes with the ability for long- and short-term repopulation could be demonstrated [9]. These findings are generally taken as evidence for a developmental hierarchy associated with a gradual but irreversible decline of self-renewing potential [3]. The hematopoietic microenvironment has been shown to play an important role in the regulation of stem cell organization [10–13]. Self-renewal depends on local growth conditions, namely, on the direct contact of stem and stroma cells [14–16]. Moreover, evidence is accumulating that gene or marker expression can be reverted and redirected. Gene expression pattern can be switched from adult to embryonic type and vice versa [17], and specific phenotypic traits show reversible changes [18,19]. The recently de-

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scribed plasticity of primitive precursors demonstrates the potential of somatic stem cells to change their tissue-specific [20,21] or lineage-specific [22] differentiation due to environmental signals. Whereas the term plasticity has been coined to describe the potential of stem cells to alter tissue or lineage characteristics, we introduce the term *within-tissue plasticity* to describe flexibility of cellular genotype and phenotype expression restricted to one tissue or cell lineage. Furthermore, clonal competition phenomena at the stem cell level have been described. Examples of competing cell populations in animal chimeras [23,24] and in human clonal disorders [25,26], as well as fluctuating contributions of individual cell clones [27–29], can be found.

Compared with the tremendous experimental effort to characterize hematopoietic stem cells, little progress has been made to establish a theoretical framework of how stem cells are organized and self-sustained. To our knowledge, there are no worked theories and dynamic models (including our own previous work) that fulfill the criteria of the above-mentioned functional stem cell definition and satisfy the demands for a consistent quantitative explanation of the listed phenomena of cell kinetic and functional heterogeneity, of microenvironmental dependence, and of clonal evolution and competition.

Here, we present a new and comprehensive model concept of stem cell organization, which is able to explain the above-mentioned classes of phenomena consistently. Stemness is not treated as an explicit cell intrinsic property but results as a dynamic system property combining individual cell potential and microenvironmental influence. The model clearly shows that it is possible to explain the observed heterogeneity of hematopoietic stem cells by a self-organizing process based on (within-tissue) plasticity properties without the assumption of a predefined differentiation hierarchy.

Methods

Model description

The basic idea of the concept is a separation of potential cell intrinsic properties and their actual use. Expression and use of these properties develop according to a specific propensity of the cells, which itself depends on the local growth environment. Hence, cellular properties or functionalities that are lost under a certain signaling scheme can be reestablished under the influence of another growth environment. Therefore, self-renewal is considered the result of fluctuating environmental influences rather than a predefined program.

To formalize the model and obtain a mathematical description, we make the following minimal set of assumptions. A1) We assume two different growth environments (GE-A, GE-Ω). A2) Each cell is characterized by two properties, the cycling status c and a property a, which describes the affinity of the cell to reside in GE-A. A3) Residing in GE-A, a cell is assumed to be nonproliferating. In contrast, cells in GE-Ω proliferate with average turnover time τ_c. A4) Whereas cells in GE-A have the propensity to increase affinity a, cells in GE-Ω tend to decrease a. A5) Cells can change from GE-A to GE-Ω and vice versa, with transition intensities (probabilities per time interval) ω and α, which depend on the actual affinity a and the cell numbers in the system. This implies a competition of cells in both growth environments in a stochastic sense. A6) Cellular development with respect to properties a and c is reversible (within-tissue plasticity). Only if a has become critically small is the cell considered to have lost the potential to stick to GE-A. Such cells we call differentiated. A schematic representation of the model is shown in Figure 1A.

With reference to the hematopoietic system, we like to think of two growth environments in the bone marrow where stem cells can either be *attached to* (GE-A) or *detached from* (GE-Ω) specific stroma components, resulting in switches between different patterns of gene expression and activation of signal transduction pathways [30–33]. Differences in the cycling behavior of different stem cell subsets [5,6] and the observation that stroma contact can prevent the loss of repopulating ability [13,14] support this interpretation.

Simulation assumptions

Each cell in the system is described by the following variables: i) attachment affinity a (stem cells: $a_{\min} \le a \le a_{\max}$; differentiated cells: $a = 0$); ii) position in the cell cycle c with $0 \le c \le \tau_c$; and iii) an indicator m specifying the membership of the cell to either GE-A or GE-Ω ($m \in \{A, \Omega\}$). To simulate the development of the system over time, all cells are synchronously updated at discrete time steps ($\Delta t = 1$ hour). Hereby, a cell transition from GE-Ω to GE-A, which is assumed to be possible during G_1 phase only ($c < g_1 =$ constant), occurs with intensity (probability per time interval Δt) α, and from GE-A to GE-Ω with intensity ω, where

$$\alpha = \frac{a}{a_{max}} \cdot f_\alpha \text{ and } \omega = \frac{a_{min}}{a} \cdot f_\omega. \quad (1)$$

Herein, function f_α models the capacity of GE-A to assimilate cells. This capacity decreases with rising numbers of cells in GE-A representing a limited resource of binding sites. Similarly, f_ω represents the cell production demand with low numbers of proliferating cells inducing an activation of dormant cells into cycle. These functions, which are modeled as sigmoid functions of the form $f(x) = 1/(A+B\cdot\exp[C\cdot x])+D$, are characteristic for a genetic background (e.g., different for mouse strains). Furthermore, both functions are modulated by the individual attachment affinity a of the cells in the sense that cells with high a are more likely to change to GE-A, whereas cells with low a tend to reside in GE-Ω (Fig. 1B and C). If cells do not undertake a transitions from one GE to the other in Δt (probability 1-α or 1-ω respectively), they develop inside the actual growth environment according to the following rules:

$m = \Omega$:

$$a(t+\Delta t) = \begin{cases} a(t)/d & \text{if } a(t)/d > a_{\min}, \text{ with } d > 1 \\ 0 & else \end{cases} \quad (2a)$$

$$c(t+\Delta t) = \begin{cases} c(t)+\Delta t & \text{if } c(t)+\Delta t < \tau_c \\ 0 & else \end{cases} \quad (2b)$$

$m = A$:

$$a(t+\Delta t) = \begin{cases} a(t)\cdot r & \text{if } a(t)\cdot r < a_{\max}, \text{ with } r > 1 \\ a_{\max} & else \end{cases} \quad (3a)$$

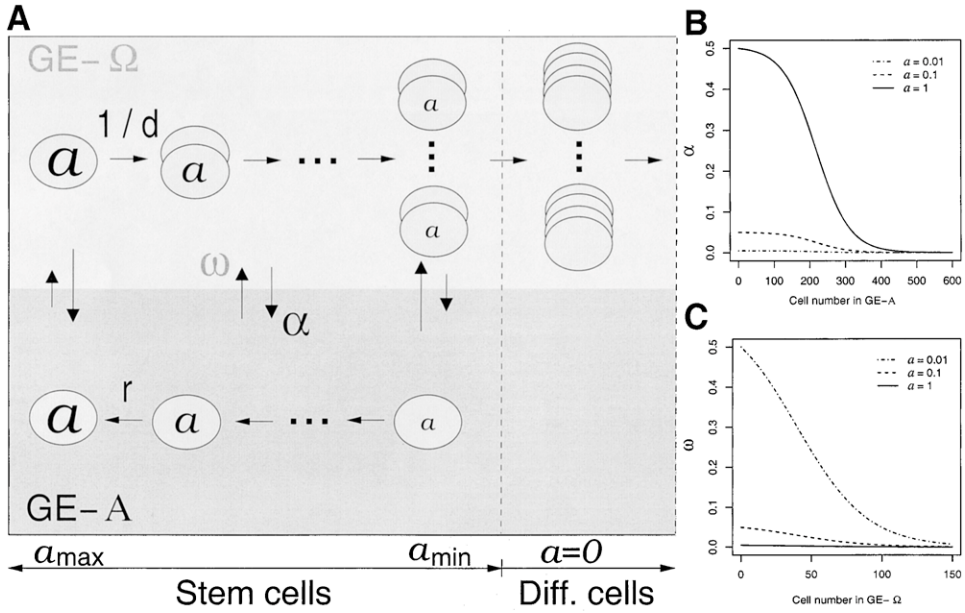

Figure 1. Schematic representation of the model concept. **(A)** The lower part represents growth environment (GE)-A and the upper part GE-Ω. Cell amplification due to proliferation in GE-Ω is illustrated by growing cell numbers (cell groups separated by vertical dots represent large cell numbers). Whereas attachment 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 sketched by different font sizes. If a fell below a critical threshold a_{min}, the cell lost its potential to switch to GE-A and a is set to 0 (represented by empty cells). These cells are called differentiated. Transition between GE-A and Ω occurs with intensities α and ω, which depend on the value of a (represented by the differently scaled vertical arrows) and on the cell numbers in the target GE. **(B,C)** Typical profiles of the transition intensities α and ω for different values of attachment affinity a.

$$c(t + \Delta t) = g_1 = \text{constant}. \quad (3b)$$

Cell division at $c = \tau_c$ is realized by a duplication of the cell, inheriting the actual state of the variables a and m, setting $c = 0$ and initializing τ_c, which in general is assumed to be a random variable. If cells have reached $a = 0$, according to Equation 2a, they are assumed to be founders of maturing clones.

Simulation procedure

To simulate individuals (e.g., mice, cats), the system was initiated with cell populations that are obtained by sampling cell subsets out of pilot simulations. Transplantation settings were simulated by adding cell subsets (sampled from another simulation) to a running system. In the case of colony growth simulation (e.g., colony assays), the system was initiated by a single cell. For each type of analysis, 1 to 100 independent simulation runs with identical parameter sets were performed. Mean statistics were obtained by averaging these individual results. Endpoints were determined by the sampling of cells at given time points and with probabilities deduced from experimental protocols.

The program is implemented in C++ on a LINUX platform. Statistics and graphics were produced using the statistical computing environment R [34].

Parameter choice

Simulation of *in vivo* situations:

- Range for attachment affinity: $a_{min} = 0.01 < a < a_{max} = 1$
- Turnover time τ_c: normal distributed with mean $\mu = 4$ days (mouse, steady state), $\mu = 12$ hours (mouse, regeneration phase, e.g., colony-forming unit spleen [CFU-S assay]), $\mu = 50$ days (cats), and variance $\sigma^2 = 0.1 \cdot \tau_c$
- Transition intensities α and ω: specific class of sigmoid functions (Fig. 1B and C)
- Differentiation coefficient $d = 1.04$; regeneration coefficient $r = 1.1$

Derivations hereof in simulation of in vitro situations:

- Different qualities of stem cell support by culture conditions: choice of $a_{max} \leq 1$
- Transition intensity for cultures without stroma contact: $\alpha = 0 = \text{constant}$

Results

General system behavior

The model is able to produce qualitatively different growth scenarios, including the capacity to fully reestablish the system from one cell and to compensate repeated damages as requested in the functional definition of stem cells. Steady-state (with respect to average cell numbers) situations are dynamically stable and robust against perturbations. System behavior differs qualitatively depending on the rates of losing and regaining attachment affinity a, which are described by the parameters d and r. Whereas small values of d produce oscillations, with the limiting case of indefinite growth ($d = 0$), large

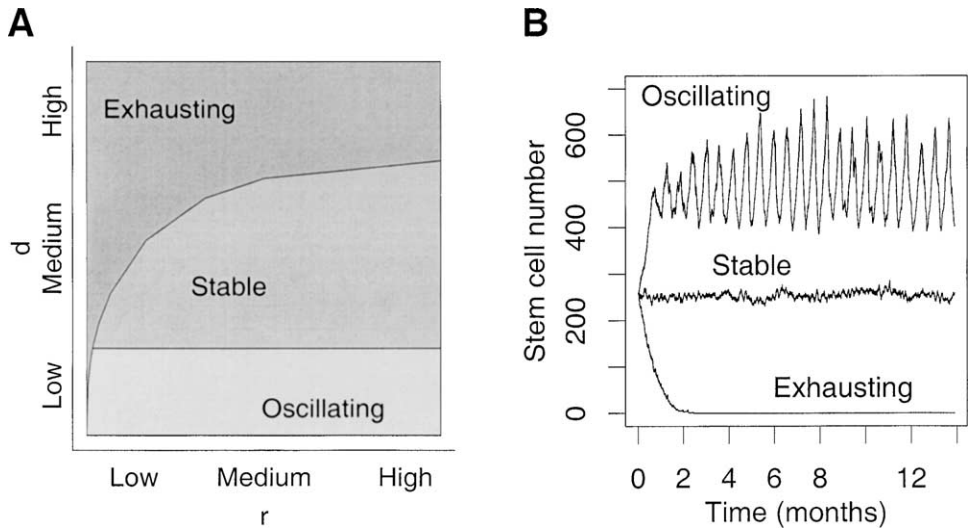

Figure 2. General system behavior. **(A)** Schematic representation of qualitative system behavior depending on model parameters *d* and *r*, which describe the decrease and regain of *a,* respectively. It is possible to distinguish regions of exhausting, stable (with respect to average cell numbers), and oscillating system behavior. **(B)** Representative simulations of stem cell number development: *d* and *r* chosen according to indicated regions in *d*-*r* parameter space of **(A)**; otherwise identical parameter configuration.

values of *d* result in exhaustion (Fig. 2). We speculate that oscillations observed in cyclic neutropenia [35] or in clonal disorders [36] relate to a stem cell alteration of the *r,d* parameter set.

Clonal fluctuation and competition

Investigating chimeric cats, Abkowitz et al. [24] showed that fluctuations in the contribution of two neutrally marked cell populations are especially pronounced shortly after transplantation. We performed simulations varying the number of initiating cells between 10 and 100. The results are similar to the experimental data (Fig. 3A).

Whereas high numbers of initiating cells produce stable chimerism (Fig. 3B), low numbers lead to substantial initial fluctuations (Fig. 3C). In the model, the degree of fluctua-

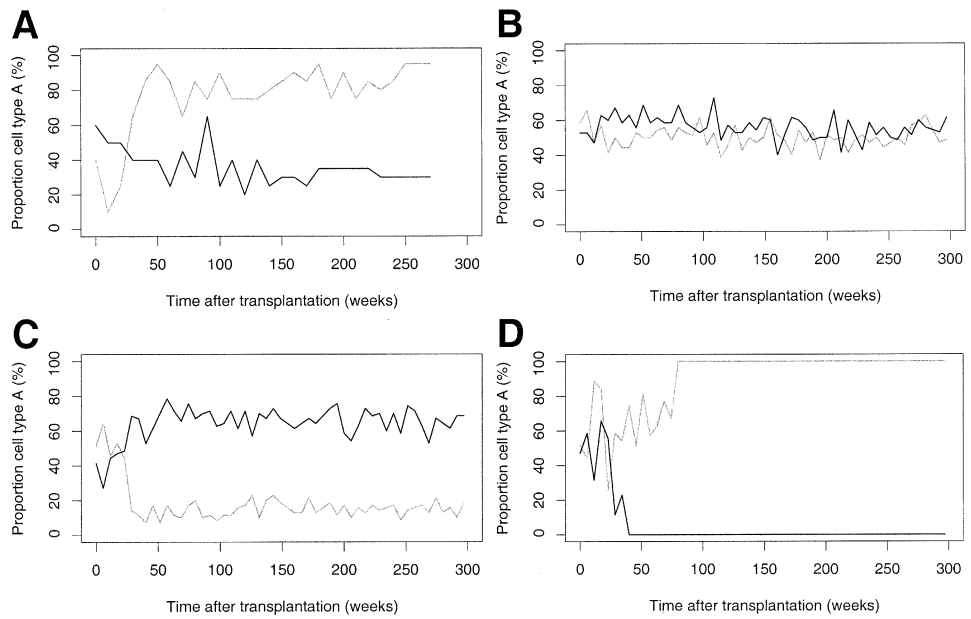

Figure 3. Simulations of clonal fluctuations. **(A)** Two typical time courses (data from [24]) showing the fluctuating contribution of cell type A to blood production after autologous bone marrow transplantation in cat chimeras that contain two differently marked but otherwise identical cell populations, called A and B. Total cell numbers of type A and B always totals 100%. **(B–D)** Representative simulation examples of this scenario for different starting situations. **(B)** Simulations initiated with 100 stem cells from each cell population. Attachment affinity *a* of initiating cells was randomly chosen between 0.1 and 1. **(C)** Like situation B, but number of initiating cells reduced to 10 cells per population. **(D)** Like situation C, using initiating cells with attachment affinities randomly chosen between 0.01 and 0.1.

tions also depends on the initial composition of the transplanted cells with respect to attachment affinity *a*. Our simulations predict an enforcement of fluctuations, with the frequent result of one exhausting cell type, if cell populations enriched for cells with small *a* are transplanted (Fig. 3D). Modifications of parameters *d*, *r*, and τ_c, as well as transition intensities α and ω, show only minor effects on the fluctuating behavior. These parameters become important in competition scenarios of kinetically different cell types, which have been described for the mouse model [23,37] and for human clonal disorders [25,26,38]. There are examples where the complete replacement of one cell type by another is reversible. Such a disappearance and reappearance of clones has been observed in DBA/2-C57BL/6 mouse chimeras [23]. In these animals, the contribution of the DBA/2 cells to blood production disappears completely after a couple of months. Reactivation can be induced by transplantation of bone marrow of these animals into lethally irradiated host mice. However, the achieved DBA/2 contribution is only transient. This behavior can be repeated in a secondary host. We simulated such a scenario (Fig. 4A)

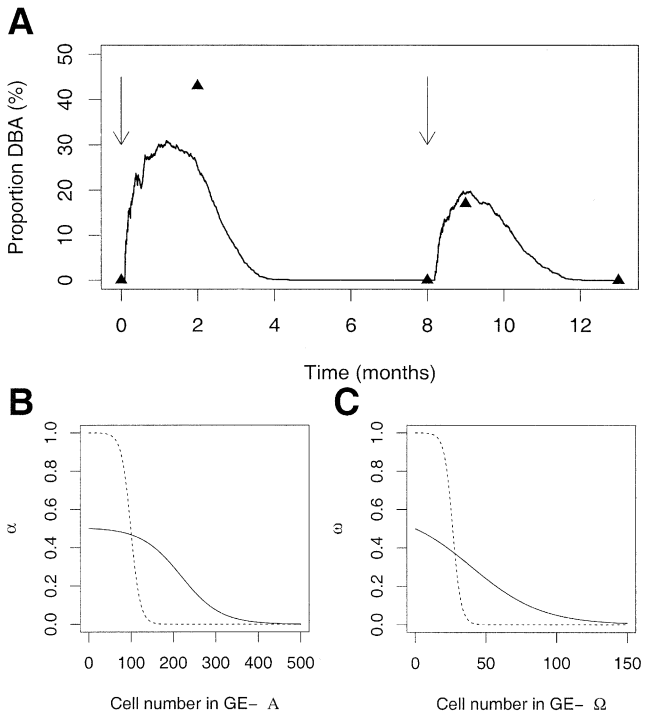

Figure 4. Simulations of clonal competition. **(A)** The data points ▲ (data from [23]) show the contribution of DBA/2 cells in the peripheral blood of irradiated mice after transplantation with bone marrow cells of a C57BL/6-DBA/2 chimera. Arrows represent time points of transplantation. The transient DBA/2 contribution, which disappeared 8 months after the first transplantation, can be reactivated by secondary transplantation of primary host bone marrow into another irradiated recipient. The solid line represents an average simulation (mean of 10 simulations) assuming shorter turnover time and more sensitively regulated GE-transition intensities of DBA/2 vs C57BL/6. The profiles of the transition intensities α (for $a = 1$) and ω (for $a = 0.01$) of DBA/2 (dashed) and C57BL/6 (solid) are given in **(B)** and **(C)**, respectively.

by making the following assumptions. First, DBA/2 cells are given a shorter average turnover time τ_c than C57BL/6 cells, implying an elevated cycling activity as previously reported [39]. Second, it is necessary to assume more sensitively regulated characteristics of the transition intensities α and ω of DBA/2 compared to C57BL/6 cells (Fig. 4B and C). This assumption induces a growth advantage of DBA/2 cells in situations of reduced cell numbers, e.g., following transplantation. After recovery, the activation/deactivation process of DBA/2 cells will be largely down-regulated and the cell production is overtaken by the C57BL/6 cells, still ensuring the persistence of a silent DBA/2 population. These cells can be reactivated if the system is driven again into a low cell number state. We predict that this reactivation of DBA/2 cells is not only achievable by transplantation but also by a reduction of cell numbers using, for example, cytotoxic agents (experiments in progress).

Individual clone tracking

The proposed single cell-based model offers the possibility of simulating competition processes on the individual clone level. Depending on the scenario, oligoclonality with large long-lived clones as well as polyclonal situations with many short-lived clones are detectable. The underlying model, however, predicts that the system always converts from a polyclonal (at the time of individual marking) to an oligoclonal and finally to a monoclonal situation in the long run. To understand experimental results on clonal composition in viral gene-marking experiments [27–29,40], it is necessary to incorporate the sampling and measurement strategies into the analysis. Figure 5A–C shows a simulation initiated with a sample of 50 individually marked cells to demonstrate the principal effect that sampling can have on the observable clonality pattern. Figure 5A illustrates the time courses of the actual cell numbers in the clones produced by the 50 founder cells, clearly showing fluctuations in the clone sizes. In contrast, Figures 5B and C depict different projections of these data. Whereas Figure 5B shows all existing clones (i.e., clone size > 0 cells), Figure 5C indicates measurable clones, assuming a certain detection threshold.

Simulations approximating two different experimental strategies are shown in Figure 5D. It shows proportions of individually marked clones observed less respectively more than 3 months. Jordan and Lemischka [27] reported a high proportion of long-lived clones (Fig. 5D, panel J) by analyzing repeated blood samples with high cell number but low detection sensitivity for the individual marker signal. On the other hand, Drize et al. [28] analyzed single cell-induced colonies. Using small cell samples but achieving a high detection sensitivity by the clonal expansion of the colony-initiating cells, they detected only clones with life spans of less than 3 months (Fig. 5D, panel D) [28]. As demonstrated by our simulations (Fig. 5D, panels SJ and SD), these seemingly contradictory observations can be explained consis-

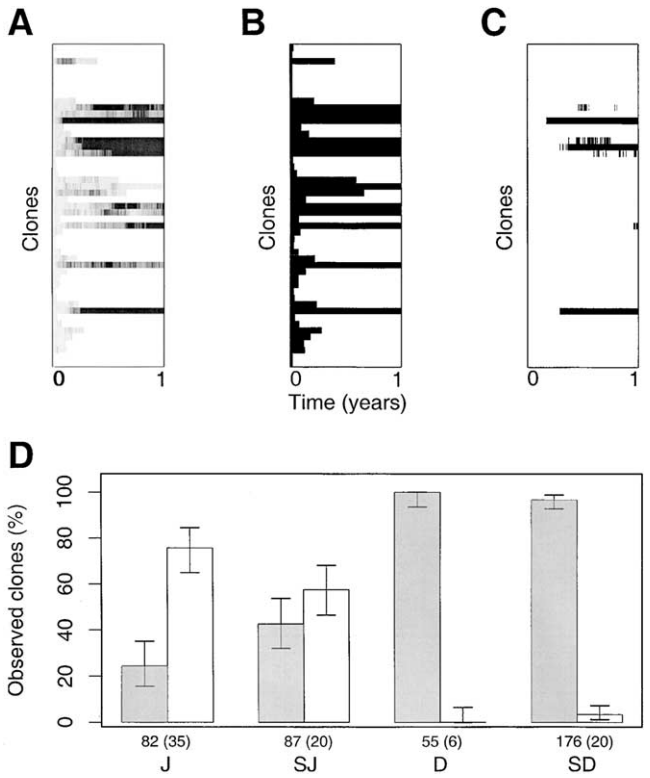

Figure 5. Simulations of individual clone development. **(A)** Simulated 1-year follow-up of stem cell clone size in a system initiated with 50 cells individually labeled with a heritable marker. Each horizontal bar represents one clone with brightness indicating the stem cell number (light gray: low cell numbers; black: high cell numbers). **(B)** See **(A)**, but here the bars illustrate the existence of the clones, i.e., all clones containing at least one cell are marked in black. **(C)** See **(A)**, but clones are only marked if the stem cell number per clone exceeds 20 cells to mimic limited detection sensitivities. **(D)** Simulation of two different assay strategies for individual clone tracking. Bars show proportions (mean, 95% confidence interval) of individually marked clones. Whereas shaded bars represent clones observed less than 3 months, empty bars represent clones present more than 3 months. The first bar-pair (panel J) shows data [27] based on the analyses of repeated blood samples, and the third bar-pair (panel D) shows data [28] based on the analysis of single cell-induced colonies. Respective simulation results (panels SJ and SD) were obtained using an identical underlying system but different sampling and measuring strategies according to the experimental protocol. The number of analyzed clones and the number of mice/simulation run (in parentheses) is given on the bottom.

tently, taking into account the different sampling techniques and sensitivities in the assays used. It should be emphasized that the different simulated sampling strategies also can be interpreted as different assay endpoints, as there are actively contributing stem cells [27] or cells that potentially induce differentiated clones [28]. A low detection threshold technique tends to miss dormant cells that did not amplify considerably since the time of individual marking, although these cells have the potential to form clones of differentiated cells. In contrast, sensitive single-cell sampling assays detect all potential clonogenic cells ($a > 0$), including the dormant ones, if the sample size is large enough.

Microenvironmental dependence

Differences in stem cell support between stroma cell lines and the effect of direct stem cell/stroma contact on in vitro preservation of repopulating ability have been demonstrated [14,15,41]. To model different stroma types, we introduced differences in the achievable maximum values (a_{max}) for the attachment affinity describing the diversity of stem cell supporting quality. Different engraftment patterns result because cell populations with a higher proportion of cells with large a will, on average, induce a better reconstitution than populations containing only cells with small values of a. This advantage, however, does not exclude the rare event that cells with low but still positive attachment affinity a can successfully engraft. Blocking of direct stem cell/stroma contact, for example, by using transwell cultures [14], was simulated by setting the transition intensity α identical to zero. This assumption inhibits the transition of cells from GE-Ω to GE-A. For that reason, all cells inevitably will lose their attachment affinity a and, therefore, their reconstituting ability over time. These assumptions can explain the differences in the repopulating ability of stem cells cultured in different in vitro settings (Fig. 6).

Cell kinetic and functional heterogeneity

Variability with respect to clone size, clonogenic potential, or time to clonal appearance has been reported for various colony-growth assays [3,7,8]. For example, the time range of appearance of colonies induced by single cells from a purified $CD34^+CD38^-$ cell population in the long-term culture-initiating cell assay varies between 7 and over 40 days [7]. Another example is the classic CFU-S assay [42]. From one CFU-S cell seeding in the spleen of an irradiated mouse, 0 to more than 60 secondary CFU-S can arise. Our proposed model is able to explain this variability without assuming a predefined heterogeneity of the stem cell popu-

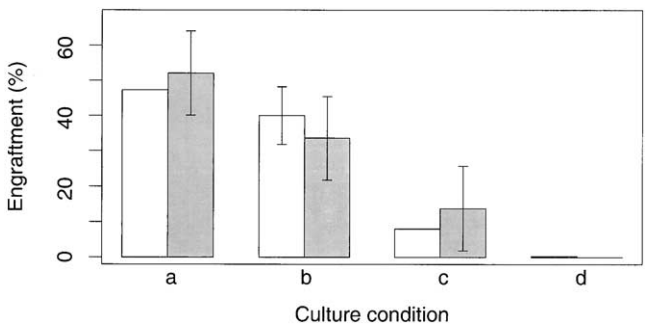

Figure 6. Simulation of microenvironmental dependence. Bone marrow cells, cultured in different stroma-dependent conditions, were cotransplanted with equal numbers of noncultured cells into lethally irradiated mice. Bars represent mean engraftment (± SD if available) of the cultured cells 3 months after transplantation. Data [14] (open bars) and simulations (shaded bars) are shown for four different conditions. (a) Stroma cell line in culture (SCL): 2017, simulation assumption (SA) $a_{max} = 1$; (b) SCL: S17, SA: $a_{max} = 0.5$; (c) SCL: AFT011, SA: $a_{max} = 0.2$; (d) SCL: S17, but without direct stem cell–stroma contact, SA: $\alpha = 0$.

lation. As an example, we simulated an CFU-S–like assay system. Figure 7A shows the distribution of the number of clonogenic cells per colony at day 12 of the assay. This distribution was obtained by initiating many systems with identical, single cells representing the founders of the primary CFU-S colonies. The variance of the produced number of clonogenic cells (i.e., potential founders of secondary colonies) purely results from the stochasticity produced by the system.

Moreover, it is well documented that the majority of stem cells is nonproliferating under steady-state conditions [3]. However, most of the primitive cells can be labeled in S-phase by continuous bromodeoxyuridine (BrdU) exposition [43,44], showing that there is no permanently silent cell population. The model is consistent with these data (Fig. 7B). The ongoing activation/deactivation process linked to repeated growth environment switches allows for sequential S-phase labeling of the entire cell pool together with a high proportion of dormant cells at each time point of observation. Hence, the model provides a natural explanation for the delayed label uptake based on the stochastic activation of dormant stem cells.

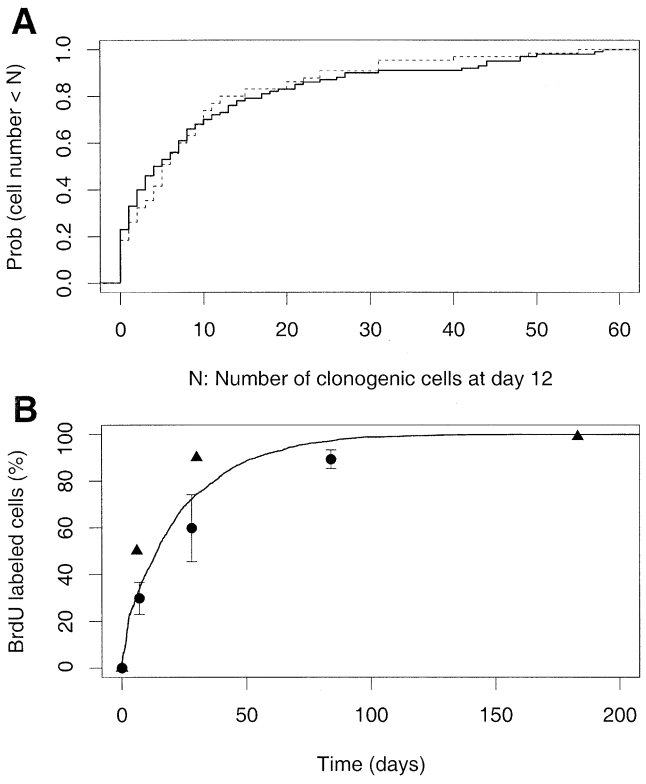

Figure 7. Simulation of cell kinetic heterogeneity. **(A)** Cumulative distribution function (i.e., probability of observing less than N clonogenic cells) of CFU-S content in 69 spleen colonies [42] (dashed line) and the number of clonogenic cells ($a > a_{min}$) in simulations of 100 single cell-induced systems (solid line). **(B)** Proportion of S-phase labeled cells during continuous BrdU administration. Data points are given by • [43] (mean ± SE if available) and ▲ [44] (mean). The solid line shows the average of 10 independent simulation runs with identical parameter configuration.

Discussion

The presented single cell-based model concept is innovative. It does not use explicit assumptions on stemness properties. Instead, each cell is characterized by two properties (attachment affinity and cell cycle status) that can reversibly be altered (within-tissue plasticity). A cell may home to one of two growth environments but will switch to the other with a certain probability. The combination of environment switching and within-tissue plasticity eventually generates the cell kinetic and functional heterogeneity as well as clonal fluctuations. To our knowledge, this is the first model to simultaneously satisfy the criteria of the functional stem cell definition [1,2,4], to conceptually link in vivo observations with the in vitro readout assays, and to be consistent with a large variety of data on functional and cell kinetic heterogeneity. The model highlights the intimate interaction between cells and the growth environment. Hence, we need to change the perspective from "stem cells" toward a "stem cell-environment system."

Our model suggests a reinterpretation of many experimental findings. First, clones originating from stem cells obtained in ideal purification protocols will not develop alike, and the outcome of an assay or a transplantation can only be predicted in a probabilistic sense. However, even though single cell fates are not predictable, the effect can average out if one looks at cell populations. Second, the observation that stem cells need specific growth conditions to sustain their potential usually is taken as evidence that the stem cell character of the cells determines the preferred growth environment. In our concept, there is no such causality because stemness respectively self-renewal is a dynamic product of interacting cell intrinsic potentials (e.g., the choice of the transition function) and extrinsic microenvironmental influences. Third, the results of many assay systems should be interpreted with caution. As an example, the cobblestone area-forming cell (CAFC) assay [45] permits observing the time sequences of in vitro colony formation that are used to estimate the number of stem cells of different qualities. These calculations are based on the assumption that one cell is initiating one colony. The concept of within-tissue plasticity, however, would allow the possibility that some cells of a newly initiated clone can reestablish their clonogenic potential and initiate other, later-appearing colonies. Therefore, the CAFC assay would have to be interpreted as a measure of clonogenic potential rather than of numbers of stem cells.

We are aware that our concept of hematopoietic stem cell development is simplistic. Feedback mechanisms from more mature cell stages [46–48] are not considered at the moment. Likewise, the spatial component of the bone marrow matrix [3] is disregarded. Another point is the reduction to two growth environments as well as the description of cells by only two functional properties. Microenvironmental influences and cellular properties are certainly high dimensional. Furthermore, the two transition functions are condensing a complex process of niche formation, cell–matrix

adhesion, and growth factor interactions. Contributions from the cells and from the environmental components should be disentangled in a later modeling step to distinguish between cell intrinsic and extrinsic contributions. However, according to "Occam's razor," we aimed to investigate the most simple model to determine the potential for covering a broad scope of phenomena.

The model has implications for a genomic definition of stem cells [49]. In this context, specific attention should be paid not only to the average pattern of genes expressed but also to the spectrum of possible gene expression patterns and how they depend on environmental signals. Our concept implies that determining whether a cell has the potential to act as a stem cell cannot be done by a simple assessment of specific gene expression patterns but, because of the predicted fuzziness, only by a complex scanning of regions of potential gene expression changes under a set of different conditions.

Furthermore, we believe that lineage commitment processes, which are not included yet, could be interpreted using an extension of the model concept by coupling within-tissue plasticity and environment dependent fluctuations. Tissue plasticity could be obtained assuming a propensity of cells to increase tissue-A–specific and to decrease tissue-B–specific properties while exposed to signals from tissue A but lacking signals from tissue B and vice versa. To experimentally prove these predictions, a detailed follow-up of tissue-specific gene expression on the clonal level is necessary.

Regarding previous stem cell models, our approach is related to, but distinct from, the classic concept of self-renewal based on a stochastic decision between different types of cell division [42,50]. In our model, which assumes only symmetric duplications of cells, the stochastic nature of cell development does not rely on the assumption of different kinds of cell divisions but on dynamically controlled switches between growth environments. Another model, which allows dynamic regulation of the self-renewing probability by tissue-specific feedback mechanisms, was introduced by Loeffler and Wichmann [46]. However, it was not able to describe single-cell fates; hence, it cannot be used to explain results of individual clone tracking experiments. This also holds true for the stochastic model of clonal succession introduced by Abkowitz et al. [24], which is suitable for investigating clonal competition phenomena on the cell population level. Microenvironmental influences were incorporated into stem cell concepts by Schofield [51], who introduced the concept of stem cell niches, or by Muller-Sieburg and Deryugina [52], who linked the binding of stem cells with stroma components to a differentiation arrest. The two latter concepts lack a mathematical representation allowing for a theoretical analysis or simulation studies. The development of our model concept has been influenced by these ideas; however, it is extended to a microscopic mechanism that explains the dynamics of stem cell self-renewal and allows for tracking of individual clones.

The classic question whether hematopoiesis is maintained by different successively activated or by identical simultaneously working clones turns out to be ill defined because clone membership can only be specified relative to the founder cell of the clone. If all cells in the system are uniquely labeled at one time point, our model predicts an asymptotic conversion to a monoclonal system. The same system can, however, be interpreted as a process of clonal succession if all cells that lost their potential for regaining a ($a < a_{min}$), and in this sense their self-renewal potential, are counted as founders of maturing clones. Hence, clonality statements need to be carefully described.

In summary, we demonstrated that a novel type of dynamic model, combining alternating homing to growth environments and within-tissue plasticity of cellular properties, is able to provide a consistent view on the hematopoietic stem cell system. To check the validity of our assumptions, the stated predictions, such as the monoclonality conversion after individual stem cell marking, the cell number dependence of attachment/detachment processes, or the reversibility of cellular properties on the clonal level, require experimental examination.

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