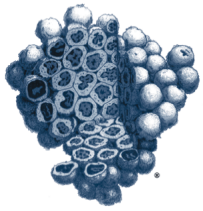

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Lineage Specification of Hematopoietic Stem Cells: Mathematical Modeling and Biological Implications

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Lineage Specification of Hematopoietic Stem Cells: Mathematical Modeling and Biological Implications

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Key Words. Lineage specification • Hematopoiesis • Stem cells • Mathematical model • Simulation • Systems biology

ABSTRACT

Lineage specification of hematopoietic stem cells is considered a progressive restriction in lineage potential. This view is consistent with observations that differentiation and lineage specification is preceded by a low-level coexpression of lineage specific, potentially antagonistic genes in early progenitor cells. This coexistence, commonly referred to as priming, disappears in the course of differentiation when certain lineage-restricted genes are upregulated while others are downregulated. Based on this phenomenological description, we propose a quantitative model that describes lineage specification as a competition process between different interacting lineage propensities. The competition is governed by environmental stimuli promoting a drift from a multipotent coexpression to the dominance of one lineage. The assumption of a context-dependent intracellular differentiation control is consistently embedded into our previously proposed model of hematopoietic stem cell organization. The extended model, which comprises self-renewal and lineage specification, is verified using available data on the lineage specification potential of primary hematopoietic stem cells and on the differentiation kinetics of the FDCP-mix cell line. The model provides a number of experimentally testable predictions. From our results, we conclude that lineage specification is best described as a flexible and temporally extended process in which lineage commitment emerges as the result of a sequence of small decision steps. The proposed model provides a novel systems biological view on the functioning of lineage specification in adult tissue stem cells and its connections to the self-renewal properties of these cells. STEM CELLS *2007;25:1791–1799*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

In a seminal series of experiments in the 1980s, Ogawa and coworkers [1, 2] studied the lineage specification potential of hematopoietic progenitor cells. From their results, they concluded that committed cells are derived from multipotent progenitors through a progressive restriction of lineage potential, in which the restriction in type and number of lineages occurs in a stochastic fashion. Although many details on the molecular mechanisms of lineage specification have been elucidated since then, it remains unclear how the specific gene expression dynamics are generated and how they are controlled by cell-cell and cell-environment interactions. However, an increasing number of reports agree upon a low-level coexpression of many lineage specific and potentially antagonistic genes in early progenitor cells [3–6]. This coexpression, commonly referred to as priming, disappears in the course of differentiation when certain lineage-restricted genes are upregulated and others are downregulated.

The question remains whether this phenomenological view is consistent with the findings on the progressive restriction of lineage potential. Moreover, it is unclear whether the proposed molecular dynamics of lineage specification agree with the observable temporal pattern of differentiating stem and progenitor cells. Therefore, it is our objective to derive a generalized analytical framework to understand lineage specification as a temporally extended process. Within our approach, the role of complex cell-extrinsic signaling events that influence the lineage specification process (e.g., by cell-cell and cell-environment interactions) is approximated by two contrary control regimes. Particularly, a regressive control regime maintains an undifferentiated priming state, and a progressive control regime promotes the process of lineage commitment. In this framework, lineage specification emerges as the result of a sequence of small decision steps (approximating a continuous process) rather than a singular decision event. The decision sequence slowly shifts the probabilities for development into a particular lineage and passes this potential on to the daughter cells. Therefore, daughter cells are identical after mitosis but continue lineage specification independently from each other.

The proposed intracellular lineage specification dynamics are embedded in the model for hematopoietic stem cell organization recently proposed by Roeder and Loeffler [7, 8]. Within this model, stem cells have the ability to change between two signaling contexts that impose different effects on the cellular development. For the model extension described here, we assume that these signals also affect the intracellular lineage specification dynamics, therefore inducing a correlation between the regulation of self-renewal and lineage specification. The extended model comprises a whole new class of phenomena in full consistency with former results on stem cell self-renewal and clonal competition [8–12].

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To verify the proposed theoretical model, we compare our simulation results with different sets of experimental data. Specifically, we apply literature data that describe the lineage contribution of single differentiating cells [13] as well as the lineage contribution within the progeny of two first generation daughter cells derived from a common parental cell (sibling analysis) [1, 13]. Furthermore, we show that the model is able to account for the typical kinetics of lineage development as observed for the differentiation of the (stem cell-like) FDCP-mix cell line, which have been measured in our lab.

Materials and Methods

We first describe the model of the intracellular lineage specification (i.e., the process that is realized within each individual cell) and, thereafter, discuss how this process is incorporated in the existing single cell-based model of hematopoietic stem cell organization. Furthermore, we outline the validation strategy of our numerical model preceded by a brief introduction of the relevant experimental data.

Intracellular Dynamics

For a conceptual understanding of the complex molecular dynamics governing lineage specification, we assume that all regulators that are specific for one common lineage fate are summarized in a single generic measure called lineage propensity. The level of each lineage propensity represents the potential of a cell to develop into the corresponding lineage. Furthermore, we assume that lineage specification can be understood as a mutual competition process between such propensities, in which every gain (or loss) in a particular lineage propensity will lead to the reduction (or increase) of the remaining propensities. Particularly, we apply a stochastic competition process leading either to the maintenance of the low-level coexpression (priming) or to the dominance of one or the other lineage propensity (commitment).

Formally, these model assumptions are specified as follows:

- The differentiation state of a model cell at any given time point is characterized by the actual levels of a number n of different lineage propensities denoted by x_i ($i = 1, \ldots, n$). The x_i, which can take values between zero and one, represents relative propensity levels for the development into the n possible lineages.
- In every time step, one randomly chosen lineage propensity x_i is updated. Herein, the probability for choosing a particular lineage i equals its propensity x_i. For the update, we assume two different control regimes that depend on the signaling context the cell is actually exposed to: (a) Regressive control regime: Deviations of the chosen lineage propensities x_i from a common mean propensity level are penalized by positive or negative rewards. (b) Progressive control regime: The chosen lineage propensity x_i is always enhanced by lineage-specific positive rewards m_i. The preferential update of lineages with high propensities (due to the coupling of update probability and actual lineage propensity) ultimately leads to the dominance of one lineage over the others. The final dominating lineage of a particular realization can only be predicted in a probabilistic sense. In the case of identical rewards m_i, the dominance is equally likely for each lineage. However, the specification of rewards m_i with different values for each lineage skews the decision process toward lineages with the higher rewards.
- To map the intracellular lineage propensities onto the phenotypic level (undifferentiated vs. committed to a certain lineage fate), individual cells are classified according to their highest actual lineage propensity $x_{max} = \max_i x_i$. Herein, the following denotation is applied: undifferentiated cells, $x_{max} < x_{com}$; cells committed to lineage i, $x_{max} > x_{com}$. For further mathematical details, see supplemental online data.

Figure 1 outlines the intracellular lineage specification dynamics for a particular model cell. The horizontal dashed line indicates the propensity level x_{com} that is used for the phenotypic separation

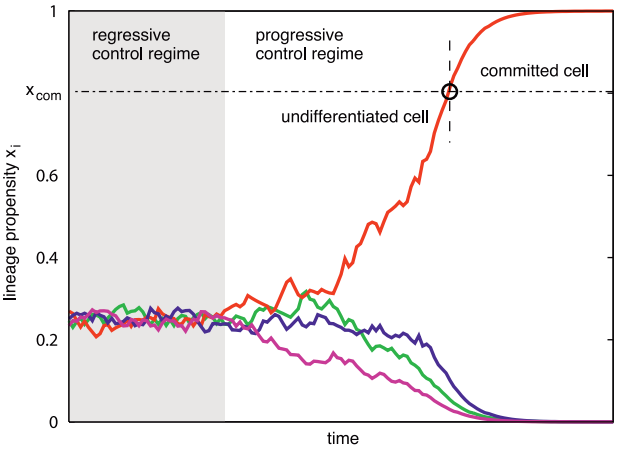

Figure 1. Intracellular lineage specification dynamics. In the regressive control regime (gray background) the $n = 4$ lineage propensities fluctuate around a common mean propensity level (in this example, $\text{mean}(x_1, \ldots, X_n) = 1/n = 0.25$). Changing to the progressive control regime (white background), one lineage becomes favored in a sequence of stochastic decision steps representing the lineage commitment (the graphic shows one particular realization of the stochastic process). The dashed line separates the regions for the phenotypic mapping (undifferentiated cells [$x_{max} < x_{com}$] vs. committed cells [$x_{com} < x_{max}$]).

of undifferentiated ($x_{max} < x_{com}$) and committed ($x_{max} > x_{com}$) cells. The specification of x_{com} is used solely for the phenotypic mapping of model results to experimental data and does not imply the irreversibility of the commitment decision. However, the probability for a change of the dominant lineage decreases considerably with increasing values of the propensity x_i.

Cell Population Dynamics

The intracellular lineage specification dynamics are embedded into our previously proposed model of hematopoietic stem cell organization [7, 8], in which intracellular properties, which characterize an individual cell, are modulated according to defined rules representing cell-cell and cell-environment interactions. Adopting the mathematical formalization introduced by Roeder and Loeffler [8], the actual status of a stem cell is characterized by its current signaling context (A or Ω), its position in the cell cycle (G1, S, G2, M, or G0), and its affinity a, which quantifies the propensity of a particular cell to reside in signaling context A. Cells in A are assumed to be nonproliferative, maintaining (e.g., in in vitro scenarios) or even regaining their affinity a up to an upper limit $a_{max} = 1$ (e.g., in in vivo scenarios). In contrast, cells in signaling context Ω are characterized as proliferative, accompanied by a gradual loss of their affinity a. It can be shown that the affinity a characterizes the cell's ability to realize long-term system repopulation. Accounting for the presumed underlying complexity, transitions between the two signaling contexts are described by a stochastic process. The probability of switching depends on the actual value of a as well as on the number of cells in the target signaling context. Transition from signaling contexts Ω to A is impaired for cells with $a < a_{min}$. These cells continue to divide throughout a proliferative phase, followed by a maturation phase without further amplification. Finally, mature cells are removed from the system to reflect their limited life span (Fig. 2A).

Extending the idea that varying microenvironmental signals differentially direct cellular development [7, 14], we assume the two signaling contexts A and Ω to impose contrary effects on the intracellular lineage specification dynamics of each individual cell. We link the progressive control regime to signaling context Ω, thereby relating the processes of lineage specification with the loss of repopulation potential. Vice versa, we link signaling context A with the regressive control regime. Therefore, cells in A experience no lineage commitment and simultaneously maintain their ability to act as stem cells. Characteristic time courses for the intracellular

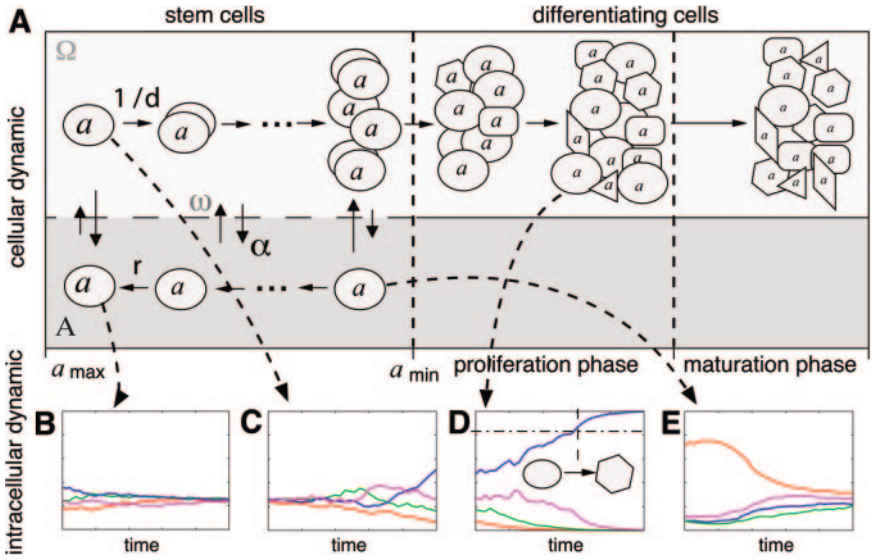

Figure 2. Extended model concept. **(A):** The affinity *a* decreases in signaling context Ω (light gray) by factor 1/d per time step and increases in signaling context A (dark gray) by factor r (the actual quantity of *a* is indicated by the different font sizes). For most in vitro scenarios, r = 1 such that the affinity *a* is maintained but not regained. Transition between A and Ω occurs with intensities α and ω, which depend on the affinity *a* and the number of cells in the target signaling context (for details see [8]). During their differentiation in Ω, undifferentiated (round) cells become committed (rectangular, triangle, polygonal, . . .). **(B–E):** Time courses of the intracellular lineage specification dynamics. **(B):** The nonproliferative, stem cell supporting signaling context A hosts multipotent cells, which are characterized by the balanced low-level coexpression of the lineage propensities. **(C):** Due to progressive control regime in signaling context Ω, the balanced coexpression is upset, and one lineage propensity is expanded at the cost of the others. **(D):** Continuation of the process in which the particular factor manifests the lineage decision and identifies the cell as committed. **(E):** Intracellular development of a cell that has been recaptured into signaling context A. Here, the regressive control regime counteracts the differentiation process and re-establishes the typical priming pattern. Abbreviations: *a*, affinity; d, differentiation coefficient; r, regeneration coefficient; α, ω, transition intensities.

lineage specification dynamics of individual cells are shown in Figure 2B–2E.

Model Comparison with Experimental Data

We compare our model with experimental data from three different types of experiments.

Differentiation of Unselected Progenitor Cells. Early quantitative approaches used hematopoietic spleen-derived mouse cells [1] and human umbilical cord blood cells [2] to examine the developmental fate of two daughter cells derived from one parent cell. The authors concluded from their results that lineage potential is progressively restricted by a sequence of stochastic commitment steps that take place at each cell division.

Differentiation of Enriched Cells. A set of results comparable in many respects with those presented by Suda et al. [1] was recently published by Takano et al. [13], using $CD34^-$ c-Kit^+ $Sca\text{-}1^+$ lin^- ($CD34^-$ KSL) cells taken from adult mouse bone marrow. The lineage composition of the progeny of these cells was analyzed in vitro using single cell differentiation experiments. Furthermore, the variability of the lineage contribution of a parental cell was evaluated by following the fate of its two daughter cells. In these experiments, the authors studied the influence of the culture conditions within the initial division assay on the subsequent asymmetry of daughter cell development.

Differentiation of FDCP-Mix Cells. The FDCP-mix cell line is a well established example of a stable cell line, derived from murine, multipotent hematopoietic progenitors, which retains the capacity to self-renew in the presence of high concentrations of Interleukin-3 (IL-3) [15, 16]. When transferred to low concentrations of IL-3 combined with other hematopoietic growth factors or injected into experimental animals, FDCP-mix cells show an apparently normal progression of lineage commitment and differentiation. FDCP-mix cells maintained in Iscove's modified Dulbecco's medium (IMDM) containing 20% horse serum and 100 U/ml IL-3 were washed and transferred at a density of 4×10^4 cells per milliliter to IMDM containing 20% fetal calf serum and either myeloid (M) or erythroid (E) growth factors as previously described [16]. The combination of growth factors supports differentiation either into a mixture of granulocytes and macrophages (M) or into a predominantly erythroid population (E). On consecutive days up to day 9, cells were harvested from replicate cultures and cytospun. Following May-Grunwald staining, differential counts were performed blind on 100–200 cells per time point. This way, we obtained a temporal pattern of the differentiation process.

Simulation Strategy

Following the experimental situations outlined above, we used three simulation protocols for model verification.

Comparative Differentiation of Paired Daughter Cells (Fig. 3A). To represent the homeostatic situation from which spleen/bone marrow cells had been isolated experimentally, we used a previously established reference parameter set that describes hematopoietic stem cell organization in unperturbed mice [10] (source assay, compare Fig. 3A). This parameter set was complemented by an appropriate representation of the intracellular lineage specification dynamics with equal rewards m_i for all lineages, intentionally neglecting any correlations between the development of certain lineages. Parameters are chosen such that a differentiating stem cell is considered "committed" ($x_{max} > x_{com}$) after 4–10 days of lineage specification in signaling context Ω. Cells used for transplantation into division assays are chosen randomly among a well defined subpopulation of the source assay (transfer pool), characterized by the range of the affinity parameter a_{trans}. The boundaries of these transfer pools are the central parameters to fit the simulation results to the experimental data by Suda et al. [1] (pool S) and Takano et al. [13] (pool T). The division assay is represented by an empty model system that mimics the culture conditions for the division of the parent cell. For simulation efficacy, all transferred cells are under the governance of signaling context Ω. The cell cycle position, the affinity *a,* and the lineage propensities x_i are preserved. After division, both daughter cells are transferred into two separate empty model systems, in which the development of the progeny is observed for 240 hours (lineage assay). Finally, the number and the lineage of cells produced in each lineage assay are evaluated. Due to an expected deficiency of a properly functioning hematopoietic

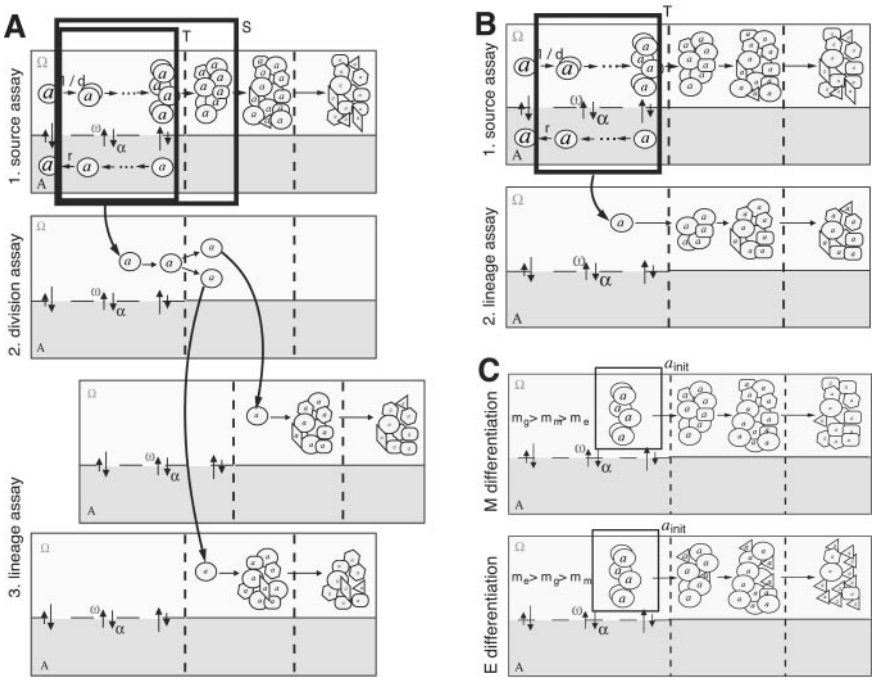

Figure 3. Simulation strategies. **(A):** Comparative differentiation of paired daughter cells. The transfer pools are indicated by the boxes in the source assay. **(B):** Single cell differentiation. **(C):** Lineage specification in cultures of differentiating cells. The affinity range of the initialized cells is indicated by the boxes marked a_{init}. Abbreviations: *a*, affinity; d, differentiation coefficient; E, erythroid; M, myeloid; r, regeneration coefficient; S, pool acccording to the data by Suda et al. [1]; T, pool according to the data by Takano et al. [13]; α, ω, transition intensities.

niche environment in cell cultures, we assume that, for all simulated in vitro assays, the signaling context A simply maintains the self-renewal ability of a cell (measured by its affinity *a*) but does not promote its regeneration.

Lineage Contribution of Single Differentiating Cells (Fig. 3B). The single cell differentiation experiments by Takano et al. [13] are incorporated into the simulation protocol with only minor adaptations. From transfer pool T of the source assay, randomly chosen cells are directly transferred into the lineage assay, where their lineage contribution is determined. All other parameters are left unchanged.

Lineage Specification in Differentiating Cell Cultures (Fig. 3C). In order to reflect the usage of relatively homogenous cells from a cell line, the differentiation assay is initialized with a population of 250 cells with a well defined initial affinity, uniformly distributed in the range $a_{init} \in [0.01,0.1]$. The fraction of undifferentiated and committed cells is evaluated hourly for a period of 9 days. A balanced expression of the $n = 3$ lineage propensities $x_1 = x_2 = x_3 = 1/3$ is assigned to the cells such that they are initially unbiased for the development in each of the three experimentally observed cell types: granulocytes, macrophages, and erythrocytes. The lineage specific rewards m_i are adjusted to meet the observed development of the cultures under granulocyte/macrophage (M) or erythrocyte (E) stimulating conditions. Particular parameter values are given in the supplemental online data.

RESULTS

Comparative Differentiation of Paired Daughter Cells

The experiments by Suda et al. [1] distinguished six different lineages, namely neutrophils, macrophages, eosinophils, mast cells, megakaryocytes, and erythrocytes. Therefore, the in silico intracellular lineage specification dynamics are constructed with $n = 6$ different lineages. For the spleen-derived mouse cells, Suda et al. [1] observed that the majority (73%) of the paired daughter cells contributed to just one lineage, identical for both daughters, suggesting that the parental cell had already been committed to one particular lineage (identical single lineage contribution). In addition, a number of paired daughter cells were observed, which contributed to more than one lineage. In 10% of the experiments, both daughter cells contributed to the same combination of lineages (identical multiple lineages), whereas in 17% the daughter cells contributed to different combinations of lineages (nonidentical multiple lineages). Under the outlined assumptions, the experimental results can be reproduced by adjusting the transfer pool S to $a_{trans} \in [0.000001,0.99]$, as shown in Figure 4A. In close correspondence to the findings of Suda et al. [1], we also observed the case in which one daughter cell develops into up to five lineages, whereas the other daughter cell is restricted to just one or two. Furthermore, some simulations generated daughters that contribute to the same overall combination of lineages with considerably different proportions of the individual cell types among their progeny.

To adapt the model system to the experimental setup presented by Takano et al. [13], the number of possible lineages is reduced to $n = 4$ (neutrophils, megakaryocytes, erythroblasts, macrophages). Due to the more sophisticated stem cell sorting procedure used in this experiment, the source population of initial parent cells is expected to contain an increased fraction of undifferentiated cells. This is reflected by the narrower transfer pool T ($a_{trans} \in [0.012,0.99]$) and marks the central difference to the setup of Suda et al. [1] (Fig. 3A). Changing no other parameters, the model reproduces the results of the experiments. Among the initial parental cells with complete lineage contribution (all four lineages), paired daughter cells with identical lineage development dominate over pairs with asymmetric development (Fig. 4B). In the simulation results, we observe additional minor contributions (0.1%–8.0%) to other combinations of lineages (data not shown). These are not described experimentally, which might be due to the limited number of observations. Takano et al. [13] also report that different combinations of cytokines in the in vitro division assay influence the lineage potential of the daughter cells and change the particular ratios of symmetric versus asymmetric development. Qualitatively similar phenomena can be observed by modifying the lineage specific rewards m_i within the division assay of the in silico model (data not shown).

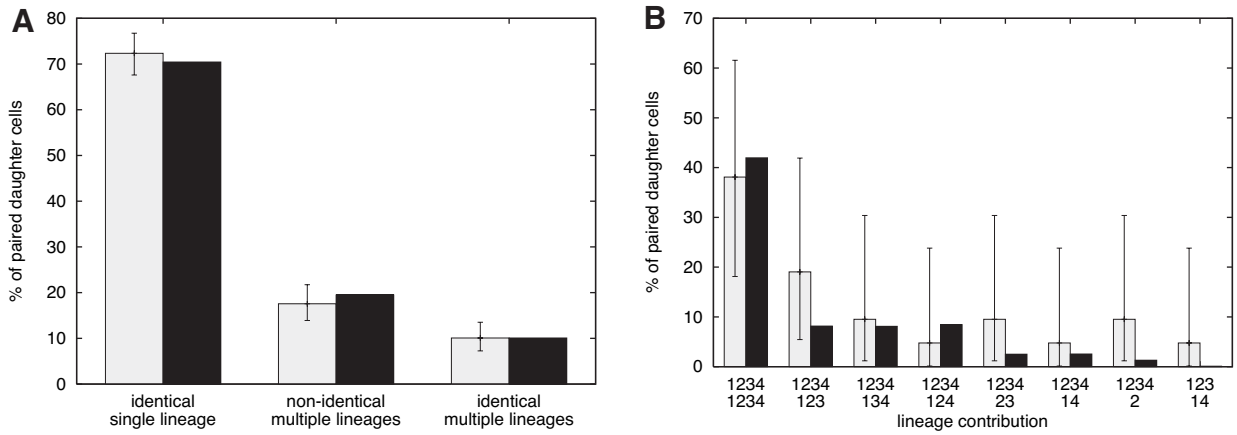

Figure 4. Lineage contribution of paired daughter cells. **(A):** Experimental results for hematopoietic spleen-derived mouse cells [1] are shown in gray (mean, 95% confidence interval [CI]). Results for the simulated cells (defined by the transfer pool S [fit for the data from Suda et al. [1]] of the source assay) are shown in black (mean of 50,000 simulation runs, CI negligible due to the high number of replicates). **(B):** Experimental results (corresponding to parental division of $CD34^-$ c-Kit^+ $Sca\text{-}1^+$ lin^- cells in medium containing stem cell factor + Interleukin-3 [13]) are shown in gray (mean, 95% CI). Results for the simulated cells (transfer pool T [fit for the data from Takano et al. [13]]) are shown in black (mean of 50,000 simulation runs). Only cells with complete lineage potential (contribution to all four lineages) are shown. The lineage contribution of the first daughter is given on top, of the second daughter below (1, neutrophil; 2, megakaryocyte; 3, erythroblast; 4, macrophage).

Lineage Contribution of Single Differentiating Cells

In single cell differentiation studies of bone marrow derived $CD34^-$ KSL cells, the majority (43%) of plated cells contributed to all four determined lineages, whereas other combinations are observed with lower frequency [13]. Applying the identical transfer pool T that has been used for the comparative differentiation of paired daughter cells and evaluating the lineage contribution of the single differentiating cells without an intermediate division step, these findings can be reproduced in silico. In particular, we find that, in the majority of cases (45.2%), the progeny contained all four lineages, whereas other combinations are reduced (Fig. 5A). It should be emphasized that this qualitative pattern is achieved even under the simplifying assumption of equal lineage potentials (i.e., equal rewards m_i). However, the precise matching of the results is incomplete. The experimental data suggest that there is a correlation between neutrophil and macrophage differentiation (see lineage combination 14 in Fig. 5A). Introduction of a moderate, positive correlation between lineages 1 and 4 (neutrophils and macrophages, respectively) in the in silico model leads to a shift in the differentiation pattern similar to the experimental observations (Fig. 5B). Progeny of single cells containing neutrophil and macrophage cells are now significantly enhanced compared with other developments. Due to the complexity of these potentially weak correlations between certain lineages, a detailed quantification of this process is hardly possible on the basis of the available experimental data.

Lineage Specification in Differentiating Cell Cultures

To simulate the differentiation of FDCP-mix populations in the presence of growth factors supporting either M (granulocytes and macrophages) or E lineages, the corresponding rewards m_i were adapted in favor of the relevant fates. This is the only difference assumed for the M and E media.

During erythroid development, erythrocytes mature from erythroblasts. Since it is possible to distinguish between these cell types morphologically, erythroid cells are subdivided for the phenotypic mapping such that the committed cell stage now comprises early committed cells (erythroblasts) and mature cells (erythrocytes).

Figure 6 shows that the simulation model is able to quantitatively account for the temporal development of the proportions of observed cell types in both M and E media. Although the lineage specific rewards m_i have been adapted to meet these particular experimental results, the agreement of simulation and experiment can be regarded as a proof of principle that the proposed model is able to adequately account for differentiation kinetics on the population level.

Discussion

The mathematical model presented here is the first to describe the individual commitment process of single cells within a whole population of stem cells and their progeny. It represents an essentially novel systems biological approach to the quantitative elucidation of lineage specification as a temporally extended, self-organizing process. The model is based on the idea of lineage commitment being coordinated by a mutual competition between different lineage specific transcription factors [3, 5]. The lineage propensities x_i used in our model are simplified representations of these regulatory complexes. The coexpression of these propensities in uncommitted cells is accommodated by the assumption of two opposing control regimes representing different microenvironmental stimuli that influence the mutual competition process. This proves sufficient to account for the key features of stem cell differentiation: the early priming of all programs and the subsequent upregulation of genes specific for one lineage at the expense of their antagonists. The proposed intracellular lineage specification dynamics are integrated into our previously described model of self-organizing hematopoietic stem cell populations [7, 8]. Consistent with previous findings on stem cell self-renewal and clonal competition [8, 10, 11, 12], our extended approach additionally accounts for the experimentally observed phenotypic heterogeneity in populations of differentiating stem and progenitor cells assayed under various conditions.

We showed that the model supports the idea of a progressive restriction in lineage potential in the course of differentiation. To make this idea more visible, we have studied the lineage contribution of single differentiating cells depending on their initial repopulation ability, characterized by the affinity parameter *a* for the example of a system with four possible lineages (Fig. 7). As expected, nearly all cells with high repopulation

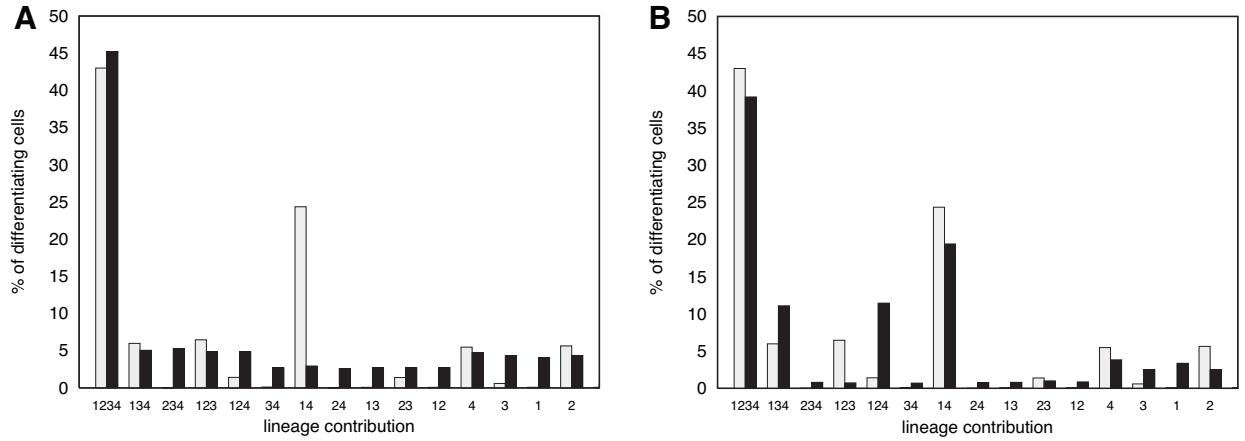

Figure 5. Lineage contribution of single cells. **(A):** Experimental results for the differentiation of single $CD34^{-}$ c-Kit^{+} Sca-1^{+} lin^{-} cells [13] are indicated by the gray bars (mean, confidence interval not available). Results for the corresponding simulated cells are shown in black (mean of 50,000 simulation runs). The lineage representation 1–4 is used as in Figure 4. **(B):** Introduction of a moderate correlation between lineages 1 and 4 in the in silico model increases the particular lineage contributions.

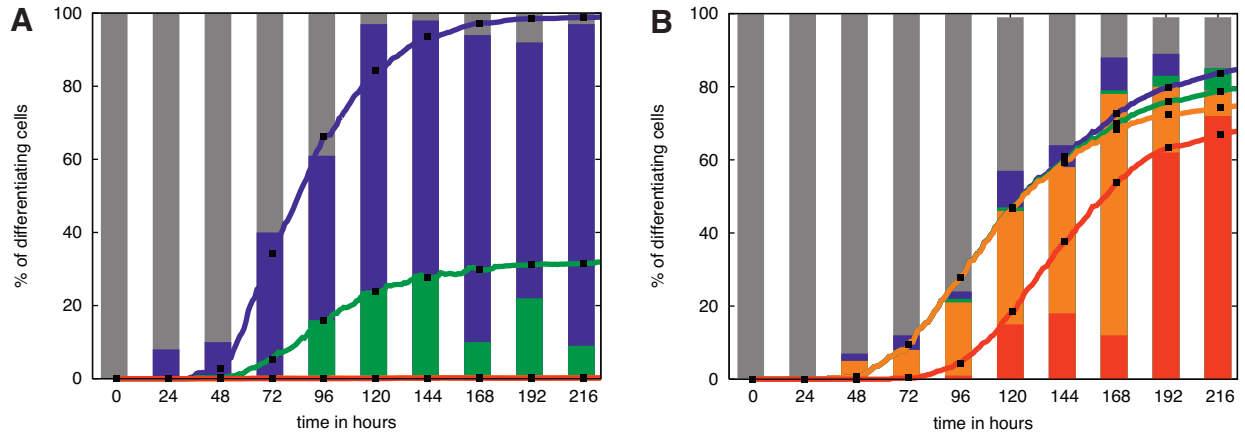

Figure 6. Lineage contribution of FDCP-mix cells versus time. Experimental results for differentiation in myeloid medium **(A)** and erythroid medium **(B)** are illustrated by the bars (assessed by morphology counts on a daily basis). The simulation results are indicated by the corresponding lines (blue, granulocytes; green, macrophages; orange, committed erythroblasts; red, mature erythrocytes; gray, undifferentiated progenitors).

ability contribute to all four lineages, whereas tri- and bipotent cells are found mostly in the population with moderate repopulation capacity. The ultimate loss of repopulation potential is associated with commitment to a single lineage. It is essential to note that this hierarchic decrease of the lineage potential is predicted by the model as an emergent system property and is not intrinsically predefined in individual cells.

In the same line of argument, lineage specification appears as a process in which particular lineages (such as neutrophils and macrophages) are more closely related than others. Relaxing the simplifying assumption that all lineages develop completely independent of each other, we showed in the Results that preferred trends in the differentiation process can be consistently incorporated in the presented model. The introduction of a small positive correlation between certain lineages (e.g., mediated by common coregulatory molecules) proved sufficient to account for the observed developmental preference. From this perspective we arrive at the same conclusion: the hierarchical appearance of the lineage specification process is not necessarily due to a cell-intrinsic, predefined developmental program, but could be well explained in the context of self-organizing and flexible stem cell populations.

Another important aspect is the distinction between lineage contribution (being the lineages actually generated by the progeny of a particular cell) and lineage potential (being the lineages to which the same progeny could have contributed). Since a single cell can only differentiate once, the lineage potential cannot be determined experimentally. However, despite this inherent uncertainty, the notion of lineage potential is important to understand the organizational principles of cell populations and tissues as well as for the characterization of the stem cell properties. As we have exemplarily shown for the lineage specification kinetics of the FDCP-mix cell population, the fluctuations in lineage potential that occur on the single cell level average out on the population level. This means that, although the outcome on the population level is robust, the particular fate of a single cell can only be predicted in a probabilistic sense. Based on this understanding, our model predicts that heterogeneity of a progenitor population is inherently generated as a consequence of the autonomous development of individual cells.

The role of asymmetric cell divisions in the process of hematopoietic lineage specification is still controversial [17–19]. Although such divisions are reported for a number of other systems [20, 21], no evidence for (functional) asymmetric cell division has yet been found within the hematopoietic system. Although we cannot rule out the possibility of asymmetric cell divisions in hematopoietic cell differentiation, our model demonstrates that a consistent explanation of the heterogeneity among differentiated cells is possible without assuming an

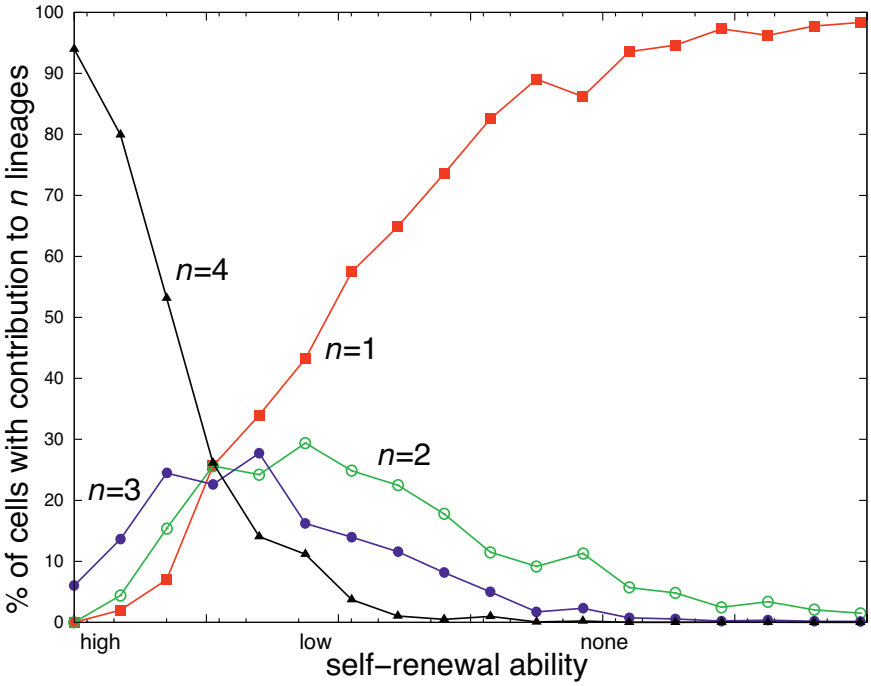

Figure 7. Lineage contribution versus self-renewal ability. The curves correspond to the fraction of cells that contribute to n out of four possible different lineages within their differentiating progeny. The curves are shown as functions of the initial self-renewal ability of the parental cell measured by the affinity a in the model simulations (50,000 simulated cells).

asymmetric division process. Technically, any simulated cell division is symmetric. Differences in the individual development of the daughter cells occur only due to their independent differentiation sequences after mitosis. Asymmetric development is thus interpreted as the asymmetry of cellular fates, not of the division process itself (see also [12]).

Our model concept supports the hypothesis that the experimentally observed priming behavior is a common molecular representation of the stem cell state (see also [14, 22, 23]), which is maintained under specific conditions (e.g., due to niche signals). Maintenance of the priming state could feasibly be achieved by the active epigenetic stabilization of chromatin structures that retain parallel developmental options. Changing microenvironmental signals destabilizes the priming state. Under these modified conditions, chromatin changes at key loci may result in a sequential shift of the expression state toward one or the other lineage specific expression pattern. This process represents a molecular view of the differentiation process with progressively decreasing probabilities for multipotent development. At an experimentally accessible level, our model predicts that targeted up- or downregulation of certain lineage specific genes upsets the balance at the priming level and, consequentially, supports or discriminates certain options in the subsequent differentiation process. A particular strength of the model is the foundation on the level of single cells. Alongside with the experimental tracing of individual cells in culture [24, 25], our model is able to identify critical phenomena of the molecular differentiation sequence (as there are asymmetric developments, the occurrence of lineage specific markers and their inheritance to the daughter cells, and the role of apoptosis and selection) and to link them to the population level.

The assumed temporal extension of losing multilineage potential is closely associated with a reversibility of the differentiation process. We model lineage specification as a process that favors a certain lineage development by progressively decreasing the probabilities for the competing options. Therefore, reversibility depends strongly on both the actual state of differentiation and on the influence of the microenvironment. The model predicts that reversibility of lineage specification is a rare event in a homeostatic system. However, it is expected to be more common in a disturbed situation with the need for system repopulation. Similar effects should be observable when cells that are primarily cultured in a particular differentiation promoting medium are transferred into a condition with different properties (e.g., promoting self-renewal or another differentiation program). The model predicts that the fraction of cells with "reverted" development is not an all-or-nothing decision but depends in the first place on the exposure time in the particular medium. A rigorous experimental test of this prediction would have to use molecular markers that are irreversibly switched on if a certain characteristic gene expression identifies a particular lineage commitment. The detection of such markers can elucidate to what extent early committed cells actually "reverse" their previous development under changing environmental conditions. The model predicts that the fraction of cells with reversible developments gradually decreases as the process of lineage specification continues.

The particular underlying mathematical process of the lineage specification dynamic was chosen because it resembles a number of desired characteristics, such as the low-level priming, the competitive lineage specification in which one lineage is favored at the expense of others, the controllability of lineage development on a predictable level involving stochastic elements, the temporal extension, and the capability for reversible events. However, the process is based on a number of simplifying assumptions that hamper the application to a directly measurable molecular process. For instance, lineage specification dynamics presumably require a set of many coregulated factors that have been summarized into one generic lineage propensity. This simplification neglects subsequent activation steps, mutual interactions between the members of each of the sets of coregulated factors, and the role of late signaling events. Similarly, the role of extrinsic signaling by cell-cell and cell-environment interactions is reduced to the influence of two antagonistic control regimes that govern the lineage specification process. Furthermore, the phenotypic mapping to classify cells as either undifferentiated or committed is only a rough approximation of the highly complex maturation process. De-

spite, or perhaps because of, this simplicity, the model proves sufficient to account for a considerable number of phenomena on the lineage specification of hematopoietic stem cells. Most notably, all these results are consistent with previous findings on self-renewal and clonal competition.

This is not to say that the explanation is either unique or complete. Indeed, a detailed quantitative understanding of lineage specification must eventually take account of the characteristics and interactions of a plethora of regulatory molecules, starting with the lineage-specific transcription factors. Modeling approaches to describing the sequential downregulation of lineage specific (transcription) factors during differentiation have previously been suggested by Preisler and Kauffman [26], Furusawa and Kaneko [27, 28], Cinquin and Demongeot [29], and Laslo et al. [30]. In a similar context, we recently showed that autostimulation and specific mutual inhibitions of the transcription factors PU.1 and Gata-1 are sufficient to explain a robust, switch-like behavior from a low-level coexpression of both transcription factors to different states of predominant expression of one of them [31]. This change in the system dynamics can be explained by alterations in transcription efficiency of the individual transcription factors and might provide a molecular basis for the differences between the two antagonistic control regimes in the model presented here.

Conclusion

There are two major conclusions from our study. First, we showed that the molecular view on lineage specification (priming vs. the dominance of one lineage) can be integrated into our previously proposed hematopoietic stem cell model [7, 8] in a conceptually consistent way. Second, we could demonstrate that this combined model of stem cell self-renewal and lineage specification accounts for the phenotypic heterogeneity that is experimentally observed in populations of differentiating stem and progenitor cells and is consistent with the assumption of a progressive restriction in lineage potential.

As outlined above, stem cell development and lineage specification are considered temporally extended processes of continuously changing cellular characteristics. This concept does not exclude certain preferred trends in the differentiation sequence, but it comprises the possibility of reversible developments for individual cells and, thus, allows the system to flexibly react to changing demands. In this sense, "stemness" is no longer understood as a cellular feature but as a system property, a perspective which has been proposed independently by us [7, 8, 12, 32, 33] and by other groups [34–39]. This concept is fundamentally different from approaches that describe stem cell organization as the consequence of a predefined, cell-intrinsic differentiation program. Such approaches assume discontinuous transitions from one confined stem cell or progenitor subpopulation to another in a predefined, strictly unidirectional differentiation sequence [40–43]. Clearly, the grouping of stem and progenitor cells according to features such as cell surface marker expression and functional characteristics remains useful for classification, selection, and enrichment, since it accurately reflects the behavior of a population under a certain set of conditions. Ultimately, however, our increasing awareness of heterogeneity, flexibility, and plasticity within stem and progenitor cell populations questions the validity of these strictly unidirectional concepts at the mechanistic level in single cells. It is here that the combination of experimental and modeling approaches, as the one presented here, may prove most productive.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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