

Emergent Properties of Feedback Regulation and Stem Cell Behavior in a Granulopoiesis Model as a Complex System*

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To identify the internally generative theoretical relationship between microscopic mechanisms and the macroscopic behavior of hematopoietic processes as a complex system, a computer simulation of granulopoiesis was exploited by developing a cellular automaton (CA) model. Hematopoietic stem cells (HSCs) distribute themselves to proliferate and differentiate in a three-dimensional analytical space. The number of mitotic events of the cells in a proliferating phase, the transit times (T) of each of 15 differential stages progressing from a HSC to a mature cell, the duplication times (T_{dup}) of HSCs, and the neighborhood rules for cell proliferation were all incorporated as analytical parameters in this space. Homeostatic granulopoiesis originating from a single HSC was successfully achieved. An important part is the stabilization of cell production induced by way of negative feedback following external perturbation of the peripheral granulocyte numbers. Single-cell kinetic analyses describe the behavior of differentiating cells and HSCs as fluctuating their T and self-renewal time (T_{dup}) in response to the feedback dynamics. Stochastic cell divisions of HSCs were recruited in a transitional manner resulting in the generation of a regulatory effect on the differentiation–commitment processes. The concept that local cellular interaction produces global dynamics in a granulopoietic system was reified by CA modeling. This approach will provide a framework for analyzing the behavior of HSCs and enable an understanding of the abnormal kinetics of hematopoietic diseases.

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1. Introduction

Mature blood cells and their precursors in bone marrow are ultimately derived from a small population of hematopoietic stem cells (HSCs) that have a high proliferative potential and maintain hematopoiesis throughout life as depicted in Figure 1 [1].

In Figure 1(a) a hematopoietic stem cell is defined as a cell with extensive self-renewal and proliferative potential, coupled with the capacity to differentiate into the progenitors of all blood lineages including red blood cells, neutrophil, eosinophil, and basophil granulocytes, mast cells, monocytes and macrophages, platelets, B lymphocytes, T lymphocytes, natural killer cells, and dendritic cells. Progenitor cells committed to specific lineages are called a *colony forming unit*. For example, CFU-GM, granulocyte-macrophage colony-forming unit cell; CFU-E, colony-forming unit erythroid; CFU-Meg, colony-forming unit megakaryocyte; CFU-Eo, eosinophil colony-forming unit cell; CFU-Ba, basophil colony-forming unit cell. Progenitors that are more primitive than CFU are called *burst-forming units* (BFUs).

Figure 1(b) shows the granulocyte differentiation pathway. Granulopoiesis following CFU-GM consists of the proliferating cell stages (CFU-G, myeloblasts, promyelocytes, and myelocytes) and the postmitotic, nonproliferating, and maturing stages (metamyelocytes, band, and segmented-form neutrophils).

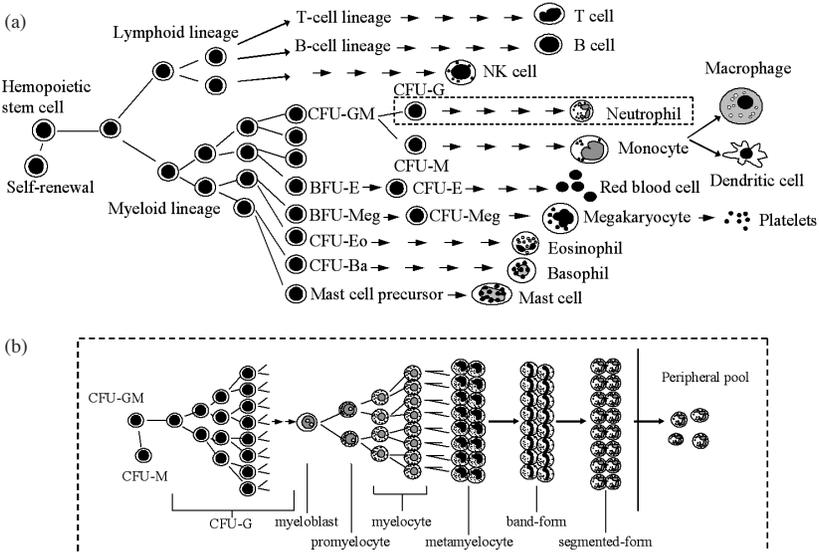


Figure 1. Sequential development of progenitor cells and mature cells from stem cells [1].

The earliest HSCs are able to differentiate into any type of blood cell (totipotent) and have a high self-renewal capacity [2, 3], although such potency is progressively lost as the stem cells differentiate. The progenitor cells are committed to one-cell lineages and proliferate to form large colonies; therefore they are called a *colony-forming unit* (CFU), of erythrocytes, granulocytes, monocytes, or megakaryocytes, as depicted in Figure 1. The number of cells of each type is maintained within a very narrow range in normal individuals: approximately 5000 granulocytes, 5×10^6 red blood cells, and 150,000 to 300,000 platelets/ μL of whole blood.

The mechanisms regulating hematopoiesis are not as well understood. Biological approaches seeking the factors that directly regulate cell proliferation and differentiation in hematopoiesis have been extensively performed. Erythropoietin for red blood cells, thrombopoietin for megakaryocytes, and the granulocyte colony-stimulating factor (G-CSF) for granulocytes are all regulatory factors that have been well-investigated [4]. Their essential roles for the growth of each lineage-committed cell have been established and they are in use clinically for the treatment of hematological patients [4]. Moreover, recent molecular analyses have opened the way for investigating the genetic events that occur at the time cells make a lineage-specific commitment [5]. Regulatory systems of lineage-specific gene activation seem even more complex, since a number of lineage-affiliated genes are activated at different levels in a cell [6, 7]. Amplification of the specific gene expression, while down regulating the other genes, should be conducted intrinsically or extrinsically *via* regulatory factors as well as microenvironmental interaction, however, all the regulatory features have yet to be clearly elucidated.

The dynamical regulatory mechanisms for maintaining the number of blood cells presumably involve negative feedback. For example, an increase (decrease) in the number of circulating granulocytes would induce a decrease (increase) in the production of granulocytes through the adjustment of a regulatory factor (e.g., G-CSF levels). However, no organs or tissues detecting such changes in number or level of the factors for granulopoiesis have been identified, therefore, the existence of feedback in the systems is still hypothetical. Furthermore, little is known about how self-maintenance of the HSC population is achieved. HSCs are usually in a resting state but are activated to proliferate in response to the demand for blood cells. Because of the difficulty of manipulating HSCs *in vitro*, the mechanisms controlling HSC kinetics are still unknown and starting to be investigated. New approaches to clarify these regulatory networks should be exploited, and mathematical model analyses have been proposed for this purpose [8–17].

Computer simulations using cellular automata (CA) may provide useful tools for understanding the systems involved [18–22]. CA models can produce complex patterns based on simple strategies describing the

behavior of elements, which are analogous to the appearance of complex systems as commonly seen in biological events [23–26]. CA consist of discrete unit elements arranged uniformly on spaces of one or more dimensions, each of which can vary within a finite set of values to express the physical state of the system components being analyzed. The time evolution of the element states is performed synchronously according to local neighbor rules, instead of governing equations, taking into account the state of the element itself and the interactions between nearby elements. Hence local interaction leads to global dynamics in a CA model.

The aim of this study is to use CA modeling to explore the theoretical relationships between microscopic events including cell division, cell proliferation and differentiation, and macroscopic regulation in a hematopoietic system. The present simulation is focused on a granulocyte differentiation process (Figure 1(b)) extracted from the hematopoietic system for simplification. We show here the dynamical feedback regulation generated as a result of local interaction of granulopoietic cells and also characterize a single stem cell behavior in our granulopoietic model system.

2. Methods

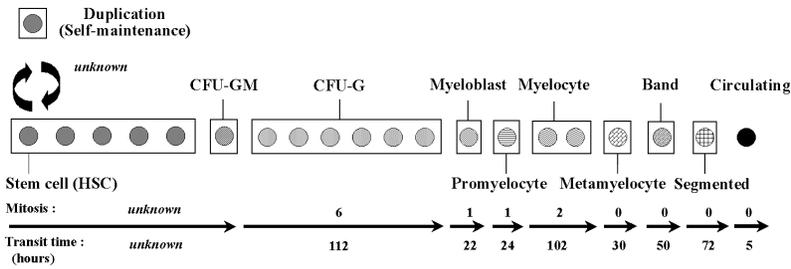
2.1 Compartmentalization of granulopoiesis

To describe human granulopoiesis, we divide the granulopoietic processes into 10 compartments in which each cell stage is represented by a model compartment characterized by the transit time T , the number of mitoses, and the fraction of actively proliferating cells (Figure 2). Stages 1 to 15, arbitrarily given, are defined as the state variables in this simulation. Fundamental transit time steps that reflect cell age (maturation) are appointed according to the transit times (hours).

The compartment for stem cells (stage 1) includes pluripotent stem cells with self-renewal capacities and cells at a very early stage of differentiating towards granulopoiesis. This early process of HSC differentiation has not been clarified, and the given transit time is thus referred to as optional. Likewise, all features of HSC self-renewal remain obscure. We defined this compartment as duplication, with the transit time T_{dup} referred to as optional.

The compartment of the committed progenitor cells (CFU-GM, stage 2) is fed by the influx of cells originating from the compartment with pluripotent stem cells. The next compartments represent the proliferating cell stages of CFU-G (6 mitoses, stages 3–8), myeloblasts (1 mitosis, stage 9), promyelocytes (1 mitosis, stage 10), myelocytes (2 mitoses, stages 11 and 12), and the postmitotic maturing stages of metamyelo-

[Experimental data]



[Numerical model]

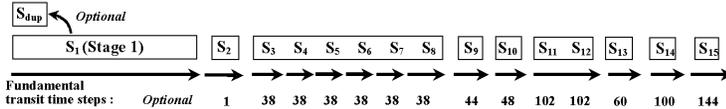


Figure 2. Compartmentalization of granulopoiesis. The granulopoietic processes were divided into 10 compartments characterized by transit times, the number of mitoses, and the fraction of actively proliferating cells.

cytes (stage 13), band (stage 14), and segmented form (stage 15). Mature granulocytes leave the bone marrow and enter the circulation compartment and the marginal pool in peripheral blood. Importantly, this model does not presuppose the presence of peripheral negative feedback loops. The model parameters, including transit times and the number of mitoses used here for normal granulopoiesis, are taken directly from the literature or deduced from published experimental data [12, 13].

■ 2.2 Stem cell division model

The stochastic model of HSC differentiation used here is shown in Figure 3(a). A HSC can replicate (self-renew), or differentiate to a CFU-GM cell. Once committed to a differentiation pathway, a HSC gives rise to a clone that contributes to granulopoiesis. Three patterns of the HSC division process were theoretically proposed for this model system; each stem cell undergoing cell division can either generate two, one, or no daughter stem cells until differentiated to CFU-GM in a stochastic situation. If these HSC differentiation processes are described with three probabilities (p , r , and q as indicated in Figure 3; $p+r+q = 1$), an expansion of stem cell numbers is induced under the situation that $p > r + q$, while an extinction for a finite population occurs if $p < r + q$. A strict steady state is maintained if $r = 1$; a stationary state is also possible if $r < 1, p = q$. The stochastic concept of single cell growth described here is in line with the classic models proposed by others [11, 27].

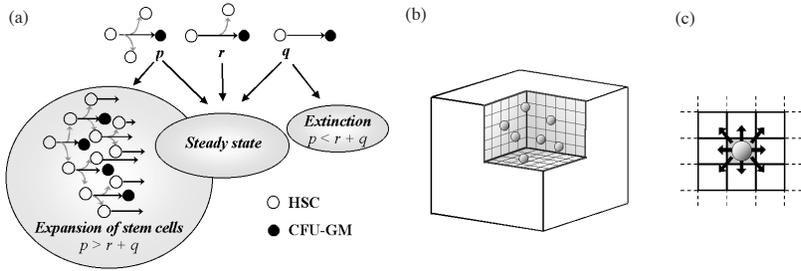


Figure 3. (a) Stem cell division models. A HSC (open circle) generates two, one, or no daughter stem cells until differentiated to a CFU-GM cell (closed circle) in a stochastic manner in this model. The probability of each type of cell division was expressed as p , r , and q , respectively. (b) Schematic presentation of a three-dimensional CA model. The CA model is developed for a three-dimensional space consisting of $100 \times 100 \times 100$ unit cubic areas with periodic boundaries. (c) A single cell moves to any of its nearby elements from 26 directions randomly selected at every time step. The two-dimensional directions for cell movement are shown with arrows.

2.3 Development of a cellular automata model

The CA model is developed for a three-dimensional space that assumes the bone marrow where the granulocyte-lineage cells are distributed. The space consists of $100 \times 100 \times 100$ unit cubic areas with periodic boundaries, the size of each corresponding to a single biological cell (Figure 3(b)). To represent cell distribution in space, state variables that represent any of a limited set of values must be assigned to each unit area. Two kinds of state variables are defined in the present simulation. First, a set of cell states is prepared so that cells are located at certain positions, and are also distinguishable by their proliferation stage. According to the assumption regarding the granulopoietic process, a total of 16 cell states including a cell absence case are required (Figure 2). As the second state variable, cell age is defined in addition to the areas where cells are present. The age is counted at every simulation step to express cell maturation until reaching their respective transit times for the next stage (see fundamental transit time steps in Figure 2).

2.4 Description of the local neighbor rules

In order to carry out spatio-temporal changes of the system during the course of simulation, the state variables must be updated synchronously according to local neighbor rules at every single calculation step. These rules, which describe cell dynamics, include the movement of cells, transition to a different stage depending on the state of the unit element itself, and the micro-environmental influence of neighbor elements. Es-

entially, the cell is able to move to any of its nearby elements from 26 directions selected randomly at every time step (Figure 3(c)). To avoid collisions with other cells at a certain neighbor site, conflicting directions are evaded during the movements. For example, each cell is mapped, and prior to movement a moving direction is randomly chosen from the available nearby vacant sites. If cells happen to conflict at a destination, an arbitrarily selected cell is then allowed to move while the others are kept in their present locations. The respective transition to the next stage in the cell lineage is fundamentally determined by the intrinsic properties of each cell, such as the transit time compared with maturation (age) counts, although the process which incorporates cell multiplication is influenced by local neighbor conditions. That is, the cells are restricted in their proliferation and cell division, and are kept as they are if there is no vacancy in an adjacent space. As a consequence, cells may overrun their transit time steps. A flow chart of a simulation is shown in Figure 4.

2.5 Assumptions in the model

The model has three assumptions: (1) HSCs behave independently, (2) all events related to HSCs (self-renewal and differentiation) occur stochastically, and (3) all clones originating from a HSC contribute equally to granulopoiesis.

3. Results

3.1 Homeostatic production of granulocyte-lineage cells

The simulation program as developed in this study yields three-dimensional distribution patterns of granulocyte-lineage cells. Since total cell numbers in bone marrow are theoretically maintained at a balance between cellular influx (production) and efflux (leaving the bone marrow), the rates of self-renewal and differentiation of HSC are critical simulation parameters. Based on the stochastic model described for HSC division, T_1/T_{dup} or $\lambda_R/\mu_R = 2$ was initially given to avoid exhausting the HSC population. Changing these parameters produced three different features of granulopoiesis: steady state, extinction, or oscillation. As shown in Table 1, an HSC differentiation time (T_1) double the sum of $T_2 - T_{15}$, was required for the successful achievement of a steady-state granulopoiesis. Also, the ratio μ_R/μ_C determined in this study is consistent with estimates using other mathematical simulations [14, 16]. Figures 5(a) and (b) show a representative steady-state granulopoiesis with a typical set of simulation parameters; fundamental transit time steps were as shown in Figure 2; optional T_1 and T_{dup} were 1, 600, and 800 steps, respectively (Table 1). Under this simulation, the total

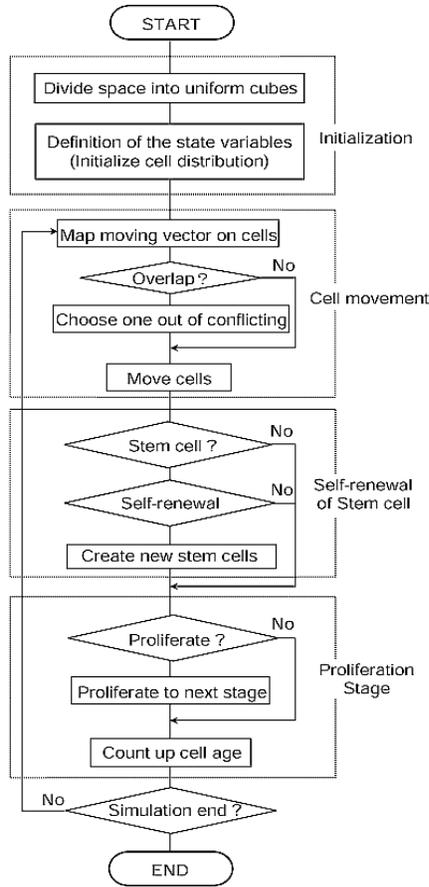


Figure 4. Flow chart of a computer simulation with CA.

Fate of granulopoiesis	Optional					Fundamental T		
	T_{dup}	λ_R	T_1	μ_R	λ_R/μ_r	$T_2 - T_{15}$	μ_C	μ_R/μ_C
Steady-state	800	1/2.4wk	1600	1/4.8wk	2.0	829	1/2.5wk	0.52
	3200	1/9.5wk	6400	1/19wk	2.0	3316	1/9.9wk	0.52
Extinction	400	1/1.2wk	800	1/2.4wk	2.0	3316	1/9.9wk	4.13
	800	1/2.4wk	1600	1/4.8wk	2.0	1658	1/4.9wk	1.02
	800	1/2.4wk	1600	1/4.8wk	2.0	3316	1/9.9wk	2.06
	800	1/2.4wk	1600	1/4.8wk	2.0	6632	1/19.7wk	4.10
Oscillation	6400	1/19wk	12800	1/38wk	2.0	3316	1/9.9wk	0.26

Table 1. Parameter analyses: initial sets of the parameters for T_{dup} of HSCs and the transit time steps of a differentiating clone. T_{dup} , HSC duplication time; λ_R , HSC duplication rate; T_1 , HSC differentiation time; μ_R , HSC differentiation rate; $T_2 - T_{15}$, the sum of the transit time of a differentiating clone; and μ_C , extinction rate of a differentiating clone. Probabilistic rates were determined with a simulation step = 0.5 hr.

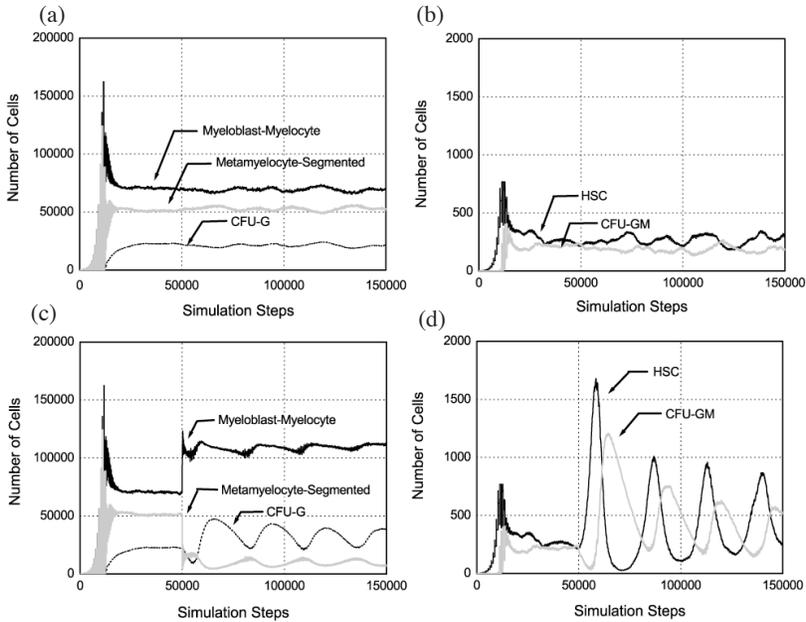


Figure 5. (a) A representative steady-state granulopoiesis model. An initially harvested single HSC developed and achieved steady-state granulopoiesis with fundamental transit times as shown in Figure 2 and optional parameters: $T_1 = 1,600$ steps and $T_{dup} = 800$ steps. (b) The compartments of stem cells and CFU-GM cells were extracted and shown in a different scale for the cell number. (c) Generation of feedback regulation. At 50,000 steps of simulation where steady-state granulopoiesis had been achieved, nonmitotic mature-stage granulocytes (stages 13–15, see Figure 2) were consecutively eliminated from the model by shortening their transit time steps (60, 100, and 144 for stages 13, 14, and 15, respectively, see Figure 2) to 10 steps each until 150,000 steps of simulation. The other simulation parameters are as in Figure 5(a). (d) The compartments of stem cells and CFU-GM cells were extracted and are shown in a different scale for their cell numbers.

cell production and stem cell numbers at the steady state were approximately 150,000 and 300 per analytical space, respectively, indicating that stem cells were maintained with a frequency of 0.2% on average in this model. The parameters producing the steady-state granulopoiesis were then applied for further analyses in this study.

3.2 Inference of feedback circuit in granulopoiesis

We questioned whether a dynamical feedback regulation could be generated as the emergent property in the model. To test this hypothesis,

we perturbed the system by eliminating nonmitotic mature-stage granulocytes (stages 13–15) from the model after 50,000 simulation steps. This was achieved by shortening their transit time steps (60, 100, and 144 steps for stages 13, 14, and 15, respectively, see Figure 2) to 10 steps each until 150,000 simulation steps. As shown in Figure 5(c), a rapidly downward shift of mature cells attributable to their export to the peripheral blood pool and the mobilization of immature cells (myeloblast–myelocyte) was observed. Meanwhile, HSCs increased in number to supply the downstream cells (Figure 5(d)). In contrast, the cell numbers in the CFU-GM compartment decreased inversely, suggesting the presence of down-regulatory kinetics. Subsequently, the cells in the HSC and CFU-GM compartments behaved characteristically to compensate for the consumption of the mature granulocytes in a time-delayed and negative feedback manner, resulting in the oscillation of cell numbers (Figures 5(c) and (d)). Based on the cellular kinetics, we could perceive at least two separate regulatory loops in this model system. One might be a HSC-peripheral feedback loop: the decrease of mature cells causes an increase in the number of HSCs resulting in a subsequent increment of the granulopoietic cell count, which then fed back to cause the suppression of HSC mitotic activity. Another regulatory loop might be a very short circuit regulating cellular kinetics between the compartments of HSCs and CFU-GM: an expansion of the HSC population induces the reduction of differentiation to CFU-GM, resulting in a decrease of the CFU-GM count. These regulatory loops fit well with the well-known hypothetical feedback regulation that has been applied to describe the hematopoietic system [11]. Since the internally generative regulatory mechanisms observed in this model are high-level functions produced as a result of combining the simple low-level rules given to the individual cells, this phenomenon could be defined as a complex emergence.

■ 3.3 Cellular kinetics of feedback regulation

To characterize the cellular behavior responding to the feedback dynamics, we examined the single cell kinetics of the transit times by marking HSCs and tracing their offspring in this model. In Figure 6, panels (a) through (c) show the representative profiles of the transit times of the offspring born at different phases, originating from a single stem cell. The transit times of the offspring from a HSC born at the initial cell expansion phase of granulopoiesis (panel (a)) were effectively consistent throughout the differentiation. They followed the fundamental transit time steps given in Figure 2, and which provided the fastest differentiation producing rapid proliferation of the cells. At a steady state (panel (b)), the transit times of the offspring in the mid-differentiation stage (stages 5–11 corresponding with CFU-G to myelocytes) became

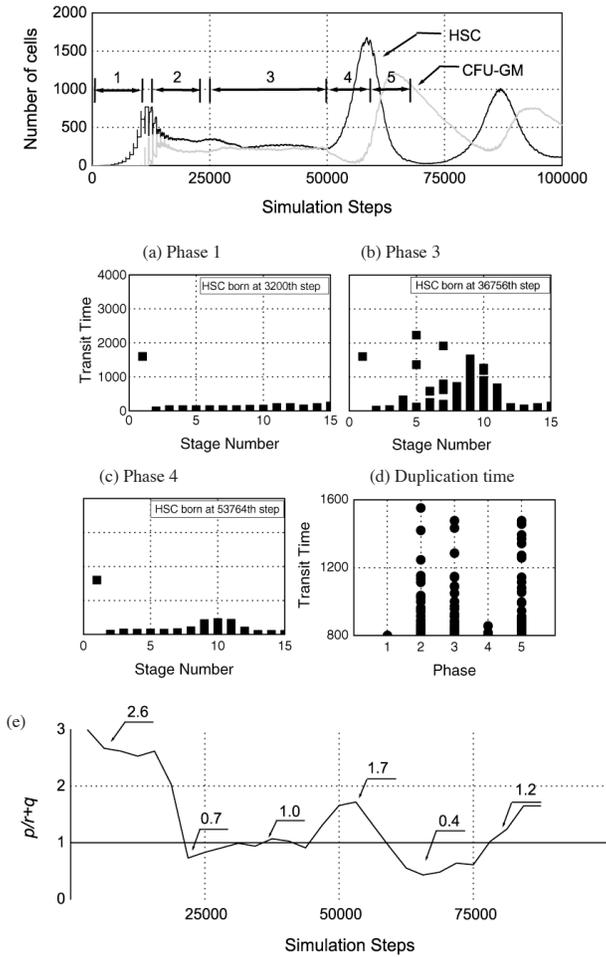


Figure 6. Kinetic analyses of feedback dynamics. Clonal discrimination was performed by labeling a stem cell and its offspring with an identification number. Cells with the same ID numbers were extracted and each of their transit times plotted for the corresponding differentiation stage (stages 1–15). Upper, 1, the initial HSC proliferating phase (simulation steps: 3,200–11,203); 2 and 3, the steady-state phase (simulation steps: 11,259–23,163 and 25,033–49,931, respectively); 4, the HSC expansion phase after mobilization (simulation steps: 50,002–56,862); and 5, the HSC reduction phase (simulation steps: 56,866–61,919) are indicated as two-way arrows with numbers. The simulation parameters are as in Figure 2 and $T_1 = 1,600$. Middle, representative transit time profiles of the offspring derived from a stem cell born at the 3,200th step in phase 1 (a), at the 36,756th step in phase 3 (b), and at the 53,764th step in phase 4 (c). (d) The duplication times of the individual stem cells born during the indicated phases were extracted and plotted for the corresponding phases. (e) Kinetic analysis of stochastic cell divisions. Numbers of newly born stem cells and newly born CFU-GM cells in every 2,000-simulation step were extracted. The ratio of symmetrical and asymmetrical division of HSCs was expressed as $p/r + q$ and plotted for the respective time periods.

Parameters	Initial set	Range
T_{dup}	800	800–1552
λ_R	1/2.4 wk	1/2.4–4.6 wk
T_1	1600	<i>constant</i>
μ_R	1/4.8 wk	<i>constant</i>
λ_R/μ_R	2.0	1.04–2.0

Table 2. Parameters describing HSC behavior in the steady-state granulopoiesis. T_{dup} , HSC duplication time; λ_R , HSC duplication rate; T_1 , HSC differentiation time; and μ_R , HSC differentiation rate. Probabilistic rates were determined with a simulation step = 0.5 hr.

widely distributed. The cell proliferation produced an uneven distribution of the cells in the analytical space resulting in the creation of a density-dependent influence of local neighbor conditions. For example, the cells located in an area with a high cellular density were restricted in cell division as well as differentiation resulting in the prolongation of their transit times. Notably, a strong restriction often caused extremely long transit times (>50-fold) relative to the corresponding fundamental transit times (see panel (b)). On the other hand, the cells followed the fundamental transit time steps in low cellular density conditions. This internally generated variance of the transit time is thought to be a dampening mechanism to compensate for the oscillatory tendency seen in the granulopoietic system [11, 13]. When cellular mobilization was primed, the cells proliferated rapidly and thus the distribution of the transit times became narrow in response to the feedback dynamics (panel (c)) resulting in a rapid expansion of the granulocytes and HSCs. Similarly, as shown in panel (d), HSC duplication times markedly fluctuated, synchronized at the expansionary phases of HSCs (phases 1 and 4), and widely distributed (ranging from 800 to 1,552 steps) at the steady state (phases 2 and 3) and the HSC-declining phase (phase 5). Changes of kinetic parameters describing HSC behavior in the steady state granulopoiesis are summarized in Table 2.

■ 3.4 Stochastic stem cell divisions for self-renewal of hematopoietic stem cells

To distinguish the kinetic frequency of HSC divisions, we traced and determined the number of HSCs producing either symmetrical (p) or asymmetrical division ($r + q$) after each 2,000 simulation steps. The ratio of p and $r + q$ was plotted for the respective time periods as shown in Figure 6(e). A ratio over 1 was observed during the HSC expansionary phase; nearly equal to 1 and below 1 were seen at the steady state and the HSC-declining phases, respectively. This kinetic change fits with the behavior of HSCs and CFU-GM cells responding to the peripheral perturbation (Figure 5). For example, increasing p

produces a greater HSC population, while decreasing r and/or q leads to a reduction of the committed CFU-GM population. Thus, recruitment of stochastic stem cell divisions in a transitional manner appeared to regulate the differentiation–commitment processes in response to the feedback dynamics.

4. Discussion

A pathomechanistic interpretation of experimental and clinical data of abnormal hematopoietic diseases is complicated by the fact that hematopoietic stem cells (HSCs) are hard to manipulate as they are in bone marrow, and they may be likely to lose “stem cellness” as measured by their repopulating ability after *in vitro* manipulation. In addition, the regulatory mechanisms underlying the complex dynamical features of normal hematopoiesis are not completely understood. Thus, further insights into unobserved issues such as *in vivo* HSC kinetics cannot be derived directly from any observed experimental data. In such circumstances, mathematical modeling can be an appropriate method.

The conventional approach using equations that describe the global characteristics of the hematopoietic system, may not express fully enough the details of the behavior of the system’s components because biological systems encompass several different levels of information. For example, hematopoiesis consists of complex elements. Each stem cell would be located in a geographical niche in the bone marrow space and interact with its environment. Cytokines produced by microenvironmental cells would give a synergistic or inhibitory influence on the cells throughout their differentiation process. The genetic programming or events initiated in a cell also have impact on differentiation decisions and proliferation. Governing equations describing each element’s role that consequently affects the global phenomenon of hematopoiesis is an extremely complicated operation. In fact, though the incorporation of the cell–cell interaction of each component cell would be possible, the quantification and integration of physical interactions as a network have not been fully developed; only probabilistic rendering could be incorporated in such models [8–17]. With the aim of focusing on the behavior of the system’s components, cellular automata (CA) modeling can provide a new lateral approach for analyzing a complex system. CA are constituted in a bottom-up approach using local rules without equations, and also include a spatio-temporal concept; the synthesized phenomena as well as behavior of the individual system components (cells) are directly viewed on the computer screen.

The embodiment, with specific emphasis through our CA modeling, of granulopoiesis was as follows.

1. Microscopic behavior of granulopoietic cells did generate feedback circuits in the system.

2. Individual cells varied their transit times in the developmental process resulting in the production of system stability.
3. HSCs dramatically changed their self-renewal times, responding to feedback dynamics.
4. The probabilistic recruitment of symmetrical and asymmetrical HSC divisions fluctuated in a transitional manner in response to the system dynamics.

Given a simulation step of 0.5 hr (see Figure 2), our preliminary estimation of the HSC replication rate would be 1 per 2.4 through 4.6 weeks based on the duplication times of HSCs, ranging from 800 to 1,552 simulation steps in normal, steady-state bone marrow (Table 2). However, these values are assumption-dependent, and therefore, they are not comparable with data from a series of simulation studies [14–16] by Abkowitz *et al.*, in which it was suggested that feline [14, 15] and murine [16] HSCs replicated an average of once every 10 and 2.5 weeks, respectively, and that human HSCs were less frequent and replicated more slowly than these animals [16, 28]. Despite these remarkable differences in the HSC replication rates among animals, the ratios λ_R/μ_R and μ_R/μ_C required to achieve homeostatic hematopoiesis were constant: 1.1 through 2.0 and 0.533 for Safari cats [14] and 1.06 through 1.97 and 0.552 for mice [16], respectively. The values corresponding to these ratios in our data (Table 2) were 1.04 through 2.0 and 0.52, respectively, fitting well with the reported data.

It should be noted that the apoptotic events of the HSCs as well as the differentiating cells were rated as 0 in this model because of a lack of data regarding actual rates of cellular apoptosis in normal hematopoiesis. However, the fundamental nature of hematopoiesis observed in this model is unlikely to be disturbed by this simplification since the cellular apoptosis could be considered as not significantly high in normal hematopoiesis, and the Abkowitz's model [14–16] supports this assumption. For further analysis of the abnormal kinetics of hematopoietic diseases, apoptotic events should be incorporated as one of the underlying mechanisms.

Our CA model is still immature and is some distance from *in vivo* hematopoiesis. Despite several simplifications contained in this model, however, the results obtained in the study will help to provide fundamental principles for the kinetic analyses of the hematopoietic system. Furthermore, it is possible to analyze the individual kinetics of several cell clones with different biological properties, for example, normal *versus* malignant stem cells. CA modeling will provide an appropriate approach for analyzing the residual leukemic cell kinetics in minimal residual disease. In addition, it is possible to distribute the virtual sites that interact with the cells, taken as microenvironments or niches, in the

analytical space. Thus, CA modeling could be applied for analyses of cellular interaction with stromal cells, niches or different lineage cells, and periodic hematopoiesis.

Acknowledgments

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