

Modeling of the Inherence of Feedback Regulation and Stem Cell Behavior in Granulopoiesis

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ABSTRACT

Long-standing controversies in hematopoiesis include the mechanisms of self-maintenance and differentiation commitment of the hematopoietic stem cells (HSC), and regulation of the peripheral control of hematopoiesis. In the present study, we have applied a three-dimensional cellular automaton (CA) model to granulopoiesis in order to identify the internally generative theoretical relationship between microscopic mechanisms and macroscopic behavior of hematopoietic processes. The number of mitotic events of the cells in a proliferating phase, the transit time of each of 15 differential stages from HSC to mature cells (T_1 to T_{15} , and T_{dup} for HSC duplication time), and the neighborhood rules for HSC self-renewal were incorporated in this model system as analytical parameters. Homeostatic granulopoiesis was achieved when the following inequalities for the transit times were fulfilled: $T_1 > \sum^{15} T_n$ and $T_{dup} \geq 1/2 T_1$. Importantly, stabilization of the cell production was induced in a negative feedback manner following external perturbation of the peripheral granulocyte numbers. The T_{dup} of individual HSC dramatically fluctuated to produce the offspring responding to this perturbation. A single cell kinetic analysis demonstrated that symmetrical or asymmetrical cell division of the HSC was recruited in a transitional manner resulting in generation of the regulatory effect on the lineage-commitment processes. The inherence of feedback regulation would be a characteristic feature of the emergent dynamical property in the hematopoietic system. The CA modeling will provide the framework to analyze the behavior of HSC and to understand the abnormal kinetics of hematopoietic diseases.

1. INTRODUCTION

Mature blood cells and their precursors in the bone marrow ultimately derive from a small population of hematopoietic stem cells (HSC) that have a high proliferative potential and sustain hematopoiesis throughout life (see Figure. 1). The earliest HSC are totipotent and have a high self-renewal capacity [2, 7], although these potencies are progressively lost as the stem cells differentiate. The progenitor cells or colony-forming units (CFUs) are committed to one cell lineage and proliferate to form large colonies of erythrocytes, granulocytes, monocytes, or megakaryocytes. 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. However, these regulatory systems in hematopoiesis are not completely understood. Well-investigated regulatory factors include lineage-specific growth factors, such as erythropoietin (EPO), thrombopoietin (TPO),

granulocyte colony-stimulating factor (G-CSF), monocyte colony-stimulating factor (M-CSF), and granulocyte-monocyte colony-stimulating factor (GM-CSF), and their essential roles have been established for the growth of each lineage-committed cell *in vitro* and *in vivo* [8]. Recent molecular analyses have opened the way to investigate the molecular events that occur at the lineage-specific commitment decisions a cell makes and the differentiation process to mature cells [12]. Regulation of the lineage-specific gene activation seems even more complex since a number of lineage-affiliated genes are activated at different levels in a cell [5, 6]. Thus, the orchestration of these gene expressions, for example, amplification of the specific gene expression while down-regulating the other genes to reduce the noise, should be conducted intrinsically or extrinsically through extracellular signaling via cytokines, growth factors, and microenvironmental interaction, but all the regulatory features have not been clearly elucidated to date. Therefore, new approaches to clarify these regulatory networks need to be exploited, and mathematical model analyses have been proposed for this purpose [3, 4, 9, 13].

Computer simulations using Cellular Automaton (CA) may provide a useful tool for understanding the system involved. CA models can produce complex patterns based on simple strategies describing behavior of elements, which are analogous to the appearance of complex systems as commonly seen in biological events. CA consists of discrete unit elements arranged uniformly on one or more dimensional space, each of which can represent a limited set of values. The time evolution of elements is performed synchronously according to the local neighbor rules, taking into account the state of the element itself and also the interaction between nearby elements. Hence the local interaction leads to global dynamics in the CA model.

In the current study, we present our preliminary efforts using CA to explore the theoretical relationship between microscopic events including cell division, cell proliferation and differentiation, and macroscopic regulation of hematopoiesis. We report here the dynamical feedback regulation generated as a feature of the emergent property and characterization of a single stem cell behavior in the hematopoietic model system.

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2. METHODS

2.1 Compartmentalization of Granulopoiesis

To describe human granulopoiesis, we divided the granulopoietic process into several compartments in which each biological cell stage was represented by a model compartment characterized by the transit time (T), the number of mitoses, and the fraction of actively proliferating cells. As shown in Figure 1, the compartment of the stem cells (stage 1) includes the pluripotent stem cells with self-renewal capacities and very early-differentiated cells toward granulopoiesis. This early process of HSC differentiation has not been clarified, and hence, the given transit time is referred to as *optional*. Likewise, the full features of the self-renewal of HSC 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 pluripotent stem cell compartment. The next compartments represent the proliferating cell stages of CFU-G (6 mitoses, stage 3-8), myeloblasts (1 mitosis, stage 9), promyelocytes (1 mitosis, stage 10), myelocytes (2 mitoses, stage 11 and 12), and the postmitotic maturing stages of metamyelocytes (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 the peripheral blood. The model parameters used here for normal granulopoiesis are taken directly from the literature or deduced from published experimental data [10, 11], and are incorporated in Figure 1.

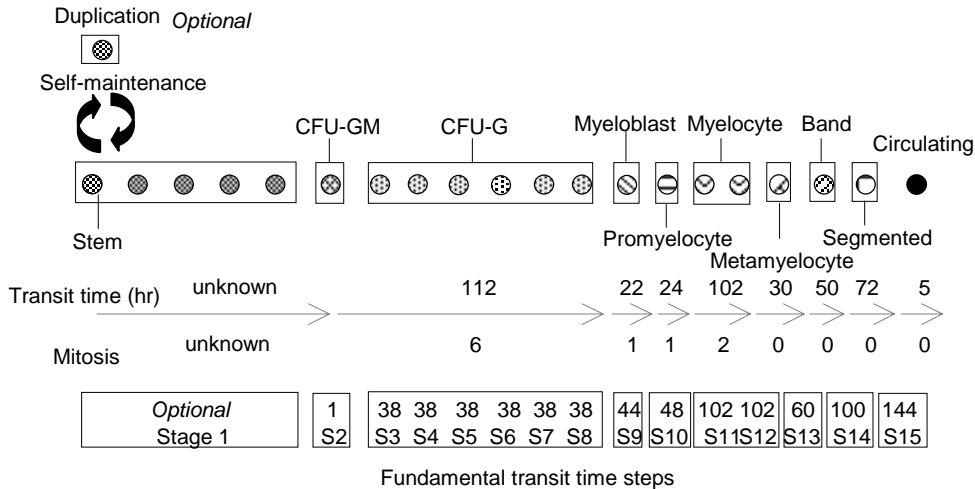


Figure 1: Compartmentalization of granulopoiesis

2.2 Development of a CA Model

The Cellular Automata model is developed for three-dimensional space assuming the bone marrow where the granulocyte-lineage cells are distributed. The space consists of 100 x 100 x 100 unit cubic areas; the size of each corresponds to a single biological cell. In order to represent cell distribution in space, the state variables must be assigned to each unit area that represents any of a limited set of values. 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 a certain position, and are also distinguishable

by their proliferation stage. According to the assumption on the granulopoietic process, a total of 16 cell states including a cell absence case are required. As the second state variable, the cell age is defined additionally to areas where the cells are present. The age is counted up every simulation step to express cell maturation until they reach the respective transit time for the next stage.

2.3 Description of the Local Neighbor Rules

In order to carry out spatio-temporal change of the system during the course of simulation, the state variables must be updated synchronously according to the local neighbor rules at every single calculation step. These rules, which describe cell dynamics, include the movement of cells, the transition to the different stage depending on the state of the unit element itself, and the micro-environmental influence of neighbor elements. Basically, the cell can move to any of its nearby element out of 26 directions selected randomly at every time step. To avoid collision of cells with other cells at a certain neighbor site, conflicting directions are avoided for the movements.

The respective transition to the next stage in the cell lineage is also determined fundamentally by 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 condition. That is, the cell proliferation and the cell division are restricted if there is no free space in the adjacent space, which consequently causes over-maturation.

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 the bone marrow are theoretically maintained on a balance between cellular influx (production) and efflux (leaving from the bone marrow), the rates of self-renewal

and differentiation of HSC are critical simulation parameters. Changing these parameters produced 3 different features of granulopoiesis: extinction, steady state, or oscillation. Figure 2 shows the steady-state granulopoiesis achieved with a typical set of stimulation parameters. This simulation model demonstrated two inequalities for the transit times: $T_1 > \sum_{n=2}^{13} T_n$ and $T_{dup} \geq 1/2 T_1$, which fit well with clinical observations and experimental results from transplantation studies [1].

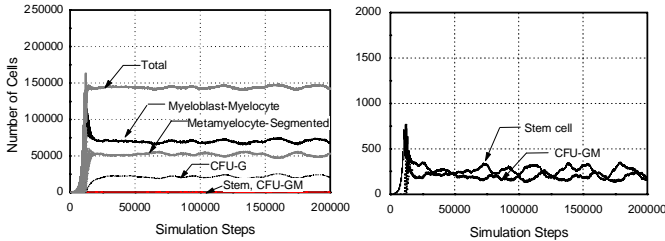


Figure 2: Homeostatic granulopoiesis

3.2 Inherence of Feedback Circuit in Granulopoiesis

We questioned whether the dynamical feedback regulation could be generated as the emergent property in this model. To test this hypothesis, we perturbed the system by eliminating non-mitotic mature-stage granulocytes (stage 13-15) at the steady state achieved. This could reflect situations characterized by increased demand for peripheral granulocytes such as bacterial infections. As shown in Figure 3, a rapidly downward shift of mature cells attributable to exporting to the outside pool and mobilization of immature cells were observed. Meanwhile, HSC increased in number to supply the downstream cells. In contrast, the cell numbers in CFU-GM compartment decreased inversely, suggesting the presence of down regulatory kinetics, presumably to expand the stem cell numbers. Thereafter, the following behavior of the cells in the compartments came to compensate for the consumption of the mature granulocytes with the characteristic feature of a time-delayed and negative feedback manner resulting in oscillation of the cell numbers. From this cellular kinetics, we could perceive at least two separate regulatory loops in this model system. One might be an HSC-peripheral feedback loop: the decrease of mature cells causing an increase of HSC number resulting in a subsequent increment of granulopoietic cell count, which then fed back to cause the suppression of HSC mitotic activity.

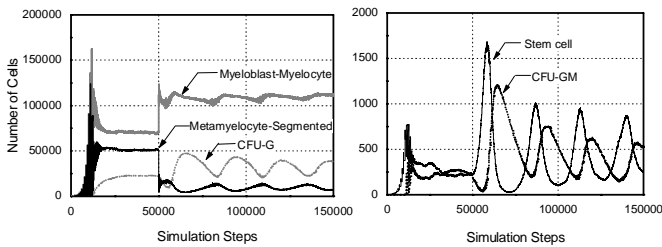


Figure 3: Feedback phenomenon

Another regulatory loop might be a very short circuit down-regulating cellular kinetics between HSC and CFU-GM: an increase in HSC suppressing itself to commit to CFU-GM resulting in a decrease of CFU-GM count. Since the internally generative regulatory mechanisms observed in this model are the high-level functions produced as a result of combining simple low-level rules given to the individual cells, this phenomenon could be defined as emergence wherein complex.

3.3 Cellular Kinetics of Feedback Regulation

To characterize the mechanical dynamics of the feedback regulation, we determined the single-cell kinetics by marking HSC and tracing their offspring in this simulation model. In Figure 4, panels A-C show the profiles of transit times of the cells derived from a single stem cell born at different phases in the feedback dynamics generated internally by peripheral perturbation. At the initial phase of granulopoiesis (panel A), the transit times of the stem cell and its offspring were virtually consistent throughout the differentiation. At steady state (panel B), the transit times of the cells in the mid-differentiation stage became widely distributed. This variance of the transit times of differentiating cells is thought to be a dampening mechanism to compensate for the oscillatory tendency seen in the granulopoietic system [11]. 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 expansion of the granulocytes and HSC. As shown in panel D, HSC duplication times markedly fluctuated as synchronized at the expansionary phase of HSC (phases 1 and 4) and were widely distributed at the steady state (phases 2 and 3) and the down-regulatory phase of HSC self-renewal (phase 5).

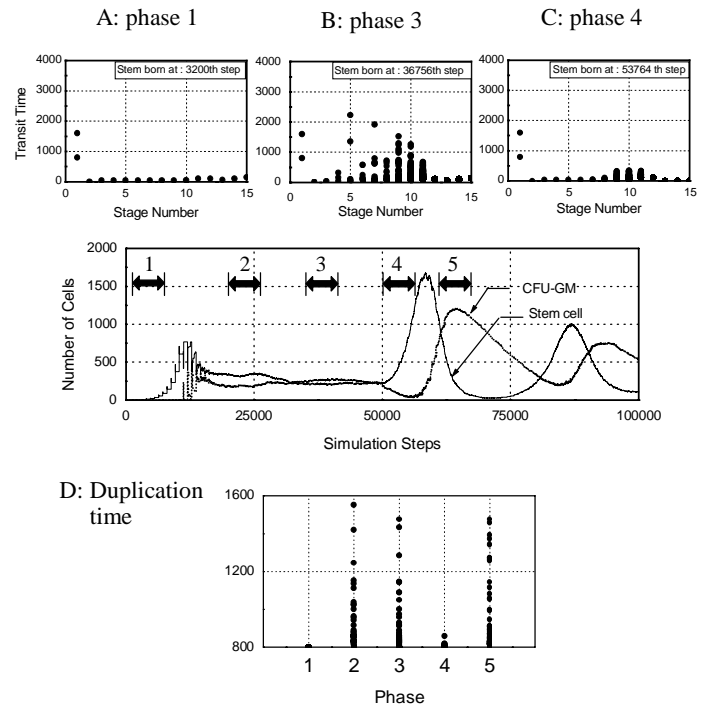


Figure 4: Kinetic analysis of feedback dynamics

3.4 Stochastic Stem Cell Divisions for Self-renewal of HSC

The description given to the stem cells in this simulation model is that a stem cell is able to produce a maximum of two daughter stem cells until committed to CFU-GM. In practice, each stem cell undergoing cell division can either generate two, one (the other is a committed cell), or no daughter stem cells (producing two committed cells) in a stochastic situation (see Figure 5).

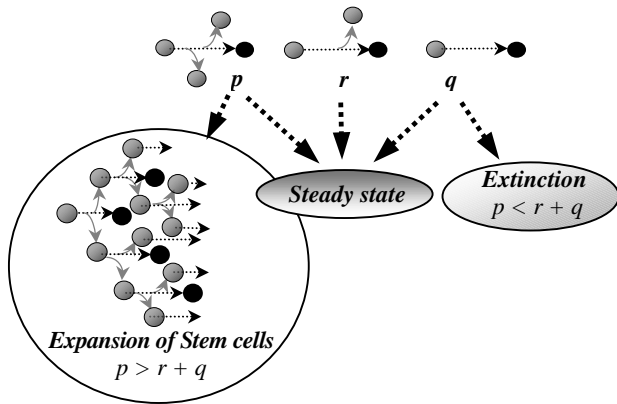


Figure 5: Stem cell division models

When we describe the cell division processes with three probabilities (p , r , and q as indicated in Figure 5), a steady state would be maintained if symmetrical and asymmetrical divisions were balanced while an expansion of stem cells could be induced under the situation that $p > r + q$. The extinction for finite population would occur if $p < r + q$. To distinguish the kinetic frequency of these stem cell divisions, we traced and determined the number of stem cells producing either symmetrical or asymmetrical division in each of 2000 simulation steps. The ratio of p and $r + q$ was plotted for the respective time period as shown in Figure 6. A ratio over 1 was observed during the cell

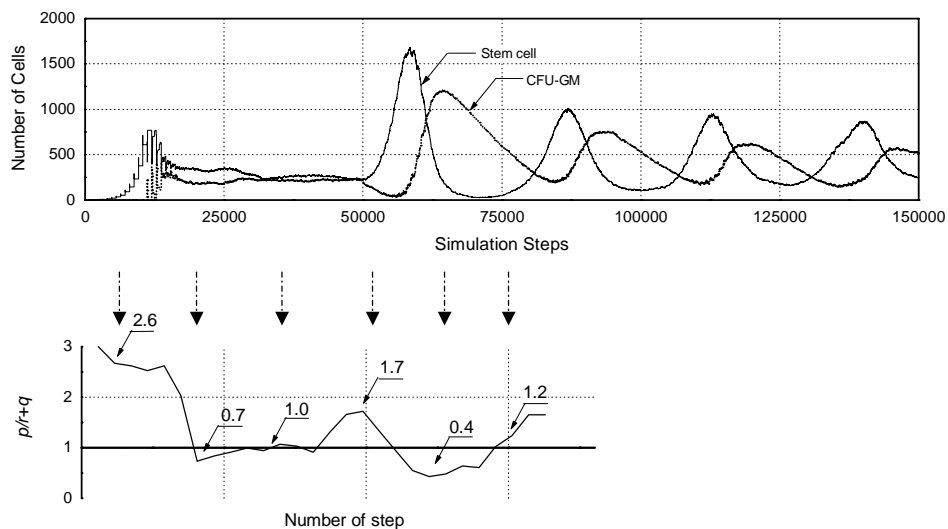


Figure 6: Frequency of stochastic cell divisions

expansionary phase; nearly equal to 1 and below 1 was seen at the steady state and the cell-declining phase, respectively. This kinetic change of stem cell divisions explanatorily fit the behavior of HSC and CFU-GM responding to the peripheral perturbation (Figure 3). For example, increasing p produces a greater HSC population, while decreasing r and/or q leads to depletion of committed CFU-GM population. Recruitment of stochastic stem cell divisions appeared to be regulated in a transitional manner responding to the feedback dynamics resulting in regulatory effects on the lineage-commitment processes.

4. DISCUSSION

The pathomechanistic interpretation of experimental and clinical data of abnormal hematopoietic diseases is complicated by the fact that hematopoietic stem cells are hard to be manipulated as they are in bone marrow and the regulatory mechanisms underlying the complex dynamical feature of hematopoiesis are not completely understood. Thus, further insights cannot be derived directly from experimental data. In such circumstances, mathematical modeling can be an appropriate method. Our CA modeling of granulopoiesis demonstrated the following: 1) homeostatic granulopoiesis was simulated and two inequalities for the transit time of the cellular developmental process were drawn in this model; 2) microscopic behavior of granulopoietic cells generated the feedback circuits in the system; 3) the transit times of individual cells were variable at the steady-state granulopoiesis and modulated in response to the feedback dynamics; 4) the frequency of HSC self-renewal dramatically fluctuated in response to the system dynamics; and 5) the stochastic cell divisions were recruited in a transitional manner in response to the feedback dynamics.

It should be noted that several assumptions were required in this preliminary model for its simplification. First, this model simulated only granulopoiesis so that the specific interaction of different lineage cells is not considered. Second, the stem cells defined in this model are not the typical HSC characterized by pluripotency. The stem cells in the model are deterministically programmed to differentiate toward granulocytes. Third, the apoptotic events of the cells are excluded in this model, and that

would be incorporated when particular conditions are considered. Last, the bone marrow cavity where the cells proliferate consists of a vast network of vascular channels as well as non-hematopoietic cells forming the microenvironment and niches, and thus it is not as simple as that in the model.

Despite these simplifications, the results obtained in this model will help to provide fundamental principles for the kinetic analyses of the hematopoietic system. This modeling could also be applied for conceptual analyses of the cellular interaction with stromal cells, niches, or different lineage cells, and the influence of apoptosis on hematopoiesis, as well as clinical investigation of residual leukemic cell kinetics in minimal residual disease and periodic hematopoiesis.

5. REFERENCES

- [1] Abkowitz, J. L., Catlin, S. N., and Gutter, P. Evidence that the hematopoiesis may be a stochastic process *in vivo*. *Nature Med* 2:190, 1996.
- [2] Becker, A., McCulloch, E., and Till, J. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* 197: 452, 1963.
- [3] Blumenson, L. E. A comprehensive modeling procedure for the human granulopoietic system: Over-all view and summary of data. *Blood* 42: 303, 1973.
- [4] Fokas, A. S., Keller, J. B., and Clarkson, B. D. Mathematical model of granulocytopenia and chronic myelogenous leukemia. *Cancer Res* 51: 2084, 1991.
- [5] Ford, A. M., Bennett, C. A., Healy, L. E., Navarro, E., Spooner, E., and Greaves, M. F. Immunoglobulin heavy-chain and CD3 delta-chain gene enhancers are DNase I-hypersensitive in hemopoietic progenitor cells. *Proc Natl Acad Sci USA* 89: 3424, 1992.
- [6] Jimenez, G., Griffiths, S. D., Ford, A. M., Greaves, M. F., and Enver, T. Activation of the beta-globin locus control region precedes commitment to the erythroid lineage. *Proc Natl Acad Sci USA* 89: 10618, 1992.
- [7] Lemishka, I. R., Raulet, D. H., and Mulligan R. C. Developmental potential and dynamic behavior of hematopoietic stem cells. *Cell* 45: 917, 1986.
- [8] Metcalf, D., and Nicola, N. A. *The Hematopoietic Colony Stimulating Factors*. Cambridge, UK, Cambridge, 1995.
- [9] Rubinow, S. I., and Lebowitz, J. Z. A mathematical model of neutrophil production and control in normal man. *J Math Biol* 1: 187, 1975.
- [10] Schmitz, S., Franke, H., Brusis, J., and Wichmann, H. E. Quantification of the cell kinetic effects of G-CSF using a model of human granulopoiesis. *Exp Hematol* 21: 755, 1993.
- [11] Schmitz, S., Franke, H., Loeffler, M., Wichmann, H. E., and Diehl, V. Reduced variance of bone-marrow transit time of granulopoiesis—a possible pathomechanism of human cyclic neutropenia. *Cell Prolif* 27: 655, 1994.
- [12] Shivdasani, R. A., and Orkin, S. H. The transcriptional control of hematopoiesis. *Blood* 87: 4025, 1996.
- [13] Wichmann, H. E., and Loeffler, M. *Mathematical Modeling of Cell Proliferation: Stem Cell Regulation in Hemopoiesis*. Boca Raton, FL, CRC, 1988.