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Hybrid Models in Erythropoiesis and in Megakaryopoiesis

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Abstract. Hematopoiesis is a complex process which results in production of erythrocytes, platelets and white blood cells from pluripotent stem cells located in the bone marrow. We will present hybrid models of hematopoiesis and will use them to study the lineage choice of bipotent erythro-megakaryocytic progenitors and erythroid lineage of hematopoiesis. Biological cells will be considered as individual objects, intracellular regulatory networks will be described with ordinary differential equations and biochemical substances in the extracellular matrix with partial differential equations.

Keywords and phrases: hybrid models, cell populations, intracellular regulation, hematopoiesis

Mathematics Subject Classification: 92C30, 35K57, 70F99

1. Introduction

Systematic investigation of hematopoiesis with mathematical modeling began in the 1970s with the study of the dynamics of hematopoietic stem cells by Mackey [33, 34]. It was supposed that aplastic anaemia and periodic hematopoiesis in humans appear due to the irreversible cell loss from the proliferating pluripotential stem cell population. A model for pluripotential stem cell population was described by delay differential equations. In more recent models, described by first order differential equations, a population dynamics of cells capable of both proliferation and maturation was analysed [35]. In Bernard et al. [5] a mathematical model was proposed to explain the origin of oscillations of circulating blood neutrophil number. The authors demonstrated that an increase in the rate of stem cell apoptosis can lead to long period oscillations in the neutrophil count. In extension of the previous model Colijn and Mackey in [16] applied mathematical model, described with a system of delay differential equations, to explain coupled oscillations of leukocytes, platelets and erythrocytes in cyclical neutropenia. The platelet production process (thrombopoiesis) attracted less attention through years [22, 47]. Cyclical platelet disease was a subject of mathematical modeling in Santillan et al. [43] and was enriched in Apostu et al. [3]. Leukemia and other blood disorders were studied in [2, 4, 27, 28, 39, 45].

The red blood cell production process (erythropoiesis) has recently been the focus of modeling in hematopoiesis. A model describing the regulation of erythropoiesis in mice and rats has been developed by Wichmann, Loeffler and co-workers [48]. The authors considered feedback controls from reticulocytes

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on progenitors and from progenitors on stem cells, confronted their models with experimental data on stress erythropoiesis and validated by comparing with experimental data during stress erythropoiesis such as hypoxia and different forms of induced anaemia [50]. Analysis of the regulating mechanisms in erythropoiesis was enriched in [49]. In 1995, Bélair et al. proposed an age-structured model of erythropoiesis where erythropoietin (EPO) caused differentiation, without taking into account erythropoietin control of apoptosis [29]. In 1998 Mahaffy et al. [36] expanded this model by the apoptosis rate dependence on the level of erythropoietin. The age-structured model is detailed in [1] with the assumption that decay rate of erythropoietin depends on the number of precursor cells. In [18] Crauste et al. included in the model the influence of EPO upon progenitors apoptosis and showed the importance of erythroid progenitor self-renewing by confronting their model with experimental data on anaemia in mice. A model of all hematopoietic cell lineages that has been proposed by Colijn and Mackey [15, 16] includes dynamics of hematopoietic stem cells, white cell lineage, red blood cell lineage and platelet lineage. A review of mathematical models and simulation studies, applied to stem cell biology, with particular interest to the hematopoietic system is proposed by Roeder [41].

All the previously mentioned approaches did not consider spatial aspects of hematopoiesis. Cellular regulation by cell-cell interaction was neither considered in these models. However, their spatial position relatively to each other should be taken into account [14, 24]. Multiscale approaches include both cell population kinetics [7] or erythroid progenitor dynamics [17, 18] and intracellular regulatory networks dynamics in the models [9] in order to give insights in the mechanisms involved in erythropoiesis. Off-lattice discrete-continuous hybrid models, applied to the erythropoiesis modeling, allow one to take into account simultaneously interactions at the cell population level, regulation at the intracellular and extracellular levels and to study the importance of the spatial structure [11,31]. This approach permitted to investigate spatial aspects of erythropoiesis and the role of macrophage in stabilizing erythroblastic island and therefore its role in normal red blood cell production [24]. Also this approach was used in modeling of leukemia and its treatments, showing the relevance of chronotherapy to cure this disease [30].

In this work we continue to develop hybrid models with off-lattice cell dynamics in order to study hematopoiesis. Cells will be considered as discrete objects which interact with each other mechanically and biochemically while intracellular and extracellular concentrations will be described with ordinary and partial differential equations. In the next section we will briefly recall the multi-scale hybrid approach. More detailed description could be found in the previous articles [11, 24, 30, 31]. Then we will apply it to study the lineage choice of erythrocytic-megakaryocytic bipotent proenitors MEP between megakaryocytic or erythrocytic lineages (Section 3) and some open questions of erythropoiesis modeling (Section 4).

2. Multi-scale hybrid model

Multi-scale hybrid models take into account dynamics of cell populations, intracellular and extracellular regulations. The intracellular regulation described by ordinary differential equations can be influenced by some substances in the extracellular matrix described by partial differential equations. Stochasticity due to random events (cell cycle duration, orientation of the mitotic spindle at division) and small population effects also plays an important role. Such discrete-continuous models are called hybrid models [7,38,40,46]. They were used to study hematopoiesis in [6, 8, 10].

In hybrid models, cells can be considered as individual objects which can divide, differentiate, die by apoptosis, move and interact mechanically with each other. Cell fate is determined by intra-cellular regulatory networks and is described by a system of ordinary differential equations

$$\frac{d\xi_i(t)}{dt} = F(\xi_i(t), \nu(x_i, t)), \tag{2.1}$$

where ξ_i is a vector of intra-cellular concentrations of cell i, ν is a vector of extra-cellular concentrations, F is the vector of reaction rates which will be specified below for intracellular regulation of erythroid

progenitors. The concentrations of the species in the extra-cellular matrix are described by the reaction-diffusion system of equations

$$\frac{\partial \nu}{\partial t} = D\ \Delta\nu + G(\nu, c), \tag{2.2}$$

where c is the local cell density, G is the rate of consumption or production of these substances by cells. These species can be either nutrients coming from outside and consumed by cells or some other bio-chemical products consumed or produced by cells. We will specify them in the following section.

When cells divide and increase their number, they can push each other resulting in cell displacement. In order to describe mechanical interaction between cells, we restrict ourselves here to the simplest model where cells are presented as elastic spheres (compressible) with an incompressible core (black core inside each cell, it can be seen in all figures with cell populations). Cell motion is determined by the sum of the mechanical forces acting on that cell from other cells. Under the assumption of small deformations, we can express the mechanical contact force acting between them as a function of the distance between their centers. The force between two particles centered at x_i and x_j is given by a function $f(d_{ij})$ of the distance d_{ij} between the centers. Cell motion is described by the displacement of its center by Newton's second law:

$$m\ddot{x_i} + \mu m\dot{x_i} - \sum_{j\neq i} f(d_{ij}) = 0, \tag{2.3}$$

where m is the mass of the cell, μ the coefficient of friction, the second term in left-hand side describes the friction due to viscosity of the surrounding medium, the third term is the potential force between cells. The force between two spherical spheres is given by the formula

$$f(d_{ij}) = \begin{cases} +\infty, & d_{ij} < d_0 - 2H_1 \\ \kappa \dfrac{d_0 - d_{ij}}{d_{ij} - d_0 + 2H_1}, & d_{ij} < d_0, \\ 0, & d_{ij} \geq d_0 \end{cases} \tag{2.4}$$

where d_0 is the sum of cell radii, κ is a positive parameter, and H_1 is the size of the outer shell that forms the compressible part of the cell. The force between the particles tends to infinity when d_{ij} decreases to $d_0 - 2H_1$. The force is repulsive if $d_{ij} \geq d_0$ and is null otherwise.

Let us recall that cell cycle progresses through G_0(quiescence)/G_1(gap 1), S (synthesis), G_2 (gap 2) and M (Mitosis) phases. We assume the duration of G_0/G_1 phase is a random variable with a uniform distribution in some given interval [26] while duration of other phases is supposed to be constant.

All newborn cells have the same radius r_0 and linearly increase in size until the end of their cycle, when they reach twice the initial radius. When a cell divides, it gives birth to two small cells side by side, the direction of division being randomly chosen, from 0 to 2π. At the end of cell cycle, the cells make a choice between self-renewal and differentiation.

For a more detailed description of hybrid models we refer the reader to previous articles [11,24,30,31]. We hereafter present how the model is defined at each scale and how the different levels interact for particular applications to hematopoiesis.

Thus, we describe blood cell production with hybrid models where cells are considered as individual objects, intracellular regulation is described by ordinary differential equations and extracellular regulation by partial differential equations. Initial concentrations of intracellular proteins are determined either by these values in the mother cell or they are considered as random variables. During cell cycle, cells grow and then divide. At the end of the cell cycle, they make a choice between self-renewal and differentiation depending on the concentrations of intracellular proteins. Cell growth and division result in their displacement. Cell motion is reduced to the motion of their centers, and it is described by Newton's second law. We use this approach to study the erythroid lineage of hematopoiesis beginning from lineage choice and then its further progression.

3. Lineage choice

All blood cells originate from the pluripotent stem cells after a cascade of differentiations regulated by different transcription factors. Recent models take into count PU.1 and GATA-1 for the regulation of differentiation of progenitors into granulocyte/macrophage and erythrocyte/megakaryocyte lineages [21]. We will begin with a model of lineage choice of bipotent erythro-megakaryocytic progenitors. In this case, cell-cell interaction can be neglected, and the model does not include extracellular regulation.

3.1. Intracellular regulation

In this section we discuss intracellular regulation of bipotent MEP (erythro-megakaryocytic progenitor) between thrombocytic and erythroid lineage. MEP can differentiate into BFU-E (burst forming unit erythroid) progenitors or BFU-MK (burst forming units-megakaryocytic) progenitors giving rise to the two lineages of blood cells. MEP cells can also self-renew. The complex mechanism that induces commitment of MEP is not completely understood but the role of some growth factors and transcription factors has been established. Indeed, the erythroid transcription factor (zinc finger factor) GATA-1 is required for the differentiation and maturation of erythroid/megakaryocytic cells [13].

Endogenous EKLF (erythroid Kruppel-like factor) promotes the erythroid lineage choice while FLI-1 (Friend Leukemia Integration 1) overexpression inhibits erythroid differentiation [13]. Interactions between FLI-1 and EKLF are also involved [13], EKLF represses FLI-1 [12, 25, 44].

We will take into account intracellular regulation of MEP with GATA-1 and transcription factors FLI-1 and EKLF. Moreover according to the biological observations there is no communication between these cells. Therefore extracellular regulation is not present in this model.

Let u be the concentration of the transcription factor EKLF, v the concentration of FLI-1 and w of GATA-1. FLI-1 can form complexes with the other two factors. We will denote them by $[uv]$ and $[vw]$, respectively. These complexes can degrade into EKLF, FLI-1. Then the intracellular regulation can be described as follows:

$$u + w \underset{k_1^-}{\overset{k_1^+}{\longleftrightarrow}} [uw], \tag{3.1}$$

$$[uw] \xrightarrow{k_2} n_1 u, \tag{3.2}$$

$$v + w \underset{k_3^-}{\overset{k_3^+}{\longleftrightarrow}} [vw], \tag{3.3}$$

$$[vw] \xrightarrow{k_4} n_2 v. \tag{3.4}$$

$$\frac{du}{dt} = -k_1^+ uw + k_1^-[uw] + k_2 n_1 [uw] \tag{3.5}$$

$$\frac{dv}{dt} = -k_3^+ vw + k_3^-[vw] + k_4 n_2 [vw] \tag{3.6}$$

$$\frac{dw}{dt} = -k_1^+ uw + k_1^-[uw] - k_3^+ vw + k_3^-[vw] \tag{3.7}$$

$$\frac{d[uw]}{dt} = k_1^+ uw - k_1^-[uw] \tag{3.8}$$

$$\frac{d[vw]}{dt} = k_3^+ vw - k_3^-[vw] \tag{3.9}$$

Taking into account the mass balance $w + [uw] + [vw] = w_0$, we obtain the system of equations for these concentrations:

$$\frac{du}{dt} = -k_1^+ u(w_0 - [uw] - [vw]) + (k_1^- + n_1 k_2)[uw], \tag{3.10}$$

$$\frac{dv}{dt} = -k_3^+ v(w_0 - [uw] - [vw]) + (k_3^- + n_2 k_4)[vw], \tag{3.11}$$

$$\frac{d[uw]}{dt} = k_1^+ u(w_0 - [uw] - [vw]) - k_1^-[uw], \tag{3.12}$$

$$\frac{d[vw]}{dt} = k_3^+ v(w_0 - [uw] - [vw]) - k_3^-[vw], \tag{3.13}$$

$$w + [uw] + [vw] = w_0. \tag{3.14}$$

where k_i^+ and k_i^- are the coefficients of production and of degradation of the different substances. n_i is the multiplicative coefficient of production.

Intracellular regulation described by this system of equations should be completed by conditions of self-renewal of the progenitors or their commitment to one of the two lineages. We will suppose that if the concentrations of FLI-1 and EKLF are less than some critical values at the end of cell cycle, then differentiation does not hold and the cell self-renews. The cell cycle duration is variable and is attributed at each new cell at the time of its birth. If at least one of these two concentrations is greater than the critical level, then the cell differentiates. In this case, it becomes BFU-MK if the concentration of FLI-1 is greater than the concentration of EKLF, and it becomes BFU-E otherwise (Figure 3.1).

In this model, transcription factors FLI-1 and EKLF influence the commitment of progenitors in a MEP cell lineage with the following rules (cf. Figure: 3.1):

- If [FLI-1] $<$ [FLI-1]$_{crit}$ and [EKLF] $<$ [EKLF]$_{crit}$ then self-renewal.
- If [FLI-1] $>$ [FLI-1]$_{crit}$ and [EKLF] $<$ [EKLF]$_{crit}$ then BFU-MK production.
- If [FLI-1] $<$ [FLI-1]$_{crit}$ and [EKLF] $>$ [EKLF]$_{crit}$ then BFU-E production.
- If [FLI-1] $>$ [FLI-1]$_{crit}$, [EKLF] $>$ [EKLF]$_{crit}$ and [FLI-1] $>$ [EKLF] then BFU-MK production else [FLI-1] $<$ [EKLF] BFU-E production.

A simple idea to achieve such a behavior is to introduce two threshold values: $[EKLF]_{crit}$, $[FLI-1]_{crit}$ that determine the choice between the two lineages for cells reaching the end of their cycle.

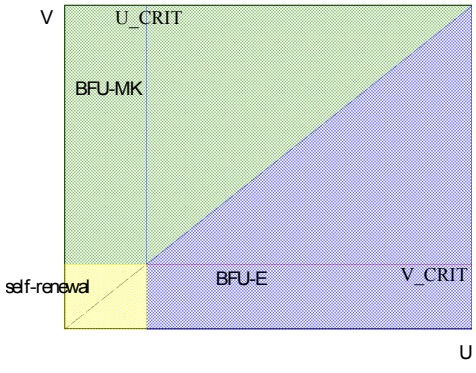
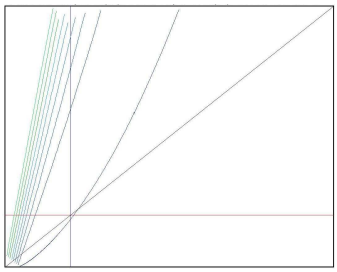
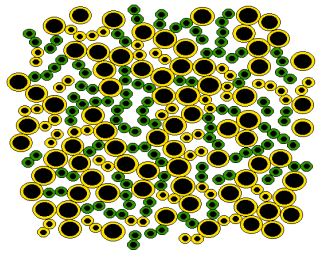


FIGURE 1. Phase space: concentration u on x-axis, v on y-axis. If the concentrations at the end of cell cycle are at a coordinate point that belongs to the yellow region, the considered cell self-renews. In blue region, the cell differentiates into BFU-E. In green region, the cell differentiates into BFU-MK. Cell fate depends on the concentrations u and v.

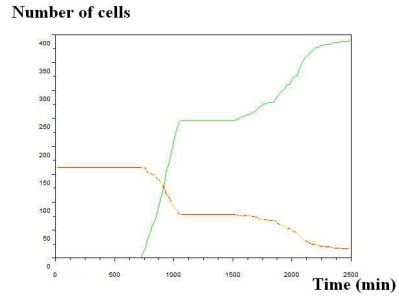
Figure Simulations show the trajectory in phase space, i.e. the behavior and the quantities of u and v in a cell during a cell cycle. Snapshot and curves allow to see the behavior of a cell culture in accordance with what we could expected.



(a) Concentrations u and v in 10 cells

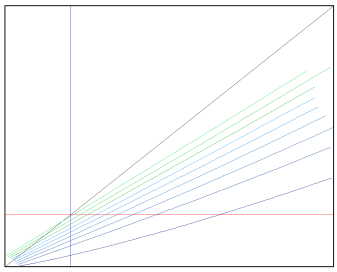


(b) Snapshot of cell population

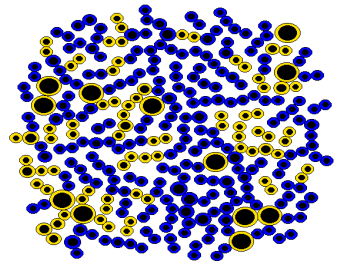


(c) Number of MEP (red) and BFU-MK (green) cells as a function of time

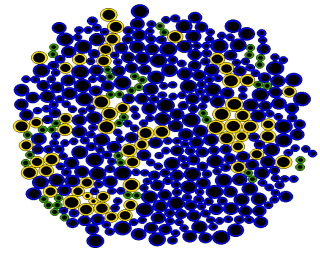
FIGURE 2. Evolution of the population of MEP cells for the values of parameters $k_1^+ = 0.005$, $k_1^- = 0.008$, $k_2 = 0.01$, $k_3^+ = 0.005$, $k_3^- = 0.008$, $k_4 = 0.1$. In Figure 2(a), curves of the phase space are in majority located above the first bisector. MEP cells self-renew and differentiate into BFU-MK cell lineage as shown in Figure 3.1. The Figures 2(b) and 2(c) show the evolution towards BFU-MK cells and gradual disappearance of self-renewal.



(a) Concentrations u and v in 10 cells



(b) Snapshot of cell population



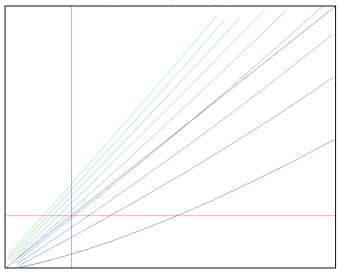
(c) The same cell population some time later

FIGURE 3. Evolution of the population of MEP cells for the values of parameters $k_1^+ = 0.005$, $k_1^- = 0.008$, $k_2 = 0.1$, $k_3^+ = 0.005$, $k_3^- = 0.008$, $k_4 = 0.2$. MEP cells self-renew and differentiate into BFU-E cell lineage. In Figure 3(a), curves of the phase space are in majority located below the first bisector, there is commitment into BFU-E lineage as shown in Figure 3.1. The Figures 3(b) and 3(c) show the evolution towards BFU-E cells and gradual disappearance of self-renewal.

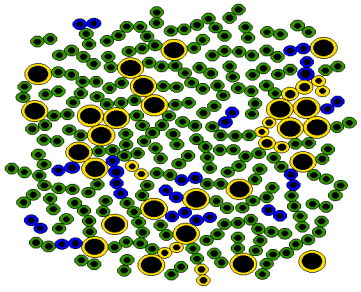
3.2. Results

We carry out numerical simulations of the model presented in the previous section. Cell cycle duration is taken to be 12 hours with a random perturbation uniformly distributed between 0 and 7 hours. Initial concentrations are equal to a random value between 0 and 0.05 for u and v, and between 0 and 0.09 for w. Initial concentrations of $[uw]$ and $[vw]$ are equal to zero. Daughter cells have the same values of intracellular concentrations as mothers cells at the moment of cell division. The critical values of u and v, for which differentiation occurs, are taken equal 0.2.

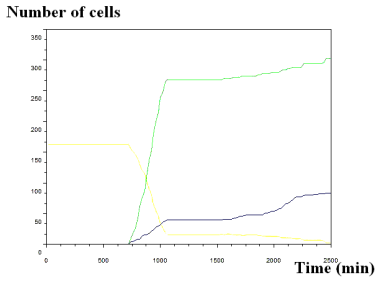
Figures 2, 3 and 4 show the results of numerical simulations. In subfigures (a) we present evolution of intracellular concentrations u and v in time in 10 different cells. These concentrations determine the cell fate according to Figure 3.1. The initial concentration of u and v in each cell is randomly distributed in



(a) Concentrations u and v in 10 cells



(b) Snapshot of cell populations



(c) Number of MEP cells (red), BFU-MK (green) and BFU-E (blue) as a function of time

FIGURE 4. Evolution of the population of MEP cells for the values of parameters $k_1^+ = 0.002$, $k_1^- = 0.003$, $k_2 = 0.1$, $k_3^+ = 0.004$, $k_3^- = 0.00329$, $k_4 = 0.1$. Both lineages of differentiated cells BFU-E and BFU-MK are present. In Figure 4(a), curves of the phase space are distributed around the first bissector, there is commitment into BFU-E and BFU-MK lineage as shown in Figure 3.1. Figure 4(b) shows the coexistence of the three type of cells. Figure 4(c) shows the evolution towards BFU-E and BFU-MK cells and gradual disappearance of self-renewal.

the square $[0, 0.05] \times [0, 0.05]$. Subfigures (b) and (c) show snapshots of cell populations and the evolution of cell number in time.

Depending on the values of parameters, different regimes of cell population dynamics are observed. If production of the intracellular concentration u (EKLF) is slower than of the concentration v (FLI-1), then cells MEP basically differentiate into the BFU-MK lineage. There is weak self-renewal observed for the values of parameters in Figure 2, and after several cell cycles all cells differentiate. An opposite situation is shown in Figure 3 where MEP cells differentiate into BFU-E. As before, the self-renewing activity is weak and after several cell cycles all cells differentiate. For the intermediate values of reaction rates, both lineages of differentiated cells are present (Figure 5). If we decrease the rate of production of the intracellular proteins EKLF and FLI-1, then some of the MEP cells can remain undifferentiated and preserve their self-renewal capacity. Let us also note that the distribution of initial protein concentrations can influence the dynamics of the cell population.

Thus we show how intracellular regulation of MEP cells influences their differentiation in one of the two lineages. The balance between them can be controlled by the hormones EPO stimulating production of erythrocytes and TPO stimulating production of platelets. Their concentrations influence the parameters of the intracellular regulation of the MEP cells and can increase production of one of the two cell types decreasing production of the other one.

4. Erythropoiesis modeling

Burst forming units (BFU-E) appeared as a result of differentiation of erythro-megakaryocytic progenitors undergo further differentiation in colony forming units (CFU) also called erythroid progenitors. These cells form erythroblastic islands, special structures where maturation of erythroid progenitors occurs. A typical structure of these islands consists of a macrophage surrounded by immature erythroid cells (progenitors), with more mature cells on the periphery of the island [14]. Erythroid progenitors can self-renew, differentiate or die by apoptosis. Their differentiation in erythroblast and then in reticulocytes leads to appearance of mature erythrocytes. For modeling erythroblastic islands we need to take into account cell-cell interaction and extracellular regulation which were not essential in the previous model.

4.1. Intracellular and extracellular regulations

4.1.1. Intracellular regulation

Intracellular regulation of erythroid progenitors determine their choice between self-renewal, differentiation and apoptosis. The model of intracellular regulation developed in [23] takes into account activated glucocorticosteroid receptor (GR), activated BMPR4 receptor (BMP-R), transcription factor GATA-1 and activated caspases. In this section we will consider a simplified regulatory network based on two proteins, Erk and Fas, responsible respectively for cell self-renewal and proliferation and cell differentiation and apoptosis [37, 42]. These proteins are antagonist, they inhibit expression of each either directly or through the extracellular regulation. One of such regulations is determined by more mature cells, reticulocytes. They produce Fas-ligand which is fixed to their exterior cell membrane. Fas-ligand activates Fas, a transmembrane protein and influences differentiation and apoptosis of erythroid progenitors. Another feedback control is related to mature erythrocytes in blood. Their quantity determines production of erythropoietin and of other hormones. Erythropoietin is known to inhibit apoptosis of erythroid progenitors [29] and to stimulate their self-renewal [19]. Other hormones, like glucocorticoids [17, 20], also increase their self-renewal by activating Erk.

The simplified model of the Erk-Fas intracellular regulation is given by the system of two ordinary differential equations for their concentrations [17, 20, 24]:

$$\frac{dE}{dt} = (\alpha + \beta E^k)(1 - E) - aE - bEF, \tag{4.1}$$

$$\frac{dF}{dt} = \gamma(1 - F) - cEF - dF, \tag{4.2}$$

where E and F denote the dimensionless concentrations of Erk and Fas. The first equation in this system describes self-accelerating production of Erk (β and k are self-activation parameters), its degradation and its suppression by Fas. Erk is linearly degraded with a rate a and is inhibited by Fas with a rate bF. The value of $\alpha = \alpha(Epo, GF)$ in the right-hand side of this equation depends on the concentration of EPO and on the growth factor GF produced by macrophages (see below). The second equation for the concentration of Fas is similar. The rate of its production depends on the concentration F_L of Fas-ligand produced by reticulocytes through the factor $\gamma = \gamma(F_L)$. In the meantime, Fas it is degraded with a rate d and inhibited by Erk with a rate cE. The critical values of Erk and Fas, denoted by E_{cr} and F_{cr}, determine self-renewal and apoptosis, respectively. If at the end of the cell cycle intracellular concentrations of Erk and Fas do not reach their critical values, then the cell differentiates.

4.1.2. Extracellular regulation

Several cell types are considered in this model. Erythroid progenitors either self-renew and give two cells of the same type, or differentiate and give two reticulocytes, or they die by apoptosis depending. Their fate is determined by the intracellular regulation discussed above. Reticulocytes are almost mature red blood cells that leave the bone marrow (computational domain) after one cell cycle. They produce Fas-ligand and influence surrounding progenitors.

Erythroid cells in mammals are organized in spatial structures called erythroblastic islands. They are formed around macrophages located at the center of erythroblastic islands. Macrophages produce growth factors which stimulate self-renewal of erythroid progenitors. Concentrations of Fas-ligand (F_L) and of growth factor (GF) are described by the reaction-diffusion equations [24]:

$$\frac{\partial F_L}{\partial t} = D_{F_L} \Delta F_L + W_{F_L} - \sigma_{F_L} F_L, \tag{4.3}$$

$$\frac{\partial GF}{\partial t} = D_{GF} \Delta GF + W_{GF} - \sigma_{GF} GF, \tag{4.4}$$

where $W_{F_L} = k_{F_L} C_{ret}$ is a source term depending on the number of reticulocytes C_{ret} and W_{GF} is a constant source term for growth factor concentration, σ_{F_L} and σ_{GF} are degradation rates, and D_{F_L} and

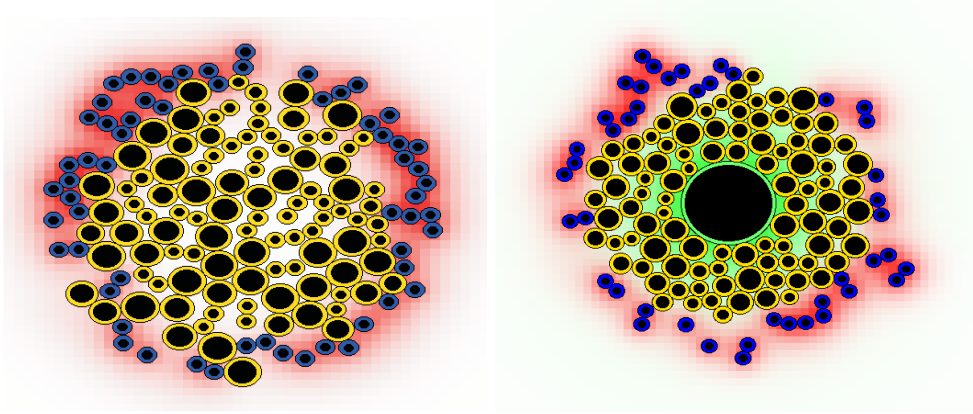


FIGURE 5. Erythroblastic island without central macrophage (left) and with macrophage (right). Progenitors (yellow cells) are surrounded by reticulocytes (blue) which produce fas-ligand (red halo). Macrophage (large green cell) produces grows factors (green halo). Black circles inside cells show their incompressible part. Reprinted from the Journal of Theoretical Biology, 298, S. Fischer, P. Kurbatova, N. Bessonov, O. Gandrillon, V. Volpert, F. Crauste, Modelling erythroblastic islands : using a hybrid model to assess the function of central macrophage, Pages No. 92-106, Copyright (2012), with permission from Elsevier.

D_{GF} are diffusion rates. We choose a sufficiently small diffusion coefficient D_{F_L}, so that Fas-ligand is concentrated in a small vicinity of reticulocytes. In this case, Fas-ligand influences erythroid progenitors when they are sufficiently close to reticulocytes, mimicking cell-cell contact interaction.

Numerical simulations are carried out in a rectangular domain. Each cell is considered as a circle with a compressible exterior part and incompressible inside. All newly born cells have the same radius r_0. They grow linearly in time until their diameter increases twice. Then they divide. The direction of cell division is chosen randomly from 0 to 2π. Examples of numerical simulations of erythroblastic islands with and without macrophage are shown in Figure 5. Macrophages effectuate local control of self-renewal of erythroid progenitors. Without them, the cell population becomes unstable with unbounded growth or extinction.

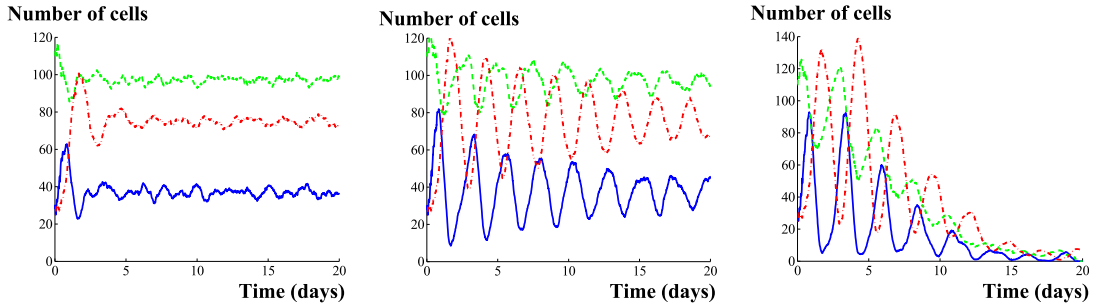
4.2. Response to stress

Different feedback controls play an important role in the model with intracellular regulation (4.1)–(4.2). We focus here on the global feedback control mediated by EPO. It is known that increasing EPO levels in the blood stream and consequently in the bone marrow, upregulates Erk and decreases erythroblast apoptosis rate [29]. We take it into account in the values of α and F_{cr} which depend now on the EPO concentration. This regulation is particularly important in the case of anaemia. Anaemia is one of the more common blood disorders, characterized by a low level of red blood cells (RBCs) in the body or less than normal quantity of hemoglobin in the blood. Anaemia can be caused either by blood loss, by excessive destruction of RBCs or by decreased or faulty red blood cell production. We include in the model the feedback based on erythropoietin production in response to anaemia. Since EPO level depends on the number N of red blood cells in blood (more precisely, on hemoglobin), then we can suppose that intracellular regulation of erythroid progenitors also depends directly on N. For the sake of simplicity we choose a linear function:

$$\alpha := \alpha(N) = \alpha_0 + k_\alpha(N_0 - N) + k_{GF}G, \tag{4.5}$$

$$F_{cr} := F_{cr}(N) = F_0 + k_F(N_0 - N), \tag{4.6}$$

where N_0 is the normal number of erythrocytes in blood. The number of erythrocytes in blood N is estimated as the number of reticulocytes which leave the bone marrow.



(a) k_F=0.002 (b) k_F=0.004 (c) k_F=0.005

FIGURE 6. Evolution of cell number for different values of the coefficient k_F which determines the influence of EPO on apoptosis of erythroid progenitors. Anaemia conditions were created by suddenly lowering the number of cells in blood to 23%. Red dash-dot curves correspond to the number of RBCs in blood, green dashed curves represent the number of progenitors and the blue solid curves the number of reticulocytes.

We used experimental data in mouse. Normal value of hematocrit is 46%. After bleeding during experiments mouse hematocrit was estimated 23%. (unpublished data). Numerical simulations of stress erythropoiesis are shown in Figure 6. Feedback control parameters are given in Table 1. Parameters for intracellular and extracellular regulations are given in Tables 2 and 3. The values of parameters for normal hematopoiesis can be found in [24]. The number of cells returns to its equilibrium value in the case of small values of k_F, (Figure 6 (a)). For larger values of k_F the number of cells oscillates in time. Strong oscillations can result in extinction of the cell population (Figure 6 (c)).

4.3. The role of stochasticity in initial conditions

Behavior of erythroid progenitors *in vivo* strongly depends on the surrounding cells. Their differentiation and apoptosis are stimulated by reticulocytes and their self-renewal by macrophages (Section 4.1). The situation is different if we model a cell culture which consists initially only of erythroid progenitors. Then the cell fate is determined only by the intracellular regulation, and it is not influenced by the extracellular regulation.

Without extracellular regulation, the fate of cells is determined by the solution of the system of ordinary differential equations which coefficients are the same for all cells. In order to obtain different fates, it is necessary to introduce stochasticity. The duration of cell cycle is partly random. We studied the consequences of added random perturbations to initial conditions. Since it is observed in the biological experiments that cells in the culture can have different fates, then we need to assume that the initial concentrations of intracellular proteins can be different.

Let us recall that the cell dies by apoptosis if the concentration of Fas reaches the critical level F_{cr} during cell cycle. If this is not the case but the concentration of Erk exceeds the critical value E_{cr}, then the cell self-renews. If neither of these conditions is satisfied, then the cell differentiates.

Example of numerical simulations is shown in Figure 7. In the beginning, there are only progenitors in the cell population. Some of these progenitors will differentiate into reticulocytes while some other will self-renew. Reticulocytes begin to produce Fas-ligand and influence the surrounding cells stimulating their differentiation and apoptosis. Therefore there is a region filled by differentiated cells. This region grows in time.

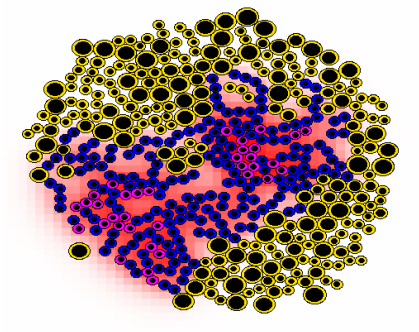


FIGURE 7. Modeling of cell population *in vitro*. In the beginning, there are only progenitors. Cells begin to differentiate. After several divisions, progenitors (yellow) become reticulocytes (blue) which produce a growth factor Fas-ligand (red halo). After one cycle, reticulocytes become erythrocytes (purple) and remain in the cells culture.

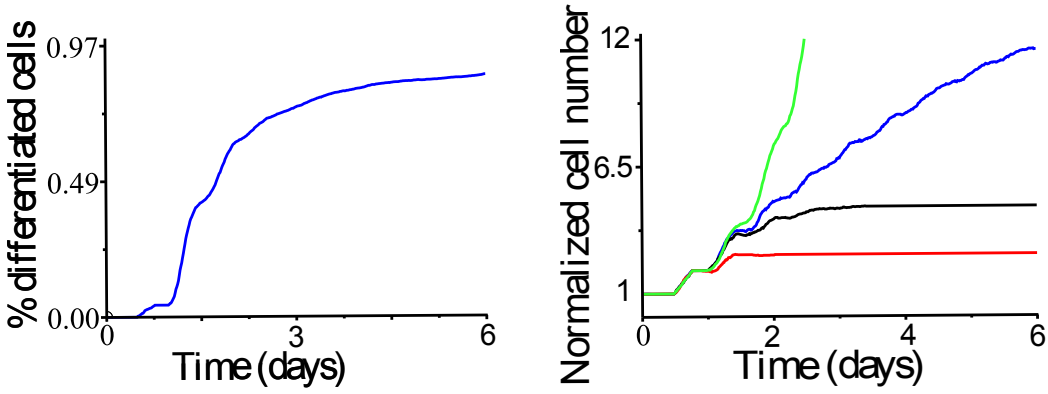


FIGURE 8. Curve of number of each type of cells in a cell culture. Left: proportion of differentiated cells (reticulocytes, erythrocytes) with respect to all cells over time. Right: total number of cells for different ranges of variation of initial concentrations. Blue curve corresponds to the case where the initial activity of ERK is between 0 and 0.015, Fas between 0 and 0.01, with a uniform probability distribution. Red, black and green curves correspond to c=0.15, 0.01, 0.007 respectively (see the explanation in the text). Normalized cell number is the ratio of the current cell number to the initial cell number. Each curve represents a mean value with respect to 3 simulations.

Thus, random variation of initial intracellular concentration is important in the beginning of the evolution of the cell population. After some time, when differentiated cells appear, they determine further development of the population. This dynamics depends on the range of stochasticity of the initial concentrations. Figure 8 shows the influence of initial conditions. In these simulations, initial quantities of Erk and Fas are randomly distributed between 0 and some value c. If $c < 0.005$, then all cells self-renew and the population contains only undifferentiated progenitors. For larger values of c, both self-renewal and differentiation are observed. Finally, for the values of c even larger, all cells will die by apoptosis. Let us note that differentiated cells remain in the cell culture and after some time become mature erythrocytes. In the previous model developed to describes erythropoiesis *in vivo*, reticulocytes leave the bone marrow (computational domain) to the blood flow.

5. Discussion

In this work we continued to develop one hybrid model of erythropoiesis. The model couples cell dynamics with intracellular and extracellular regulations. Cell movement and their spatial position play an

important role in cell choice between self-renewal, differentiation and apoptosis because the extracellular regulation influences concentration of ERK and Fas and depends on the position of each cell. It is important to emphasize that we do not impose cell fate as a given parameter (deterministic or stochastic) as it is conventionally done in cell population dynamics. Cell fate is determined by intracellular regulation of protein concentrations and by its environment.

Erythropoiesis begins with differentiation of erythro-megakaryocytic progenitors into one of two lineages, BFU-E and BFU-MK. This simulation focuses on the evolution of a bipotent cell: erythro-megakaryocytic progenitors which can give rise to two lineages of blood cells. This model shows the possibility to obtain two lineages, according to the concentration of the erythroid transcription factor GATA-1 and hormones FLI-1 and EKLF.

Burst forming units of erythroid lineage give rise to colony forming units (CFU) or erythroid progenitors which form erythroblastic islands. Their functioning is determined by a balance between self-renewal, differentiation and apoptosis which depends on the intracellular regulation of erythroid progenitors, on the local extracellular regulation by the surrounding cells and on the global extracellular regulations by the hormones including erythropoietin. Structure and functioning of erythroblastic islands were studied in detail in the previous works [17], [23]. In this work, we studied the response to stress situations (anaemia, hypoxia). In this case, the amount of produced erythrocytes should be rapidly increased without losing the control of the process. The model shows robustness of erythroblastic islands and their capacity to rapidly increase production of erythrocytes in response to the increased EPO level.

Another important question discussed in this work is the role of stochasticity in the intracellular regulation. In Section 4.3, concerning modeling of cell culture *in vitro*, there is no extracellular regulation. Therefore we need to impose random variation of the initial intracellular concentrations to obtain two behaviors (self-renewal and differentiation) of the cells in culture. The range of this random variation influences the behavior of the whole cell population.

Hybrid models presented and studied in this work represent a basis for further investigation of normal hematopoiesis and of various blood diseases. We used those models to produce a mathematical model of multiple myeloma and T lymphoblastic lymphoma.

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6. Appendix

Parameters of intracellular regulation are not known from the experiments. We choose them in such a way that: 1. the bistable dynamics is preserved, 2. the hybrid system shows qualitatively correct and robust behavior with a correct proportion of cells of different types, 3. the results of the simulations fit the experimental curves. The values of parameters are given in the following tables.

Parameter	Value	Unit
α_0	0.6	h^{-1}
k_α	0	h^{-1} / reticulocyte
k_{GF}	6	h^{-1} / GF molecules
N_{target}	70	reticulocyte
N_0	42	reticulocyte
F_0	0.6	NU

TABLE 1. Feedback control parameters. NU is a normalized quantity unit for the intracellular molecules (Section 4.2).

Parameter	Value	Unit
Cell cycle length	12	h
Cell cycle variations	3	h
r_0	0.01	L
m	1	M
μ	6.10^5	h^{-1}
K	$3.6.10^6$	$M.h^{-2}$
D_{F_L}	6.10^{-4}	$L^2.h^{-1}$
k_{F_L}	6.10^{-3}	F_L molecules / reticulocyte / h
σ_{F_L}	1.2	h^{-1}
D_{GF}	6.10^{-3}	$L^2.h^{-1}$
W_{GF}	6.10^{-2}	GF molecules / L^2 / h
σ_{GF}	0.6	h^{-1}

TABLE 2. Extracellular parameters: M is a dimensionless mass unit, L a dimensionless length unit (Section 4.2).

Parameter	Value	Unit
β	120	$h^{-1}.NU^{-1}$
k	2	-
a	31.8	h^{-1}
b	3.6	$h^{-1}.NU^{-1}$
k_γ	1.8	$h^{-1}.NU^{-2}$ / F_L molecule
c	6	$h^{-1}.NU^{-1}$
d	3	h^{-1}
E_{cr}	0.3	NU
F_{cr}	0.4	NU

TABLE 3. Internal parameters when cells are on the hysteresis cycle (Section 4.2).

Parameter	Value	Unit
Cell cycle length	720	min
Cell cycle variation	420	min
α	0.000358	h^{-1}
β	0.05	$h^{-1}.NU^{-1}$
a	0.0041	h^{-1}
b	0.0139	$h^{-1}.NU^{-1}$
c	0.00139	h^{-1}
d	0.00011	h^{-1}
kg	0.0002	$h^{-1}.NU^{-2}$
E_{cr}	0.8	NU
F_{cr}	0.19	NU
E_0 Value of initial quantity E	0.015	NU
F_0 Value of initial quantity F	0.01	NU

TABLE 4. Intracellular parameters (Section 4.3).

Parameter	Value	Unit
r_0	0.01	L
m	1	M
μ	6.10^5	h^{-1}
K	$0.9.10^6$	$M.h^{-2}$
D_{F_L}	3.10^{-4}	$L^2.h^{-1}$
k_{F_L}	3.10^{-3}	F_L molecules / reticulocyte / h
σ_{F_L}	0.6	h^{-1}

TABLE 5. Extracellular parameters (Section 4.3).