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Stochastic control of proliferation and differentiation in stem cell dynamics

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Abstract In self-renewing tissues, cell lineages consisting of stem cell and classes of daughter cells are the basic units which are responsible for the correct functioning of the organ. Cell proliferation and differentiation in lineages is thought to be mediated by feedback signals. In the simplest case a lineage is comprised of stem cells and differentiated cells. We create a model where stem cell proliferation and differentiation are controlled by the size of cell populations by means of a negative feedback loop. This two-dimensional Markov process allows for an analytical solution for the mean numbers and variances of stem and daughter cells. The mean values and the amounts of variation in cell numbers can be tightly regulated by the parameters of the control loop.

Keywords Stem cells · Stochastic modeling · Tissue homeostasis · Regulation · Control

Mathematics Subject Classification 92D25 · 92C99 · 60G10 · 93E15

1 Introduction

Cell lineages consist of classes of cells that differ by their properties such as the degree of differentiation and the capability of self-renewal. In the simplest case a lineage can be comprised of stem cells and differentiated cells. Several intermediate classes of daughter cells can also be present, giving rise to multistage cell lineages. For self-renewing tissues such as the epithelial tissue, cell lineages are considered to be basic units that ensure the correct functioning of the organ.

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In self-renewing tissues, the process of homeostasis (or maintaining a constant population size) is an important objective. For a system of stem and daughter cells to maintain a nearly-constant size, regulatory loops have to be in place to prevent instability. Cell proliferation and differentiation in cell lineages is thought to be mediated by feedback signals. It has been suggested that negative control loops play an important role in development and maintenance of many tissues, including olfactory epithelium (Wu et al. 2003), skin (Yamasaki et al. 2003), liver (Endo et al. 2006), bone (Daluiski et al. 2001), central nervous system (Platel et al. 2008), blood cells (Marshall and Lord 1996), retina (Close et al. 2005), and hair (Plikus et al. 2008), see a nice review in Lander et al. (2009). Many of these factors are members of the TGFb family (Newfeld et al. 1999).

Studying tissue regulation mechanisms, both biologically and mathematically, is an area of active interest of many researchers. Understanding how control loops are set up, and how various processes are regulated is part of getting a handle on tissue engineering, and can hopefully lead to new treatment strategies for various diseases. The mechanisms underlying the maintenance and regulation of cell lineages have been studied experimentally by many groups, see for example Novak and Tyson (2003), Tyson et al. (2003), Khammash (2008), Tsankov et al. (2006), Morrison and Spradling (2008), Discher et al. (2009), Pera and Tam (2010), and the references therein. Conceptual theoretical issues for the studies of stem cells have been identified in Loeffler and Roeder (2002), d'Inverno et al. (2006), Roeder et al. (2006). Discrete and continuous models relevant for studying carcinogenesis have been proposed (Tomlinson and Bodmer 1995; d'Onofrio and Tomlinson 2007; Johnston et al. 2007; Boman et al. 2008; Hardy and Stark 2002; Yatabe et al. 2001; Enderling and Hahnfeldt 2011; Piotrowska et al. 2008; Michor 2008; Tomasetti and Levy 2010). Evolutionary modeling of stem cells in systems other than cancer was introduced in Mangel and Bonsall (2008). Modeling of stem cells in the hematopoietic system was proposed by several authors (Glauche et al. 2007; Colijn and Mackey 2005; Adimy et al. 2006). In the mathematical and computational literature, both deterministic and stochastic models have been introduced and studied. The deterministic (ODE) approach provides useful analytical insights into the dynamics and long-term behavior of cell lineages. The stochastic approach allows to quantify the role of fluctuations in the behavior of the system of interest. While a comprehensive review of the rapidly growing stem cell modeling literature is beyond the scope of this study, the papers which are most closely related to our present paper are Lander et al. (2009) and Lo et al. (2009). The focus of these papers is proliferative control of cell lineages by means of feedback loops. An insightful deterministic model for the dynamics of multistage lineages was introduced and validated in the context of olfactory systems.

In the present paper, we focus on stochastic modeling of a stem/daughter cell population with negative feedback. In our previous work (Sun and Komarova 2012), we have explored the simplest scenario where the total number of cells in a lineage is kept constant by some exogenous means, and that proliferation/differentiation decisions of stem cells are subject to internal control. This choice was partly motivated by the results of Lander et al. (2009) where it was concluded that differentiation control loops are at the core of tissue maintenance. In Sun and Komarova (2012) we were able to analytically calculate the expected values and variances of the number of stem cells in

a system with nonlinear control, and compare different types of regulatory loops for robustness and for tightness of variance control. Still the question remains how these results change if the tissue size is a variable, and both divisions and differentiations are controlled endogenously.

In the present paper we expanded our methodology to describe the stochastic dynamics of stem cell lineages where both death and division events are decoupled, and the total size of the colony (together with the number of stem cells) is an independent stochastic variable, leading to a two-dimensional Markov process. Despite this increase of dimensionality of the problem, we were able to solve the system analytically (by using a moment closure method) and to find the mean values and the variances of the total number of cells, as well as the stem/daughter cell populations.

This paper is organized as follows. In Sect. 2 we define our model, and discuss the biological information which motivated our assumptions. In Sects. 3.1 and 3.2 we calculate the means and the variances of the stem cells and daughter cells analytically. In Sect. 4 we offer a biological interpretation of our results, provide comparison of the analytical results with numerical simulations, and discuss the robustness and other properties of the model. Section 5 is reserved for discussion and conclusions.

2 Model description

In this paper we will consider the simplest type of a lineage which only consists of two types of cells: stem cells and terminally differentiated daughter cells. Let us denote by i the number of stem cells, by j the number of daughter cells and by $N = i + j$ the total number of cells. We assume that daughter cells can die and that stem cells can divide symmetrically. Each division can either be a differentiation event (resulting in two daughter cells) or a proliferation event (resulting in two stem cells).

We will further assume that the total population is somewhat close to its carrying capacity, in the sense that the proliferation and death probabilities are not proportional to the total number of cells available for death or division, but are defined by population-dependent homeostatic signaling mechanisms. We set a discrete-time stochastic process, whereby at each time step, the following events can happen, see Fig. 1:

1. With probability $1 - Q(i, j)$, one daughter cell dies:

$$\Rightarrow N \to N - 1, i \to i, j \to j - 1$$

2. With probability $Q(i, j)$, one stem cell divides. In this case, we have two possibilities:
   (a) With probability $P(i, j)$, the stem cell divides into two daughter cells:

$$\Rightarrow N \to N + 1, i \to i - 1, j \to j + 2.$$

   (b) With probability $1 - P(i, j)$, the stem cell divides into two stem cells:

$$\Rightarrow N \to N + 1, i \to i + 1, j \to j$$

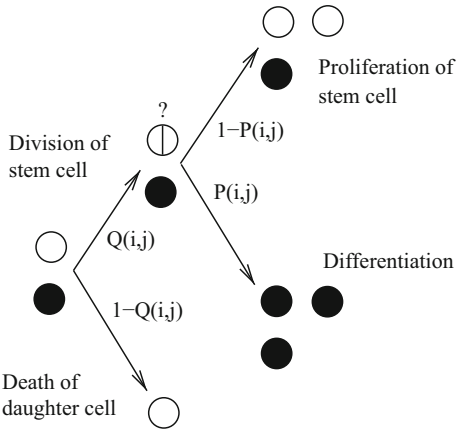

Fig. 1 A schematic showing the structure of the stochastic process and the definitions of the probability functions $Q(i, j)$ and $P(i, j)$. *Black circles* are daughter cells, and *white circles* are stem cells. We start with one stem and one daughter cell. The first decision that the system makes at each time-update is whether a stem cell divides (this happens with probability $Q(i, j)$), or a daughter cell dies. If division is chosen, the next decision concerns differentiation/proliferation pathway that the cell follows. With probability $P(i, j)$, the stem cell divides into two daughter cells (differentiation event), and with probability $1 - P(i, j)$ it divides into two stem cells (proliferation event)

Thus, the quantities $0 \leq Q(i, j) \leq 1$ and $0 \leq P(i, j) \leq 1$ are probabilities of division and differentiation respectively. These quantities contain information about the feedback loops that control division and differentiation processes.

Note that for the studies of moments in steady-state, the discrete-time formulation introduced here is sufficient, as it produces exactly the same equations as a continuous-time process, as explained in the next section.

A model with no control In this model, we assume that P and Q are constants. In order for the mean number of cells to remain finite and positive, we must require that

$$P(i, j) = Q(i, j) = \frac{1}{2}.$$

To show this, let us assume that $Q(i, j) = a \neq \frac{1}{2}$. Then

$$\begin{aligned} E[N_t \mid N_{t-1}] &= a(N_{t-1} + 1) + (1 - a)(N_{t-1} - 1) \\ &= N_{t-1} + 2a - 1 \end{aligned}$$

Therefore,

$$\begin{aligned} E[N_t] &= E[N_{t-1}] + 2a - 1 = \cdots = E[N_0] + t(2a - 1) \\ E[N_t] &\to \begin{cases} \infty & : \ t \to \infty, a > \frac{1}{2} \\ 0 & : \ t \to \infty, a < \frac{1}{2} \end{cases} \end{aligned}$$

Therefore, $Q(i, j) = \frac{1}{2}$. Similarly, we can prove that $P(i, j) = \frac{1}{2}$. For these particular choices of P and Q, we have $E[N_t] = N_0$, $E[i_t] = i_0$, for all t. However, large fluctuations in the system make these results for the expectations meaningless.

A model with control: biological motivation If a certain degree of endogenous control is present in the system, the probabilities Q and P become functions of i and j, the population numbers of stem and daughter cells. In the experimental literature, a fast developing area is the study of mechanisms of stem cell regulation. The exact ways in which divisions and division/differentiation decisions are controlled are far from being understood. At present, there are a lot of data pertaining to different biological systems, which describe possible regulatory mechanisms that may play a role in the functioning of stem cells. In this paper, we used a particular set of evidence that suggests that divisions of stem cells are negatively regulated by the population, and differentiation decisions are negatively regulated by the number of differentiated cells.

It has been argued that cell numbers can negatively control stem cell divisions. Crowding and contact inhibition play an important role in determining the fate of stem cells. Cell shape (rounded or flat), as well as mechanical stress received from surrounding cells, control proliferation decisions (Guilak et al. 2009). It has further been proposed that increased densities of neuronal precursors are sensed by an increase in the proportion of the cell surface that is occupied by adherens junctions and leads to a downregulation of Hedgehog signaling, resulting in decreased proliferation (Lien et al. 2006). Similar mechanisms have been reported in colon (Ordóñez-Morán and Huelsken 2012) and neural system (Hsieh 2012). Therefore, in the present model we chose the division probability, Q, to be a decreasing function of the total cell number.

A crucial component in regulating organ size is the balanced regulation of proliferation and cell death that is required to achieve the correct number of specific cell types within an organ. While it is not known whether cell death triggers regeneration or whether regeneration activates cell death, the two processes are not independent (see e.g. Anversa et al. 2006 for cardiac stem cells). The regulation and timing of programmed cell death is achieved by many of the same factors that are crucial in cell division regulation (e.g. the p53/p63/p73 family in neural stem cell regulation, see Joseph and Hermanson 2010). Therefore in the present paper we chose the programmed cell death probability to be regulated as $1 - Q(N)$, in accord with the probability of divisions.

Finally, the differentiation/proliferation decisions of stem cells have been proposed to be controlled from downstream. Once generated, neural stem cell descendants can trigger a feedback mechanism (possibly through Notch signaling) to stop stem cell differentiation (Lavado et al. 2010). Hematopoietic stem cells have also been reported to be regulated by their mature progeny (de Graaf et al. 2010). Further, in Lander et al. (2009) it is reported that in the olfactory epithelium, differentiated cells produce a factor, GDF11, which specifically affects the differentiation/proliferation decisions of the intermediate compartment cells by decreasing their probability to proliferate. Therefore, in this paper we assume that the probability of differentiation, P, is a decreasing function of the number of differentiated cells, j.

A model with control: mathematical implementation Based on the above arguments, it is reasonable to assume that the probability of stem cell division, $Q(i, j) = Q(N)$, is a decreasing function of the population size, N, and the probability of differentiation, $P(i, j) = P(j)$, is a decreasing function of the number of differentiated cells. In this paper we will consider the following functional form for the probabilities:

$$Q(N) = \frac{b}{1+hN}, \quad P(j) = \frac{r}{1+gj}.$$

These functions contain constant parameters b, h, r, and g. The parameters h and g define the strength of control of the divisions and differentiation, respectively. In particular, the cases with $h = 0$ and $g = 0$ correspond to a model without control. The constants b and r define the magnitude of division and differentiation probabilities in the absence of control.

This model is a generalization of the constant-population model considered in Sun and Komarova (2012). Before we present the analysis of the present model, we will review the results for a simpler, one-dimensional model of Sun and Komarova (2012).

A constant-population model with control: a review Let us assume that the population size, N, is kept constant, and the number of stem cells and daughter cells vary such that $i + j = N$. At each time-step, one differentiated cell is deleted, and one stem cell reproduces. Upon reproduction, a stem cell can differentiate, that is, produce two daughter cells, with probability p_i. Otherwise, it proliferates, that is, produces two stem cells, with probability $1 - p_i$. If there are no stem cells or no daughter cells, the process stops. We further assume that there is a certain degree of control in the system. Namely, the probability for a stem cell to differentiate is a non-decreasing function of the total number of stem cells, i. The more stem cells there are in the system, the lower must be the probability to produce more of the stem cells, and thus the higher the probability to differentiate, p_i. A particular model of control which was analyzed (along with other models) in Sun and Komarova (2012), is given by

$$p_i = \frac{\beta}{1-\nu i}, \tag{1}$$

where parameter ν measures the strength of control, and parameter β defines the magnitude of the differentiation probability in the absence of control. We referred to this model as the "hyperbolic law" of differentiation control. The following results for the mean and the variance of the number of stem cells were obtained:

$$E[i] = \sum_i \varphi_i i = \frac{1-2\beta}{\nu} - \frac{1}{2}, \tag{2}$$

$$Var[i] = \frac{\beta}{\nu} + \frac{1}{4}. \tag{3}$$

In what follows we will consider the more general, and a more realistic case where both differentiation and proliferation are subject to feedback control.

3 Hyperbolic control

Let us denote by $\varphi^t_{j,N}$ the probability to have j daughter cells and $N - j$ stem cells at time t. We have the following Kolmogorov forward equation:

$$\varphi_{j,N}^{t+1} - \varphi_{j,N}^{t} = \varphi_{j+1,N+1}^{t}(1 - Q(N+1)) + \varphi_{j-2,N-1}^{t} Q(N-1)P(j-2) + \varphi_{j,N-1}^{t} Q(N-1)(1 - P(j)) - \varphi_{j,N}^{t}, \tag{4}$$

where the left hand side represents temporal change in the probability distribution function, the first term on the right hand side represents a death of a daughter cell, the second term is a differentiation event, and the third term is a proliferation event. As mentioned before, this process is different from the conventional linear birth-death process, because we assume that the system is close to its carrying capacity, and the probabilities of divisions are negatively correlated with the population size, rather than being positively (linearly) correlated with the number of cells of the appropriate class.

The process described by Eq. (4) is a discrete-time Markov process. Since we are interested in the moments of the probability distribution in steady state, this description is equivalent to a continuous-time process, where the function $\varphi_{j,N}(t)$ continuously depends on time. In such a process, during an infinitesimal time-interval Δt, a daughter cell dies with probability $(1 - Q(N))\Delta t$, a stem cell differentiates with probability $Q(N)P(j)\Delta t$, and a stem cell proliferates with probability $Q(N)(1 - P(j))\Delta t$. In this case, the master equation is identical to Eq. (4), except on the left hand side we have a time derivative, $\dot{\varphi}_{j,N}(t)$.

3.1 Expectations

It is convenient to introduce the function

$$f_{j,N} = \frac{1}{(1+hN)(1+gj)}\varphi_{j,N}.$$

The Kolmogorov forward equation for $\varphi_{j,N}$ can be rewritten as follows:

$$\varphi_{j,N}^{t+1} - \varphi_{j,N}^{t} = [b(1+gj) - br] f_{j,N-1} + [(1+h(N+1))(1+g(j+1)) - b(1+g(j+1))] f_{j+1,N+1} + br f_{j-2,N-1} - [(1+hN)(1+gj)] f_{j,N}, \tag{5}$$

Our goal is to find the expectations of N and j, $E[N]$ and $E[j]$, in steady state [zero time-change in Eq. (5)]. We have

$$E[N] = \sum_j \sum_N N\varphi_{j,N} = \sum_j \sum_N N(1+hN)(1+gj) f_{j,N},$$
$$E[j] = \sum_j \sum_N j\varphi_{j,N} = \sum_j \sum_N j(1+hN)(1+gj) f_{j,N}.$$

Let us denote the moments

$$x_{st} = \sum_j \sum_N j^s N^t f_{j,N}.$$

Then we can express the mean numbers of cells in terms of the variables x_{st}:

$$E[N] = x_{01} + gx_{11} + hx_{02} + ghx_{12}, \tag{6}$$
$$E[j] = x_{10} + gx_{20} + hx_{11} + ghx_{21}. \tag{7}$$

Let (s, r) denote the operation whereby we multiply Eq. (5) in steady state by $j^s N^r$, and then sum over j and N. Then we can get the following equations for the relevant variables x_{st}:

$$\begin{aligned}
(1,0):&\ (b-1+2br)x_{00} + (bg-g)x_{10} - hx_{01} - ghx_{11} = 0,\\
(0,1):&\ (2b-1)x_{00} + (2bg-g)x_{10} - hx_{01} - ghx_{11} = 0,\\
(0,2):&\ x_{00} + gx_{10} + (-2+4b+h)x_{01} + (-2g+4bg+gh)x_{11}\\
&\ -2hx_{02} - 2ghx_{12} = 0,\\
(2,0):&\ (1-b+4br)x_{00} + (-2+2b+g-bg+4br)x_{10} + hx_{01} + (-2h+gh)x_{11},\\
&\ +(-2g+2bg)x_{20} - 2ghx_{21} = 0,\\
(1,1):&\ (1-b+2br)x_{00} + (-1+2b+g-bg)x_{10} + (-1+b+h+2br)x_{01} - hx_{02},\\
&\ +(-g+bg-h+gh)x_{11} + (-g+2bg)x_{20} - ghx_{21} - ghx_{12} = 0.
\end{aligned}$$

These are 5 equations for 8 unknowns, x_{00}, x_{10}, x_{01}, x_{11}, x_{02}, x_{12}, x_{20}, and x_{21}. To obtain a closed system, we need to come up with three more equations for these variables. First we note that because $\sum_j \sum_N \varphi_{j,N} = 1$, we have the identity

$$x_{00} + gx_{10} + hx_{01} + ghx_{11} = 1.$$

Since x_{00} is positive and $\sum_j \sum_N f_{j,N} = x_{00}$, then $\frac{f_{j,N}}{x_{00}}$ is a probability distribution.

In order to formulate the two remaining equations, we will use the method of cumulant closure (Nasell 2003; Singh and Hespanha 2007; Lloyd 2004; Socha 2008). In general, use of moment equations requires the deployment of an approximation technique - a moment/cumulant closure approximation. This technique truncates an infinite set of coupled equations at some order. The standard cumulant closure methods assume that the distribution of states follows some given distribution and then use the known relationship between the moments of that distribution to truncate the set of moment equations. Here we assume that the third-order cumulants vanish:

$$\kappa_{2,1} = 0, \quad \kappa_{1,2} = 0.$$

After simplification, we get the following two equations:

$$\begin{aligned}
2x_{10}x_{01}^2 - 2x_{00}x_{01}x_{11} - x_{00}x_{10}x_{02} + x_{00}^2 x_{12} = 0,\\
2x_{01}x_{10}^2 - 2x_{00}x_{10}x_{11} - x_{00}x_{01}x_{20} + x_{00}^2 x_{21} = 0.
\end{aligned}$$

Thus, we have eight equations for eight unknowns. After solving the equation system, we obtain the following result for the expectations:

$$E[N] = \frac{1}{2} + \frac{2b-1}{h}, \tag{8}$$

$$E[i] = \frac{-8g+14bg-3gh+8h-20hr+\sqrt{4b^2g^2+h^2(g-4r)^2+4bgh(4r-5g)}}{8gh}, \tag{9}$$

$$E[j] = \frac{2bg-8h+7gh+20hr-\sqrt{4b^2g^2+h^2(g-4r)^2+4bgh(4r-5g)}}{8gh}. \tag{10}$$

3.2 Variances

Next, our goal is to find the variance for i, j and N. We have for the two second moments,

$$\begin{aligned} E[N^2] &= x_{02} + gx_{12} + hx_{03} + ghx_{13}, \\ E[j^2] &= x_{20} + gx_{30} + hx_{21} + ghx_{31}. \end{aligned} \tag{11}$$

Therefore, we need at least 4 more equations to solve for the 4 additional unknowns, x_{03}, x_{13}, x_{30}, and x_{31}. By the method described above, we write down the following four equations:

$$\begin{aligned}
&(3,0): (b-1+8br)x_{00} + (3-3b-g+bg+12br)x_{10} - hx_{01} + (3h-gh)x_{11} \\
&\quad +(-3+3b+3g-3bg+6br)x_{20} + (-3h+3gh)x_{21} + (-3g+3bg)x_{30} \\
&\quad -3ghx_{31} = 0 \\
&(2,1): (b-1+4br)x_{00} + (2-2b-g+bg+4br)x_{10} + (1-b-h+4br)x_{01} \\
&\quad +(-2+2b+g-bg+2h-gh+4br)x_{11} + (-1+2b+2g-2bg)x_{20} + hx_{02} \\
&\quad +(-g+2bg)x_{30} + (-2g+2bg-h+2gh)x_{21} + (-2h+gh)x_{12} - ghx_{31} \\
&\quad -2ghx_{22} = 0 \\
&(1,2): (b-1+2br)x_{00} + (1-g+bg)x_{10} + (2-2b-h+4br)x_{01} \\
&\quad +(-2+4b+2g-2bg+h-gh)x_{11} + gx_{20} + (-1+b+2h+2br)x_{02} \\
&\quad +(-2g+4bg+gh)x_{21} + (-g+bg-2h+2gh)x_{12} - hx_{03} \\
&\quad -2ghx_{22} - ghx_{13} = 0 \\
&(0,3): (2b-1)x_{00} + (2bg-g)x_{10} + (3-h)x_{01} + (3g-gh)x_{11} \\
&\quad +(-3+6b+3h)x_{02} + (-3g+6bg+3gh)x_{12} - 3hx_{03} - 3ghx_{13} = 0
\end{aligned}$$

We notice that a new unknown, x_{22}, appears in these 4 equations. Therefore, we need one more equation, which we obtain by the truncation method for the cumulant $\kappa_{0,3}$,

$$2x_{01}^3 - 3x_{02}x_{01}x_{00} + x_{00}^2x_{03} = 0.$$

After solving the resulting equation system, we obtain the following results for the variances:

Table 1 The mean and the variance of the total cell population, as well as limiting values of the mean and the variance of the daughter cell population

Mean total number of cells	$E[N]$	$\frac{2b-1}{h}+\frac{1}{2}$	
Variance of the total number of cells	$Var[N]$	$\frac{b}{h}+\frac{1}{4}$	
		$h\to 0$	$g\to 0$
Mean number of daughter cells	$E[j]$	$\frac{2r-1}{g}+1$	$\frac{2r-1}{g}+\frac{3}{2}$
Variance of the number of daughter cells	$Var[j]$	$\frac{r}{g}+\frac{b}{h}+\frac{1}{2}$	$\frac{3r}{g}-\frac{3}{4}$

$$Var[N]=\frac{1}{4}+\frac{b}{h}, \tag{12}$$

$$Var[i]=\frac{3}{32}+\frac{3b^2}{8h^2}+\frac{3b}{8h}+\frac{7r}{4g}+\frac{br}{2gh}-\frac{r^2}{2g^2} +\frac{-6bg-5gh+4hr}{32g^2h^2}\sqrt{(4b^2g^2+h^2(g-4r)^2+4bgh(4r-5g))}, \tag{13}$$

$$Var[j]=\frac{23}{32}-\frac{b^2}{8h^2}-\frac{b}{8h}-\frac{r}{4g}+\frac{br}{2gh}+\frac{3r^2}{2g^2} +\frac{2gb+7gh-12hr}{32g^2h^2}\sqrt{(4b^2g^2+h^2(g-4r)^2+4bgh(4r-5g))}. \tag{14}$$

4 Interpretation of the results and numerical simulations

4.1 Limiting cases and model applicability

From formulas (8, 12) we notice that the mean and the variance for the total number of cells, N, are independent of r and g, the regulation of differentiation (see also Table 1, the top two rows). They only depend on the parameters b and h which control growth. It turns out that the mean and the variance of the number of differentiated cells [formulas (10) and (14)] are at large defined by the parameters regulating differentiation decisions, r and g, and only weakly depend on b and h, as demonstrated below.

To study $E[j]$ and $Var[j]$, let us consider the limiting cases as $h\to 0$ and $g\to 0$. The results are summarized in Table 1. These approximate expressions show that the dynamics of daughter cells is mostly regulated by parameters r and g. In Fig. 2 we present a contour plot of the full expressions for $E[j]$ and $\sqrt{Var[j]}$ [formulas (10) and (14)] as functions of the two control parameters h and g (the values r and b are fixed). We can see that the dependence of both the mean and the standard deviation on the parameter h is weak compared to the dependence on g.

Approximations for the mean and the variance of the number of stem cells presented in Table 1 provide a convenient way to set the parameters corresponding to a desired value of the mean. Let us suppose that we have some target values for the mean of the

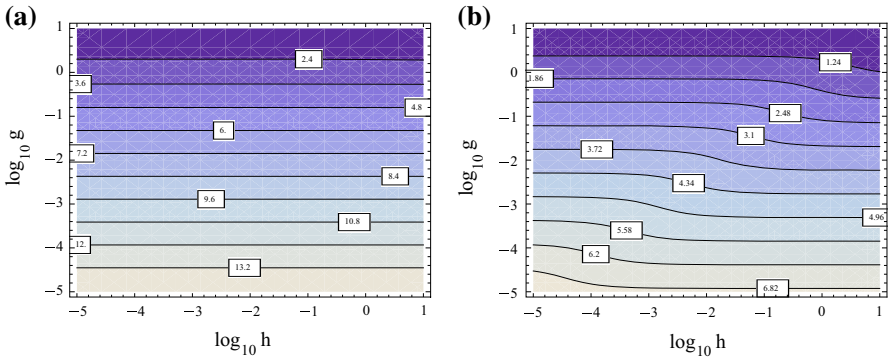

Fig. 2 Contour plots of the functions $\ln E[j]$ (**a**) and $\ln \sqrt{Var[j]}$ (**b**) are presented as functions of parameters g and h. The parameters are $b = 20$ and $r = 10$

numbers of stem and differentiated cells, such that $E[N] \approx N_0$ and $E[j] \approx J_0$ for some given $J_0 < N_0$. Then by setting

$$b = \frac{N_0 h + 1}{2}, \quad r = \frac{J_0 g + 1}{2} \tag{15}$$

we can get the mean of the variables N and j to be near the target values N_0 and J_0 respectively.

Next we examine the model applicability. Analysis of simplified expressions given in Table 1,

$$E[i] \approx \frac{2b-1}{h} - \frac{2r-1}{g} - \frac{1}{2}, \quad E[j] \approx \frac{2r-1}{g} + 1,$$

suggests that the following conditions must hold:

(i) $r > 1/2$, assuring that the mean number of daughter cells is greater than one;
(ii) $h < \frac{2g(2b-1)}{2(2r-1)+3g}$, assuring the the mean number of stem cells is greater than one. In addition, in most biological systems, the number of daughter cells exceeds the number of stem cells, which suggests the following condition,
(iii) $h > \frac{2g(2b-1)}{4(2r-1)+3g}$.

The analytical expressions for the means and the variances obtained in this paper are in good agreement with numerical simulations. In Fig. 3 we present results of numerical simulations and a comparison with the obtained analytical expressions. In the simulations, we fixed parameters b and r, and varied parameters g and h over several orders of magnitude. In order to stay in the regime where the number of stem cells is significantly smaller than the number of daughter cells, we made h depend on g in the following way. Using condition (ii) above, we required that h is near the threshold level specified by that condition. This guarantees that the number of stem cells is positive, and at the same time it is significantly smaller than the number of daughter cells [condition (iii)] above.

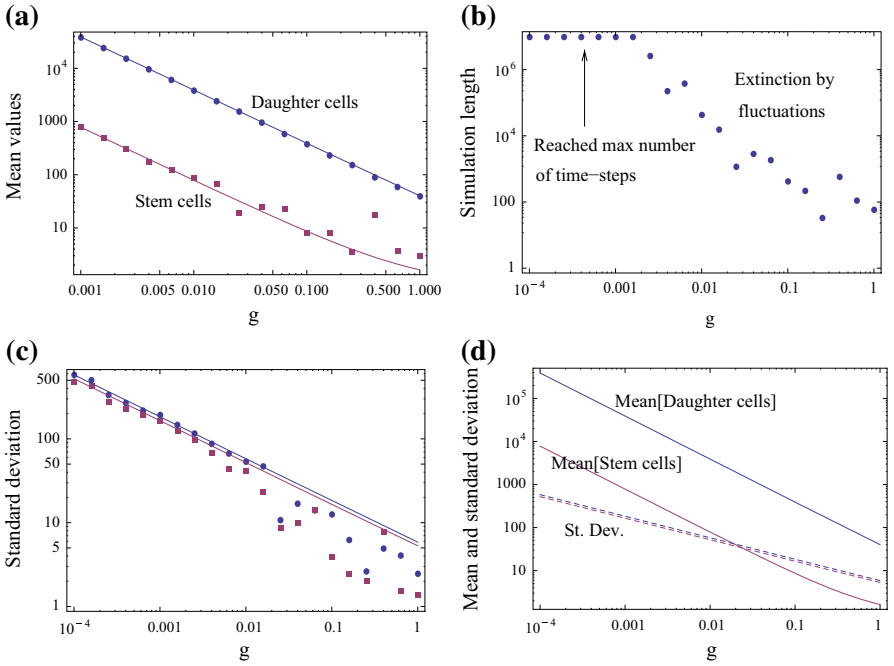

Fig. 3 The behavior of the mean numbers and the standard deviations of stem and daughter cells. **a** Comparison of the obtained formulas (*solid lines*) with the numerical approximations (*dots*) for the mean values of stem and daughter cells. The numerical estimates are obtained from stochastic simulations running up to 10^7 steps, with initial conditions for i and j given by the integer part of $E[i]$ and $E[j]$ respectively. **b** The time to extinction of the simulation, as a function of parameter g. **c** Comparison of the formulas (*solid lines*) with the numerical approximations (*dots*) for the standard deviations of stem and daughter cells. **d** A plot of predicted values of the means (*solid lines*) and standard deviations (*dashed lines*) of the stem and daughter cells. In all plots, the value of g is varied, and h is given by $h = 2g(2b-1)/(4r+3g-2)/k$, with the parameters $b = 10$, $r = 20$, $k = 1.02$

For each value of g, we ran one stochastic simulation, starting at the expected values of the stem and daughter cells (rounded up), and finishing either when the number of time-steps reached 10^7, or if any of the cell types went extinct. We then calculated the mean values of the cell populations over the time-course of the simulation, and also calculated the standard deviations.

In Fig. 3a we compare the mean values for stem and daughter cells obtained by formulas (9), (10), with their numerical estimates, and observe a very good agreement. In Fig. 3c we show the comparison of the analytical values for the standard deviations [formulas (14), (12)] with their numerical estimates. The standard deviation plots demonstrate a good agreement of the theory and the numerical simulations up to approximately $g = 10^{-2}$, and for larger values of g we observe a systematic deviation of the theoretical values from the numerical estimates. This is because in that region, standard deviation for the stem cell size becomes as large or larger than the predicted mean (see Fig. 3d). We also observe a drastic drop in the durations of the simulations (Fig. 3b), when the fluctuation amplitude approaches the magnitude of the mean. For

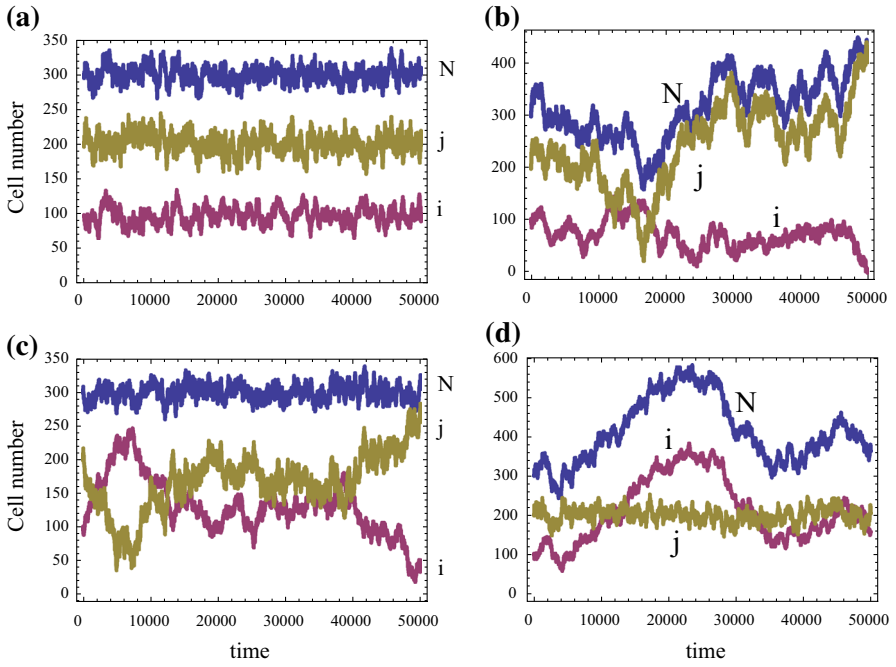

Fig. 4 Typical time-series of cell populations (N, the total number of cells, i, the stem cells, and j, the daughter cells). The control parameters are: **a** $h = g = 10$, **b** $h = g = 10^{-5}$, **c** $h = 10$, $g = 10^{-5}$, **d** $h = 10^{-5}$, $g = 10$. Other parameters are $N_0 = 300$, $J_0 = 200$, and b and r are defined by formula (15)

the given parameter values, for g greater than approximately 10^{-2} the fluctuations drive the system extinct, naturally leading to a numerical underestimation of the standard deviation, observed in Fig. 3c.

In Fig. 4 we show typical time-series for the stem cells, at different levels of control. We fixed $N_0 = 300$ and $J_0 = 200$, and set parameters b and r according to formulas (15). The parameters g and h are different in the four panels in Fig. 4. In Fig. 4a, we have $h = g = 10$, which corresponds to strong control of both division and differentiation. We can see that the standard deviations of all the three cell types in this case are relatively small, and the cell numbers oscillate near their predicted mean values. Figure 4b shows the opposite extreme where very little control is present in both division and differentiation processes, $h = g = 10^{-5}$. All three populations are characterized by large fluctuations, and the population experiences extinction relatively quickly compared to the simulations in Fig. 4a. The bottom two pictures show mixed regimes. In Fig. 4c cell division is strongly controlled ($h = 10$) while differentiation is controlled weakly ($g = 10^{-5}$). As a result, the total population number is very stable, but both the stem cells and daughter cells fluctuate with large amplitudes. Finally, in Fig. 4d we have $g = 10$ and $h = 10^{-5}$: the number of daughter cells is maintained nearly constant, but the number of stem cells fluctuates, and as a consequence the total population is also characterized by large variances.

4.2 Connection with the constant-population model

The results for the mean and the variance of the total number of cells, N, are given in Table 1. These results can be obtained from a one-dimensional system reviewed in Sect. 2, Eqs. (2–3). The connection can be seen in the following way. In the one-dimensional system, the probability to differentiate is given by Eq. (1) and can be rewritten in terms of the number of daughter cells as

$$p_i = \frac{\tilde{\beta}}{1+\tilde{h}j},$$

where we used $j = N - i$ and $\tilde{\beta} = \frac{\beta}{1-N\nu}$, $\tilde{h} = \frac{\nu}{1-N\nu}$. This formula tells us that the probability for the number of daughter cells to increase by one is a decreasing function of their total number. The expectation and the variance for the number of daughter cells follow from Eqs. (2–3):

$$E[j] = N + \frac{2\beta-1}{\nu} + \frac{1}{2} = \frac{2\tilde{\beta}-1}{\tilde{h}} + \frac{1}{2}, \quad Var[j] = \frac{\beta}{\nu} + \frac{1}{4} = \frac{\tilde{\beta}}{\tilde{h}} + \frac{1}{4}.$$

In the two-dimensional model, we have $Q(N) = b/(1+hN)$, which is the probability for the total number of cells to increase by one. Again, it is negatively regulated by the total number of cells, N. We can see that if we do not distinguish between cell and daughter cells, the two-dimensional process becomes a one-dimensional process for the variable N, and it is identical to the process described by Eq. (1), if we set $b = \tilde{\beta}$ and $h = \tilde{h}$. Therefore, the expectation and the variance for N are given by

$$E[N] = \frac{2b-1}{h} + \frac{1}{2}, \quad Var[N] = \frac{b}{h} + \frac{1}{4}.$$

From above considerations we can see that the formula for the statistics of the variable N in the two-dimensional process is closely related to the formula for i derived for the one-dimensional process. However, the two processes have some important differences.

1. In the one-dimensional process, we fix the total number of cells, assuming that it is regulated externally, through some unspecified mechanism. As a consequence, reproduction of a stem cell is always coupled to a daughter-cell death. In the two-dimensional process, the total number of cells varies.
2. The meaning of the stochastic variable in the 1D process is the number of daughter cells. In the two-dimensional process, the meaning of the stochastic variable is the total number of cells.
3. As the amount of control (parameter ν) increases, the probability p_i in formula (1) becomes negative (this happens for $\nu > 1/N$). This does not happen in the two-dimensional process. This can be interpreted as a consequence of an endogenous regulation of the total cell number, which leads to a more robust process compared to the fixed-size (exogenously regulated) process.

4. If the desired number of cell is fixed to N_0 in the 2D process, from Eq. (15) we can obtain the expressions for the resulting variance:

$$Var[N] = \frac{N_0}{2} + \frac{1}{2h}, \quad Var[j] = \frac{J_0}{2} + \frac{1}{2g}.$$

While increasing the control (the values of h and g) will lead to a decrease in the variations of the cell populations, the magnitude of variance has a lower bound given by $N_0/2$ and $J_0/2$ for the total number of cells and the daughter cells respectively. This means that no values of control parameters can reduce the variance below this theoretical minimum. For the constant-population system, if we want to keep the mean number of stem cells at n_0, we must take $\beta = \frac{1}{2} - \frac{(n_0+1/2)\nu}{2}$. Therefore, the variance becomes $Var[i] = \frac{1}{2\nu} - \frac{n_0}{2}$. One can see that ν cannot exceed the value $(n_0+1/2)^{-1}$, which sets the theoretical minimum for the variance to $1/4$, a significantly lower number.

A mathematical explanation of the difference between the two models is simple. Note that in the 1D model there are two distinct parameter regimes. In the first regime, the model is robust in the sense that all the probabilities are well defined for the full range of the values of i, the number of stem cells. This happens if $\nu < 1/N$. In this regime, the minimum variance achieved by the 1D model is $n_0/2$. In the second regime, which corresponds to values $1/N < \nu < 1/(n_0 + 1)$, the variance can decrease to numbers of the order one, but the robustness of the model decreases, as the probabilities are negative in some parts of the space $0 \leq i \leq N$. The second, non-robust regime, corresponds to negative values of the parameters b and h in the 2D model.[1] This can be seen if we recall that the two models' probabilities of a population increase are identical under the transformation $b = \frac{\beta}{1-N\nu}, h = \frac{\nu}{1-N\nu}$, where N is the total number of cells in the 1D process.

The above argument shows that in principle, if we allow for negative control parameters, the 2D model has exactly the same properties as the 1D models. The mathematical framework presented here allows any sign of the parameters. Therefore, we can say that the negative control of the form

$$Q(N) = \frac{|b|}{|h|N - 1}$$

can yield arbitrarily small values of the variance under a fixed mean, and has the same robustness problems as the 1D model (1). However, as ν increases through $1/N$, the functions $b(\nu)$ and $h(\nu)$ experience a type II discontinuity. Biologically, negative values of b and h probably correspond to a different mechanism of control.

[1] Probabilities Q and P in this model are functions of i and j, which are both stochastic variables. If the probability in the stochastic process attains a negative value, it means that the model fails. One can calculate the probability and timing of the failure event for a particular choice of parameters. There is a non-zero probability for this model to fail for any parameter regimes, but if the probability of failure is very small, this means that the model is robust. This corresponds to the regime where all the probabilities are in the (0, 1) interval for values of i and j within several standard deviations of their means.

If we restrict ourselves to only positive values of b and h in the case studied here, the variance is bounded from below by $N_0/2$. This is an artifact of the control function chosen here. Other types of control such as $Q(N) = be^{-\Gamma N}$ or $Q(N) = \frac{N^{-\alpha}}{k^{-\alpha}+N^{-\alpha}}$ allow to vary the control parameters such that the variance is reduced to arbitrarily low numbers, while keeping the mean value fixed and equal to N_0. In particular, in the two examples mentioned here the variance is given by $1/(2\Gamma)$ and k/α respectively, and in the limit of $\Gamma \to \infty$ and $\alpha \to \infty$ it tends to zero.

5 Discussion and conclusions

In this paper we considered stochastic dynamics of stem cells and daughter cells, where proliferation and differentiation decisions were both under endogenous control mediated by negative regulatory loops. This gave rise to a two-dimensional Markov process. We were able to obtain analytical solutions for the means and variances of stem and daughter cells, and investigated the system's properties.

We have shown that in the absence of control, a stochastic system of stem and daughter cells becomes unstable, and cannot maintain finite, nonzero populations in the lineage.

Negative control was defined by two specific functions, such that the probability for the stem cells to divide is negatively correlated with the total number of cells, and the probability for the stem cells to give rise to two daughter cells is negatively correlated with the number of daughter cells. In this case, the stochastic system is capable of maintaining a given number of stem and daughter cells with the variance that decreases as a function of the control parameters.

It must be noted that the system studied here is a gross simplification of reality. For example, the system is assumed to be close to its carrying capacity. Thus the model does not include the possibility to recover from a severe injury, or develop tissue from a small number of daughter cells. For such large deviations from the equilibrium, the probability of division would have to include a linear dependence on the number of stem cells. In our model we only consider the regime close to the equilibrium. This is what makes the model analytically tractable (but necessarily reduces its applicability).

Also, the current model does not include phenomena such as de-differentiation and asymmetric divisions, which have been reported to occur in various healthy and cancerous tissues. While the model can definitely be extended to include such phenomena, obtaining an analytical solution in this case may prove a more difficult task.

Next, we emphasize that the type of control explored here is not the only possibility. In the present paper we assumed the following patterns: (1) the probability of stem cell divisions, Q, decreases with the total population size, (2) the probability of differentiated cell death increases with the total population size, and (3) the probability of differentiation, P, decreases with the number of differentiated cells. While these assumptions are consistent with what is known about many stem cell systems (see Sect. 2 for the discussion), other possibilities have been proposed. In particular, it is possible that both the number of stem and differentiated cells participate in the regulation of divisions and proliferation/differentiation decisions. It is also possible

that positive control loops may play a role. In the present paper we only concentrate on one type of regulation.

Given the type of regulation assumed here, we further note that the present model is restricted to a particular functional choice for all the controls, which of course does not exhaust all the set of possibilities. The particular functional form adopted in the paper is only one way of reflecting the biological facts in a model. A subject of current work is developing a methodology which would work for a larger class of functional forms of control.

Furthermore, this paper does not aim to quantitatively model a particular tissue. This task would require measuring model parameters, which at this stage is hardly possible, because experimental literature is only starting to uncover the structure of control loops in stem cells. Further experimental work on correlations between various population sizes and the intensity of divisions, deaths, and proliferation/differentiation events will help to fine-tune the model for a more quantitative description.

Understanding tissue architecture can be key to numerous medical applications, including treatment of cancer. In the recent years it has been suggested (Clevers 2011; Petersen and Polyak 2010; Wang et al. 2010; Al-Hajj et al. 2003) that cancer has the architecture which resembles that of healthy epithelium, such that a relatively small number of cells (so-called cancer stem cells) possesses self-renewal capacities, while the rest of the cancerous tissue only has a limited division potential. It is possible that in carcinogenesis, one or more of control loops become dysfunctional which may lead to the tissue breaking out of homeostatic control (Rodriguez-Brenes et al. 2011). A possible interesting extension of our present work would be to consider the stochastic dynamics of this type of models in the presence of mutants whose regulatory loops are disabled.

Another extension of this work is to consider multistage lineages, where apart from stem cells and daughter cells, several intermediate classes of cells are present that differ by their degrees of differentiation. More complicated types of control loops can be envisaged (Lo et al. 2009). It would be interesting to see how control loop architecture influences the stochastic properties of the system. Finally, spatial considerations can be very important and a stochastic theory of spatial stem cell regulation needs to be developed, in analogy with a recent paper by Chou et al. (2010).

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