Cellular feedback networks and their resilience against mutations

LORA D. BAILEY and NATALIA L. KOMAROVA

Department of Mathematics, University of California Irvine, Irvine CA 92697

Abstract. Many tissues undergo a steady turnover, where cell divisions are on average balanced with cell deaths. Cell fate decisions such as stem cell differentiations, proliferations, or differentiated cell deaths, may be controlled by cell populations through cell-to-cell signaling. Here we examine a class of mathematical models of turnover in stem cell lineages to understand engineering design principles of control (feedback) loops, that may operate in such systems. By using ordinary differential equations that describe the co-dynamics of stem cells and differentiated cells, we study the effect of different types of mutations that interfere with feedback present within cellular networks. For instance, we find that mutants that do not participate in feedback are less dangerous in the sense that they will not rise from low numbers, whereas mutants that do not respond to feedback signals could rise and replace the wild type population. Additionally, we asked if different feedback networks can have different degrees of resilience against such mutations. We found that all minimal networks, that is networks consisting of exactly one feedback loop that is sufficient for homeostatic stability of the wild type population, are equally vulnerable. Mutants with a weakened/eliminated feedback parameter might expand from lower numbers and either enter unlimited growth or reach an equilibrium with an increased number of stem and differentiated cells. Therefore, from an evolutionary view point, it appears advantageous to combine feedback loops, creating redundant feedback networks. Interestingly, from an engineering prospective, not all such redundant systems are equally resilient. For some of them, any mutation that weakens/eliminates one of the loops will lead to a population growth of stem cells. For others, the population of stem cells can actually shrink as a result of "cutting" one of the loops, thus slowing down further unwanted transformations.

1 Introduction

Many healthy tissues, such as epithelial tissues, undergo a steady turnover, where cell divisions are on average at balance with cell deaths. Such tissues are often characterized by a hierarchical structure, whereby cells differ by their degree of maturity/differentiation. Stem cells, which are the least mature, undergo the processes of self-renewal and differentiation, giving rise to more differentiated offspring. Nevertheless the cells of different levels of maturation maintain approximately constant populations. The functioning of stem cell lineages at homeostasis is thought to be subject to cell-to cell regulation, whereby different cell fate decisions are made with probabilities that are influenced by the cells' environment. This idea was proposed decades ago in the context of "chalones", which are secreted tissue-specific substances that inhibit proliferation to keep the organ from over-growing [1, 2].

Identifying the exact nature of the regulatory circuits that can stably maintain tissue homeostasis is an area of active experimental research. There are several examples of systems where stem cell (SC) reproduction rates are negatively regulated by the number of differentiated cells (DCs) through soluble factors. These include bone morphogenetic protein regulating dynamics of hair follicles [3], myostatin controlling muscle growth by acting upon myoblasts and satellite cells [4], and neutrophil elastase antagonizing the effect of granulocyte-colony-stimulating factor G-CSF [5, 6].

Several mechanisms have been proposed to describe feedback loops that act in SC lineages to maintain homeostasis. In [7], tissue turnover in a range of organisms is discussed, and a particular model organism, fresh-water planarian *Schmidtea mediterranea*, is used to study homeostasis regulation. Two mechanisms of homeostatic regulation are proposed. One assumes that dividing SCs send a signal, which evokes DC death and enhances SC's own differentiation, whereby guaranteeing the removal of DCs and the maintenance of a constant population size. The second mechanism involves dying DCs that send a signal to SCs to activate their compensatory proliferation. The latter process has also been proposed in

the context of Drosophila studies, where it was described in terms of "social control" in cellular dynamics [8, 9]. In humans, the death of DCs has been shown to trigger cell divisions in bladder cancer, where a PGE2-mediated wound-healing type response was implicated [10, 11]. Thus, chemotherapy-induced death of DCs resulted in a positive feedback signal that induced SC proliferation.

Given the evidence of feedback loops acting in SC lineages, tissue turnover in healthy and cancerous systems has attracted considerable attention of mathematical and computational scientists. Mathematical models of SC lineages have been created, in particular, in the context of hematopoiesis, see e.g. [12, 13, 14, 15, 16, 17, 18]. These models consider the dynamics of lineages that contain a number of compartments that differ by their degree of differentiation, and include cell fate decisions such as cell self-renewal, differentiation, and death. At the basis of the modeling is often a set of ordinary differential equations, or integro-differential equations for more sophisticated, continuously structured models [19]. Different assumptions on the control (feedback) loops acting in the systems have been investigated, see also [20, 21]. Mutant dynamics in stem cell lineages have often been studied in the context of leukemias, see also [22, 23, 24, 25, 26]. Other models adopted a spatial approach, which allows more realism but is more difficult for analysis, see e.g. [27, 28, 29, 30]. These models are aimed at revealing spatial mechanisms of tumor development in the presence of stem and non-stem cell co-dynamics.

In our previous work we built on the tradition of Kimmel and Marciniak-Czochra groups and adopted an axiomatic approach to studying SC regulation in tissues undergoing turnover. Defining a "control network" as a network of cellular decisions with specific feedback loops present, in [31] we established a way to classify all possible such possible networks with the minimal number of feedback loops in two- and three-compartment systems containing symmetric divisions (resulting in either self-renewal or differentiation) and death. If the total (and not per-cell rates) are considered, we found that exactly two feedback loops are necessary in two-compartment models and three loops are required in three-compartment models. In [32] we applied this theory to experimental data on colonic crypts to deduce possible feedback mechanisms that are responsible for regulating cell dynamics in the lineages. Note that as the number of compartments increases, the number of theoretically possible feedback networks increases dramatically. In [33] we attempted to make sense of this complexity. We used the formalism of digraphs and established the requirements on the feedback networks (which are locally the Jacobians of the ODEs and referred to as 'control networks' in the paper) that guarantee stability.

A question that came up but was not resolved in previous work is whether all stable networks are equally "good". From the engineering prospective, is there the best way to arrange feedback loops such that the system is stable and is somehow harder to "break"? This is an important issue given that the feedback loops, like all other protective adaptations, are at risk of being altered or destroyed by mutations leading to selfish, malignant cell growth. Therefore, there is an evolutionary pressure to solve the engineering problem of optimizing the resilience of the feedback network.

In the present paper we attempted to tackle this problem by comprehensively studying all the control networks of a given complexity that are stable within a given system. Our base ODE model contains only two compartments, but allows for a degree of complexity by including not only self-renewals, differentiations, deaths, but also other processes such as de-differentiation of DCs and asymmetric divisions of SCs. We introduce a very wide class of mutations that alter different aspects of the system, including the way cells signal to other cells, the way cells react to feedback signals, and also other phenotypic changes. We study both minimal and redundant feedback networks and investigate if they differ by their resilience properties. For the purposes of the current study, resilience of a feedback network is equivalent to being able to keep the populations as low as possible and avoid large fluctuations in population size, even in the presence of selfish mutants with altered properties that are fitter than the wild type cells.

This paper is organized as follows. Section 2 presents the modeling framework and exhaustively finds all the minimal feedback networks, that is, the regulation systems that have the minimum possible number of feedback loops present. Section 3 introduces different types of mutations and studies the co-dynamics of wild type and mutant cells; it identifies dangerous types of mutations and compares the minimal feedback network properties in the presence of mutations. Section 4 studies all the 2-loop

redundant feedback networks and compares their resilience properties in the presence of mutants. Section 5 contains discussion and conclusions.

2 Framework

Our model uses a very general two-compartment deterministic system with x denoting the number of stem cells (SCs) and y the number of differentiated cells (DCs). The following processes are included, see Table 1: SCs can divide and differentiate into two DCs with rate R, divide and self-renew with rate S_{SC}, divide asymmetrically with rate A, or die with rate D_{SC}, while DCs die with rate D_{DC}, de-differentiate[1] with rate B, or self-renew with rate S_{DC}. Note that the self-renewal rate of DCs has been added in this description, because the DC compartment may represent a conglomerate compartment that contains cells that are not fully differentiated (such as transit amplifying cells). Figure 1(a) shows all the processes schematically, and Table 1 lists the changes occurring in the populations of SCs and DCs as a result of a single instance of each process. For example, a differentiation of a SC leads to a decrease in the total number of SCs ($\Delta x = -1$) and an addition of two differentiated cells ($\Delta y = 2$).

Note that each rate could be affected by the presence, and the level, of SCs and/or DCs, resulting in determination of cell fate decisions by the cellular populations, see [31]. Since the exact mechanisms governing cell fate decisions are often unknown, we consider many possible cellular processes and make no assumptions on functional forms that define feedback parameters. Instead, we use an axiomatic model to account for the extensive possibilities [34, 35].

Rate	Process	Δx	Δy
R	Differentiation division of SCs	-1	2
A	Asymmetric division of SCs	0	1
B	De-differentiation of DCs	1	-1
S_{SC}	Self-renewal of SCs	1	0
S_{DC}	Self-renewal of DCs	0	1
D_{SC}	Death of SCs	-1	0
D_{DC}	Death of DCs	0	-1
S	(Combined) self-renewal of SCs, $S = S_{SC} - D_{SC}$	1	0
D	(Combined) death of DCs, $D = D_{DC} - S_{DC}$	0	-1

Table 1: Cellular processes: per cell rate notation, along with the variable names and resulting changes in populations of SCs and DCs.

The general ordinary differential equations (ODEs) describing the dynamics are given by

$$\dot{x} = -x\left[R(x,y) + S_{SC}(x,y) - D_{SC}(x,y)\right] + yB(x,y), \tag{1}$$

$$\dot{y} = x\left[2R(x,y) + A(x,y)\right] - y\left[D_{DC}(x,y) + B(x,y) - S_{DC}(x,y)\right]. \tag{2}$$

Let us simplify and denote

$$S = S_{SC} - D_{SC}, \quad D = D_{DC} - S_{DC}.$$

In other words, the process of SC death is absorbed in SC proliferation rate by reducing it, and the process of DC proliferation is absorbed in DC death rate by reducing it. While SCs may die (D_{SC}) and DCs may self-renew (S_{DC}), these processes are much less common than DC death (D_{DC}) and SC proliferation (S_{SC}), so going forward we assume combined rates, S and D, are non-negative, unless otherwise specified. Figure 1(b) shows a schematic of the processes in this model with the concatenated rates.

[1]Note that we use symbol B for de-differentiation to signify that DCs are turning 'back' into SCs, as symbol D is already taken by the death rate.

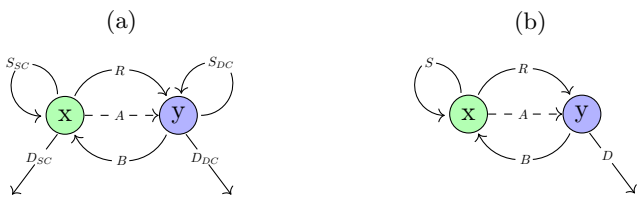


Figure 1: A schematic showing the kinetic processes included in the (a) full model and (b) after using the concatenated rates. The SCs and DCs are denoted by x and y circles, and black arrows represent the kinetic rates that originate at the relevant population. Asymmetric divisions are marked by dashed lines, and all rates are marked next to the corresponding arrows.

To further simplify the notations, let us assume each rate, R, S, D, A, B, is a function of SCs (x) and DCs (y), unless otherwise specified, and drop the arguments. The ODE model then becomes

$$\dot{x} = x(-R+S) + yB, \tag{3}$$
$$\dot{y} = x(2R+A) - y(D+B). \tag{4}$$

2.1 The healthy equilibrium and the regulation of feedback

System (3-4) describes the dynamics of cell fate decisions in a healthy organ, therefore we will assume that this system has a positive equilibrium, which we denote by $(\bar{x}, \bar{y})$. This equilibrium satisfies

$$(-R+S)\bar{x} + B\bar{y} = 0, \tag{5}$$
$$(2R+A)\bar{x} - (D+B)\bar{y} = 0. \tag{6}$$

The Jacobian, J_0, is given by

$$J_0 = \begin{pmatrix} -R+S+\bar{x}(-R_x+S_x)+\bar{y}B_x & \bar{x}(-R_y+S_y)+B+\bar{y}B_y \\ 2R+A+\bar{x}(2R_x+A_x)-\bar{y}(D_x+B_x) & \bar{x}(2R_y+A_y)-(D+B)-\bar{y}(D_y+B_y) \end{pmatrix} \tag{7}$$

where subscripts denote partial derivatives, and all rate functions R, S, B, D, A and their derivatives are evaluated at the equilibrium $(\bar{x}, \bar{y})$. The stability conditions are

$$Tr\, J_0 < 0, \quad Det\, J_0 > 0. \tag{8}$$

For the rest of this study we assume these stability conditions are satisfied.

The dynamics of the system is then (locally) defined by the values of the partial derivatives evaluated at the equilibrium:

$$R_x, R_y, S_x, S_y, B_x, B_y, D_x, D_y, A_x, A_y. \tag{9}$$

We will refer to these quantities as "feedback parameters". The total absence of feedback results in the linear system (3-4) with constant rates; in this case no positive equilibrium exists.

Note that in this study, the rates R, S, B, D, A are defined per cell. If in the vicinity of the equilibrium, the rate is a constant (that is, the derivatives with respect to x and y are zero), then we say that the corresponding process has *no feedback* from the cell populations. In this case, the process happens with a constant per cell intensity, and the total intensity is a linear function of the population size (x or y, depending on which cell population is engaged is this process). We do not consider this dependence of the total rate on the population as a "feedback parameter", which is different from the terminology we used in previous publications [31, 34, 35], where any dependence of *total* rates on any population was regarded as "feedback", or what in those papers was referred to as "control".

If a per cell rate depends on x only (that is, the derivative with respect to x is nonzero while the derivative with respect to y is zero in the vicinity of the equilibrium), we say that the *process has a feedback loop depending on* x, or *is regulated by* x. Additionally, we say a feedback loop on the cellular process Q that is mediated by x is *positive (negative)*, that is x *positively (negatively)* regulates Q, when the derivative of Q with respect to x is positive (negative). Similarly, with y, or with both x and y. We make several assumptions about the equilibrium:

- If a derivative is zero at equilibrium, then we assume that the function is a constant, at least in a finite vicinity of the equilibrium. In Appendix A.1 we consider the case where the equilibrium occurs at a critical point, such that while the derivative is zero at the equilibrium, the derivative is nonzero everywhere in a (one-sided) vicinity of the equilibrium.
- We also assume that if the process takes place, then its intensity is nonzero at the equilibrium. That is, if for example $B(x, y) = 0$ at the equilibrium, then we assume that B is (locally) a constant. In Appendix A.2 we consider the case where this does not hold, for example, $B(x, y) = 0$ at the equilibrium (no de-differentiation at the equilibrium), but there is a positive de-differentiation intensity on one or both sides of the equilibrium.

2.2 Minimal feedback

A minimal feedback network is a system with the smallest number of nonzero elements in list (9), for which a positive equilibrium is stable. As mentioned, the total absence of feedback cannot lead to a stable equilibrium, and thus at least one feedback loop is necessary for stable homeostasis.

In order to find all minimal feedback networks, we consider two cases: (1) $B = 0$ (no DC de-differentiation) and (2) $B > 0$.

(1) In the absence of DC de-differentiation: $B = 0$. In this case, expressions are particularly simple. At equilibrium we have

$$R(\bar{x}, \bar{y}) = S(\bar{x}, \bar{y}), \tag{10}$$

that is, the rate of self-renewals is equal to the rate of differentiation divisions. It follows at least one of these quantities should be non-constant, because we assume that having two constant rates exactly equal to each other is a degenerate case. The Jacobian (7) simplifies to

$$J_0 = \begin{pmatrix} \bar{x}(-R_x + S_x) & \bar{x}(-R_y + S_y) \\ 2R + A + \bar{x}(2R_x + A_x) - \bar{y}D_x & \bar{x}(2R_y + A_y) - D - \bar{y}D_y \end{pmatrix}.$$

From the requirement that $Det\, J_0 > 0$ it again follows that at least one of the quantities R_x, R_y, S_x, S_y has to be nonzero, that is, self-renewals or differentiations of SCs must be regulated by a feedback loop. Further, for stability we need $R > 0, S > 0$ and $D > 0$.[2] The minimal feedback can then be found by assuming SC self-renewal or differentiation is influenced by either population and then solving for the feedback parameters that satisfy the stability conditions given in (8). They are as follows:

(i) *SCs positively regulate their differentiation divisions :* $R_x > 0$, and no other dependencies exist, such that $R_y = 0$, and S, A, D are constants.
In this case, the eigenvalues are real and negative: $-\bar{x}R_x, -D$.

(ii) *DCs positively regulate the differentiation divisions of SCs:* $0 < R_y < D/(2\bar{x})$, and no other dependencies exist, such that $R_x = 0$, and S, A, D are constants.
For this minimal case, we have

$$Tr(J_0)^2 - 4Det(J_0) = (D - 2\bar{x}R_y)^2 - 4\bar{x}R_y(2R + A). \tag{11}$$

If (11) is negative, the eigenvalues are complex and the system will oscillate around the equilibrium. Otherwise if (11) is positive, the system will be non-oscillatory. Figures 2 (a) and (b) show oscillatory and non-oscillatory examples of this minimal feedback network.

(iii) *SCs negatively regulate their self-renewal divisions:* $S_x < 0$, and no other dependencies exist, such that $S_y = 0$, and R, A, D are constants.
The eigenvalues $xS_x, -D$ are real and negative.

[2] If $R \equiv 0$ or $S \equiv 0$, then at equilibrium S (R) must also be zero, and by our previous assumption then both rates are identically 0, which violates the requirement that at least one of these rates must have feedback. Further, $D > 0$ at the equilibrium because equation (6) must be satisfied and $(2R + A)\bar{x} > 0$.

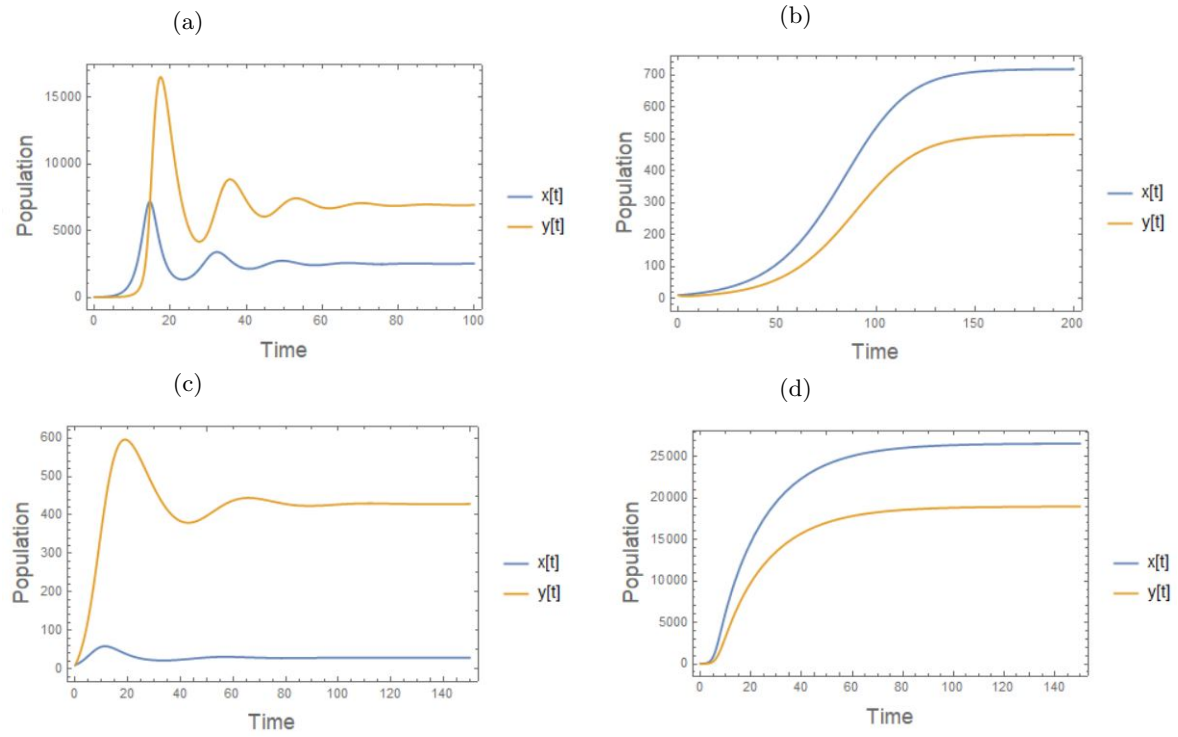


Figure 2: Minimal feedback examples in the absence of de-differentiation, where the system can be either oscillatory or non-oscillatory. (a) and (b) are examples of minimal feedback network (ii) where R is positively regulated by y, $0 < R_y < D/(2\bar{x})$, and (c) and (d) are examples for minimal feedback networks (iv) when S is negatively regulated by y, $S_y < 0$. (a) We take $R(y) = 1 - e^{-0.0001y}$, $S = .5$, $D = .4$, $A = .1$, along with initial conditions $x(0) = 10$ and $y(0) = 10$. (b) We take the same function for $R(y)$ along with $S = .05$, $D = .7$, $A = .4$, and the same initial conditions. (c) Here $S(y) = \frac{1}{0.001y+1}$, $R = .7$, $D = .1$, $A = .1$, $x(0) = 10$ and $y(0) = 10$. (d) Here $S(y)$ is the same as before with $R = .05$, $D = .7$, $A = .4$ and the same initial conditions.

(iv) *DCs negatively regulate the self-renewal divisions of SCs:* $S_y < 0$, and no other dependencies exist, such that $S_x = 0$, and R, A, D are constants.
For this minimal case, we have

$$Tr(J_0)^2 - 4Det(J_0) = D^2 + 4\bar{x}S_y(2R + A). \tag{12}$$

If (12) is negative, the system if oscillatory. Figures 2 (c) and (d) show oscillatory and non-oscillatory examples in this minimal feedback network.

To summarize, in the absence of de-differentiation, the system can maintain stability through a positive regulation of SC differentiation or a negative regulation of their self-renewal, by either SC or a DC populations, that is, increasing either population must result in an increase in differentiations or a decrease or self-renewals. Note that when de-differentiation is absent, regulation of the DC death rate D or the rate of asymmetric division A alone are not sufficient for stability. Numerical examples of oscillatory and non-oscillatory minimal networks for cases (ii) and (iv) are shown in Figure 2.

(2) In the presence of DC de-differentiation: $B > 0$**.** Now, when evaluating the Jacobian in (7), instead of (10), we have inequality

$$R > S, \tag{13}$$

that is, the rate of differentiation divisions is higher than the rate of self-renewals (which increase the number of SCs along with de-differentiations from the DC compartment). The following identity holds:

$$\frac{\bar{x}}{\bar{y}} = \frac{B}{R-S} = \frac{D+B}{2R+A}. \tag{14}$$

In this case, feedback on R and S is also sufficient for stability, except the conditions become slightly more complicated. In addition, there are several other possibilities, as listed below. In the descriptions (i-x) below, we implicitly assume that all the kinetic rates are independent of x and y, except the specific dependencies listed.

(i) *SCs positively regulate their differentiation divisions:* $R_x > 0$. This minimal network additionally requires that $B < D$ for stability, that is DCs are more likely to die than to de-differentiate. To prove this inequality, we rearrange the equilibrium equations

$$\bar{x}(-R(\bar{x}) + S) + \bar{y}B = 0, \quad \bar{x}(2R(\bar{x}) + A) - \bar{y}(D+B) = 0,$$

solve both for $\bar{x}/\bar{y}$, and set them equal to each other to obtain

$$0 = R(\bar{x})(B-D) + AB + SD + SB,$$

which implies $B < D$.

(ii) *DCs positively regulate differentiation divisions of SCs:* $0 < R_y < \frac{\bar{x}(D+B)+\bar{y}B}{2\bar{x}^2}$. As in (i), this case also requires $B < D$ for stability.

(iii) *SCs negatively regulate their self-renewal divisions:* $S_x < 0$.

(iv) *DCs negatively regulate self-renewal divisions of SCs:* $S_y < 0$

Note that these are generalizations of the feedback parameters obtained with $B = 0$, to positive values of B. Additionally, we have the following new possibilities:

(v) *SCs negatively regulate de-differentiation of DCs:* $B_x < 0$.

(vi) *SCs negatively regulate de-differentiation of DCs:* $-\frac{\bar{x}(D+B)+\bar{y}B}{\bar{x}\bar{y}} < B_y < 0$.

(vii) *SCs positively regulate death of DCs:* $D_x > 0$.

(viii) *DCs positively regulate death of DCs:* $D_y > 0$.

(ix) *SCs negatively regulate the rate of asymmetric SC divisions:* $A_x < 0$.

(x) *DCs negatively regulate the rate of asymmetric SC divisions:* $A_y < 0$.

Table 2 presents all the cases of minimum feedback and illustrates them with the corresponding schematic. Interestingly, in the presence of de-differentiation, stability of an equilibrium can be achieved through regulation of any rate, by either of the populations. In particular, to stabilize the system, SC differentiation or DC death should be regulated positively (increase with cell populations), and SC self-renewals and asymmetric divisions as well as de-differentiations should be regulated negatively (decrease with cell populations). These results are not too surprising if one uses the following intuition. SC differentiation decreases the number of SCs, and thus increasing its rate will prevent the system from uncontrolled growth. DC death can also decrease the number of SCs indirectly, by reducing the flow of de-differentiating DCs that replenishes the SC pool. Therefore, increasing DC death can prevent uncontrolled growth, a mechanism that is only possible in the presence of de-differentiation. On the other hand, SC self-renewals, asymmetric divisions, and DC de-differentiation all directly increase the number of SCs, and thus these processes must be regulated negatively for homeostasis.

Note that we have assumed the rate of DC cell death is greater than that of DC self-renewal, that is $D > 0$. If this assumption is reversed, corresponding to DCs behaving more like SCs, then the minimal feedback loops above will change. These new minimal feedback loops are described in Appendix A.3.

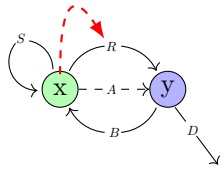
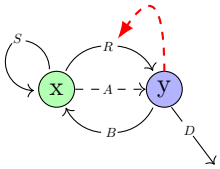
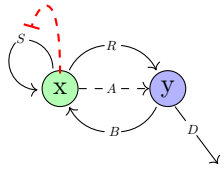
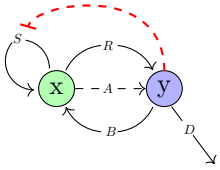
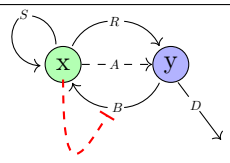
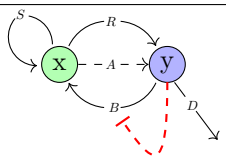
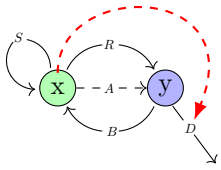
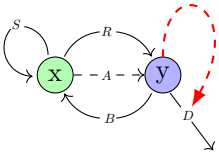
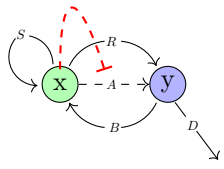
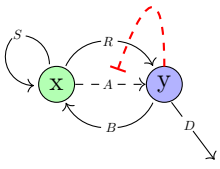
	Stability Condition	Network		Stability Condition	Network
(i)	$R_x > 0$		(ii)	$0 < R_y < \frac{\bar{x}(D+B)+\bar{y}B}{2\bar{x}^2}$	
(iii)	$S_x < 0$		(iv)	$S_y < 0$	
The following cases require $B > 0$:					
(v)	$B_x < 0$		(vi)	$-\frac{\bar{x}(D+B)+\bar{y}B}{\bar{x}\bar{y}} < B_y < 0$	
(vii)	$D_x > 0$		(viii)	$D_y > 0$	
(ix)	$A_x < 0$		(x)	$A_y < 0$	

Table 2: Minimum feedback cases (i-x). In the diagrams, the SCs and DCs are denoted by x and y circles, and black arrows represent the kinetic rates. The red positive and negative arrows indicate feedback loops; they originate at the population that mediates the feedback and point toward the process whose rate is being regulated.

2.3 Mutations

Thus far we have modeled healthy tissue in a state of homeostasis and determined that at least one feedback loop is necessary to maintain that homeostasis. Next, we introduce mutations and investigate their effect on the network. These mutations will create phenotypically different populations that have the same basic hierarchical structure (that is, SCs and DCs) as the wild type cells, but do not "play by the same rules".

To introduce mutations, we have to adapt our ODE model to formulate a coupled system that describes the co-dynamics of four sub-populations: (x_1, y_1, x_2, y_2). Subscript 1 represents the wild type, or normal, cells, such that we have wild type SCs (x_1) and wild type DCs (y_1), and subscript 2 represents the mutant populations consisting of mutant SCs (x_2) and mutant DCs (y_2). We will assume that the sub-populations are separate in the sense that cells of the wild type populations can only give rise to wild type cells, and cells of the mutant populations can only give rise to mutant cells. No continuous mutation process is assumed, and the existence of mutants will be investigated by introducing a small number of such cells as an initial condition. Mutant cells may differ from the wild type cells by their cellular kinetic rates; below we denote the mutant rates by tildes:

$$\begin{aligned}
\dot{x}_1 &= x_1[-R(x_1,x_2,y_1,y_2) + S(x_1,x_2,y_1,y_2)] + y_1 B(x_1,x_2,y_1,y_2), && (15)\\
\dot{y}_1 &= x_1[2R(x_1,x_2,y_1,y_2) + A(x_1,x_2,y_1,y_2)] \\
&\qquad -y_1[D(x_1,x_2,y_1,y_2) + B(x_1,x_2,y_1,y_2)], && (16)\\
\dot{x}_2 &= x_2[-\tilde{R}(x_1,x_2,y_1,y_2) + \tilde{S}(x_1,x_2,y_1,y_2)] + y_2 \tilde{B}(x_1,x_2,y_1,y_2), && (17)\\
\dot{y}_2 &= x_2[2\tilde{R}(x_1,x_2,y_1,y_2) + \tilde{A}(x_1,x_2,y_1,y_2)] \\
&\qquad -y_2[\tilde{D}(x_1,x_2,y_1,y_2) + \tilde{B}(x_1,x_2,y_1,y_2)], && (18)
\end{aligned}$$

Further, in the absence of mutants, the first two equations should be identical to Equations (3-4), which means that we must assume

$$Q(x_1, 0, y_1, 0) = Q(x_1, y_1).$$

Here Q stands for any of the kinetic rates R, S, etc, the left hand side contains a rate from system (15-18), and the right hand side contains the corresponding rate from system (3-4). This requirement guarantees that solution

$$x_1 = \bar{x}, y_1 = \bar{y}, x_2 = y_2 = 0 \qquad (19)$$

is an equilibrium of system (15-18). While solution $(\bar{x}, \bar{y})$ is assumed to be stable in system (3-4), stability of solution (19) in the 4-component system (15-18) will depend on the rates. In this framework we will study the stability properties of this equilibria in the presence of mutants under different assumptions on their properties.

As already mentioned, no *de novo* mutations are considered here. The concept of linear stability analysis of solution (19) implies a perturbation, which has the meaning of introducing a small number of mutants in a system that is mostly comprised of wild type cells at equilibrium. Analysis will reveal under what conditions mutants have a chance of taking off and replacing the resident wild type cells.

The Jacobian evaluated at equilibrium (19) is given by

$$J = \begin{pmatrix} J_0 & * \\ 0 & J_{\text{mut}} \end{pmatrix},$$

where J_0 is the original Jacobian of the normal cells given in (7) and evaluated at the same equilibrium (because $x_2 = y_2 = 0$), and the star denotes entries that are not important for the stability analysis. Matrix J_{mut} is given by

$$J_{\text{mut}} = \begin{pmatrix} -\tilde{R} + \tilde{S} & \tilde{B} \\ 2\tilde{R} + \tilde{A} & -\tilde{D} - \tilde{B} \end{pmatrix}, \qquad (20)$$

where each rate is evaluated at equilibrium (19). Since matrix J_0 is stable (that is, has eigenvalues with negative real parts), stability of solution (19) is defined by the eigenvalues of matrix J_{mut}. We have

$$\begin{aligned}
Tr(J_{\text{mut}}) &= -\tilde{R} + \tilde{S} - \tilde{D} - \tilde{B}, && (21)\\
Det(J_{\text{mut}}) &= (\tilde{R} - \tilde{S})(\tilde{D} + \tilde{B}) - \tilde{B}(2\tilde{R} + \tilde{A}), && (22)
\end{aligned}$$

evaluated at (19). If $Tr(J_{\text{mut}}) < 0$ and $Det(J_{\text{mut}}) > 0$, the homeostatic solution is stable and the mutant will go extinct. If either of these inequalities reverses, then the corresponding mutant can rise from low numbers. Note that $Tr(J_{\text{mut}}) > 0$ implies that

$$\tilde{S} > \tilde{R} + \tilde{D} + \tilde{B},$$

but $Det(J_{\text{mut}}) < 0$ implies that

$$\tilde{S} > \tilde{R} - \frac{\tilde{B}(2\tilde{R} + \tilde{A})}{\tilde{D} + \tilde{B}}, \tag{23}$$

which is a weaker condition. Therefore, a necessary and sufficient condition for instability is inequality (23). Note that if $\tilde{B} = 0$, it simplifies to

$$\tilde{S} > \tilde{R}. \tag{24}$$

Below we introduce several classes of mutants and investigate whether they are "dangerous", that is, if they have an ability to invade.

A a note on the methodology. The dynamical system describing lineage regulations is non-linear. Below we use linear analysis to explore the possibility of initial mutant growth. We also go beyond local analysis to investigate whether the population will increase or decrease as a result of an outgrowth of a mutant that enjoys a selective advantage. In this context, we will compare two stable equilibria: the initial one that corresponds to the system in the absence of mutations, and the mutant equilibrium that is reached after the mutant is introduced and takes over.

3 Different classes of mutants and their invasion properties

Mutant properties are reflected in two different aspects of system (15-18):

1. The way rates Q depend on their arguments and x_2 and y_2, and
2. The way the rates $\tilde{Q}$ differ from the corresponding rates of the wild type cells, Q.

The following represents the special case of neutral mutants, whose properties are identical to the properties of the wild type cells:

$$Q(x_1, x_2, y_1, y_2) = Q(x_1 + x_2, y_1 + y_2), \quad \tilde{Q}(x_1, x_2, y_1, y_2) = Q(x_1, x_2, y_1, y_2) \quad \forall Q, \tilde{Q}.$$

This means that all rates depend on total numbers of SCs and DCs, and the mutant rates are the same as wild type rates. In this case, because of inequality (13), the trace in Equation (21) is negative. Further, because of identity (14), the determinant in Equation (22) is identically zero, which corresponds to the neutrality of mutants. In other words, an infinite family of equilibrium solutions exist where

$$x_1 + x_2 = \bar{x}, \quad y_1 + y_2 = \bar{y},$$

for any positive values of the mutant and wild type sub-populations.

Next we examine several cases of mutants whose properties differ from those of the wild type cells.

3.1 Mutants that do not participate in feedback

The first type of mutation that we consider is where the mutant populations do not participate in regulating one or more rates. Take $0 \leq \alpha_Q \leq 1$ and $0 \leq \beta_Q \leq 1$ where Q represents the rate functions, and assume that

$$Q(x_1, x_2, y_1, y_2) = \tilde{Q}(x_1, x_2, y_1, y_2) = Q(x_1 + \alpha_Q x_2, y_1 + \beta_Q y_2).$$

In other words, referring back to the list of mutant properties at the beginning of Section 3, we can say that

1. The rates Q depend on weighted sums of SCs and DCs, instead of the total SC and DC populations, such that mutants can contribute disproportionately little to the regulation of cell fate processes;

2. The rates $\tilde{Q}$ are not different from the corresponding rates of the wild type cells, Q.

If the mutant does not contribute to the regulation of process Q, then we set $\alpha_Q = 0$ and $\beta_Q = 0$. Full contribution of population x_2 (y_2) corresponds to setting $\alpha_Q = 1, \beta_Q = 1$, resulting in a neutral mutant. Note that this flexible framework allows for partial loss of contribution (intermediate values of α_Q, β_Q).

At the equilibrium of interest, Equation (19), we can see that the rates in expressions (21-22) are given by $Q(\bar{x}, \bar{y})$ and thus the same argument holds as used above for neutral mutants, that is, because of inequality (13), the trace in Equation (21) is negative, and because of identity (14), the determinant in Equation (22) is identically zero. Note also that as α_Q and β_Q do not appear in J_{mut}, even if mutants were to increase participation in function regulation ($\alpha_Q > 1$ or $\beta_Q > 1$) there would be no effect on equilibrium and the solution would remain neutrally stable.

Thus mutants that do not participate in regulating one or more rates do not cause any biological problems. In a stochastic system, they do not expand but remain drifting, and the system with such mutants remains at homeostasis. This is consistent with the results reported in [36] for a different class of models.

3.2 Mutants that lose response to feedback

The previous mutation type where mutants do not participate in one or more feedback loops was non-harmful, so we consider another type of mutation where the mutant populations lose some or all response to one or more existing feedback loops. Again referring to the list of mutant properties at the beginning of Section 3, we assume here that

1. The rates Q depend on the total SC and DC populations, denoted as

$$x = x_1 + x_2, \quad y = y_1 + y_2.$$

2. For rates Q that are under regulation in the wild-type system, one or more mutant rates, $\tilde{Q}$, are different from the corresponding rates of the wild type cells in that cell fate decisions are not regulated to the same degree. Assuming $0 \leq \gamma_Q \leq 1$ and $0 \leq \delta_Q \leq 1$, we set

$$\tilde{Q}(x, y) = Q(\gamma_Q x, \delta_Q y).$$

These assumptions result in the following system:

$$\begin{aligned}
\dot{x}_1 &= x_1(-R(x,y) + S(x,y)) + y_1 B(x,y), && (25)\\
\dot{y}_1 &= x_1(2R(x,y) + A(x,y)) - y_1(D(x,y) + B(x,y)), && (26)\\
\dot{x}_2 &= x_2(-R(\gamma_R x, \delta_R y) + S(\gamma_S x, \delta_S y)) + y_2 B(\gamma_B x, \delta_B y), && (27)\\
\dot{y}_2 &= x_2(2R(\gamma_R x, \delta_R y) + A(\gamma_A x, \delta_A y)) - y_2(D(\gamma_D x, \delta_D y) + B(\gamma_B x, \delta_B y)). && (28)
\end{aligned}$$

The mutant rate $\tilde{Q}$'s response to the total SC population x is governed by weight γ_Q, and its response to the total DC population y is governed by weight δ_Q. If the mutant does not respond to any SC regulation, then $\gamma_Q = 0$, and if the mutant does not respond to any DC regulation, then $\delta_Q = 0$. If the mutant's response to regulation by the population x (y) is unaffected, then $\gamma_Q = 1$ ($\delta_Q = 1$). Similar to mutants that do not participate in feedback, this framework allows for the possibility that only partial regulation of feedback is lost. Additionally, this framework allows for the possibility that regulation is increased, if we assume $\gamma_Q > 1$ or $\delta_Q > 1$.

Again we consider the equilibrium of interest, (19). We can see that depending on the shape of the rate functions and the values of γ_Q and δ_Q, we could have different outcomes. If the determinant in (22) is positive, then this mutated network is non-neutral, the equilibrium of interest is stable, and the mutant populations will not grow. On the other hand, that is, if condition (23) is satisfied, this system is no longer stable and the mutant populations may grow. Thus, this type of mutation where mutants lose response to regulated rates, that is rates influenced by a feedback loop, may result in mutant populations growing from low numbers.

3.2.1 Specific Case: No feedback

In the most extreme case, we assume that mutant populations do not obey any of the feedback loops, so $\gamma_Q = \delta_Q = 0$ for each rate Q. Then the system of ODEs becomes

$$\begin{aligned}
\dot{x}_1 &= x_1(-R(x,y) + S(x,y)) + y_1 B(x,y), && (29)\\
\dot{y}_1 &= x_1(2R(x,y) + A(x,y)) - y_1(D(x,y) + B(x,y)), && (30)\\
\dot{x}_2 &= x_2(-\bar{R} + \bar{S}) + y_2 \bar{B}, && (31)\\
\dot{y}_2 &= x_2(2\bar{R} + \bar{A}) - y_2(\bar{D} + \bar{B}), && (32)
\end{aligned}$$

where each mutant rate has argument $(0,0)$ and is a constant, and we denoted $Q(0,0) \equiv \bar{Q}$. The (linear) equations for the mutant populations decouple from the rest, and we can see that the system behavior is determined by the matrix (20) with $\tilde{Q} = \bar{Q}$. The solution tends to infinity if condition (23) is satisfied. Otherwise, the linear system has a global zero equilibrium, corresponding to mutant extinction. In the case where $\bar{B} = 0$, we have a simpler condition (24) for instability. In other words, mutant SCs must self-renew faster than they differentiate in order for the mutant populations to grow from low numbers.

3.2.2 Effect of mutation on minimal feedback cases

As shown above, losing response to feedback may or may not result in mutant growth, depending on the feedback parameters within the network. To obtain more concrete results, let us apply this theory to the specific minimal feedback networks found in Section 2.2.

Assume we have a minimal feedback network, and a mutation affects the response to the (single) existing feedback loop. In the case where the loss of response is complete ($\gamma_Q = 0$ or $\delta_Q = 0$ for the single regulated rate, Q), results of the previous section apply, and the mutant will enter unbounded growth.

Next, let us assume that mutants are characterized by partial loss of regulation. For instance, in the absence of DC de-differentiation ($B = 0$) the minimal feedback network (i) requires R to be an increasing function of x, and so if mutants partially lose response to R, (i.e. $0 < \gamma_R < 1$), then $\bar{R}$ will be less than it was pre-mutation and $DetJ_{\text{mut}}$ will decrease from 0. Thus $DetJ_{\text{mut}} < 0$ (condition (24)), and the system is unstable. In other words, any partial loss of feedback in this situation confers advantage to the mutant, resulting in the instability of the wild-type homeostatic solution. The system however may have a different, stable steady state (which we will denote as $(0,0,\hat{x},\hat{y})$). This new state is characterized by a system similar to Equations (5-6), except the function R is evaluated at $\gamma_R\hat{x}$:

$$\begin{aligned}
(-R(\gamma_R\hat{x}) + S)\hat{x} + B\hat{y} &= 0, && (33)\\
(2R(\gamma_R\hat{x}) + A)\hat{x} - (D + B)\hat{y} &= 0. && (34)
\end{aligned}$$

With $B = 0$, the first equation above ($R(\gamma_R\hat{x}) = S$) allows us to find the new value of the SC population, which, given $R_x > 0$ and $0 < \gamma_R < 1$, is higher than $\bar{x}$, the old equilibrium. From Equation (34) we can then see that $\hat{y} > \bar{y}$.

The minimal feedback networks (ii), (iii), and (iv) respond similarly, such that any partial loss of response breaks stability and results in mutant population growth, and a new stable equilibrium (this can be shown by an argument almost identical to the one for case (i)). In the presence of DC de-differentiation ($B > 0$), for each of the minimal feedback networks (i) - (x) the loss of response to feedback will again result in $DetJ_{\text{mut}} < 0$ (condition (23)), loss of stability of the wild-type equilibrium (19), and gain of stability of a new, mutant equilibrium with populations $(\hat{x},\hat{y})$. In this case, we can prove again that $\hat{x} > \bar{x}, \hat{y} > \bar{y}$ (that is, the populations at the new equilibrium are always larger than those at the original healthy solution). Again, let us use the example of feedback network (i). From Equations (33-34) we can express

$$\begin{aligned}
\frac{\hat{y}}{\hat{x}} &= \frac{R(\gamma_R\hat{x}) - S}{B}, && (35)\\
\frac{\hat{y}}{\hat{x}} &= \frac{2R(\gamma_R\hat{x}) + A}{D + B}. && (36)
\end{aligned}$$

Then setting them equal, we can solve for $R(\gamma_R \hat{x})$:

$$R(\gamma_R \hat{x}) = \frac{AB + S(D+B)}{D-B}.$$

On the other hand, at the wild type equilibrium (19), we have

$$R(\bar{x}) = \frac{AB + S(D+B)}{D-B}.$$

From $R(\gamma_R \hat{x}) = R(\bar{x})$ we obtain $\hat{x} > \bar{x}$, and furthermore, from (36) and an equivalent identity at the old equilibrium,

$$\frac{\bar{y}}{\bar{x}} = \frac{2R(\bar{x}) + A}{D+B},$$

we conclude that

$$\frac{\hat{y}}{\hat{x}} = \frac{\bar{y}}{\bar{x}},$$

and therefore $\hat{y} > \bar{y}$. A very similar proof holds for any other feedback loop (not shown). Thus we must have both $\hat{x} > \bar{x}$ and $\hat{y} > \bar{y}$, that is, the populations at the mutant equilibrium are greater than the wild type populations at the original equilibrium.

For completeness, we note also that for these minimal networks, if either γ_Q or δ_Q is greater than one, indicating more response to regulation for mutants rather than loss of response to regulation, the stability conditions are satisfied, and thus solution (19) is stable (i.e. the mutant of this type will go extinct).

To summarize, all the minimal cases are equally easy to "break" by a mutation conferring a total loss of the existing feedback, resulting in an unbounded growth of mutants. Partial loss of feedback will always result in an outgrowth of mutants that have an advantage, but the new system will contain a stable equilibrium. The total populations of mutant SCs and DCs are always larger than the original wild type populations.

3.3 Other phenotypic changes

So far, we introduced mutation types that changed one or more existing feedback loops. In particular, in Section 3.1 the dependence of a rate function on the mutant populations was different than the dependence on the wild type populations, and in Section 3.2, mutants were subject to weaker regulation. While one was ineffective, the other could result in mutant growth under specific conditions. Next, we investigate mutations that change kinetic rates, not one or more existing feedback loops. Therefore, we assume here that

1. As in Section 3.2, mutants contribute equally to feedback, i.e. the rates Q depend on the total SC and DC populations.

2. We assume that the mutant rate, $\tilde{Q}$, is different from the corresponding wild-type rate, Q, where Q may or may not be regulated in the original system. In particular, if a certain rate is constant for the wild type cells, it could become a function of x or y (or become a different constant); if it is a function of x and/or y for the wild type cells, it could become a different function (or a constant). More then one such rate could be affected.

Instability (that is, mutants' ability to grow from small numbers) requires that condition (23) is satisfied, which can be reformulated as follows:

$$(\tilde{R} - \tilde{S})(\tilde{D} + \tilde{B}) - \tilde{B}(2\tilde{R} + \tilde{A}) < 0, \tag{37}$$

where the expressions above are evaluated at the equilibrium of interest (19). Let us assume that a mutation only alters a single kinetic rate. The following changes can satisfy (37):

(1) Decrease SC differentiation, R;

(2) Increase SC self-renewal, S;

(3) Increase the DC de-differentiation rate, B;

(4) Decrease DC death rate, D;

(5) Increase asymmetric SC divisions, A – but only in the presence of de-differentiations ($B > 0$).

Mutations of type (4) above may have a different nature depending on whether or not de-differentiation takes place in the system. If $B = 0$ (no de-differentiation), decreasing the rate D can only cause instability if D becomes negative. This results in DCs becoming more likely to self-renew than they are to die, which would biologically correspond to DCs evolving into more stem-like cells. In the presence of de-differentiations ($B > 0$) a change in D may cause instability even if D is still positive.

3.3.1 Minimal networks

Let us assume that the healthy system is regulated by one of the minimum feedback loops (i-x), and mutants are characterized by a change of type (1-5) above, which affects a single rate. It is convenient to define the following function:

$$H(R, S, B, D, A) = (R - S)(D + B) - (2R + A)B. \tag{38}$$

At the wild type equilibrium, we have

$$H(R, S, B, D, A)|_{\bar{x},\bar{y}} = \Big[(R - S)(D + B) - (2R + A)B\Big]_{\bar{x},\bar{y}} = 0. \tag{39}$$

This identity can be obtained by treating the equations at the equilibrium as a linear algebraic system for $\bar{x}$ and $\bar{y}$, and requiring its degeneracy for a nontrivial solution. This is equivalent to stating that $Det\ J_{\text{w.t.}} = 0$, where $J_{\text{w.t.}}$ is similar to matrix J_{mut}, expect all the functions correspond to those of wild type cells. To guarantee that the wild type solution is unstable, we need to require that $Det\ J_{\text{mut}} < 0$, which is identical to the inequality

$$H(\tilde{R}, \tilde{S}, \tilde{B}, \tilde{D}, \tilde{A})|_{\bar{x},\bar{y}} = \Big[(\tilde{R} - \tilde{S})(\tilde{D} + \tilde{B}) - (2\tilde{R} + \tilde{A})\tilde{B}\Big]_{\bar{x},\bar{y}} < 0. \tag{40}$$

Let us suppose that only one of the rates $\tilde{Q}$ in expression (40) is different from the corresponding wild type rate, Q. What kind of changes will guarantee the correct sign of inequality (40)? To answer this question we notice that H is monotonic in all the rates, and (under $B > 0$)

$$\frac{\partial H}{\partial R} > 0, \quad \frac{\partial H}{\partial S} < 0, \quad \frac{\partial H}{\partial B} < 0, \quad \frac{\partial H}{\partial D} > 0, \quad \frac{\partial H}{\partial A} < 0. \tag{41}$$

Next recall that minimum feedback loops are characterized by certain signs of the derivatives of the regulated functions with respect to population sizes, see Table 2 (here we denote by z any variable x and y):

$$R_z > 0, \quad S_z < 0, \quad B_z < 0, \quad D_z > 0, \quad A_z < 0. \tag{42}$$

Notice that positive derivative signs are associated with the rates that influence H in a positive way, and negative signs corresponds to the rates that influence H negatively. Combining these signs of the derivatives, we obtain that

$$\frac{\partial H}{\partial x} > 0, \quad \frac{\partial H}{\partial y} > 0. \tag{43}$$

From Inequalities (41), we can see that to decrease H below zero, we can decrease R, increase S, increase B, decrease D, or increase A. These are exactly the mutation mechanisms (1-5) above.

Suppose that one of these five phenotypic changes have occurred. Note that if the minimal feedback was altered to be a constant that satisfied (40), then the mutant populations would satisfy a linear system and undergo unlimited growth. This possibility has already been considered in Section 3.2. In order to investigate the long term effect of other phenotypic changes, we make the following assumptions:

1. If the original minimal network is regulated by x (y), then the mutated one is also regulated by the same population x (y).

2. All the rates are monotonic. In particular, the single minimal feedback loop is a monotonic function, that is, the inequality satisfied by the derivative holds globally, and not just at the equilibrium. Also, rates characterizing the mutant network are monotonic.

3. The affected mutant rate satisfies one of the conditions (1-5) above, and the inequalities are global. For example, if it is rate R that is affected, we require $\tilde{R}(z) < R(z)$ for all relevant values of the variable z (here z stands for x or y).

Given these assumptions, we show that if a new equilibrium exists, and if the minimal feedback network contains a dependence on x (y), then the new SC (DC) population is larger than the corresponding equilibrium wild type population. In any minimal network, only one of the rates is a function of x or y. By (43), H is a monotonically increasing function. Let us assume that a new mutant equilibrium exists. Then it satisfies

$$H(\tilde{R},\tilde{S},\tilde{B},\tilde{D},\tilde{A})|_{\hat{x},\hat{y}} = \left[(\tilde{R}-\tilde{S})(\tilde{D}+\tilde{B}) - (2\tilde{R}+\tilde{A})\tilde{B}\right]_{\hat{x},\hat{y}} = 0. \tag{44}$$

Let us assume that the minimum feedback loop contains an x-dependence of a rate Q, the rate that is regulated for the wild type cells. The function H with wild-type rates, $H(R,S,B,D,A)$, is an increasing function of x, which crosses 0 at $x=\bar{x}$. The function H with modified (mutant) rates, $H(\tilde{R},\tilde{S},\tilde{B},\tilde{D},\tilde{A})$, is a function of x that lies below $H(R,S,B,D,A)$ (assumption 3 above). Therefore, if the mutant function crosses 0, it happens at a value $\hat{x} > \bar{x}$. Similarly, one can show that if the dependence is on y, then $\hat{y} > \bar{y}$. We conclude that **if a system with a minimum feedback loop experiences a mutation and a new equilibrium is reached, the population that mediates the minimum feedback loop will be larger at the new equilibrium.**

Further, **if the modified rate coincides with the regulated rate, then it is easy to show that both populations will increase.** For example, if the minimal feedback involves rate $Q(x)$ regulated by the SCs, and the modified rate is $\tilde{Q}(x)$, then comparing (39) and (44) we obtain $\tilde{Q}(\hat{x}) = Q(\bar{x})$, which in turn gives us that $\frac{\hat{x}}{\hat{y}} = \frac{\bar{x}}{\bar{y}}$. Then inequality $\hat{x} > \bar{x}$ that was proven above, implies $\hat{y} > \bar{y}$. The argument for minimal feedback loops regulated by the DCs is very similar.

If the mutants are characterized by a rate that is different from the rate regulated by the minimal network, then we can only conclude that in the resulting equilibrium, either $\hat{x} > \bar{x}$ or $\hat{y} > \bar{y}$ (depending on whether it is x or y that is involved in the minimal feedback); the other population of mutants at the new equilibrium may be smaller or larger than the corresponding original wild type population.

3.3.2 Numerical examples

Figure 3 shows examples of dangerous phenotypic changes described above, in the context of minimum network (i) with $B = 0$. The original system's function $R(x)$ and (constant) S are shown in panel (a) with solid lines, as functions of x. The equilibrium value of x (under $B = 0$) is given by the intersection of these two functions.

Mechanism (1) above requires a decrease in $R(x)$. This is implemented in panels (b-1) and (b-2). In these plots, we show four populations: wild type SCs and DCs (black solid and dashed lines respectively), and mutant SCs and DCs (red solid and dashed lines respectively). In each plot, we start with the wild type equilibrium solution (in the absence of mutants, $x_2 = y_2 = 0$), and then at time $t = 100$ introduce a small amount of mutant cells. In panel (b-1), the mutants are characterized by rate R given by the dashed line in (a). Since the mutant's rate $\tilde{R}(x)$ still has an intersection with S, a new equilibrium is reached, as we can see from the behavior of the red lines in panel (b-1). As expected, the new equilibrium population levels are larger than the wild type equilibrium ones. In panel (b-2), we study a phenotypic change of the same type, but the new function $R(x)$ is given by the dotted line in panel (a) and does not contain an intersection with S. In this case, the mutants take off and enter unbounded growth.

Mechanism (2) is characterized by an increase in self-renewal and is presented in panels (c-1) ($\tilde{S} = 0.7$) and (c-2) ($\tilde{S} = 1.1$), see the dashed and dotted horizontal lines in panel (a). In the former case, an

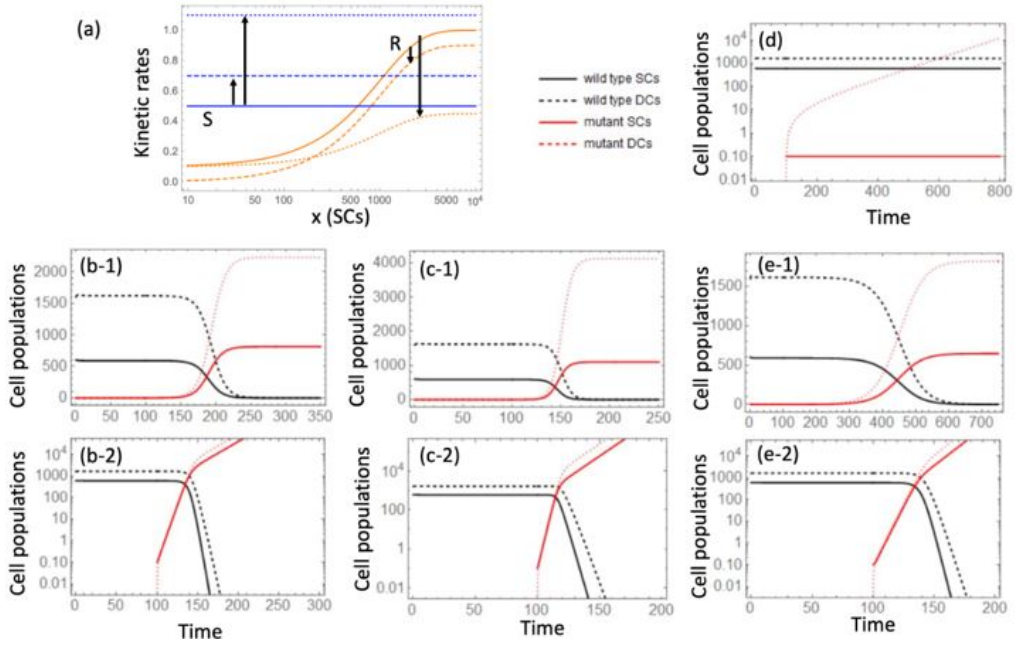


Figure 3: Examples of mutations described in Section 3.3, that can grow from low numbers, using minimum network (i) with $R(x) = 0.1 + 0.9(1 - e^{-0.001x}), S = 0.5, A = 0.1, D = 0.4, B = 0$, with wild type steady state $\bar{x}_1 = 587.8, \bar{y}_1 = 1616.4$. (a) Rates $R(x)$ and a constant S are shown as functions of x by solid lines. Changes in these functions are depicted as dashed ($\tilde{R}(x) = 0.9(1 - e^{-0.001x})$, $\tilde{S} = 0.7$) and dotted ($\tilde{R}(x) = 0.1 + 0.35(1 - e^{-0.001x}), \tilde{S} = 1.1$) lines. (b-e) The rest of the panels show the ODE simulations of the wild type (black) and mutant (red) populations of SCs (solid) and DCs (dashed). Starting from the wild-type equilibrium in the absence of mutants, a small amount of mutants are introduced at $t = 100$. The mutants are characterized by (b-1) $\tilde{R}(x) = 0.9(1 - e^{-0.001x})$, (b-2) $\tilde{R}(x) = 0.1 + 0.35(1 - e^{-0.001x})$, (c-1) $\tilde{S} = 0.7$, (c-2) $\tilde{S} = 1.1$, (d) $\tilde{D} = -0.01$, (e-1) $\tilde{B} = 0.01$, (e-2) $\tilde{B} = 0.2$.

intersection with $R(x)$ exists, and the mutant populations grow from low numbers and reach a new equilibrium. In the latter case, $R(x)$ does not intersect $\tilde{S}$, and the mutants grow to infinity.

Mechanism (3) is demonstrated in panels (e-1) and (e-2) of Figure 3, where mutants engage in de-differentiations, $\tilde{B} > 0$. Again, the two examples show mutant growth to a new equilibrium and unbounded growth, depending on whether or not an equilibrium solution under for mutant rates.

Mechanism (4) is characterized by a decrease in the rate D, and in the absence of B, it must become negative to create instability, which corresponds to mutant DCs regaining a degree of stemness: $S_{DC} > D_{DC}$. This is demonstrated in Figure 3(d). In this case, mutant DCs grow without bounds, while other populations remain finite.

Finally, mechanism (5) requires de-differentiations of wild types and is not presented here.

4 Redundant Networks

In Section 2.2 we discussed that in order to maintain homeostasis, a single feedback loop is enough. We have listed all 10 such minimal feedback loops, and shown in Sections 3.2.2 and 3.3.1, which types of mutations were required to "break" such a minimal network. We can "break" the network by causing lessened response to the existing feedback, or through five other phenotypic changes ((1-5) in Section 3.3). We saw that minimal feedback networks are not resilient in that they can be "broken" by a single mutation. Logically, a biological network will likely have redundant feedback loops so as to be more resilient to mutations. Thus it is useful to investigate redundant networks.

In this section, we assume that two minimal feedback loops are in effect, and a mutant appears that "breaks" one of the feedback loops, as found in Section 3.2.2 (the influence of other phenotypic changes described in Section 3.3 is studied in Appendix B). Such mutants will start growing from low numbers, but since a second feedback loop is still intact in the system, a new equilibrium may be reached, preventing unlimited growth.

For the analysis below, we will make some simplifying assumptions. First, we again assume that the local conditions on the rate functions stated in (i-x) of Section 2.2 hold globally. For example, minimum feedback loop (i) requires that $\frac{\partial R}{\partial x}|_{x=\bar{x}} > 0$; here we will require that $\frac{\partial R}{\partial x} > 0$ for all x. Further, we assume that all the non-constant regulated rates are functions of the total populations, $x = x_1 + x_2$ and $y = y_1 + y_2$. Finally, we will assume that the mutations lead to a complete or partial loss of one of the existing feedback loops, as described in Section 3.2.

4.1 General analysis of redundant networks

At an equilibrium,

$$\frac{y}{x} = \frac{2R + A}{D + B} = \frac{R - S}{B}. \tag{45}$$

(Note that in the case of $B = 0$, this is restricted to the first equality). We will use the notation

$$\mu = \frac{y}{x}, \quad \bar{\mu} = \frac{\bar{y}}{\bar{x}}, \quad \hat{\mu} = \frac{\hat{y}}{\hat{x}}.$$

Taking the derivative of μ in (45) with respect to all the rates we obtain that

$$\frac{\partial \mu}{\partial R} > 0, \quad \frac{\partial \mu}{\partial S} < 0, \quad \frac{\partial \mu}{\partial B} < 0, \quad \frac{\partial \mu}{\partial D} < 0, \quad \frac{\partial \mu}{\partial A} > 0. \tag{46}$$

Using Inequalities (46) and the information on the signs of stable feedback parameters, Inequalities (42), we can see how each of the feedback loops affect the fraction μ. For each minimal feedback loop, we can calculate the signs of the change in μ as the relevant population increases (again, z here is either x or y):

$$\frac{\partial \mu}{\partial R} R_z > 0, \quad \frac{\partial \mu}{\partial S} S_z > 0, \quad \frac{\partial \mu}{\partial B} B_z > 0, \quad \frac{\partial \mu}{\partial D} D_z < 0, \quad \frac{\partial \mu}{\partial A} A_z < 0. \tag{47}$$

These inequalities are important for distinguishing two classes of feedback. We can see that the first three quantities in (47) are positive. This means that regulation of R, S, and B increase μ, which is DC

to SC ratio. To explain this, note that for stability, a feedback loop must increase differentiations R as a reaction to population growth, which results in an increase of DCs and an increase of μ. Similarly, S (self-renewals) and B (de-differentiations) must decrease resulting in a decrease in SCs and an increase in μ. On the other hand, the last two quantities in (47) are negative, that is, regulation of D and A decrease μ. Indeed, for stability, an increase in the population must result in an increase in D or a decrease in A (increased DC death or decreased asymmetric divisions will reduce the DC pool, slow the total rate of de-differentiations and thus reduce the SC population). This change alone acts as to decrease y and thus decrease μ.

Below we show that **at least one of the populations will be larger at the mutant equilibrium compared to the wild type equilibrium,** and also derive some useful inequalities. We consider two cases: in the first one, the redundant network contains a single rate regulated by two different populations, and in the second one, there are two different rates regulated by the same or different populations.

The redundant network is characterized by a single rate, regulated by two populations. Let us denote this rate by $Q(x, y)$, where both partial derivatives of function Q are nonzero, and H in (38) is a function of two variables, x and y, through rate Q. At the wild type equilibrium, the function H in (38) is evaluated at $Q(\bar{x}, \bar{y})$; we will denote this quantity $\bar{H}$, where $\bar{H} = 0$. At the mutant equilibrium, it is evaluated at $Q(\gamma\hat{x}, \delta\hat{y})$, where one of the quantities γ and δ is in the interval $[0, 1)$ and the other is 1. We will denote the corresponding quantity $\hat{H}$, where again $\hat{H} = 0$, since this is an equilibrium. Note that because of Inequalities (43), lowering one of the arguments of the function H will require an increase in the other argument, if the equality $H = 0$ were to be kept. It follows that for redundant networks that involve a single rate regulated by two populations, one of the following inequalities must hold:

$$\text{If feedback by } x \text{ is weakened, } \gamma\hat{x} < \bar{x},\ \hat{y} > \bar{y} \ \text{ or } \ \gamma\hat{x} > \bar{x},\ \hat{y} < \bar{y}, \tag{48}$$

$$\text{If feedback by } y \text{ is weakened, } \delta\hat{y} < \bar{y},\ \hat{x} > \bar{x} \ \text{ or } \ \delta\hat{y} > \bar{y},\ \hat{x} < \bar{x}. \tag{49}$$

In particular, it is easy to see that we cannot have simultaneously $\hat{x} < \bar{x}$ and $\hat{y} < \bar{y}$. This is because if these two inequalities were true simultaneously, then we would have $\gamma\hat{x} < \bar{x}$ and $\delta\hat{y} < \bar{y}$. This implies $\hat{H} < \bar{H}$, which contradicts the fact that both of these quantities are equal to 0.

The redundant network is characterized by two distinct rates regulated by the same or different populations. Before we perform the analysis, we notice that there is a general pattern of rates' dependencies that will be useful in the analysis below. Recall that for function H in (38) at an equilibrium, the equation $H = 0$ holds. From this equation, any rate can be expressed in terms of the remaining four rates. Let us make the following notations:

$$w_1 = R, \quad w_2 = 1/(1 + S), \quad w_3 = 1/(1 + B), \quad w_4 = D, \quad w_5 = 1/(1 + A). \tag{50}$$

Note that in this definition, the rates appear in the denominator whenever the rate is a decreasing function of the population in a minimal feedback parameter, see (42). We have

$$\frac{\partial H}{\partial w_i} > 0, \quad (w_i)_z > 0, \quad 1 \le i \le 5. \tag{51}$$

Let us solve equation $H = 0$ for each of w_i, obtaining

$$w_1 = \mathcal{W}_1(w_2, w_3, w_4, w_5),\ \ldots,\ w_5 = \mathcal{W}_5(w_1, w_2, w_3, w_4).$$

One can show that

$$\frac{\partial \mathcal{W}_i}{\partial w_j} < 0, \quad i \neq j, \tag{52}$$

whenever $\mathcal{W}_i > 0$. Intuitively, since the function H increases with each w_i, increasing one of the w_i's will lead to a decrease in another, as long as $H = 0$ holds.

Suppose that the feedback network consists of feedback on rates Q_1 and Q_2, and that the rates Q_1 and Q_2 are different. Without loss of generality, we assume that it is regulation of Q_1 that is subsequently eliminated/weakened by a mutation. Rate Q_1 may be regulated by x or y, and rate Q_2 can be regulated by x or y. Again, to make the description more concrete, let us suppose that Q_1 is regulated by population z_1 and rate Q_2 is regulated by population z_2, where z_1 and z_2 may be the same or different populations.

Let us switch to the description given in (50), refer to the obtained functions as q_1 and q_2, and only keep the explicit dependence on these rates (because the other ones are constant).[3] We have at the wild type and mutant equilibria respectively,

$$H(q_1(\bar{z}_1), q_2(\bar{z}_2)) = 0, \quad H(q_1(\epsilon\hat{z}_1), q_2(\hat{z}_2)) = 0,$$

where again, the quantity $\epsilon < 1$ represents γ or δ and measures the degree of reduction of the feedback. Solving for q_2, we obtain:

$$q_2(\bar{z}_2) = \Omega_2(q_1(\bar{z}_1)), \quad q_2(\hat{z}_2) = \Omega_2(q_1(\epsilon\hat{z}_1)).$$

Suppose that $\epsilon\hat{z}_1 < \bar{z}_1$. According to the second inequality in (51), we have $q_1(\bar{z}_1) > q_1(\epsilon\hat{z}_1)$. Then, according to (52), $\Omega_2(q_1(\bar{z}_1)) < \Omega_2(q_1(\epsilon\hat{z}_1))$, and consequently, $q_2(\bar{z}_2) < q_2(\hat{z}_2)$. Thus, by the second inequality in (51), this implies that $\hat{z}_2 > \bar{z}_2$.

If, on the other hand, $\epsilon\hat{z}_1 > \bar{z}_1$, then through a similar chain of inequalities, we obtain that $\hat{z}_2 < \bar{z}_2$.

In the case where $z_1 = z_2$, that is, if both rates are regulated by the same population, the second case is impossible, because it suggests that, for example, $\gamma\hat{x} > \bar{x}$ (which implies $\hat{x} > \bar{x}$), and $\hat{x} < \bar{x}$. These results are summarized in Table 3.

	$Q_2(x)$	$Q_2(y)$
$Q_1(x)$	$\gamma\hat{x} < \bar{x},\ \hat{x} > \bar{x}$	$\gamma\hat{x} < \bar{x},\ \hat{y} > \bar{y}$ or $\gamma\hat{x} > \bar{x},\ \hat{y} < \bar{y}$
$Q_1(y)$	$\delta\hat{y} < \bar{y},\ \hat{x} > \bar{x}$ or $\delta\hat{y} > \bar{y},\ \hat{x} < \bar{x}$	$\delta\hat{y} < \bar{y},\ \hat{y} > \bar{y}$

Table 3: Possible inequalities for the equilibrium populations in the case where the redundant network contains two distinct rates, Q_1 and Q_2, and the regulation of Q_1 is weakened by the mutation.

Note that again, we cannot have simultaneously $\hat{x} < \bar{x}$ and $\hat{y} < \bar{y}$. We conclude that **in all cases, that is, if the redundant feedback network contains one or two rates regulated by one or both populations, at least one of the populations will increase as a result of reaching a mutant equilibrium.**

The results summarized in Inequalities (48-49) and Table 3 have an important consequence for the case of complete loss of feedback. In this case we have $\gamma = 0$ or $\delta = 0$, and the appropriate inequalities are guaranteed:

$$\gamma\hat{x} < \bar{x} \text{ or } \delta\hat{y} < \bar{y}. \tag{53}$$

This means that **if a single rate or two different rates are involved in a redundant network, and if one of the feedback loops is completely lost by a mutation, then the population mediating the feedback loop that remains intact will be higher at the mutant equilibrium, compared to the wild type equilibrium.**

In the analysis below, we will split all the two-loop networks into several classes and compare the population sizes at the mutant equilibrium to those at the wild type equilibrium. In what follows, in the context of the population x or y (or their fraction, μ), if we say that this quantity "does not change," this means that it is the same for the wild type and the mutant equilibrium. If we say it "increases" ("decreases"), this means that this population is larger (smaller) at the mutant equilibrium compared to the wild type equilibrium.

[3]Please note that functions w_i are related to particular rates, as defined by definition (50). Functions q_1 and q_2 can refer to any rates, as long as those rates correspond to the rates Q_1 and Q_2 that are involved in the feedback network. In other words, q_1 and q_2 do not necessarily correspond to w_1 and w_2 of (50).

4.2 The fraction y/x does not change, $\hat{\mu} = \bar{\mu}$

From expressions for the fraction μ (45), we can see that if rates R, S, and B are not regulated (that is, they are constant), then μ does not change. Similarly, if rates R, A, D, and B are not regulated, then μ does not change. These situations correspond to the redundant networks where the two feedback loops affect (a) only S, (b) only D, (c) only A, (d) both A and D.

There are two separate cases that one can distinguish.

[1] **The regulated rates are A and D, and the dependence of both feedback loops is on the same population.** This can happen only if two different rates are regulated, which in this case are A and D; both can be functions of x only or of y only. Let us suppose that both are functions of x. Let us compare H evaluated at $Q_1(x), Q_2(x)$ and H evaluated at $Q_1(\gamma x), Q_2(x)$ (where $\gamma < 1$; we will refer to this function as H_γ). The wild type equilibrium value of x, $\bar{x}$, is given by the intersection of H with the constant 0. The mutant equilibrium $\hat{x}$ is given by the intersection of H_γ with 0. Function H_γ is strictly smaller than H, and thus its intersection with the constant 0 occurs at a larger value of x, which means that $\hat{x} > \bar{x}$. Since the ratio μ is preserved in the present case, we also have $\hat{y} > \bar{y}$, that is, both populations increase in this case. The proof when both rates depend on y is similar. We conclude that **both populations will increase**.

[2] **The regulated rates are (a) only S, (b) only D, (c) only A, (d) both A and D, and the dependence is on different populations.** In this case, the rates Q_1 and Q_2 that are regulated may be the same or different. Let us first suppose that it is the feedback mediated by population x that is weakened by the mutation. According to Inequalities (48) for a single rate, and the top right cell in Table 3 for two different rates, there are two possibilities:

- $\gamma\hat{x} > \bar{x}, \hat{y} < \bar{y}$. Since $\gamma < 1$ it follows that $\hat{x} > \bar{x}$, which contradicts the fact that $\hat{\mu} = \bar{\mu}$. This case is impossible.
- $\gamma\hat{x} < \bar{x}, \hat{y} > \bar{y}$. There is no contradiction in the case, and from $\hat{\mu} = \bar{\mu}$ we obtain that $\hat{x} > \bar{x}$.

Next, suppose that the mutation weakens the feedback on y. By Inequalities (49) for a single rate and the lower left entry of Table 3, there are again two possibilities:

- $\hat{x} < \bar{x}, \delta\hat{y} > \bar{y}$. Since $\delta < 1$ it follows that $\hat{y} > \bar{y}$, which contradicts the fact that $\hat{\mu} = \bar{\mu}$. This case is impossible.
- $\hat{x} > \bar{x}, \delta\hat{y} < \bar{y}$. There is no contradiction in the case, and from $\hat{\mu} = \bar{\mu}$ we obtain that $\hat{y} > \bar{y}$.

To conclude, in this case like in the previous one, **both populations will increase**.

It turns out that apart from the cases that correspond to all the rates in one of the expressions in (45) being constants, there is an additional situation where the fraction μ is preserved. This happens when only a single rate is regulated (by both x and y). While rates S, D, or A are considered above, we discuss rates R and B next:

[3] **A single rate R or B is regulated by x and y.** In this case, since $\bar{H} = \hat{H} = 0$, and the first inequality in (51) holds, we must have $Q(\bar{x}, \bar{y}) = Q(\gamma\hat{x}, \delta\hat{y})$ (where only one of constants γ and δ is < 1). Therefore, $\hat{\mu} = \bar{\mu}$. The argument from case [2] applies here, resulting in the same conclusion: **both populations will increase.**

These results are summarized in Figure 4. In these tables, the notation Q_z indicates regulation of rate Q by population z; the rows correspond to the feedback that is weakened or lost by the mutation, and columns to the remaining feedback. The cases presented here are located near the diagonal and are marked with "=", that is, quantity μ stays constant.

4.3 The fraction y/x is different under the mutant and under the wild type equilibria, $\hat{\mu} \neq \bar{\mu}$

Dependence on a single population. Consider function H, definition (38). Suppose the two different rates are functions of x ($Q_1(x)$ and $Q_2(x)$ for the wild type function $H(x)$), and one of these

dependencies becomes weakened by a mutation $(Q_1(\gamma x), Q_2(x)$ for the "mutant" function $H_\gamma(x))$. We observe that weakening the dependence of rates R and D decreases them (see Inequalities (42)) and consequently decreases H. Weakening the dependence of rates S, B, A increases them (again by (42)) and consequently decreases H, leading to the inequality $H_\gamma(x) < H(x)$. Since $dH/dx > 0$, the solution $\hat{x}$ of $H_\gamma(x) = 0$ is larger than the solution $\bar{x}$ of $H(x) = 0$. Therefore, in the case where the rates depend on x only, we have $\hat{x} > \bar{x}$. Similarly, one can show that in the case of the dependence on y, we have $\hat{y} > \bar{y}$. That is, **if both feedback loops are mediated by a single population, this population will increase as a result of weakening of one of those feedback loops.**

Next, we notice that $H(\bar{x}) = H_\gamma(\hat{x}) = 0$. There are two differences between these two quantities. On the one hand, rate Q_2 is evaluated at $\bar{x}$ and at $\hat{x}$ in the two functions H and H_γ respectively. On the other hand, Q_1 is evaluated at $\bar{x}$ and at $\gamma\hat{x}$. Since $\hat{x} > \bar{x}$, the former difference leads to an increase of the mutant function H_γ with respect to the wild type H. Because both of them must be equal to 0, the second difference must lead to a decrease of the mutant H_γ. This implies that $\gamma\hat{x} < \bar{x}$. A similar result can be derived in the case of y dependence. To summarize, we have for x-dependent rates,

$$\hat{x} > \bar{x}, \quad \gamma\hat{x} < \bar{x}, \tag{54}$$

and for y-dependent rates,

$$\hat{y} > \bar{y}, \quad \delta\hat{y} < \bar{y}. \tag{55}$$

Consider pairs Q_1, Q_2, where for the wild types, both rates are functions of the same variable, and the first rate is weakened by the mutation. We can determine the change in the quantity μ from the wild type to the mutant equilibrium. Below we denote by z any variable, x or y, because the calculations are identical for both.

- Suppose that Q_1 is R or B and $Q_2 = S$. We can use the first expression for μ in (45), which does not contain S, and write, e.g. for $Q_1 = R$,

  $$\hat{\mu} = \frac{2R(\gamma\hat{z}) + A}{D + B} < \frac{2R(\bar{z}) + A}{D + B} = \bar{\mu},$$

  in other words, μ decreases after the mutation. The result is the same for $Q_1 = B$ and follows from the fact that μ increases with the populations size by (47), and the fact that $\gamma\hat{z} < \bar{z}$.

- If $Q_1 = R, Q_2 = B$ or $Q_1 = B, Q_2 = R$, both expressions for μ contain both rates, and while $Q_1(\gamma\hat{z})$ decreases μ, $Q_2(\hat{z})$ increases it, such that we could have $\hat{\mu}$ greater or smaller than $\bar{\mu}$.

- Next we consider $Q_1 = S$, and Q_2 given by R or B. The first expression in (45) does not contain S, and thus we can determine the change in μ by looking at its dependence on Q_2. Since μ increases with Q_2 (47), and $\hat{z} > \bar{z}$, we obtain $\hat{\mu} > \bar{\mu}$. For example, in the case of $Q_2 = R$, we have

  $$\hat{\mu} = \frac{2R(\hat{z}) + A}{D + B} > \frac{2R(\bar{z}) + A}{D + B} = \bar{\mu}.$$

- If Q_1 is one of the rates R, S, B, and Q_2 is given by D or A, we can use the second expression in (45), which only depends on Q_1. μ increases with z through Q_1 (Inequalities (47)), and the argument of Q_1 is $\gamma\hat{z} < \bar{z}$, which implies that $\hat{\mu} < \bar{\mu}$.

- If Q_1 is D or A, and Q_2 is R, S, or B, we again use the second expression in (45), which now depends on Q_2. μ increases with z through Q_2 (Inequalities (47)), whose argument is $\hat{z} > \bar{z}$, which implies that $\hat{\mu} > \bar{\mu}$.

- Finally, if $Q_1 = A, Q_2 = D$ or $Q_2 = A, Q_1 = D$, we are back to a case considered in the previous section, where μ remains constant. This is the consequence of the fact that the second expression in (45) does not contain Q_1 or Q_2.

(a) Partial loss of feedback.

	R_x	R_y	S_x	S_y	B_x	B_y	D_x	D_y	A_x	A_y
R_x		=	↓	↓	?	?	↓	↓	↓	↓
R_y	=		?	↓	?	?	?	↓	?	↓
S_x	↑	?		=	↑	?	↓	↓	↓	↓
S_y	?	↑	=		?	↑	?	↓	?	↓
B_x	?	?	↓	↓		=	↓	↓	↓	↓
B_y	?	?	?	↓	=		?	↓	?	↓
D_x	↑	?	↑	?	↑	?		=	=	=
D_y	↑	↑	↑	↑	↑	↑	=		=	=
A_x	↑	?	↑	?	↑	?	=	=		=
A_y	↑	↑	↑	↑	↑	↑	=	=	=	

(b) Complete loss of feedback.

	R_x	R_y	S_x	S_y	B_x	B_y	D_x	D_y	A_x	A_y
R_x		=	↓	↓	?	?	↓	↓	↓	↓
R_y	=		?	↓	?	?	?	↓	?	↓
S_x	↑	?		=	↑	?	↓	↓	↓	↓
S_y	?	↑	=		?	↑	?	↓	?	↓
B_x	?	?	↓	↓		=	↓	↓	↓	↓
B_y	?	?	?	↓	=		?	↓	?	↓
D_x	↑	?	↑	?	↑	?		=	=	=
D_y	↑	↑	↑	↑	↑	↑	=		=	=
A_x	↑	?	↑	?	↑	?	=	=		=
A_y	↑	↑	↑	↑	↑	↑	=	=	=	

Figure 4: Population trends for mutant equilibria compared to wild type equilibria. The rows indicate the feedback that becomes weakened/removed by a mutation, and the columns are the feedback that remains intact. The arrows indicate whether the fraction y/x will increase, decrease, or remain the same. A question mark means that the fraction could increase or decrease. Red means that both populations will increase, yellow that SCs must increase, blue that DCs must increase, and green that at least one population must increase. (a) Mutation weakens the feedback. (b) Mutation eliminates the feedback.

These results on the behavior of μ are summarized in Figure 4, by using upward arrows if $\hat{\mu} > \bar{\mu}$, downward arrows if $\hat{\mu} < \bar{\mu}$, "=" if $\hat{\mu} = \bar{\mu}$, and the question mark if the inequality can be either way.

Given the behavior of the fraction μ, we can determine the trends in the population sizes of SCs and DCs. Note that

$$\hat{\mu} > \bar{\mu} \Rightarrow \frac{\hat{y}}{\bar{y}} > \frac{\hat{x}}{\bar{x}}, \quad \hat{\mu} < \bar{\mu} \Rightarrow \frac{\hat{y}}{\bar{y}} < \frac{\hat{x}}{\bar{x}}. \tag{56}$$

If the system is x-dependent, then $\hat{x} > \bar{x}$, and $\hat{\mu} > \bar{\mu}$ implies

$$\frac{\hat{y}}{\bar{y}} > \frac{\hat{x}}{\bar{x}} > 1,$$

i.e. both populations will increase. A decrease in μ means that no additional inequalities exist for y. Similarly, in the y-dependent system, $\hat{\mu} < \bar{\mu}$ implies that both populations increase, and the opposite inequality does not give a condition for x. Table (a) in Figure 4 shows the trends in the populations by color-coding the four possible outcomes.

Dependence on two populations. Next we assume that Q_1 and Q_2 are regulated by different populations, and as before, the regulation on Q_1 is weakened by the mutation. This case is different from the one considered above in that we cannot necessarily assume that a given population will increase (although we know that at least one population will increase). Instead, we have the following possibilities. If the regulation of x is weakened by the mutation, then we have by the top right entry of Table 3,

$$(1)\ \ \gamma\hat{x} > \bar{x},\ \ \hat{y} < \bar{y} \text{ or } (2)\ \ \gamma\hat{x} < \bar{x},\ \ \hat{y} > \bar{y}. \tag{57}$$

Similarly, if the regulation of y is weakened, we have by the left bottom entry of Table 3,

$$(1)\ \ \delta\hat{y} > \bar{y},\ \ \hat{x} < \bar{x} \text{ or } (2)\ \ \delta\hat{y} < \bar{y},\ \ \hat{x} > \bar{x}. \tag{58}$$

Using these options and Inequalities (54-55), we proceed to group the redundant feedback loops in several classes.

- Suppose Q_1 is given by R or B and $Q_2 = S$. We will use the first expression in (45) which depends on Q_1 only. If regulation by x is weakened, by Inequalities (57), we obtain in case (1) that $\hat{\mu} > \bar{\mu}$, which by (56) implies $\hat{x} < \bar{x}$, which in turn is in contradiction with $\gamma\hat{x} > \bar{x}$. Thus case (1) is impossible. On the other hand, in case (2) of (57), we obtain $\hat{\mu} < \bar{\mu}$ and thus by (56), $\hat{x} > \bar{x}$. We conclude that both populations will increase.

  Next, let us assume that regulation by y is weakened. We can see that for both cases in (58), we arrive at inequalities that provide no additional information on the growth of x. For example, in case (1), we get $\hat{y}/\bar{y} > \hat{x}/\bar{x}$, but $\hat{x}/\bar{x} < 1$, so y could increase or decrease. A similar argument applies in case (2). We conclude that for mutations affecting regulation by y, we only have the most basic condition that at least one of the populations must grow.

- If $Q_1 = R, Q_2 = B$ or $Q_1 = B, Q_2 = R$, both expressions for μ contain both rates, and thus x or y might decrease, as long as at least one of them increases.

- If $Q_1 = S$ and Q_2 is given by R or B, first we consider the case where regulation of x is weakened/lost. In case (1) of (57), we obtain $\hat{y}/\bar{y} < \hat{x}/\bar{x}$ together with $\hat{y}/\bar{y} < 1$, which means that x could increase or decrease. In case (2), y grows, and x again can increase or decrease.

  If on the other hand we assume that regulation by y is weakened/lost, we can see that case (1) in (58) is impossible, and case (2) implies that both populations grow.

- Next we turn to the group of networks where Q_1 is one of the rates R, S, B, and Q_2 is given by D or A. We will use the second expression in (45), which only depends on Q_1. The results are exactly the same as in the very first case considered here, that is, under the weakened x regulation, both populations will increase, and under the weakened y regulation, at least one population must increase.

- If Q_1 is D or A, and Q_2 is R, S, or B, we again use the second expression in (45), which now depends on Q_2. If x-dependence is disrupted, we obtain no definite result on the growth of the populations, apart from the usual condition that at least one population must increase. If y-dependence is weakened by the mutation, in case (1) of (58) we get $\hat{y}/\bar{y} < \hat{x}/\bar{x} < 1$, which is impossible (at least one of the populations must grow). In case (2), we have $\hat{y}/\bar{y} > \hat{x}/\bar{x} > 1$, i.e. both populations must grow.

- The last case $Q_1 = A, Q_2 = D$ or $Q_2 = A, Q_1 = D$ corresponds to μ that does not change, and has already been considered.

These results are summarized in Table (a) of Figure 4. Interestingly, if we assume that $\gamma = 0$ or $\delta = 0$, that is, if feedback loops are completely lost, we have more restrictive conditions. Indeed, in such cases, Inequalities (53) are guaranteed, and we have a stronger result summarized after conditions (53). The implications for all the cases are shown in Table (b) of Figure 4.

4.4 Numerical examples

Figure 5 shows examples of two different redundant networks found in Section 4.3, where a mutation weakens one of the feedback loops and causes the system to reach a new equilibrium. The interesting feature of these examples is that at the new, mutant equilibrium, the number of SCs is smaller than that at the original, wild type equilibrium, that is, $\hat{x} < \bar{x}$. Both examples are quite simple, as they are set in the absence of de-differentiation ($B = 0$).

The first example uses a redundant network that depends on a single population, and involves feedback loops (iv) and (ii). Here, the rate of SC self-renewal is regulated by DCs ($S(y)$, blue solid line in Figure 5(a)), and its regulation is significantly reduced by a mutation with $\delta_S = 0.01$ (the dashed blue line in Figure 5(a)). The rate of SC differentiation, $R(y)$, is also regulated by DCs and is presented by the yellow line in the same panel. This rate remains unaffected by the mutation. In panel (c) of Figure 5 we plot the population sizes before and after a mutant is introduced at time 100. We observe that the mutants rise (the red lines), displace the original population (the black lines), and after some oscillations settle to a new equilibrium characterized by an increase in DCs (the red dashed line) and a decrease in SCs (the solid red line).

The second example uses feedback loops (iii) and (ii), that is, the rate of SC self-renewal is regulated by SCs, while the rate of SC differentiations is regulated by DCs. It utilizes he same function $R(y)$, and the function $S(x)$ is given by the solid line in panel (b) of Figure 5. It is slightly weakened by a mutation ($\gamma_S = 0.9$), see the dashed line in panel (b). Panel (d) presents the population dynamics caused by this mutation. Again, the mutants rise from low numbers, drive the wild type population extinct, and establish a new equilibrium characterized by a decreased SC population.

5 Discussion and conclusions

In this paper, we study the turnover dynamics of SC lineages where cell fate decisions, modeled as per cell rates, are influenced by the current cell population via cell-to-cell signaling. We formulate a general two-compartment, deterministic model that consists of SCs and DCs and which includes the following processes: SC symmetric differentiation divisions, SC symmetric self-renewal divisions, SC asymmetric divisions, DC death, and DC de-differentiation, as well as SC death and DC self-renewal.

We first establish that for stability (that is, to be able to maintain homeostasis), a SC-DC system must involve at least one feedback loop, that is, at least one of the rates has to be regulated by one of the populations. We call the networks with exactly one feedback loop "minimal feedback networks". In the absence of DC de-differentiation, there are exactly four such minimum feedback networks. Two of these minimal networks involve the positive regulation of SC differentiations by either the SC or DC population, and the other two involve the negative regulation of SC self-renewals by either SCs or DCs; regulation of DC death or SC asymmetric divisions alone is not enough for stability. In the presence of DC de-differentiations, the class of minimal feedback networks is larger. It contains 10 minimum networks, adding regulation of asymmetric SC divisions, DC deaths, and DC de-differentiation by either

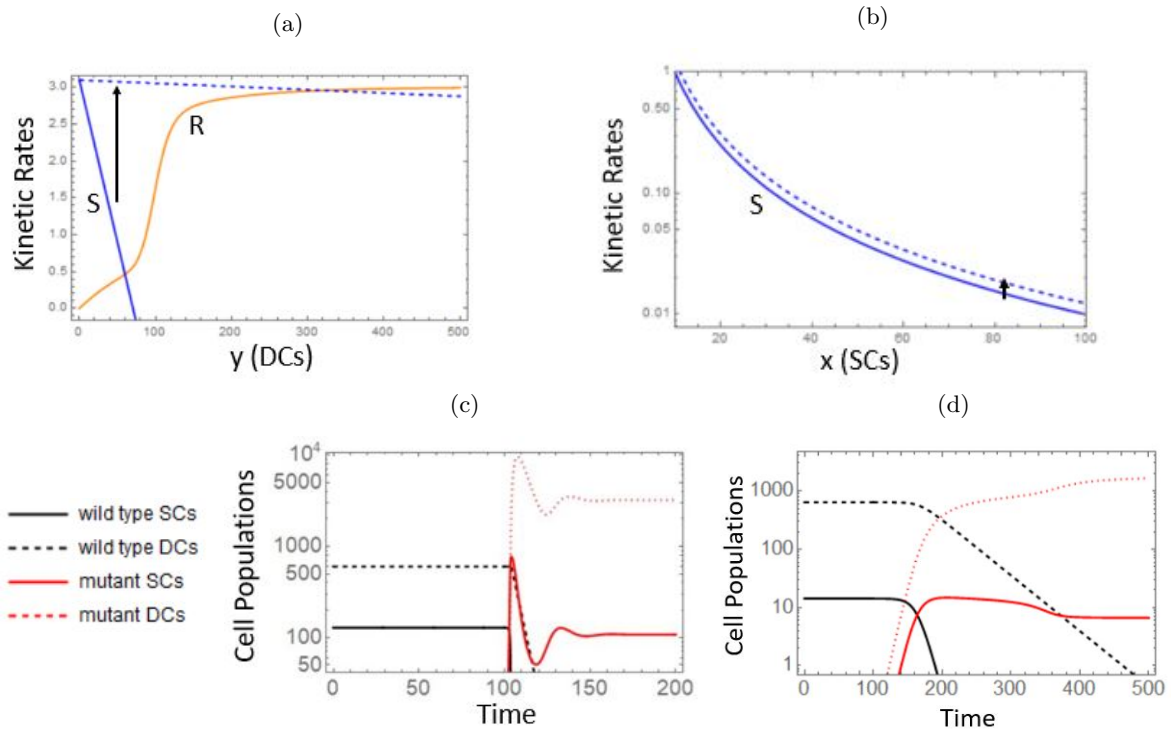


Figure 5: Examples of redundant networks found in Section 4.3, where two minimal feedback loops are in effect and one of them is weakened by a mutation. Each redundant network results in $\hat{x} < \bar{x}$ and $\hat{\mu} > \bar{\mu}$. The system in panel (c) is regulated by the redundant network consisting of minimal feedback loops (ii) and (iv) with $R(y) = 1 - e^{-0.001y} + \frac{2\times(10y)^{10}}{1+(0.001y)^{10}}$ and $S(y) = 3.1 - 0.0044y$, shown in panel (a) by solid lines; feedback on $S(y)$ is weakened by $\delta_S = 0.01$, shown in (a) as the dashed line. The other rates are $A = 0.01, B = 0$, and $D = 0.2$. Panel (d) uses the redundant network consisting of minimal feedback loops (ii) and (iii) with the same rate of differentiation $R(y)$, and $S(x) = \frac{100}{x^2}$ shown in panel (b) by the solid line; feedback on $S(x)$ is weakened using $\gamma_S = 0.9$, pictured by the dashed line in (b). The other rates $A = 0, B = 0$, and $D = 0.022$. In (c) and (d), the mutants are introduced at time 100.

of the populations to the list of possible minimum feedback networks. A summary of all the minimal feedback networks is given in Table 2.

The focus of this paper is to investigate the resilience of various feedback networks with respect to mutant populations. Such knowledge is necessary to improve our understanding of the effectiveness of feedback loops in healthy tissues, as well as the pathways to cancer that may take place in real biological systems. Three types of mutants are considered: (1) mutants whose participation in feedback signaling is altered; (2) mutants whose response to feedback is altered; (3) mutants characterized by other phenotypic changes.

We find that mutants that do not participate in signaling will not grow from low numbers (consistent with [36]). In the ODE (deterministic) system, homeostatic equilibrium is neutrally stable with respect to a perturbation comprised of adding such a mutant population; in a stochastic system, this corresponds to neutral drift. On the other hand, mutants that do not respond to a feedback loop, or are characterized by a weaker response to that feedback, can rise from low numbers and take over the system, displacing the wild type populations. These are "dangerous" mutants that can be thought of as representing malignant or pre-malignant change. Finally, mutations that do not alter the regulated rates, but affect other aspects of the cell dynamics (or alter the regulated rate in a manner other than a weakened response), can also be advantageous and rise from low numbers. These mutations are those that decrease the differentiation rate, increase the self-renewal rate, increase the asymmetric division rate, decrease the DC death rate, or increase the DC de-differentiation rate.

The first question we ask is whether, among the 10 minimal feedback systems, any particular choice is "better" than others in a sense of being able to "resist" mutant invasion, or being more difficult to "break". Our analysis shows that for all 10 minimal feedback networks, a mutant that is characterized by a weakened or non-existent response to the feedback will rise from low numbers and take over, leading to either a larger population at an equilibrium (if a degree of regulation remains) or unlimited growth if the feedback is absent in the mutants. In other words, if only a single feedback loop is used, there is no better or worse way to choose this feedback loop in terms of evolutionary advantage.

Interestingly, if we expand the types of mutations under consideration, then we find that not all minimal feedback loops are created equal. For all 10 minimal feedback networks, a mutant that alters a non-regulated rate will rise from low numbers and either lead to unlimited mutant growth, or to a new equilibrium where only the regulating population must be larger, while the non-regulating population may increase or decrease. Thus, the five minimal networks regulated by DCs can result in a new equilibrium under this type of mutation with a depleted SC population, potentially giving them an advantage in that the smaller SC population may slow down further evolution.

Next, we combine two different minimal feedback loops to create all possible two-loop redundant feedback networks (45 networks in total). We investigate the dynamics in the presence of potentially dangerous mutants. We focus on the mutations that confer advantage to the affected cells by weakening or completely eliminating the effect of one of the existing feedback loops. Interestingly, while cells containing this type of mutation always grow from low numbers, not all of the redundant networks behave in the same way. As a measure of resilience we decided to track equilibrium population numbers of SCs and DCs, corresponding to the wild-type (homeostatic, healthy) equilibrium, and to the new, mutant equilibrium that is reached after one of the feedback loops is severed by a mutation. The following patterns are observed.

First, let us consider the DC to SC ratio, μ, see Figure 4, where the trends are indicated by arrows; a decrease in μ means SC enrichment, see [37] for analysis of enrichment correlates in healthy tissues and tumors. In the present setting, we can see that the DC to SC ratio remains constant if only a single rate is involved in the two-loop feedback network (and it is regulated by both SC and DC populations). If one of the feedback loops is diminished, the mutant population will grow, but the proportion of SCs will stay the same. The same result is observed if the only feedback in the system comes from the regulation of DC death rates and the regulation of asymmetric SC divisions.

To explain the next result, it is useful to separate all the rates into two groups. Group I contains

symmetric SC divisions (self-renewal and differentiation divisions) and DC de-differentiations. Group II contains asymmetric divisions and DC deaths:

$$\text{Group I rates: } R, S, B.$$
$$\text{Group II rates: } D, A.$$

If a redundant feedback loop contains regulation of a rate from group I and a rate from group II, and if the regulation of the Group I rate is weakened by a mutation, then the proportion of SCs will likely increase. On the other hand, if the regulation of the Group II rate is weakened, then the proportion of SCs will likely decrease.[4]

Next we examine the change in the numbers of cells in the individual populations, the SCs and DCs. In the tables of Figure 4, red cells represent cases where both populations will always increase, yellow (blue) when SCs (DCs) will always increase, and green when at least one of the populations must increase while the other could increase or decrease. There are two types of redundant networks for which the number of SCs will always increase as a result of a weakening of one of the feedback loops. First, if both regulations of the feedback come from Group II, then the numbers of both SCs and DCs will increase. Further, if one regulated rate is from Group I and the other is from Group II, and the Group I rate is regulated by the SCs, then the SCs will always increase.

These results are hardly surprising, as weakening or eliminating a feedback loop (which is there to keep the population from growing) seems to be directly associated with an increase in a population size. Partially, this intuition is correct: weakening or removing a feedback loop will always lead to an increase in at least one of the populations. There are however cases where one of the populations may decrease as a result of mutant invasion, and it may even happen that it is the number of SCs that decreases. For example, if one of the regulated rates comes from Group II (and this is the rate affected by the mutation), and the other rate comes from Group I and it is regulated by DCs, then it is possible that as a result of weakening/removing the first feedback loop, the population of SCs decreases compared to the homeostatic equilibrium. In a way, we can say that such a redundant feedback network has an advantageous design, because, if a mutation hits the "right" feedback (the group II feedback), then the pool of mutant SCs may shrink, delaying further evolution. For comparison, under feedback networks with both regulations from Group II, a mutation weakening one of the feedback loops will always result in the population growth for both SCs and DCs, expanding the mutant population and making further dangerous changes more likely. Another good design "idea" is to combine two different rates from Group I, such that one of them is R or B, regulated by the DCs. Then if this regulation remains intact, the population of SCs could shrink after a mutation weakens/destroys the other feedback loop. There is even a redundant network (the one consisting of R and B rates both regulated by DCs) such that no mater which of the feedback loops is damaged, the number of SCs could decrease.

These results show that a single feedback loop, even if it is enough to keep a stable equilibrium, is vulnerable to mutations. For example, mutants with a weakened/eliminated feedback will expand from low numbers and either enter unlimited growth or reach an equilibrium with an increased number of SCs and DCs. Therefore, from an evolutionary viewpoint, it appears advantageous to combine multiple feedback loops, creating redundant feedback networks. Interestingly, from an engineering prospective, not all such redundant systems are equally resilient to mutations. For some of them, any mutation that weakens/eliminates one of the loops will lead to a population growth of SCs. For others, the population of SCs can actually shrink as a result of "cutting" one of the loops, thus slowing down further unwanted transformations. Similar results could be demonstrated for a wider class of mutations that e.g. affect non-regulated rates of the system.

The framework adopted for this study is necessarily limited. While the number of cell fate decisions allowed in the model is relatively large, and the functional forms for all the dependencies are kept completely general, we only considered a two-compartment, non-spatial, deterministic system. Including

[4]This rule holds in all cases except when the Group I rate is regulated by DCs and the Group II rate is regulated by SCs, in which case the SC proportion could increase or decrease.

longer lineages (such as allowing for intermediate cell types, e.g. transit amplifying cells) is the next logical step. Formulating this problem in a spatial, stochastic setting is also subject of future work.

Another limitation of the model is that it does not include any effects of the immune system. At present, we assume that if a homeostatic equilibrium exhibits linear instability in the presence of a mutant, this means that mutants can expand in the face of (implicit) actions of the immune system. Conversely, if the homeostatic equilibrium is stable, this corresponds to the mutants' inability to expand, which may be a consequence of the immune system killing the mutants, as well as the mutants' genetic deficiencies. The present description could be a stepping stone for further, more detailed analyses that include more realism, such as the co-dynamics with immune cells.

While the exact biological mechanisms regulating cell fate decisions remain largely unknown, there are some examples of factors that have been shown to play an important role in cell fate decisions. Notch signaling, which requires cellular contact, is believed to be integral in cellular decision making, and may explain how cells combine information from neighboring cells as well as their extracellular environment [38, 39]. The Wnt signaling pathway is another important player. An investigation of hair follicle stem cell lineage found evidence that Wnt signaling may be responsible for SC maintenance, activation and proliferation, and transitioning from proliferation to differentiation [40]. Cell fate decisions are further influenced by other factors such as metabolism, reactive oxygen species, intracellular pH, and cell morphology [41]. The role of the immune system in determining cell fate decisions has also been recently highlighted [42]. While further data and analysis are still required, biological evidence of this kind combined with mathematical analysis may help us determine which feedback mechanisms are present in specific tissue. This in turn would give insights on how these mechanisms can be overcome by mutations and eventually may help determine treatment strategies to counter these mutations, given knowledge about the cellular behavior they cause.

Acknowledgements. Support of grants NIH 1 U01 CA187956-01, NIH/NCI U54-CA217378, and The NSF-Simons Center for Multiscale Cell Fate Research is gratefully acknowledged.

References

[1] Bullough WS. The actions of the chalones. Agents and actions. 1971;2(1):1–7.

[2] Iversen O. The chalones. In: Tissue growth factors. Springer; 1981. p. 491–550.

[3] Plikus MV, Mayer JA, de La Cruz D, Baker RE, Maini PK, Maxson R, et al. Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. Nature. 2008;451(7176):340–344.

[4] Lee SJ. Regulation of muscle mass by myostatin. Annu Rev Cell Dev Biol. 2004;20:61–86.

[5] El Ouriaghli F, Fujiwara H, Melenhorst JJ, Sconocchia G, Hensel N, Barrett AJ. Neutrophil elastase enzymatically antagonizes the in vitro action of G-CSF: implications for the regulation of granulopoiesis. Blood, The Journal of the American Society of Hematology. 2003;101(5):1752–1758.

[6] Corcos D. Toward a universal treatment for cancer: cell inflation assisted chemotherapy. Cancer medicine. 2013;2(4):421–426.

[7] Pellettieri J, Alvarado AS. Cell turnover and adult tissue homeostasis: from humans to planarians. Annu Rev Genet. 2007;41:83–105.

[8] Raff MC. Social controls on cell survival and cell death. Nature. 1992;356(6368):397–400.

[9] Gallant P. Myc, cell competition, and compensatory proliferation. Cancer research. 2005;65(15):6485–6487.

[10] Kurtova AV, Xiao J, Mo Q, Pazhanisamy S, Krasnow R, Lerner SP, et al. Blocking PGE 2-induced tumour repopulation abrogates bladder cancer chemoresistance. Nature. 2015;517(7533):209–213.

[11] Chan KS. Molecular pathways: targeting cancer stem cells awakened by chemotherapy to abrogate tumor repopulation. Clinical Cancer Research. 2016;22(4):802–806.

[12] Arino O, Kimmel M. Stability analysis of models of cell production systems. Mathematical Modelling. 1986;7(9-12):1269–1300.

[13] Dingli D, Traulsen A, Pacheco JM. Compartmental architecture and dynamics of hematopoiesis. PloS one. 2007;2(4).

[14] Marciniak-Czochra A, Stiehl T, Ho AD, Jäger W, Wagner W. Modeling of asymmetric cell division in hematopoietic stem cells—regulation of self-renewal is essential for efficient repopulation. Stem cells and development. 2009;18(3):377–386.

[15] Stiehl T, Marciniak-Czochra A. Characterization of stem cells using mathematical models of multistage cell lineages. Mathematical and Computer Modelling. 2011;53(7-8):1505–1517.

[16] Nakata Y, Getto P, Marciniak-Czochra A, Alarcón T. Stability analysis of multi-compartment models for cell production systems. Journal of biological dynamics. 2012;6(sup1):2–18.

[17] Stiehl T, Marciniak-Czochra A. Mathematical modeling of leukemogenesis and cancer stem cell dynamics. Mathematical Modelling of Natural Phenomena. 2012;7(1):166–202.

[18] Weekes SL, Barker B, Bober S, Cisneros K, Cline J, Thompson A, et al. A multicompartment mathematical model of cancer stem cell-driven tumor growth dynamics. Bulletin of mathematical biology. 2014;76(7):1762–1782.

[19] Lorenzi T, Marciniak-Czochra A, Stiehl T. A structured population model of clonal selection in acute leukemias with multiple maturation stages. Journal of mathematical biology. 2019;79(5):1587–1621.

[20] Lander AD, Gokoffski KK, Wan FY, Nie Q, Calof AL. Cell lineages and the logic of proliferative control. PLoS biology. 2009;7(1).

[21] Lo WC, Chou CS, Gokoffski KK, Wan FYM, Lander AD, Calof AL, et al. Feedback regulation in multistage cell lineages. Mathematical biosciences and engineering: MBE. 2009;6(1):59.

[22] Traulsen A, Pacheco JM, Dingli D. On the origin of multiple mutant clones in paroxysmal nocturnal hemoglobinuria. Stem Cells. 2007;25(12):3081–3084.

[23] Traulsen A, Lenaerts T, Pacheco JM, Dingli D. On the dynamics of neutral mutations in a mathematical model for a homogeneous stem cell population. Journal of the Royal Society Interface. 2013;10(79):20120810.

[24] Werner B, Dingli D, Lenaerts T, Pacheco JM, Traulsen A. Dynamics of mutant cells in hierarchical organized tissues. PLoS computational biology. 2011;7(12).

[25] Werner B, Dingli D, Traulsen A. A deterministic model for the occurrence and dynamics of multiple mutations in hierarchically organized tissues. Journal of The Royal Society Interface. 2013;10(85):20130349.

[26] Wang W, Stiehl T, Raffel S, Hoang VT, Hoffmann I, Poisa-Beiro L, et al. Reduced hematopoietic stem cell frequency predicts outcome in acute myeloid leukemia. Haematologica. 2017;102(9):1567–1577.

[27] Enderling H, Park D, Hlatky L, Hahnfeldt P. The importance of spatial distribution of stemness and proliferation state in determining tumor radioresponse. Mathematical Modelling of Natural Phenomena. 2009;4(3):117–133.

[28] Morton CI, Hlatky L, Hahnfeldt P, Enderling H. Non-stem cancer cell kinetics modulate solid tumor progression. Theoretical Biology and Medical Modelling. 2011;8(1):48.

[29] Poleszczuk J, Hahnfeldt P, Enderling H. Evolution and phenotypic selection of cancer stem cells. PLoS computational biology. 2015;11(3).

[30] Poleszczuk J, Macklin P, Enderling H. Agent-based modeling of cancer stem cell driven solid tumor growth. In: Stem Cell Heterogeneity. Springer; 2016. p. 335–346.

[31] Komarova NL. Principles of regulation of self-renewing cell lineages. PloS one. 2013;8(9).

[32] Yang J, Axelrod DE, Komarova NL. Determining the control networks regulating stem cell lineages in colonic crypts. Journal of theoretical biology. 2017;429:190–203.

[33] Komarova NL, Van Den Driessche P. Stability of control networks in autonomous homeostatic regulation of stem cell lineages. Bulletin of mathematical biology. 2018;80(5):1345–1365.

[34] Yang J, Plikus MV, Komarova NL. The role of symmetric stem cell divisions in tissue homeostasis. PLoS computational biology. 2015;11(12).

[35] Sun Z, Plikus MV, Komarova NL. Near equilibrium calculus of stem cells in application to the airway epithelium lineage. PLoS computational biology. 2016;12(7).

[36] Rodriguez-Brenes IA, Komarova NL, Wodarz D. Evolutionary dynamics of feedback escape and the development of stem-cell–driven cancers. Proceedings of the National Academy of Sciences. 2011;108(47):18983–18988.

[37] Komarova NL, Weiss LD, Van Den Driessche P, Lowengrub JS, Wodarz D. Determinants of stem cell enrichment in healthy tissues and tumors: implications for non-genetic drug resistance. BioRxiv. 2019;p. 663146.

[38] Lloyd-Lewis B, Mourikis P, Fre S. Notch signalling: sensor and instructor of the microenvironment to coordinate cell fate and organ morphogenesis. Current opinion in cell biology. 2019;61:16–23.

[39] Ali N, Zirak B, Rodriguez RS, Pauli ML, Truong HA, Lai K, et al. Regulatory T cells in skin facilitate epithelial stem cell differentiation. Cell. 2017;169(6):1119–1129.

[40] Lowry WE, Blanpain C, Nowak JA, Guasch G, Lewis L, Fuchs E. Defining the impact of β-catenin/Tcf transactivation on epithelial stem cells. Genes & development. 2005;19(13):1596–1611.

[41] Tatapudy S, Aloisio F, Barber D, Nystul T. Cell fate decisions: emerging roles for metabolic signals and cell morphology. EMBO reports. 2017;18(12):2105–2118.

[42] Biton M, Haber AL, Rogel N, Burgin G, Beyaz S, Schnell A, et al. T helper cell cytokines modulate intestinal stem cell renewal and differentiation. Cell. 2018;175(5):1307–1320.

A Generalizations of the analysis: equilibria and minimal feedback

A.1 The equilibrium occurs at a critical point

Under the blanket of minimal feedback in a healthy, wild type network where we consider the least number of feedback loops necessary to achieve an initially stably system, we now consider the situation where the equilibrium occurs at a critical point, so a derivative of zero does not guarantee that the function is (locally) a constant. For example, the rate R is regulated only by x in the vicinity of the equilibrium, but $R_x = 0$ at equilibrium and $R_x \neq 0$ everywhere in a (one-sided) vicinity of the equilibrium, that is while $R_x = 0, R_{xx} \neq 0$ at equilibrium. Continuing the assumption that $D > 0$, we are able to find exact solutions to only a few of the differential equations. We take $(\bar{x}, \bar{y})$ to be the point of equilibrium, and perturb the system by some unknown $(x_0(t), y_0(t))$ so that $x(t) = \bar{x} + x_0(t)$ and $y(t) = \bar{y} + y_0(t)$. Then stability requires the perturbations $x_0(t)$ and $y_0(t)$ to approach a constant or approach zero. We split the analysis into two cases, (1) in the absence of DC de-differentiation ($B = 0$) and (2) in the presence of DC de-differentiation ($B > 0$). In each case, only the described rate is regulated while all others are constants. We get the following results.

(1) In the absence of DC de-differentiation: $B = 0$**.**

(i) *SCs regulate their differentiation divisions, equilibrium occurs at a local minimum:* $R_{xx} > 0$. The system of differential equations for the perturbations at $(\bar{x}, \bar{y})$ are:

$$\begin{aligned} \dot{x}_0 &= -\frac{\bar{x}}{2} R_{xx} x_0^2, \\ \dot{y}_0 &= (2R + A)x_0 + \bar{x} R_{xx} x_0^2 - D y_0. \end{aligned}$$

It is clear that if $R_{xx} < 0$, then x_0 will grow, and so stability requires the critical point be a local minimum. This system is solvable with solution:

$$\begin{aligned} x_0(t) &= \frac{2x_0(0)}{2 + (\bar{x} x_0(0) R_{xx})t}, \\ y_0(t) &= \frac{-4x_0(0)}{2 + \bar{x} x_0(0) R_{xx} t} + (2x_0(0) + y_0(0))e^{-Dt} + \\ &\quad + \frac{2(2R + 2D + A) Exp[-D(t + \frac{2}{\bar{x} x_0(0) R_{xx}})]}{\bar{x} R_{xx}} \left[Ei\left(D(t + \frac{2}{\bar{x} x_0(0) R_{xx}}) \right) - Ei\left(\frac{2D}{\bar{x} x_0(0) R_{xx}} \right) \right], \end{aligned}$$

where $Ei(z) = -\int_{-z}^{\infty} \frac{e^{-p}}{p}\, dp$. We prove that both $x_0(t)$ and $y_0(t)$ go to 0 as $t \to \infty$.

Proof: Since this analysis is at the local equilibrium point, R_{xx} is a constant. Clearly $\lim_{t\to\infty} x_0(t) = \lim_{t\to\infty} \frac{2x_0(0)}{2+(\bar{x} x_0(0) R_{xx})t} = 0$. Similarly,

$$\lim_{t\to\infty} \frac{-4x_0(0)}{2 + \bar{x}_0 R_{xx} t} + (2x_0(0) + y_0(0))e^{-Dt} = 0.$$

Since $Ei\left(\frac{2D}{\bar{x} x_0(0) R_{xx}}\right)$ is a constant, then

$$\lim_{t\to\infty} Exp\left[-D(t + \frac{2}{\bar{x} x_0(0) R_{xx}}) \right] Ei\left(\frac{2D}{\bar{x} x_0(0) R_{xx}} \right) = 0.$$

We now evaluate $\lim_{t\to\infty} Exp\left[-D(t + \frac{2}{\bar{x} x_0(0) R_{xx}}) \right] Ei\left(D(t + \frac{2}{\bar{x} x_0(0) R_{xx}}) \right)$. Taking $a = D$ and $b = \frac{2}{\bar{x} x_0(0) R_{xx}}$, by L'Hospital's Rule we have

$$\begin{aligned} \lim_{t\to\infty} Exp\left[-a(t+b)\right] Ei\left(a(t+b)\right) &= \lim_{t\to\infty} \left(\int_{-a(t+b)}^{\infty} \frac{e^{-p}}{p}\, dp \right) / \left(Exp\left[a(t+b)\right] \right) \\ &= \lim_{t\to\infty} \left(\frac{Exp[a(t+b)]}{-a(t+b)} \right) / \left(aExp\left[a(t+b)\right] \right) \\ &= \lim_{t\to\infty} \frac{1}{-a^2(t+b)} \\ &= 0. \end{aligned}$$

Thus $\lim_{t\to\infty} x_0(t)$ and $\lim_{t\to\infty} y_0(t) = 0$.

(ii) *DCs regulate differentiation divisions, equilibrium occurs at a local minimum:* $R_{yy} > 0$. The system of differential equations for the perturbations are:

$$\begin{aligned} \dot{x}_0 &= -\frac{\bar{x}}{2} R_{yy} y_0^2, \\ \dot{y}_0 &= (2R + A)x_0 + \bar{x} R_{yy} y_0^2 - D y_0. \end{aligned}$$

While this system is not easily solved symbolically, if $R_{yy} < 0$ then x_0 will grow. If $R_{yy} > 0$ and if we can assume $x_0 \to 0$, then the second equation becomes

$$\dot{y}_0 = \bar{x} R_{yy} y_0^2 - D y_0,$$

which has the solution

$$y_0(t) = \frac{D e^{-Dt}}{\bar{x} R_{yy} e^{-Dt} + \frac{D}{y_0(0)} - \bar{x} R_{yy}}.$$

Note that as long as $\frac{D}{y_0(0)} \neq \bar{x} R_{yy}$, then $y_0 \to 0$ as $t \to \infty$.

(iii) *SCs regulate their self-renewal, equilibrium occurs at a local maximum:* $S_{xx} < 0$.
The perturbations are described by:

$$\begin{aligned} \dot{x}_0 &= \frac{\bar{x}}{2} S_{xx} x_0^2, \\ \dot{y}_0 &= (2R + A) x_0 - D y_0. \end{aligned}$$

This system has the solution:

$$\begin{aligned} x_0(t) &= \frac{-2x_0(0)}{-2 + (\bar{x} x_0(0) S_{xx}) t} \\ y_0(t) &= \frac{e^{-Dt}}{\bar{x} S_{xx}} \left[-2(2R + A) e^{\frac{2D}{\bar{x} x_0(0) S_{xx}}} \left[Ei\left(D(t - \frac{2}{\bar{x} x_0(0) S_{xx}}) \right) - Ei\left(\frac{-2D}{\bar{x} x_0(0) S_{xx}} \right) \right] \right. \\ &\qquad \left. + \bar{x} y_0(0) S_{xx} \right] \end{aligned}$$

where $x_0(t)$ and $y_0(t)$ both go to 0 as $t \to \infty$. The proof is similar to the proof given in (i).

(iv) *DCs regulate SC self-renewal, equilibrium occurs at a local maximum:* $S_{yy} < 0$.
The system of differential equations for the perturbations are:

$$\begin{aligned} \dot{x}_0 &= \frac{\bar{x}}{2} S_{yy} y_0^2, \\ \dot{y}_0 &= (2R + A) x_0 - D y_0. \end{aligned}$$

Similarly to (ii), this system is not easily solved symbolically, however if $S_{yy} > 0$ then x_0 will grow. On the other hand, if $S_{yy} < 0$ and $x_0 \to 0$, then the second equation becomes $\dot{y}_0 = -D y_0$, which has solution

$$y_0(t) = y_0(0) e^{-Dt}.$$

It would then follow that $y_0 \to 0$ as $t \to \infty$.

(2) In the presence of DC de-differentiation: $B > 0$**.** Here, since $B > 0$, the equations become much more complex, and thus only some cases have conclusive results. If the network is stable, then the perturbed system evaluated at the equilibrium point of $(0, 0)$ must be stable or neutrally stable, so we analyze the stability of $x_0(t), y_0(t)$ at $(0, 0)$ by first ignoring every squared term. The perturbed system of differential equations for each of the ten cases below then becomes

$$\begin{aligned} \dot{x}_0 &= (-R + S) x_0 + B y_0, \\ \dot{y}_0 &= (2R + A) x_0 - (D + B) y_0, \end{aligned}$$

and at equilibrium this is neutrally stable since

$$R - S + D + B > 0, \tag{59}$$

$$(R - S)(D + B) - B(2R + A) = 0. \tag{60}$$

Then adding the squared term back into the system of differential equations, we may be able to determine the sign of the second derivative and thus whether a minimal network is stable if the equilibrium occurs at a local extrema. We find that when R or B is regulated and the equilibrium occurs at a critical point, the critical point cannot be classified as a local maximum or minimum, while regulation of S or A results in an equilibrium which occurs at a local maximum, and regulation of D at a local minimum. We discuss each case below.

(i) *SCs regulate their differentiation divisions, equilibrium occurs at a critical point:* Unclassified. The system of differential equations for the perturbations are:

$$\begin{aligned} \dot{x}_0 &= (-R+S)x_0 + By_0 - \frac{\bar{x}}{2}R_{xx}x_0^2, \\ \dot{y}_0 &= (2R+A)x_0 - (D+B)y_0 + \frac{\bar{x}}{2}R_{xx}x_0^2. \end{aligned}$$

Due to the different signs of the $R_{xx}x_0^2$ terms in the two equations, we are unable to determine whether stability requires $R_{xx} > 0$ or $R_{xx} < 0$. If $R_{xx} > 0$, then the population x_0 would decrease but the population y_0 would increase, and if $R_{xx} < 0$ then vice versa. Thus this critical point remains unclassified.

(ii) *DCs regulate SC differentiation divisions, equilibrium occurs at a critical point:* Unclassified. The system of differential equations are the same as in (i) where $R_{xx}x_0^2$ becomes $R_{yy}y_0^2$, and thus by the same reasoning this critical point also remains unclassified.

(iii) *SCs regulate their self-renewal, equilibrium occurs at a local maximum:* $S_{xx} < 0$. The system of differential equations for the perturbations are:

$$\begin{aligned} \dot{x}_0 &= (-R+S)x_0 + By_0 + \frac{\bar{x}}{2}S_{xx}x_0^2, \\ \dot{y}_0 &= (2R+A)x_0 - (D+B)y_0. \end{aligned}$$

If $S_{xx} > 0$, then the population x_0 and then y_0 to grow from small numbers. Thus in order to be stable, we require $S_{xx} < 0$, and the critical point is a local maximum.

(iv) *DCs regulate SC self-renewal, equilibrium occurs at a local maximum:* $S_{yy} < 0$. The differential equations are given in case (iii) where $S_{xx}x_0^2$ is replaced by $S_{yy}y_0^2$, and the results are the same.

(v) *SCs regulate DC de-differentiation, equilibrium occurs at a critical point:* Unclassified. The differential equations are the same as in (i) where $\frac{\bar{x}}{2}R_{xx}x_0^2$ is replaced by $-\frac{\bar{y}}{2}B_{xx}x_0^2$. Then by similar reasoning as (i), this critical point also remains unclassified.

(vi) *DCs regulate their de-differentiation, equilibrium occurs at a critical point:* Unclassified. The system is the same as in (i), except $\frac{\bar{x}}{2}R_{xx}x_0^2$ is replaced by $-\frac{\bar{y}}{2}B_{yy}y_0^2$, and by similar reasoning the critical point is unclassified.

(vii) *SCs regulate DC death, equilibrium occurs at a local minimum:* $D_{xx} > 0$. The system of differential equations at the perturbations are:

$$\begin{aligned} \dot{x}_0 &= (-R+S)x_0 + By_0, \\ \dot{y}_0 &= (2R+A)x_0 - (D+B)y_0 - \frac{\bar{y}}{2}D_{xx}x_0^2. \end{aligned}$$

By reasoning similar to that in case (iii), the critical point is a local minimum for stability.

(viii) *DCs regulate their death, equilibrium occurs at a local minimum:* $D_{yy} > 0$. The system of differential equations is the same as in (vii) with $D_{xx}x_0^2$ replaced by $D_{yy}y_0^2$, and the same reasoning results in a local minimum for stability.

(ix) *SCs regulate asymmetric divisions, equilibrium occurs at a local maximum:* $A_{xx} < 0$.
The differential equations are the same as in case (vii) except $\frac{\bar{y}}{2} D_{xx} x_0^2$ is replaced by $-\frac{\bar{x}}{2} A_{xx} x_0^2$. Thus by similar reasoning, we have a local maximum.

(x) *DCs regulate asymmetric divisions, equilibrium occurs at a local maximum:* $A_{yy} < 0$.
The system of differential equations are the same as in case (vii), except $\frac{\bar{y}}{2} D_{xx} x_0^2$ is replaced by $-\frac{\bar{x}}{2} A_{yy} y_0^2$, and thus the critical point is a local maximum for stability.

A.2 Relaxing the assumption of nonzero intensity at equilibrium

Still looking at minimal feedback for the original network of wild type cells, here we consider the situation where $f(x, y) = 0$ at equilibrium, but $f(x, y) > 0$ on one or both sides of the equilibrium. For example, we could have $R(x) = 0$ but $R_x \neq 0$ at equilibrium. The only feedback loops affected are those where the regulated rate appears as part of the inequality required for feedback. Thus, the only case affected is when SCs negatively regulate de-differentiation of DCs, in which case the inequality simplifies to $-\frac{D}{\bar{y}} < B_y < 0$.

A.3 Relaxing the assumption of the positivity of D

We consider the unlikely event that $D < 0$ as it applies to the minimal feedback necessary for stability of the original wild type network, that is we assume the proliferation rate of DCs is greater than the death rate of DCs. This corresponds to DCs that have stem-like characteristics. If we again assume $B = 0$, then stability requires $D > 0$ for all four possible minimal feedback loops, so we restrict to the case $B > 0$. We have

(i) $-\frac{\bar{x}(D+B)+\bar{y}B}{\bar{x}^2} < R_x < 0$.

(ii) $R_y < 0$ and $R_y < \frac{\bar{x}(D+B)+\bar{y}B}{2\bar{x}^2}$.

(iii) If $D + B > 0$, we have $S_x < 0$. Otherwise, that is if $D + B < 0$, we require

$$0 < S_x < \frac{\bar{x}(D + B) + \bar{y}B}{\bar{x}^2}.$$

(iv) If $D+B > 0$, then $S_y < 0$. Otherwise if $D+B < 0$, we have both $S_y > 0$ and $\bar{x}(D+B)+\bar{y}B > 0$.

For cases involving feedback of de-differentiation (B), the assumptions change to

(v) $0 < B_x < \frac{\bar{x}(D+B)+\bar{y}B}{\bar{x}\bar{y}}$.

(vi) $B_y > 0$ and $B_y > -\frac{\bar{x}(D+B)+\bar{y}B}{\bar{x}\bar{y}}$.

For the additional possibilities (vii) - (x), the assumptions remain the same but additional assumptions are needed. We have

(vii) $D_x > 0$ and $\bar{x}(D + B) + \bar{y}B > 0$.

(viii) $D_y > 0$ and $D_y > -\frac{\bar{x}(D+B)+\bar{y}B}{\bar{x}\bar{y}}$.

(ix) $A_x < 0$ and $\bar{x}(D + B) + \bar{y}B > 0$.

(x) $A_y < 0$ and $A_y < \frac{\bar{x}(D+B)+\bar{y}B}{\bar{x}^2}$.

B Redundant networks, further analysis

In Section 4 we considered redundant networks where mutations attack the existing feedback such that mutants had a weakened response to that feedback. In Section 3.3, we found five additional mechanisms that alter the network phenotype and allow mutant cells to grow from low numbers. Thus we want to do similar analysis of redundant networks under these five mechanistic changes.

We assume two minimal feedback loops found in 2.2 are in effect, and a mutant appears that has a decreased rate of SC differentiation (R) or DC death (D), or an increased rate of SC self-renewal (S), DC de-differentiation (B), or asymmetric SC divisions (A). While the mutant population will grow initially, since feedback loops may still be intact, a new equilibrium may be reached, preventing unlimited growth.

For the analysis below, we make several simplifying assumptions.

1. The local conditions on the two minimal feedback loops stated in (i-x) of Section 2.2 hold globally. For example, minimal feedback loop (i) becomes $R_x > 0$ for all x instead of just at the equilibrium $(\bar{x}, \bar{y})$.

2. All the non-constant rates regulated by feedback are functions of the total populations, $x = x_1 + x_2$ and $y = y_1 + y_2$.

3. The affected mutant rate satisfies one of the five phenotypic changes described above and in Section 3.3, and the inequalities are global. For example, if R is the affected rate, then we require $\tilde{R}(x, y) < R(x, y)$ for all relevant values of x and y.

Let us consider the function $H(R, S, B, D, A)$ defined in (38), which as seen in (39) is zero at the wild type equilibrium. Under one of the five alterations, the inequality in (40) holds, that is $H(\tilde{R}, \tilde{S}, \tilde{B}, \tilde{D}, \tilde{A})|_{\bar{x},\bar{y}} < 0$. If a new equilibrium is reached, then it will satisfy (44). However, since $H(R, S, B, D, A)$ is an increasing function of x and y by (43), and H at the mutated rates lies below H at the original rates by (40) and assumption 3, then a **new equilibrium cannot have both populations decrease.** That is, it is not possible that both $\hat{x} < \bar{x}$ and $\hat{y} < \bar{y}$.

Similar to Section 4, we can determine information about the population sizes at a new mutant equilibrium to those at the wild type equilibrium. Now however, the mutated rate does not have to match one of the regulated rates. We take $\bar{H} = H(R, S, B, D, A)|_{\bar{x},\bar{y}}$, $\hat{H} = H(\tilde{R}, \tilde{S}, \tilde{B}, \tilde{D}, \tilde{A})|_{\hat{x},\hat{y}}$, and the expression for μ in (45) where $\hat{\mu} = \hat{y}/\hat{x}$, and $\bar{\mu} = \bar{y}/\bar{x}$. Let us denote Q_1 and Q_2 as the minimally regulated rates if they are different, Q as the minimally reguated rate if both populations regulate Q, and $\tilde{V}$ as the altered rate that satisfies one of the five phenotypic changes. Consider the following general scenarios.

- The redundant network is characterized by **a single rate regulated by both populations, and the mutated rate is the same as the regulated rate.** That is, $Q = V$. Then at the two equilibria we have $\bar{H} = \hat{H} = 0$, and rearranging results in $\tilde{Q}|_{\hat{x},\hat{y}} = \tilde{Q}(\bar{x}, \bar{y})$. Thus we have that $\hat{\mu} = \bar{\mu}$, and since one population must increase, then **both populations must increase**.

- **The regulated wild type and altered mutant rates are functions of the same variable.** Without loss of generality, let us assume the network is dependent on x only. Then, by previous analysis, since $H(\tilde{R}, \tilde{S}, \tilde{B}, \tilde{D}, \tilde{A})$ lies below $H(R, S, B, D, A)$ and both are functions on x, we will have $\hat{x} > \bar{x}$. The result is similar if the network is y-dependent. Thus, if both feedback loops are mediated by a single population and the altered mutant feedback loop is also mediated by this same population, then **the regulating population will increase as a result of the alteration of the mutant rate.**

- **The regulated rates and the altered mutant rate are any combination of A and D.** For example, both regulations can be of asymmetric division while the altered rate is the DC death rate ($Q = A(x, y)$ and $V = D$), or both A and D are regulated by either population while D is the altered rate ($Q_1 = A$, $Q_2 = D$, and $V = D$). No matter the combination, neither A or D appear in the second definition of μ in (45), and so $\hat{\mu} = \bar{\mu}$. This means that since at least one population must increase, we have that **both populations will increase**.

The rest must be done case by case. As there are 45 possible redundant networks in the presence of de-differentiation and each can undergo five possible phenotypic alterations to cause initial mutant growth, we limit this analysis to the redundant networks possible in the absence of de-differentiation. That is, the rest of our analysis assumes $B = 0$.

B.1 Redundant networks under other phenotypic changes in the absence of de-differentiation, $B = 0$

There are now only four minimal feedback loops possible, and thus we have 6 possible redundant networks. Recall that altering A is only an effective mutation in the presence of de-differentiation, and so there are only four alterations for each redundant network. Let us use the same numbering as in Section 3.3 for these changes:

(1) decrease SC differentiation R,

(2) increase SC self-renewal S,

(3) increase the DC de-differentiation rate from 0 (B),

(4) decrease the DC death rate D such that it becomes negative at the equilibrium.

Note that these alterations may change functions into constants, constants into functions, or keep constants as constants or functions as functions. Additionally, we use only the first equality for μ in (45), so $\mu = \frac{2R+A}{D+B}$ and we do not divide by zero.

For alteration (4), if $\tilde{D} < 0$ for all x, y, then the mutant population of DCs will grow to infinity while the other populations remain finite, and a new equilibrium will never be reached. Let us instead make the global assumption that $\tilde{D} < D$ for all x, y, and only require $\tilde{D}|_{\bar{x},\bar{y}} < 0$. Then we use the same assumptions made at the beginning of this section, and consider case each separately.

For the case-by-case analysis, we will use the fact that both populations cannot decrease. If $\hat{\mu} = \bar{\mu}$, then both populations must increase. If $\hat{\mu} > \bar{\mu}$, we cannot have $\hat{y} < \bar{y}$ as that will make $\hat{x} < \bar{x}$ as well, however x can increase or decrease. Similarly, if $\hat{\mu} < \bar{\mu}$ we cannot have $\hat{x} < \bar{x}$, but y can increase or decrease.

R_x, R_y: Minimal feedback loops (i) and (ii). Here we assume that R is positively regulated by both populations. We consider the four possible alterations below.

(1) Here the mutant rate of differentiation is smaller than the wild type rate, $\tilde{R} < R(x, y)$ for all x, y. In this case, $Q = V$ and so the result is already described above. We have $\hat{\mu} = \bar{\mu}$ and both populations increase.

(2) SC self-renewal is increased so that $\tilde{S} > S$ for all x, y. Then since S is a constant,

$$R(\bar{x}, \bar{y}) = S < \tilde{S}|_{\hat{x},\hat{y}} = R(\hat{x}, \hat{y}),$$

so we have $\hat{\mu} > \bar{\mu}$. Since both populations cannot decrease, we must have $\hat{y} > \bar{y}$, while x can increase or decrease.

(3) De-differentiation increases from 0 so that $\tilde{B} > 0$ for all x, y. Rearranging the equations at equilibrium gives $S = R(\bar{x}, \bar{y}) = R(\hat{x}, \hat{y}) - \hat{\mu}\tilde{B}$, and thus we know $R(\bar{x}, \bar{y}) < R(\hat{x}, \hat{y})$. However, since $\tilde{B} > B = 0$, $R(\hat{x}, \hat{y})$ increases μ but $\tilde{B}$ decreases μ, and so μ can increase or decrease. Additionally that means we know only that both populations cannot decrease.

(4) DC death decreases such that $\tilde{D}|_{\bar{x},\bar{y}} < 0$. If $\tilde{D} \geq 0$ for some x, y and $\tilde{D} < D$ for all x, y, then the network can reach a new equilibrium. In this case, $S = R(\bar{x}, \bar{y}) = R(\hat{x}, \hat{y})$, so since $\tilde{D} < D$ then $\hat{\mu} > \bar{\mu}$. Thus $\hat{y} > \bar{y}$, and x could increase or decrease.

S_x, S_y: **Minimal feedback loops (iii) and (iv).** Here we assume that S is negatively regulated by both populations. We consider the four possible alterations below.

(1) Here the mutant rate of differentiation is smaller than the wild type rate, $\tilde{R} < R$ for all x, y. Then $\hat{\mu} < \bar{\mu}$, and so x must increase whereas y can increase or decrease.

(2) SC self-renewal is increased so that $\tilde{S} > S(x, y)$ for all x, y. In this case, $Q = V$ and so the result is already described above. We have $\hat{\mu} = \bar{\mu}$ and both populations increase.

(3) De-differentiation increases from 0 so that $\tilde{B} > 0$ for all x, y. Since R is a constant, then $\hat{\mu} < \bar{\mu}$. Thus x must increase whereas y can increase or decrease.

(4) DC death decreases such that $\tilde{D}|_{\bar{x},\bar{y}} < 0$. If $\tilde{D} \geq 0$ for some x, y and $\tilde{D} < D$ for all x, y, then the network can reach a new equilibrium. In this case, since R is a constant, $\hat{\mu} > \bar{\mu}$, so y must increase and x can increase or decrease.

R_x, S_y: **Minimal feedback loops (i) and (iv).** Here we assume that differentiation is positively regulated by SCs and SC self-renewal is negatively regulated by DCs. We consider the four possible alterations below.

(1) Differentiation is decreased for mutants such that $\tilde{R} < R(x)$ for all x, y. Assume y decreases such that $\hat{y} < \bar{y}$. Then, since S is a decreasing function of y, we have

$$R(\bar{x}) = S(\bar{y}) < S(\hat{y}) = \tilde{R}|_{\hat{x},\hat{y}} < R(\hat{x}).$$

Since since R is an increasing function of x, then we must also have that $\hat{x} > \bar{x}$. Since $R(\bar{x}) < \tilde{R}|_{\hat{x},\hat{y}}$, then $\hat{\mu} > \bar{\mu}$. However, this is not possible if $\hat{y} < \bar{y}$ and $\hat{x} > \bar{x}$, so there is a contradiction and we have $\hat{y} > \bar{y}$. Then $R(\bar{x}) > \tilde{R}|_{\hat{x},\hat{y}}$ gives $\hat{\mu} < \bar{\mu}$, and so we must also have $\hat{x} > \bar{x}$. Thus both populations increase.

(2) SC self-renewal is increased so that $\tilde{S} > S(y)$ for all x, y. Similarly to (1), $\hat{x} < \bar{x}$ leads to $\hat{y} > \bar{y}$ and $\hat{\mu} < \bar{\mu}$, which is a contradiction. Thus $\hat{x} > \bar{x}$. Then $R(\hat{x}) > R(\bar{x})$ and so $\hat{\mu} > \bar{\mu}$, which gives that $\hat{y} > \bar{y}$. Thus both populations increase.

(3) De-differentiation increases from 0 so that $\tilde{B} > 0$ for all x, y. Rearranging the equations at equilibrium gives $R(\bar{x}) = S(\bar{y})$ and $R(\hat{y}) = \hat{\mu}\tilde{B} + S(\hat{y})$. If $\hat{x} < \bar{x}$, then $R(\bar{x}) > R(\hat{x})$ and so $S(\bar{y}) > S(\hat{y})$. Since S is a decreasing function, then $\hat{y} > \bar{y}$. However, $R(\bar{x}) > R(\hat{x})$ and $\tilde{B} > 0$ also give us that $\hat{\mu} < \bar{\mu}$. This contradicts the fact that $\hat{x} < \bar{x}$ and $\hat{y} > \bar{y}$, so instead we must have $\hat{x} > \bar{x}$. This means that μ can increase or decrease, and thus y can increase or decrease.

(4) DC death decreases such that $\tilde{D}|_{\bar{x},\bar{y}} < 0$. If $\tilde{D} \geq 0$ for some x, y and $\tilde{D} < D$ for all x, y, then the network can reach a new equilibrium. In this case, since R is an increasing function, S is a decreasing function, if $\hat{x} > \bar{x}$ then $\hat{y} < \bar{y}$. This also means that $R(\hat{x}) > R(\bar{x})$, and so $\hat{\mu} > \bar{\mu}$. However, this is a contradiction with the fact that $\hat{x} > \bar{x}$ and $\hat{y} < \bar{y}$. Thus we must have $\hat{x} < \bar{x}$ and $\hat{y} > \bar{y}$. This also gives us that $\hat{\mu} > \bar{\mu}$.

R_y, S_x: **Minimal feedback loops (ii) and (iii).** Here we assume that differentiation is positively regulated by DCs and SC self-renewal is negatively regulated by SCs. We consider the four possible alterations below.

(1) Differentiation is decreased for mutants such that $\tilde{R} < R(y)$ for all x, y. In this case, an increase in either population results in the other population either increasing or decreasing, and so we have only the most basic requirement that both populations cannot decrease.

(2) SC self-renewal is increased so that $\tilde{S} > S(x)$ for all x, y. Similarly to (1), we have only the most basic requirement that both populations cannot decrease.

(3) De-differentiation increases from 0 so that $\tilde{B} > 0$ for all x, y. Similar rearranging as the other redundant networks gives two possibilities: (a) if $\hat{y} < \bar{y}$, then $\hat{x} > \bar{x}$ and $\hat{\mu} < \bar{\mu}$, and (b) if $\hat{y} > \bar{y}$ then $\hat{x} < \bar{x}$ and $\hat{\mu} > \bar{\mu}$. Thus, μ could increase or decrease, and we have only that both populations cannot decrease.

(4) DC death decreases such that $\tilde{D}|_{\bar{x},\bar{y}} < 0$. If $\tilde{D} \geq 0$ for some x, y and $\tilde{D} < D$ for all x, y, then the network can reach a new equilibrium. Similarly to (3), we get the same two cases, and thus μ could increase or decrease and we have only that both populations cannot decrease.

R_x, S_x: Minimal feedback loops (i) and (iii). Here we assume that differentiation is positively regulated by SCs and SC self-renewal is negatively regulated by SCs. We consider the four possible alterations below.

(1) Differentiation is decreased for mutants such that $\tilde{R} < R(x)$ for all x, y. Assume x decreases such that $\hat{x} < \bar{x}$. Then, since S is a decreasing function of x, we have

$$R(\bar{x}) = S(\bar{x}) < S(\hat{x}) = \tilde{R}|_{\hat{x},\hat{y}} < R(\hat{x}).$$

However, since R is an increasing function of x, then we have that x must be increasing, so there is a contradiction and we have $\hat{x} > \bar{x}$. Thus the equality above changes and we have $R(\bar{x}) > \tilde{R}|_{\hat{x},\hat{y}}$, so $\hat{\mu} < \bar{\mu}$, and y can increase or decrease.

(2) SC self-renewal is increased so that $\tilde{S} > S(x)$ for all x, y. Similarly to (1), if $\hat{x} < \bar{x}$ then

$$S(\bar{x}) = R(\bar{x}) > R(\hat{x}) = \tilde{S}|_{\hat{x},\hat{y}} > S(\hat{x})$$

leads to a contradiction. So $\hat{x} > \bar{x}$. Thus $R(\bar{x}) < R(\hat{x})$ gives $\hat{\mu} > \bar{\mu}$, and so we must also have $\hat{y} > \bar{y}$. Thus both populations increase.

(3) De-differentiation increases from 0 so that $\tilde{B} > 0$ for all x, y. Rearranging the equations at equilibrium gives $R(\bar{x}) = S(\bar{x})$ and $R(\hat{x}) = \hat{\mu}\tilde{B} + S(\hat{x})$. If $\hat{x} < \bar{x}$, then $S(\bar{x}) < S(\hat{x})$ and

$$S(\bar{x}) = R(\bar{x}) > R(\hat{x}) = \hat{\mu}\tilde{B} + S(\hat{x})$$

leads to a contradiction, since $\hat{\mu}\tilde{B} > 0$. Thus $\hat{x} > \bar{x}$. However, this gives that μ can increase or decrease, and thus y can increase or decrease.

(4) DC death decreases such that $\tilde{D}|_{\bar{x},\bar{y}} < 0$. If $\tilde{D} \geq 0$ for some x, y and $\tilde{D} < D$ for all x, y, then the network can reach a new equilibrium. In this case, since R is an increasing function, S is a decreasing function, $R(\bar{x}) = S(\bar{x})$ and $R(\hat{x}) = S(\hat{x})$, then $\hat{x} = \bar{x}$. Then $\hat{\mu} > \bar{\mu}$, and so $\hat{y} > \bar{y}$.

R_y, S_y: Minimal feedback loops (ii) and (iv). Here we assume that differentiation is positively regulated by DCs and SC self-renewal is negatively regulated by DCs. We consider the four possible alterations below.

(1) Differentiation is decreased for mutants such that $\tilde{R} < R(y)$ for all x, y. Similar to the redundant network consisting of minimal feedback loops (i) and (iii), since S is a decreasing function, if y decreases it will lead to a contradiction. Thus $\hat{y} > \bar{y}$. Then $R(\bar{y}) > \tilde{R}|_{\hat{x},\hat{y}}$, so $\hat{\mu} < \bar{\mu}$. This gives that $\hat{x} > \bar{x}$, and thus both populations increase.

(2) SC self-renewal is increased so that $\tilde{S} > S(x)$ for all x, y. Similarly to redundant network (i) and (iii), as well as case (1), $\hat{y} < \bar{y}$ leads to a contradiction, so we have $\hat{y} > \bar{y}$. Then $R(\hat{y}) > R(\bar{y})$, and so $\hat{\mu} > \bar{\mu}$. Thus, while y must increase, x can increase or decrease.

(3) De-differentiation increases from 0 so that $\tilde{B} > 0$ for all x, y. Rearranging the equilibria and using a similar argument as in case (2) of redundant network (i) and (iii), if $\hat{y} < \bar{y}$ there is a contradiction. Rather we have $\hat{y} > \bar{y}$, and μ can increase or decrease. Thus x can also increase or decrease.

	(1) $\tilde{R} < R$	(2) $\tilde{S} > S$	(3) $\tilde{B} > 0$	(4) $\tilde{D} < D$
R_x , R_y	=	↑	?	↑
S_x , S_y	↓	=	↓	↑
R_x , S_y	↓	↑	?	↑
R_y , S_x	?	?	?	?
R_x , S_x	↓	↑	?	↑
R_y , S_y	↓	↑	?	↑

Figure 6: Population trends for mutant equilibria compared to wild type equilibria for redundant networks in the absence of de-differentiation ($B = 0$) where the phenotype is altered one of four ways. Note that if the regulated rate coincides with the altered rate, then it is a different change than a weakened response to feedback. The rows represent which feedback loops are present while the columns show which rate is altered. The arrows indicate whether the fraction y/x will increase, decrease, or remain the same. A question mark means that the fraction could increase or decrease. Red means that both populations will increase, yellow that SCs must increase, light blue that DCs must increase, and green that at least one population cannot decrease. For the light blue and yellow cells, one population must increase while the other can increase or decrease, however the dark blue indicates that the DCs must increase and the SCs must decrease. The white indicates that the SCs remain the same while the DCs increase, and the purple that the DCs remain the same while the SCs decrease.

(4) DC death decreases such that $\tilde{D}|_{\bar{x},\bar{y}} < 0$. If $\tilde{D} \geq 0$ for some x, y and $\tilde{D} < D$ for all x, y, then the network can reach a new equilibrium. Similarly to (4) in redundant network (i) and (iii), since R is an increasing function and S is a decreasing function, then we must have $\hat{y} = \bar{y}$. Then $\hat{\mu} > \bar{\mu}$, and x must decrease. Note that while both populations do not decrease, neither population increases. This is this only case where one population remains the same, while the other decreases.

Each redundant network behaves differently. For instance, in redundant network R_x, S_y, we only know the most basic result that both populations cannot be decreasing for each mutation, whereas for redundant network S_x, S_y we know exactly which population must be decreasing for each mutation. For mutation type (1) where R decreases, five redundant networks must have SCs increase. On the other hand, for mutation type (2) where S increases, five redundant networks must have DCs increase. Mutation type (3), B increases from zero, half of the redundant networks result in an increase of SCs, only must result in an increase of DCs, while the other two cannot have both populations decrease but either could be non-decreasing.

Mutation type (4) is much more varied than the other mutations, and gives unique results. For instance, redundant network R_x, S_y mutated in this manner requires SCs to decrease, and it is the only mutation type and redundant network with this result. When both rates are regulated by SCs, that is R_x and S_x are regulated, we have the unique result that the SC population remains the same while the DCs increase, and when both rates are regulated by DCs, that is R_y and S_y are regulated, the DCs remain the same while the SCs actually decrease. That is, under this mutation, both rates regulated by the same population leads to one population remaining the same, and DCs as the regulating population is the only case where neither population increases.

These population trends can be clearly seen in Figure 6. We compare the populations at the new mutant equilibrium to the original wild type equilibrium, where a population increases if it is larger at the new equilibrium. The arrows and colors have the same meanings as in Figure 4, with three additional colors: the dark blue is the case where the SCs must decrease, the white is the case when the SCs stay the same while the DCs increase, and the purple is the only case where neither population increases. We can clearly see that not all redundant networks behave the same way.