

RESEARCH ARTICLE

Stability analysis of multi-compartment models for cell production systems

Yukihiko Nakata^a, Philipp Getto^a, Anna Marciniak-Czochra^b and Tomás Alarcón^c

^aBCAM - Basque Center for Applied Mathematics, Bizkaia Technology Park, Building 500, E-48160

Derio, Spain

^bInterdisciplinary Center for Scientific Computing (IWR), Institute of Applied Mathematics and BIOQUANT

^cCentre de Recerca Matemàtica, Campus de Bellaterra, Edifici C 08193 Bellaterra, Barcelona, Spain

(Received 00 Month 200x; final version received 00 Month 200x)

We study two- and three-compartment models of a hierarchical cell production system with cell division regulated by the level of mature cells. We investigate the structure of equilibria with respect to parameters as well as local stability properties for the equilibria. To interpret the results we adapt the concept of reproduction numbers, which is well-known in ecology, to stem cell population dynamics. In the two-compartment model the positive equilibrium is stable wherever it exists. In the three-compartment model we find that the intermediate stage of differentiation is responsible for the emergence of an instability region in the parameter plane. Moreover we prove that this region shrinks as the mortality rate for mature cells increases and discuss this result.

1. Introduction

Stem cells are central to the regulatory mechanisms governing development, tissue regeneration and tissue homeostasis. They are characterised by their ability to self-renew and differentiate. These two processes are, to some extent, complementary: whereas self-renewal refers to the ability of stem cells to produce progeny with the same features as its progenitor, thereby ensuring the persistence of a pool of stem cells, differentiation, on the contrary, refers to the process in which the progeny becomes more specialised and loses the characteristics of stem cells, in particular, their ability to self-renew [15].

The starting point of our investigation is a multi-compartment model of a discrete collection of cell subpopulations proposed by Marciniak-Czochra and colleagues to investigate possible mechanisms of regulation and stabilisation of blood cell production, following perturbations such as bone marrow transplantation [11, 13]. Different plausible regulatory feedback mechanisms lead to different types of non-linearities in the model equations. In [3] it was suggested that two simultaneous feedback loops (short- and long-range) are necessary to stabilise such hierarchical cell system. On the contrary to the models investigated by Arino and Kimmel, [3], the model proposed in [11] is based on a single negative feedback loop describing regulation of the whole process by the mature cells. In particular, in [11] two hypotheses concerning regulation of haematopoiesis by the external signalling in response to a shortage of mature blood cells were studied. Hypothesis 1 assumes that the differentiation

E-mail address: nakata@bcamath.org, getto@bcamath.org, anna.marciniak@iwr.uni-heidelberg.de, talarcon@crm.cat

is governed by enhancing the rate of proliferation only, while in hypothesis 2, the ratio of the rate of self-renewal to the rate of differentiation is regulated by external signals. Numerical simulations presented in [11] suggest that a single negative regulatory loop (corresponding to hypothesis 1 or to hypothesis 2) may be enough to control the system. In the present paper we aim to validate this observation and perform qualitative analysis of two- and three-compartment models under hypothesis 1. The system under hypothesis 2 was analysed in [13] and the conditions for the existence of positive and semi-trivial steady states for a multi-compartment model were formulated. Mathematical analysis of the structure of steady states led to a characterisation of stem cell population by the following properties: (i) for some cytokine levels the death rate is smaller than the reproduction rate and (ii) signal intensity (cytokine level) needed for maintenance of the population size is smaller than that of all other cell populations.

In this paper, we focus on two essential issues concerning the cell maturation process and its stability. In some hierarchical cell systems, such as the haematopoietic system [14], the cascade of events leading from stem cells to fully differentiated cells is quite well described. On the contrary, in other tissues, such as the mammary gland [8], the situation is much less clear, as the number of maturation stages is not known. Therefore, we study the impact of the intermediate stages between stem and mature cells on the stem cell dynamics by formulating mathematical models with and without progenitor cells.

The second problem we aim to address is the issue of the stability of the pool of stem cells: Is the stem cell population constant over the life time of an organism or does it decay as the organism ages? A modification of the model from [11] to investigate the dynamics of the system under the assumption that all cells, including stem cells, undergo replicative senescence was given in [12]. A full answer to this question is obviously beyond the scope of this paper. However, we hope that we can offer some insight into this important issue by analysing the stability of a positive equilibrium solution to the compartmental models. This leads to the general conditions under which a stem cell population persists in time.

The remainder of the paper is organised as follows. In Section 2, we recall the model and the different processes taken into consideration. In Section 3, we discuss the existence of equilibrium and stability results for a two-compartment model (stem and mature cells). Section 4 is devoted to the local stability analysis of a three-compartment model. Finally, in Section 5, we outline our results, provide their biological interpretation and discuss directions for future research.

2. Model formulation

We follow closely the model formulation in [11] and consider two approximations of the chain of maturation stages by two- and three-compartment models: a two-compartment model where we only include stem cells and mature cells (Section 3), and a three-compartment model (Section 4) where in addition to stem and mature cells, we consider an intermediate population (the so-called progenitor cells) that corresponds to an intermediate stage between stem cells and fully-differentiated cells.

Following [11], mentioned above hypothesis 1, we assume that the rates of cell proliferation are regulated by the mature cells population. In particular, we assume that the growth of the population of mature cells inhibits this process. The biological rationale for this is clear: once the number of mature cells has reached the level necessary to fulfill the needs of the particular tissue, self-renewal and differentiation of stem cells and progenitor cells must be adjusted just to compensate the

loss of cells due to cell death [11]. In fact, deregulation of this homeostatic mechanism may lead to cancer [1, 2, 4]. The actual mechanisms involved in this negative feedback are, in general, unclear and may be tissue-dependent: for example in the haematopoietic system, it seems to be mediated by some growth factors [9].

We consider the following two systems: a two-compartment model which describes the time evolution of the populations of stem cells, $w(t)$ and mature cells, $v(t)$:

$$\begin{cases} w'(t) &= (2a_w - 1)d_w(v(t))w(t) - \mu_w w(t), \\ v'(t) &= 2(1 - a_w)d_w(v(t))w(t) - \mu_v v(t), \end{cases} \quad (1)$$

studied in Section 2 and a three-compartment model in which we consider progenitor cells, whose density is referred to as $u(t)$:

$$\begin{cases} w'(t) &= (2a_w - 1)d_w(v(t))w(t) - \mu_w w(t), \\ u'(t) &= (2a_u - 1)d_u(v(t))u(t) + 2(1 - a_w)d_w(v(t))w(t) - \mu_u u(t), \\ v'(t) &= 2(1 - a_u)d_u(v(t))u(t) - \mu_v v(t), \end{cases} \quad (2)$$

studied in Section 3. In Eqs. (1) and (2) a_w and a_u are the fraction of self-renewal of stem cells and progenitor cells. a_w and a_u describe the fraction of the corresponding progeny cells, produced by stem and progenitor cells, respectively, which is of the same type as its mother (see also [13]). $d_w(v)$ and $d_u(v)$ are the division rates of the stem cell and progenitor cell populations, and, finally, μ_w , μ_u , and μ_v the death rates of the corresponding population. The explicit dependence of the division rates refers to the regulation of these two processes by the mature cell population. It is assumed $a_u, a_w \in [0, 1)$ and $\mu_w, \mu_u, \mu_v > 0$ (see Fig. 1).

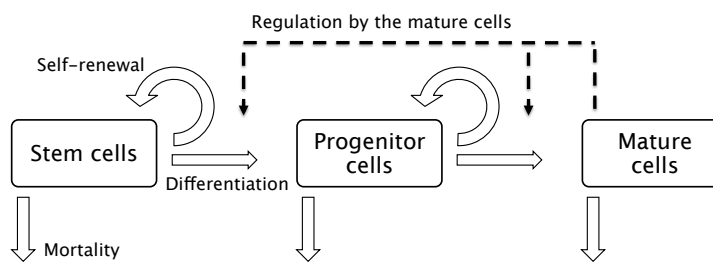


Figure 1. Compartmental diagram for the chain of maturation stages

Following [11], we assume that the division rates of stem and progenitor cells are controlled by extracellular signaling molecules such as cytokines. In particular, the division rates of stem and progenitor cells are given by $p_w s(t)$ and $p_u s(t)$, respectively, where p_w and p_u are unregulated division rates of stem and progenitor cells, respectively, and $s(t)$ is the signal intensity at time t . We assume that cytokines are secreted by specialized cells and that this secretion is regulated by mechanisms sensitive to the amount of mature cells such that

$$s(t) = \frac{1}{1 + kv(t)},$$

where k is a positive constant to take into account sensitivity to the amount of mature cells. This dependence can be justified using a quasi-steady state approximation of the plausible dynamics of the cytokine molecules [11]. This expression

reflects the heuristic assumption that signal intensity achieves its maximum under absence of mature cells and decreases asymptotically to zero if level of mature cells increases. Therefore, the division rates of stem cells and progenitor cells depend on the density of mature cells:

$$d_w(v) = \frac{p_w}{1 + kv}, \quad d_u(v) = \frac{p_u}{1 + kv},$$

where p_w and p_u are positive constants.

We interpret the fraction of self-renewal as the probability that a progeny cell remains at the same stage of differentiation as the parent cell. Upon division, the average flow of cells joining the stem cell pool equals $(2a_w - 1)d_w(v)w$. On the other hand, the flow in the pool of progenitor cells due to differentiation of stem cells equals $2(1 - a_w)d_w(v)w$, i.e. the fraction of stem cells which do not self-renew upon division. The remaining terms in Eqs. (1) and (2) have the same biological interpretation. We are further assuming that mature cells do not proliferate and therefore the only flow into the mature cell compartment correspond to differentiation of either stem cells (two-compartment model) or progenitor cells (three-compartment model).

Finally, we express our model equations in dimensionless terms by dividing by μ_w and introducing the non-dimensional time $\tilde{t} := \mu_w t$:

$$\begin{aligned} \tilde{w}(\tilde{t}) &:= w\left(\frac{\tilde{t}}{\mu_w}\right), \quad \tilde{u}(\tilde{t}) := u\left(\frac{\tilde{t}}{\mu_w}\right), \quad \tilde{v}(\tilde{t}) := v\left(\frac{\tilde{t}}{\mu_w}\right), \\ r_w &= \frac{p_w}{\mu_w}, \quad r_u = \frac{p_u}{\mu_w}, \quad m_u = \frac{\mu_u}{\mu_w}, \quad m_v = \frac{\mu_v}{\mu_w}. \end{aligned}$$

Dropping the tilde for convenience, (1) and (2) becomes

$$\begin{cases} w'(t) &= (2a_w - 1) \frac{r_w}{1+kv(t)} w(t) - w(t), \\ v'(t) &= 2(1 - a_w) \frac{r_w}{1+kv(t)} w(t) - m_v v(t), \end{cases} \quad (3)$$

and

$$\begin{cases} w'(t) &= (2a_w - 1) \frac{r_w}{1+kv(t)} w(t) - w(t), \\ u'(t) &= (2a_u - 1) \frac{r_u}{1+kv(t)} u(t) + 2(1 - a_w) \frac{r_w}{1+kv} w(t) - m_u u(t), \\ v'(t) &= 2(1 - a_u) \frac{r_u}{1+kv(t)} u(t) - m_v v(t), \end{cases} \quad (4)$$

respectively. As the initial conditions, we assume that

$$w(0) = w_0 > 0, \quad u(0) = u_0 \geq 0 \quad \text{and} \quad v(0) = v_0 \geq 0 \quad (5)$$

for (1) and (2). Then, similar to Lemma 4.1 in [13], a positive solution exists for all $t > 0$.

Lemma 2.1: *Every solution of (3) and (4) with (5) is positive and bounded for any $t > 0$.*

3. Stem cells and mature cells dynamics

In this section, we consider the two-compartment model (3). To characterize the existence and stability of equilibria we introduce *the reproduction number of stem*

cells as the number of stem cells coming in via self-renewal minus the number of stem cells going out via division in the expected lifetime of a stem cell. We denote this number by R_w and get that

$$R_w = (2a_w - 1)r_w. \quad (6)$$

3.1. Existence of two possible equilibria

In this subsection, we formulate the result on existence of equilibria in terms of R_w . We give the following result without proof.

Theorem 3.1:

- (1) There always exists a trivial equilibrium $E_0 = (0, 0)$ of (3)
 (2) There exists a positive equilibrium $E_1 = (w_1, v_1)$ of (3) given by

$$E_1 = \left(\frac{m_v R_w}{2(1 - a_w)r_w} v_1, \frac{1}{k} (R_w - 1) \right),$$

if

$$R_w > 1. \quad (7)$$

3.2. Stability of equilibria

In this subsection, we study the stability of equilibria. We present the stability of the trivial equilibrium without the proof.

Theorem 3.2: *The trivial equilibrium E_0 of (3) has two real eigenvalues $R_w - 1$ and $-m_v$ and is locally asymptotically stable if*

$$R_w < 1 \quad (8)$$

and unstable if (7).

Next, we study the stability of the positive equilibrium. Let us assume that (7) holds. For the eigenvalues associated to the positive equilibrium, we introduce positive constants

$$\zeta_1 := m_v \left(2 - \frac{1}{R_w} \right) \text{ and } \zeta_2 := m_v \left(1 - \frac{1}{R_w} \right). \quad (9)$$

We prove the following result in Appendix A.

Theorem 3.3: *Let us assume that (7) holds. The positive equilibrium E_1 of (3) has two eigenvalues $\lambda_i, i = 1, 2$ where*

$$\lambda_{1,2} = \frac{1}{2} \left\{ -\zeta_1 \pm (\zeta_1^2 - 4\zeta_2)^{\frac{1}{2}} \right\} \quad (10)$$

and $\lambda_{1,2}$ lie in the left half plane. Hence, the positive equilibrium E_1 is locally asymptotically stable.

We have checked that for the two-compartment model, the stability of the positive equilibrium on its whole existence region can also be shown for division rates with *general* negative feedback. In this sense we could exclude the possibility of

oscillations for two-compartment models with more general negative feedback than discussed in this paper. For the three-compartment model, however, existence of such results is less obvious and beyond the scope of this paper. Since our objective is comparison of the two models, we then do not present the more general results for the two-compartment model either.

4. Stem cells and mature cells dynamics with progenitor cells

In this section, we consider the three-compartment model (4). We introduce *the reproduction number of progenitor cells* R_u in an analogous manner to R_w . R_u is given by

$$R_u = \frac{(2a_u - 1)r_u}{m_u}. \tag{11}$$

The combination of R_w and R_u characterizes the existence and stability of equilibria.

4.1. Existence of three possible equilibria

In this subsection, we consider existence of equilibria. The following theorem describes that (4) admits three possible equilibria, a trivial, no stem cell and a positive equilibrium. We omit the proof.

Theorem 4.1 :

- (1) *There always exists a trivial equilibrium $E_0 = (0, 0, 0)$ of (4).*
- (2) *There exists a no stem cell equilibrium $E_1 = (0, u_1, v_1)$ of (4) given by*

$$E_1 = \left(0, \frac{m_v R_u}{2(1 - a_u) r_u} v_1, \frac{1}{k} (R_u - 1) \right)$$

if

$$R_u > 1. \tag{12}$$

- (3) *There exists a positive equilibrium $E_2 = (w_2, u_2, v_2)$ of (4) given by*

$$E_2 = \left(\frac{m_u}{2(1 - a_w) r_w} (R_w - R_u) u_2, \frac{m_v R_w}{2(1 - a_u) r_u} v_2, \frac{1}{k} (R_w - 1) \right)$$

if

$$R_w > 1 \tag{13}$$

and

$$R_w > R_u. \tag{14}$$

4.2. Stability of equilibria

In this subsection, we study the stability of each equilibrium. We present the stability of the trivial equilibrium without the proof.

Theorem 4.2: *The trivial equilibrium E_0 of (4) has three real eigenvalues $R_w - 1$, $m_u(R_u - 1)$ and $-m_v$ and is locally asymptotically stable if*

$$\max\{R_u, R_w\} < 1 \quad (15)$$

and unstable if

$$\max\{R_u, R_w\} > 1. \quad (16)$$

Now, we study the stability of the no stem cell equilibrium. Let us assume that (12) holds. For the eigenvalues associated to the no stem cell equilibrium, we introduce positive constants

$$\zeta_1^u := m_v \left(2 - \frac{1}{R_u}\right) \text{ and } \zeta_2^u := m_v m_u \left(1 - \frac{1}{R_u}\right). \quad (17)$$

Then we prove the following theorem in Appendix A.

Theorem 4.3: *Let us assume that (12) holds. The no stem cell equilibrium E_1 of (4) has three eigenvalues λ_i , $i = 1, 2, 3$ where*

$$\lambda_1 = \frac{R_w}{R_u} - 1 \text{ and } \lambda_{2,3} = \frac{1}{2} \left\{ -\zeta_1^u \pm \left((\zeta_1^u)^2 - 4\zeta_2^u \right)^{\frac{1}{2}} \right\} \quad (18)$$

and $\lambda_{2,3}$ lie in the left half plane. The no stem cell equilibrium E_1 is locally asymptotically stable if

$$R_u > R_w \quad (19)$$

and unstable if (14).

Let us investigate the stability of the positive equilibrium. We assume that (13) holds and define

$$\alpha := (2a_u - 1)r_u \text{ and } \rho(a) := \frac{a}{R_w}, \quad a \in \mathbb{R}. \quad (20)$$

Then,

$$m_u > \max\{0, \rho(\alpha)\} \quad (21)$$

defines the existence region in a parameter space (α, m_u, m_v) as well as (α, m_u) . In Appendix B, we will construct the stability boundary in the parameter space (α, m_u, m_v) and represent it in terms of two functions $\xi_+(\alpha, m_v)$ and $\xi_-(\alpha, m_v)$. As the representation of $\xi_+(\alpha, m_v)$ and $\xi_-(\alpha, m_v)$ is somewhat complex and unintuitive, we here restrict ourselves to stating their existence and presenting some of their qualitative properties. To this aim, we introduce positive parameters

$$q := \frac{1 - \frac{1}{R_w}}{2 - \frac{1}{R_w}} \text{ and } \nu := \frac{q}{3 - \frac{2}{R_w}}$$

and functions

$$\delta_1(\alpha) := \frac{1}{2 - \frac{1}{R_w}} (q + \rho(\alpha)) \text{ for } \alpha \in (-qR_w, 0),$$

$$\delta_2(\alpha) := \nu \left(\sqrt{\rho(\alpha)} - 1 \right)^2 \text{ for } \alpha \in (0, R_w).$$

Note that δ_1 and δ_2 are positive on their respective domains. Next, in the (α, m_v) plane, we introduce the sets

$$\Omega := \{(\alpha, m_v) \mid \alpha \in \mathbb{R}, m_v \in (0, \infty)\}, \tag{22}$$

$$A_1 := \{(\alpha, m_v) \mid \alpha \in (-qR_w, 0), m_v \in (0, \delta_1(\alpha))\} \cup \{(0, m_v) \mid m_v \in (0, \nu)\}, \tag{23}$$

$$A_2 := \{(\alpha, m_v) \mid \alpha \in (0, R_w), m_v \in (0, \delta_2(\alpha))\}. \tag{24}$$

Here we note that $\lim_{\alpha \rightarrow 0^-} \delta_1(\alpha) = \frac{q}{2 - \frac{1}{R_w}} > \nu$. Then we prove the following proposition in Appendix B.

Proposition 4.4: *Let us assume that (13) holds. There exist two functions $\xi_-(\alpha, m_v)$ and $\xi_+(\alpha, m_v)$ such that*

(1) for $(\alpha, m_v) \in A_1$

$$\xi_+(\alpha, m_v) > 0, \tag{25}$$

(2) for $(\alpha, m_v) \in A_2$

$$\xi_+(\alpha, m_v) \geq \xi_-(\alpha, m_v) > \rho(\alpha) \tag{26}$$

and equality holds only for $m_v = \delta_2(\alpha)$.

Now we can determine the exact regions of stability and instability in the parameter space (α, m_u, m_v) in terms of the functions $\xi_-(\alpha, m_v)$ and $\xi_+(\alpha, m_v)$.

Theorem 4.5: *Let us assume that (13) holds.*

(1) For $(\alpha, m_v) \in \Omega \setminus (A_1 \cup A_2)$ the positive equilibrium E_2 is locally asymptotically stable if (21) holds.

(2) For $(\alpha, m_v) \in A_1$ the positive equilibrium E_2 is locally asymptotically stable if

$$m_u > \xi_+(\alpha, m_v) \tag{27}$$

and is unstable if

$$0 < m_u < \xi_+(\alpha, m_v). \tag{28}$$

(3) For $(\alpha, m_v) \in A_2$ the positive equilibrium E_2 is locally asymptotically stable if

$$m_u > \xi_+(\alpha, m_v) \text{ or } \xi_-(\alpha, m_v) > m_u > \rho(\alpha) \tag{29}$$

and is unstable if

$$\xi_-(\alpha, m_v) < m_u < \xi_+(\alpha, m_v). \tag{30}$$

It is shown that the positive equilibrium can be unstable, contrary to the two-compartment model. The instability region for the positive equilibrium shrinks as m_v increases.

Theorem 4.6: *Let us assume that (13) holds.*

(1) For $(\alpha, m_v) \in A_1$

$$\frac{\partial \xi_+(\alpha, m_v)}{\partial m_v} < 0. \quad (31)$$

(2) For $(\alpha, m_v) \in A_2$

$$\frac{\partial \xi_-(\alpha, m_v)}{\partial m_v} > 0 \text{ and } \frac{\partial \xi_+(\alpha, m_v)}{\partial m_v} < 0. \quad (32)$$

In Fig. 2, we show regions of stability and instability of the positive equilibrium and no stem cell equilibrium in the parameter space (α, m_v) for numerical examples including six different values of m_v . The figure shows how the instability region of the positive equilibrium shrinks as m_v increases as stated in the conclusion of Theorem 4.6.

5. Discussion

We have analysed two models of stem cell maturation that account for self-renewal, differentiation, cell death and regulation of cell division by the mature cell population. We have considered two approximations of the chain of maturation stages: one in which only stem cells and fully mature cells are taken into account (the two-compartment model (3)) and one in which we also consider an intermediate differentiation stage between stem cells and fully differentiated cells, the so-called progenitor cells, (the three-compartment model (4)). We have focused on the existence of equilibrium points and their local stability properties.

The extension of the concept of reproduction numbers, that is well known in ecology and epidemiology [7], facilitates our analysis. To our knowledge, this is the first time that the concept of reproduction number is used in the context of stem cell population dynamics. We have shown in Theorems 3.1 and 4.1 that the reproduction numbers of stem cells and progenitor cells can be used to characterise existence boundaries for equilibria and we will discuss in the following that these characterisations allow for interpretations of the boundaries.

Regarding the two-compartment model (3), we have found that a positive equilibrium exists when $R_w > 1$. To understand this, we introduce the *regulated* reproduction number of stem cells as

$$S_w(v) = \frac{R_w}{1 + kv}.$$

It is easy to see that $S_w(v) = 1$ defines the equilibrium condition for the mature cells. Since *regulation* means *reduction* of division, R_w should exceed $S_w(v)$ and thus $R_w > 1$. Furthermore, we have proved that the positive equilibrium is stable wherever it exists (see Theorem 3.3).

The behaviour exhibited by our three-compartment model is, expectedly, richer and more interesting. In this case we can distinguish two scenarios: $R_w < 1$ and $R_w > 1$. In the former case, for similar reasons as in the two-compartment model,

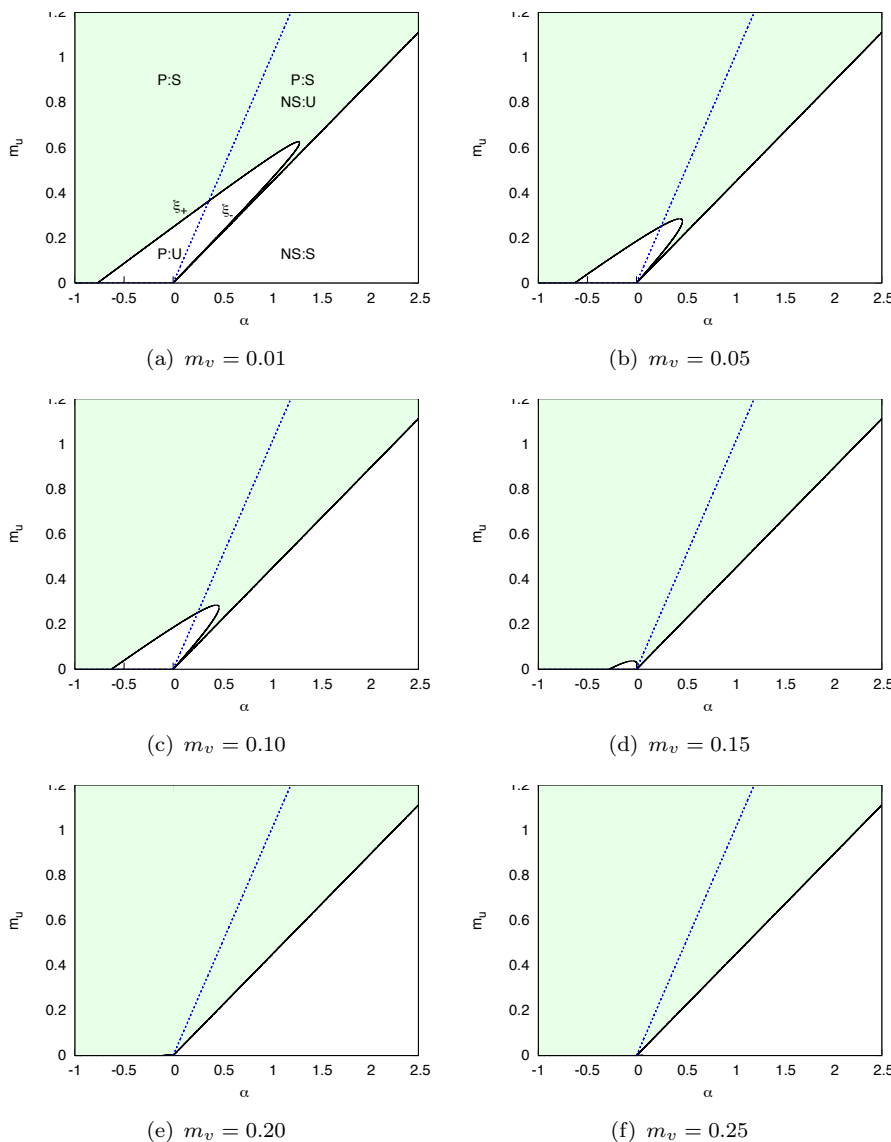


Figure 2. Stability region of the positive and no stem cell equilibrium for $R_w = 2.25$ in the parameter space (α, m_u) . The straight solid line and the dotted line represent $m_u = \rho(\alpha)$ and $m_u = \alpha$, that is, the existence boundary of the positive and no stem cell equilibrium, respectively. The curve represents $m_u = \xi_{\pm}(\alpha, m_v)$, that is, the stability boundary of the positive equilibrium. P:S and P:U denote the stability and instability region of the positive equilibrium, respectively and, similarly, NS:S and NS:U denote the stability and instability region of the no stem cell equilibrium, respectively.

there cannot be a positive equilibrium. However, a no stem cell equilibrium exists provided that $R_u > 1$, i.e. if the reproduction number of the progenitor cells is larger than one. If $R_w > 1$ a positive equilibrium may exist. Now we introduce the *regulated* reproduction number of progenitor cells:

$$S_u(v) = \frac{R_u}{1 + kv}.$$

The equilibrium condition for the mature cells is defined by $S_w(v) = 1$. On the other hand, $S_u(v) < 1$ holds at the equilibrium condition because if $S_u(v) \geq 1$ then, together with inflow of stem cells, the progenitor cells would certainly grow. Therefore, it follows that

$$S_w(v) > S_u(v),$$

i.e., in equilibrium progenitor cells should be less productive than the stem cells, as the former have additional inflow from the latter. Thus $R_w > R_u$ is necessary for the existence of the positive equilibrium.

The first interesting result concerning the positive equilibrium for the three-compartment model is related to its co-existence with the trivial equilibrium. Whenever in parameter space the positive equilibrium exists, the trivial equilibrium is unstable. This implies that, in biological terms, the cell population cannot be eradicated by de-stabilising the positive equilibrium.

Contrary to the behaviour observed in the two-compartment model, the stability region for the positive equilibrium is, generally speaking, smaller than its existence region (see Fig. 2). An interesting aspect regarding the instability region for the positive equilibrium is proved in Theorem 4.6: The instability region shrinks as the mortality rate of the mature cells, m_v increases. Theorem 4.6 and Fig. 2 show that the area of the instability region is maximal when $m_v \rightarrow 0$ and collapses to a point and eventually disappears as m_v increases. The physical rationale for this behaviour can be understood as follows. Consider a sufficiently small m_v . For a steady and finite v to be maintained the flux from the progenitor cell compartment into the mature cell compartment must vanish. This, in turn, implies that death and inflow within the progenitor cell compartment must exactly balance. On the other hand, as m_v increases, the steady and finite v can be sustained even when there is flow of population between the progenitor cell and the mature cell compartment, which removes the requirement that inflow and cell death are perfectly balanced in the progenitor cell compartment. It is clear that the region of the parameter space in which the former condition is satisfied is smaller than the region corresponding to the latter situation.

Another interesting result concerning the instability region of the positive equilibrium in our three-compartment model is that within this region no equilibrium, including the trivial equilibrium, is stable. This means that within that region the system either exhibits oscillations or unbounded growth. In the latter case, our model could thus be re-interpreted as a model for studying the emergence of malignancies such as cancer. Oscillations might also be considered in terms of pathological situations as it is the cases in several diseases of haematopoietic system [5, 6]. This is left as a subject for future research.

Regarding the two questions we posed in the Introduction, our two models indicate significant differences in the behaviour of the system as a function of the number of intermediate steps between stem and mature cells. Homeostasis can be reproduced by two- and three-compartment models (models with and without progenitor cells). However, instability can be found only in the three-compartment model. Therefore, the intermediate stage of differentiation is responsible for the emergence of an instability region. On the other hand, we have found that the no stem cell equilibrium is unstable whenever the positive equilibrium exists (see Theorem 4.3 and Fig. 2). Since the parameter region where the positive equilibrium exists can be regarded as the more realistic parameter region, our model seems to point out to a scenario in which a (steady) pool of stem cells is maintained throughout the lifetime of the organism.

Acknowledgement

The authors are very grateful to the anonymous referees for carefully reading and constructive comments which led to an improvement of the manuscript. AM-C was supported by ERC Starting Grant “Biostruct”, Emmy Noether Programme of German Research Council (DFG) and SFB 873 “Maintenance and Differentiation

of Stem Cells in Development and Disease”.

References

- [1] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter. *Molecular Biology of the Cell*. 4th Ed. Garland Science. New York, USA (2002)
- [2] M. Al-Hajj and M. F. Clarke. *Self-renewal and solid tumor stem cells*. *Oncogene* 23 (2004), pp 7274-7282.
- [3] O. Arino and M. Kimmel. *Stability analysis of models of cell production systems*. *Math. Modelling*. 7 (1986), pp 1269-1300.
- [4] P. A. Beachy, S. S. Karhadkar and D. M. Berman. *Tissue repair and stem cell renewal in carcinogenesis*. *Nature* 432 (2004), pp 324-331
- [5] C. Colijn, and M. C. Mackey. *A mathematical model of hematopoiesis: I. Periodic chronic myelogenous leukemia..* *J. Theor. Biol.* 237 (2) (2005), pp 117-132.
- [6] C. Colijn, and M. C. Mackey. *A mathematical model of hematopoiesis: II. Cyclical neutropenia..* *J. Theor. Biol.* 237 (2) (2005), pp 133-146.
- [7] K. Dietz and J. A. P. Heesterbeek. *The concept of R_0 in epidemic theory*. *Statistica neerlandica* 50 (1) (1996), pp 89-110.
- [8] G. Dontu, M. Al-Hajj, W. M. Abdallah, M.F. Clarke and M.S. Wicha. *Stem cells in normal breast development and breast cancer*. *Cell Prolif.* 36 (Supp 1) (2003), pp 59-72.
- [9] C. Foley, S. Bernard and M. C. Mackey. *Cost-effective G-CSF therapy strategies for cyclical neutropenia: Mathematical modelling based hypotheses*. *J. Theor. Biol.* 238 (2006), pp 754-763.
- [10] F. R. Gantmacher. *The Theory of Matrices*. Vol. 2, Chelsea, NewYork, 1959.
- [11] A. Marciniak-Czochra, T. Stiehl, W. Jaeger, A. Ho and W. Wagner. *Modelling asymmetric cell division in haemtopoietic stem cells - regulation of self-renewal is essential for efficient re-population*. *Stem Cells Dev.* 17 (2008), pp 1-10.
- [12] A. Marciniak-Czochra, T. Stiehl and W. Wagner. *Modeling of replicative senescence in hematopoietic development*. *Aging* 1 (2009), pp 723-732.
- [13] T. Stiehl and A. Marciniak-Czochra. *Characterization of stem cells using mathematical models of multistage cell lineages*. *Math. Comp. Modelling* (2010), doi:10.1016/j.mcm.2010.03.057
- [14] A. J. Wagers, J. L. Christensen and I. L. Weissman. *Cell fate determination from stem cells*. *Gene Ther.* 10 (2002), pp 606-612.
- [15] I. L. Weissman. *Stem cells: Units of development, units of regeneration, and units of evolution*. *Cell*. 100 (2000), pp 157-168.

Supplementary material: Stability analysis of multi-compartment models for cell production systems

Yukihiko Nakata, Philipp Getto, Anna Marciniak-Czochra and Tomás Alarcón

Appendix A. Proof of Theorems 3.3 and 4.3

In this subsection, we study the stability of the positive equilibrium for (3) and the no stem cell equilibrium for (3). We introduce the following

$$f_{w,1}(v) := (2a_w - 1) \frac{r_w}{1 + kv}, \quad f_{w,2}(v) := 2(1 - a_w) \frac{r_w}{1 + kv}, \quad (\text{A1})$$

$$f_{u,1}(v) := (2a_u - 1) \frac{r_u}{1 + kv}, \quad f_{u,2}(v) := 2(1 - a_u) \frac{r_u}{1 + kv}. \quad (\text{A2})$$

Proof of Theorem 3.3: For the positive equilibrium $E_1 = (w_1, v_1)$ we have

$$f_{w,1}(v_1) - 1 = 0 \quad \text{and} \quad w_1 = \frac{m_v v_1}{f_{w,2}(v_1)}.$$

By dropping the index from w_1 and v_1 , we obtain the characteristic equation:

$$0 = \lambda \left\{ \lambda + m_v \left(1 - \frac{f'_{w,2}(v)}{f_{w,2}(v)} v \right) \right\} - f'_{w,1}(v) m_v v. \quad (\text{A3})$$

By a direct calculation, it follows that

$$-\frac{f'_{w,2}(v)}{f_{w,2}(v)} v = -f'_{w,1}(v) v = 1 - \frac{1}{R_w},$$

and hence, (A3) becomes $0 = \lambda^2 + \zeta_1 \lambda + \zeta_2$. Then $\lambda_{1,2}$ are as stated in (10) and lie in the left half plane. \square

Proof of Theorem 4.3: Since we have

$$f_{u,1}(v_1) - m_u = 0 \quad \text{and} \quad u_1 = \frac{m_v v_1}{f_{u,2}(v_1)},$$

by dropping the index from u_1 and v_1 , we obtain the characteristic equation:

$$0 = \{ \lambda - (f_{w,1}(v) - 1) \} \left[\lambda \left\{ \lambda + m_v \left(1 - \frac{f'_{u,2}(v)}{f_{u,2}(v)} v \right) \right\} - f'_{u,1}(v) m_v v \right].$$

We obtain

$$\lambda_1 = f_{w,1}(v) - 1 = \frac{R_w}{R_u} - 1$$

from (A2) and $1 + kv = R_u$. By a direct calculation, it follows that

$$-\frac{f'_{u,2}(v)}{f_{u,2}(v)} v = 1 - \frac{1}{R_u} \quad \text{and} \quad -f'_{u,1}(v) v = f_{u,1}(v) \left(1 - \frac{1}{R_u} \right) = m_u \left(1 - \frac{1}{R_u} \right).$$

Then other roots are determined as $\lambda_{2,3}$ which lie in the left half plane, similar to the proof of Theorem 3.3. Therefore, the stability of the no stem cell equilibrium E_1 is determined by λ_1 and we obtain the conclusion. \square

Appendix B. Proof of Proposition 4.4 and Theorems 4.5 and 4.6

In this subsection, we prove Proposition 4.4 and Theorems 4.5 and 4.6.

Since we have

$$f_{w,1}(v_2) - 1 = 0, \quad u_2 = \frac{m_v v_2}{f_{u,2}(v_2)} \quad \text{and} \quad w_2 = \frac{(m_u - f_{u,1}(v_2)) m_v v_2}{f_{w,2}(v_2) f_{u,2}(v_2)},$$

by dropping the index from w_2 , u_2 and v_2 , we obtain the characteristic equation:

$$\begin{aligned} 0 = \lambda & \left[(\lambda - f_{u,1}(v) + m_u) \left\{ \lambda - m_v \left(\frac{f'_{u,2}(v)}{f_{u,2}(v)} v - 1 \right) \right\} \right. \\ & \left. - m_v v \left\{ f'_{u,1}(v) + (m_u - f_{u,1}(v)) \frac{f'_{w,2}(v)}{f_{w,2}(v)} \right\} \right] - f'_{w,1}(v) (m_u - f_{u,1}(v)) m_v v. \end{aligned} \quad (\text{B1})$$

By a direct calculation, we have the following relations:

$$\begin{aligned} -\frac{f'_{u,2}(v)}{f_{u,2}(v)} v &= -\frac{f'_{w,2}(v)}{f_{w,2}(v)} v = 1 - \frac{1}{R_w}, \\ -f'_{u,1}(v) v &= f_{u,1}(v) \left(1 - \frac{1}{R_w} \right), \\ -f'_{w,1}(v) v &= f_{w,1}(v) \left(1 - \frac{1}{R_w} \right) = 1 - \frac{1}{R_w}, \\ -f_{u,1}(v) + m_u &= m_u \left(1 - \frac{R_u}{1 + kv} \right) = m_u \left(1 - \frac{R_u}{R_w} \right) \end{aligned}$$

and then, (B1) becomes

$$\begin{aligned} 0 = \lambda & \left[\left\{ \lambda + m_u \left(1 - \frac{R_u}{R_w} \right) \right\} \left\{ \lambda + m_v \left(2 - \frac{1}{R_w} \right) \right\} + m_v m_u \left(1 - \frac{1}{R_w} \right) \right] \\ & + m_u m_v \left(1 - \frac{R_u}{R_w} \right) \left(1 - \frac{1}{R_w} \right). \end{aligned} \quad (\text{B2})$$

Now we rewrite the equation (B2) with using $m_u \frac{R_u}{R_w} = \rho(\alpha)$ as

$$0 = \lambda^3 + b_1 \lambda^2 + b_2 \lambda + b_3, \quad (\text{B3})$$

where

$$\begin{cases} b_1 &= m_u - \rho(\alpha) + \left(2 - \frac{1}{R_w} \right) m_v, \\ b_2 &= m_v \left\{ \left(3 - \frac{2}{R_w} \right) m_u - \left(2 - \frac{1}{R_w} \right) \rho(\alpha) \right\}, \\ b_3 &= m_v \left(1 - \frac{1}{R_w} \right) (m_u - \rho(\alpha)). \end{cases} \quad (\text{B4})$$

By the Routh–Hurwitz theorem (see [10]) for (B3), all roots have negative real parts if and only if $b_1 > 0$, $b_3 > 0$ and $b_1 b_2 - b_3 > 0$. We see that b_1 and b_3 are positive, since we have $R_w > 1$ and (21). Therefore, we focus only on the sign of $b_1 b_2 - b_3$.

Let us introduce the functions

$$\begin{cases} \varphi_1(x, y) &= -x - \left(\frac{2 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} x + \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} \right) + \left(2 - \frac{1}{R_w} \right) y, \\ \varphi_2(x, y) &= x \left\{ \left(\frac{2 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} x + \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} \right) - \frac{\left(2 - \frac{1}{R_w} \right)^2}{3 - \frac{2}{R_w}} y \right\}. \end{cases} \quad (\text{B5})$$

From (B4) we have

$$b_1 b_2 - b_3 = m_v \left(3 - \frac{2}{R_w} \right) \{ m_u^2 + \varphi_1(\rho(\alpha), m_v) m_u + \varphi_2(\rho(\alpha), m_v) \}. \quad (\text{B6})$$

Hence, we consider (B6) under (21). Now we transform variables as

$$x = \rho(\alpha) \quad \text{and} \quad y = m_v \quad (\text{B7})$$

and then introduce a parameter set

$$\Omega_x := \{(x, y) \mid x \in \mathbb{R}, y \in (0, \infty)\}.$$

Consequently, we consider the set of quadratic polynomials

$$F_{(x,y)}(m_u) = m_u^2 + \varphi_1(x, y) m_u + \varphi_2(x, y), \quad \text{for } (x, y) \in \Omega_x \quad (\text{B8})$$

under

$$m_u > \max\{0, x\}. \quad (\text{B9})$$

At first, we determine the existence of real roots of

$$F_{(x,y)}(m_u) = 0. \quad (\text{B10})$$

We define functions

$$D(x, y) := \varphi_1(x, y)^2 - 4\varphi_2(x, y) \quad \text{for } (x, y) \in \Omega_x \quad (\text{B11})$$

and for $x > 0$

$$\eta_2(x) := (\sqrt{x} - 1)^2 \nu \quad \text{and} \quad \eta_3(x) := (\sqrt{x} + 1)^2 \nu.$$

We introduce a set

$$B_d := \{(x, y) \mid x > 0, y \in (\eta_2(x), \eta_3(x))\}.$$

In the following lemma, we show that (B10) admits no real roots for $(x, y) \in B_d$ and one or two real roots for $(x, y) \in \Omega_x \setminus B_d$.

Lemma B.1: *The following holds.*

- (1) $D(x, y) < 0$ if and only if $(x, y) \in B_d$.
- (2) $D(x, y) \geq 0$ if and only if $(x, y) \in \Omega_x \setminus B_d$. In particular, $D(x, y) = 0$ if and only if $y = \eta_j(x)$, $j = 2, 3$.

Moreover, for $(x, y) \in \Omega_x \setminus B_d$ there exist one or two real roots of (B10) given by

$$\gamma_{\pm}(x, y) := \frac{1}{2} \left\{ -\varphi_1(x, y) \pm \sqrt{D(x, y)} \right\} \tag{B12}$$

with $\gamma_-(x, y) = \gamma_+(x, y)$ for $y = \eta_j(x)$, $j = 2, 3$.

Proof: We study the sign of $D(x, y)$. For simplicity, we put

$$\bar{x} = \frac{2 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} x + \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}}.$$

From (B5), we obtain

$$\begin{aligned} D(x, y) &= \left(2 - \frac{1}{R_w}\right)^2 y^2 + \left\{ 2(-x - \bar{x}) + 4x \frac{2 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} \right\} \left(2 - \frac{1}{R_w}\right) y + (x - \bar{x})^2 \\ &= \left(2 - \frac{1}{R_w}\right)^2 y^2 - 2(x + 1) \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} \left(2 - \frac{1}{R_w}\right) y + \left\{ (x - 1) \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} \right\}^2 \\ &= \left(2 - \frac{1}{R_w}\right)^2 \left[\{y + (x - 1)\nu\}^2 - 4\nu xy \right]. \end{aligned} \tag{B13}$$

Therefore, it follows that

$$D(x, y) > 0 \text{ for } (x, y) \in \{(x, y) \mid x < 0, y > 0\}. \tag{B14}$$

Let us consider the case $x \geq 0$. From (B13), we obtain

$$D(x, y) = \left(2 - \frac{1}{R_w}\right)^2 (y - \eta_2(x))(y - \eta_3(x)), \quad x \geq 0.$$

Therefore, we see

$$D(x, y) \begin{cases} < 0 & \text{for } (x, y) \in B_d, \\ \geq 0 & \text{for } (x, y) \in \{(x, y) \mid x \geq 0, y > 0\} \setminus B_d, \end{cases} \tag{B15}$$

Now we easily obtain the formula of the roots as (B12), since (B10) is a quadratic polynomial. Then, from (B14) and (B15), we obtain the conclusion of the lemma. We also see that $D(x, y) = 0$ if and only if either $y = \eta_2(x)$ or $y = \eta_3(x)$ holds. Hence, the proof is complete. \square

Next we study the polynomial $F_{(x,y)}(m_u)$ to determine the sign.

Lemma B.2:

- (1) For $x < 0$ the following hold.
 - a) If $\varphi_2(x, y) \geq 0$, then $F_{(x,y)}(m_u) > 0$ for any $m_u > 0$.

b) If $\varphi_2(x, y) < 0$,

$$\gamma_+(x, y) > 0. \quad (\text{B16})$$

(2) For $x = 0$ the following hold.

a) If $\varphi_1(0, y) \geq 0$, then $F_{(0,y)}(m_u) > 0$ for any $m_u > 0$.

b) If $\varphi_1(0, y) < 0$, then (B16) holds.

(3) For $x > 0$ the following hold.

a) If either $2x + \varphi_1(x, y) \geq 0$ or $(x, y) \in B_d$ holds, then $F_{(x,y)}(m_u) > 0$ for any $m_u > x$.

b) If $2x + \varphi_1(x, y) < 0$ and $(x, y) \in \Omega_x \setminus B_d$, then

$$\gamma_+(x, y) \geq \gamma_-(x, y) > x. \quad (\text{B17})$$

Proof: (1) a) By substituting $m_u = 0$ in (B8), we obtain $F_{(x,y)}(0) = \varphi_2(x, y)$. We claim that if $\varphi_2(x, y) \geq 0$ then $F'_{(x,y)}(0) > 0$ for $x < 0$. Since $F_{(x,y)}(m_u)$ is a quadratic polynomial with the positive coefficient of m_u^2 , this claim shows that if $\varphi_2(x, y) \geq 0$ then $F_{(x,y)}(m_u)$ is monotone increasing for $m_u > 0$ with $F_{(x,y)}(0) \geq 0$ and hence the conclusion holds.

We show that the claim holds. From (B5) $\varphi_2(x, y) \geq 0$ implies

$$\frac{2 - \frac{1}{R_w}}{3 - \frac{2}{R_w}}x + \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} \leq \frac{\left(2 - \frac{1}{R_w}\right)^2}{3 - \frac{2}{R_w}}y. \quad (\text{B18})$$

Then, by (B5) and (B8) it follows that

$$F'_{(x,y)}(0) = \varphi_1(x, y) \geq -x + \left(2 - \frac{1}{R_w}\right) \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}}y > 0.$$

Hence, the claim holds and we obtain the conclusion. b) There exist one or two real roots, $\gamma_-(x, y)$ and $\gamma_+(x, y)$, of (B10) by Lemma B.1. Since we have $F_{(x,y)}(0) = \varphi_2(x, y)$, $\varphi_2(x, y) < 0$ implies that

$$\gamma_-(x, y) < 0 < \gamma_+(x, y)$$

holds and hence (B16) follows.

(2) a) By substituting $m_u = 0$ in (B8), we have

$$F_{(0,y)}(0) = \varphi_2(0, y) = 0 \quad (\text{B19})$$

and $F'_{(0,y)}(0) = \varphi_1(0, y)$. Therefore, $\varphi_1(0, y) \geq 0$ implies that $F_{(0,y)}(m_u)$ is monotone increasing for $m_u > 0$ with $F_{(0,y)}(0) = 0$. Hence, we obtain the conclusion.

b) By Lemma B.1 we have that $\gamma_-(x, y)$ and $\gamma_+(x, y)$, which are the roots of (B10), are real. From (B19), one of the roots is given by 0. On the other hand, since $F'_{(0,y)}(0) = \varphi_1(0, y)$, $\varphi_1(0, y) < 0$ and $F_{(0,y)}(0) = 0$ imply that other root is positive. Thus, we obtain (B16).

(3) a) Since $F_{(x,y)}(m_u)$ is a quadratic polynomial with the positive coefficient of m_u^2 and $F'_{(x,y)}(x) = 2x + \varphi_1(x, y)$, $2x + \varphi_1(x, y) \geq 0$ implies that $F_{(x,y)}(m_u)$ is monotone increasing for $m_u > x$. On the other hand, $(x, y) \in B_d$ implies $D(x, y) < 0$

by Lemma B.1 and, hence, that there exists no real roots of (B10). Therefore, if $F_{(x,y)}(x) > 0$ then, we immediately obtain the conclusion. Now we see

$$F_{(x,y)}(x) = \left(2 - \frac{1}{R_w}\right) \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} xy > 0, \quad (\text{B20})$$

by substituting $m_u = x$ in (B8). Hence, we obtain the conclusion. b) By Lemma B.1, we have $D(x, y) \geq 0$ for $(x, y) \in \Omega_x \setminus B_d$ and, hence, that there exist one or two real roots, $\gamma_-(x, y)$ and $\gamma_+(x, y)$, of (B10). Since $F'_{(x,y)}(x) = 2x + \varphi_1(x, y)$ holds, $2x + \varphi_1(x, y) < 0$ and (B20) imply (B17).

Hence, the proof is complete. \square

Let us identify the parameter space in Ω_x which ensures each conditions in Lemma B.2. We introduce a function

$$\eta_1(x) := \frac{1}{2 - \frac{1}{R_w}}(q + x) \text{ for } x \in (-q, 0)$$

In the (x, y) plane we introduce the sets (cf. (23) and (24))

$$\begin{aligned} B_1 &:= \{(x, y) \mid x \in (-q, 0), y \in (0, \eta_1(x))\} \cup \{(0, y) \mid y \in (0, \nu)\}, \\ B_2 &:= \{(x, y) \mid x \in (0, 1), y \in (0, \eta_2(x))\}. \end{aligned}$$

Note that η_1 is positive on the domains and $\lim_{x \rightarrow 0^-} \eta_1(x) > \nu$.

Lemma B.3:

- (1) If $(x, y) \in \Omega_x \setminus (B_1 \cup B_2)$ then $F_{(x,y)}(m_u) > 0$ for any $m_u > \max\{0, x\}$.
- (2) If $(x, y) \in B_1$ then (B16) and

$$F_{(x,y)}(m_u) \begin{cases} < 0 & \text{for } m_u < \gamma_+(x, y), \\ > 0 & \text{for } m_u > \gamma_+(x, y). \end{cases} \quad (\text{B21})$$

hold.

- (3) If $(x, y) \in B_2$ then (B17) and

$$F_{(x,y)}(m_u) \begin{cases} > 0 & \text{for } m_u > \gamma_+(x, y) \text{ or } \gamma_-(x, y) > m_u > x, \\ < 0 & \text{for } \gamma_-(x, y) < m_u < \gamma_+(x, y), \end{cases} \quad (\text{B22})$$

hold.

Proof: (1) At first, we divide the set $\Omega_x \setminus (B_1 \cup B_2)$ into three sets

$$\begin{aligned} L_1 &:= \{(x, y) \mid y \geq \eta_1(x), x \in (-q, 0)\} \cup \{(x, y) \mid x \leq -q\}, \\ L_2 &:= \{(x, y) \mid y \geq \nu, x = 0\}, \\ L_3 &:= \{(x, y) \mid y > \eta_2(x), x \in (0, 1)\} \cup \{(x, y) \mid x \geq 1\}, \end{aligned}$$

and verify the conditions of Lemma B.2 for $(x, y) \in L_j, j = 1, 2, 3$.

For $(x, y) \in L_1$, we have $x < 0$ and

$$\frac{2 - \frac{1}{R_w}}{3 - \frac{2}{R_w}}x + \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} - \frac{\left(2 - \frac{1}{R_w}\right)^2}{3 - \frac{2}{R_w}}y \leq 0.$$

Hence, $\varphi_2(x, y) \geq 0$ holds.

For $(x, y) \in L_2$, it holds

$$\varphi_1(0, y) = -\frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} + \left(2 - \frac{1}{R_w}\right)y = \left(2 - \frac{1}{R_w}\right)(y - \nu) \geq 0.$$

Let us consider the case $(x, y) \in L_3$. Since it holds that

$$2x + \varphi_1(x, y) = \frac{1 - \frac{1}{R_w}}{3 - \frac{2}{R_w}}(x - 1) + \left(2 - \frac{1}{R_w}\right)y, \quad (\text{B23})$$

$2x + \varphi_1(x, y) \geq 0$ holds for

$$(x, y) \in L_{31} := \{(x, y) | y \geq \nu(1 - x), x \in (0, 1)\} \cup \{(x, y) | x \geq 1\}.$$

Since it holds that

$$\eta_2(x) < \nu(1 - x) < \eta_3(x) \text{ for } x \in (0, 1),$$

we obtain that $L_3 = L_{31} \cup B_d$.

(2) For $(x, y) \in \{(x, y) | x \in (-q, 0), y \in (0, \eta_1(x))\}$ we obtain $\varphi_2(x, y) < 0$ from the above discussion. For $(x, y) \in \{(0, y) | y \in (0, \nu)\}$, we also see that $\varphi_1(0, y) < 0$.

(3) For $(x, y) \in B_2$, we obtain $2x + \varphi_1(x, y) < 0$ and $(x, y) \in \Omega_x \setminus B_d$. \square

Finally, by using the transformation (B7) again, from Lemma B.3, we obtain the roots of (B10) given by

$$\gamma_{\pm}(\rho(\alpha), m_v) \text{ for } (\alpha, m_v) \in A_1 \cup A_2, \quad (\text{B24})$$

where A_1 and A_2 are defined by (23) and (24), respectively. Then, we can define the functions

$$\xi_{\pm}(\alpha, m_v) := \gamma_{\pm}(\rho(\alpha), m_v) \text{ for } (\alpha, m_v) \in A_1 \cup A_2. \quad (\text{B25})$$

We prove Proposition 4.4.

Proof of Proposition 4.4: By (B25), it is obvious that there exist $\xi_{\pm}(\alpha, m_v)$ for $(\alpha, m_v) \in A_1 \cup A_2$. From Lemma B.3 and (B16) and (B17) in Lemma B.2, we obtain (25) and (26), respectively. Since $\{(\alpha, m_v) | m_v = \delta_3(\alpha)\} \not\subset A_2$, $\xi_+(\alpha, m_v) = \xi_-(\alpha, m_v)$ holds only for $m_v = \delta_2(\alpha)$ by Lemma B.1. Hence the proof is complete. \square

Proof of Theorem 4.5: By the Routh–Hurwitz theorem (see [10]) for (B3), all roots have negative real parts if and only if $b_1 > 0$, $b_3 > 0$ and $b_1b_2 - b_3 > 0$. We have $b_1 > 0$ and $b_3 > 0$ from (B4). By Lemma B3, we obtain the sign of $b_1b_2 - b_3$ for any $(\alpha, m_v) \in \Omega$ after the transformation of variables (B7) again. Hence, the proof is complete. \square

Proof of Theorem 4.6: (1) Let us assume that $(x, y) \in B_1$. We consider $\gamma_+(x, y)$ and show that

$$\frac{\gamma_+(x, y + \epsilon) - \gamma_+(x, y)}{\epsilon} < 0 \quad (\text{B26})$$

for any $\epsilon > 0$ such that $(x, y + \epsilon) \in B_1$. To show that (B26), we substitute $m_u = \gamma_+(x, y + \epsilon)$ in $F_{(x,y)}(m_u)$ and then consider the sign. If the sign is negative, then (B26) holds, since $F_{(x,y)}(m_u)$ is a quadratic polynomial with positive coefficient of m_u^2 and $F_{(x,y)}(m_u) = 0$ with $m_u = \gamma_+(x, y)$.

From (B5), it follows

$$\varphi_1(x, y) = \varphi_1(x, y + \epsilon) - \left(2 - \frac{1}{R_w}\right) \epsilon \text{ and } \varphi_2(x, y) = \varphi_2(x, y + \epsilon) + \frac{\left(2 - \frac{1}{R_w}\right)^2}{3 - \frac{2}{R_w}} x \epsilon.$$

Therefore, by using $F_{(x,y+\epsilon)}(\gamma_+(x, y + \epsilon)) = 0$, we obtain

$$F_{(x,y)}(\gamma_+(x, y + \epsilon)) = \epsilon \left(2 - \frac{1}{R_w}\right) \left(\frac{2 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} x - \gamma_+(x, y + \epsilon)\right) < 0, \quad (\text{B27})$$

since we have $x \leq 0$ and $\gamma_+(x, y + \epsilon) > 0$ by Lemmas B.2 and B.3. This shows (B26) holds.

(2) Let us assume that $(x, y) \in B_2$. Similar to (1), we consider $\gamma_{\pm}(x, y)$ and show that

$$\frac{\gamma_-(x, y + \epsilon) - \gamma_-(x, y)}{\epsilon} > 0 \text{ and } \frac{\gamma_+(x, y + \epsilon) - \gamma_+(x, y)}{\epsilon} < 0 \quad (\text{B28})$$

for any $\epsilon > 0$ such that $(x, y + \epsilon) \in B_2$. To show that (B28), we substitute $m_u = \gamma_{\pm}(x, y + \epsilon)$ in $F_{(x,y)}(m_u)$. We obtain (B27) and

$$F_{(x,y)}(\gamma_-(x, y + \epsilon)) = \left(2 - \frac{1}{R_w}\right) \epsilon \left(\frac{2 - \frac{1}{R_w}}{3 - \frac{2}{R_w}} x - \gamma_-(x, y + \epsilon)\right) < 0$$

since $x < \gamma_-(x, y + \epsilon)$ by Lemmas B.2 and B.3. Hence, (B28) holds and the proof is complete. \square