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Mathematical modelling of stem cell differentiation: the PU.1–GATA-1 interaction

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Abstract The transcription factors PU.1 and GATA-1 are known to be important in the development of blood progenitor cells. Specifically they are thought to regulate the differentiation of progenitor cells into the granulocyte/macrophage lineage and the erythrocyte/megakaryocite lineage. While several mathematical models have been proposed to investigate the interaction between the transcription factors in recent years, there is still debate about the nature of the progenitor state in the dynamical system, and whether the existing models adequately capture new knowledge about the interactions gleaned from experimental data. Further, the models utilise different formalisms to represent the genetic regulation, and it appears that the resulting dynamical system depends upon which formalism is adopted. In this paper we analyse the four existing models, and propose an alternative model which is shown to demonstrate a rich variety of dynamical systems behaviours found across the existing models, including both bistability and tristability required for modelling the undifferentiated progenitors.

Keywords Bifurcation · Attractor · Auto-regulation · Gene regulatory network · Lineage specification

Mathematics Subject Classification (2000) 37M20 · 37N25 · 92B05

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1 Introduction

A stem cell is a cell that has the ability to continuously divide and differentiate (develop) into various other kinds of cells/tissues. A related concept is that of a progenitor cell that can differentiate into any of several different cell types. During development, the decision to leave the progenitor state, and the selection of a differentiation pathway, are regulated by transcription factors. The two transcription factors PU.1 and GATA-1 regulate the differentiation of a particular branch of blood cells. In the erythrocyte/megakaryocite lineage high levels of GATA-1 and low levels of PU.1 are detected (Shivdasani and Orkin 1996; Akashi et al. 2000). Conversely, in the granulocyte/macrophage lineage higher levels of PU.1 are found (Shivdasani and Orkin 1996). The initial progenitor state has low level activation of both PU.1 and GATA-1 genes. This initial state is referred to as an indeterminate state in this study. Thus the understanding of how PU.1 and GATA-1 interact is important in the study of this differentiation process, and accurate modelling of the process promises opportunities to control stem cell development for significant therapeutic benefits.

It is believed that both PU.1 and GATA-1 'autoregulate' themselves, i.e. they stimulate their own production. They are also mutually antagonistic, i.e. they repress the production of each other (Nishimura et al. 2000; Okuno et al. 2005; Liew et al. 2006). As a progenitor cell differentiates it transitions from an initial indeterminate state into one of two differentiated states. The important aspects that any mathematical model of PU.1 and GATA-1 must include are therefore: an indeterminate state; differentiated lineages represented as two stable attractors of the dynamical system; and modelling of the autoregulation and mutual antagonism between the transcription factors. The gradual changes in gene expression levels arise from the production of transcription factors, a process involving transcription, transportation and translation. Since these processes typically occur on a much faster time scale compared to the rate of change of gene expression levels, the various stages of transcription factor production are usually not modelled individually, and are considered as a single process.

Within these broad considerations, a number of different dynamical system models have been developed using different formalisms. The Shea–Ackers formalism (Shea and Ackers 1985) is a thermodynamic approach to represent the gene expression based on the structure of transcription machinery. In the case of a single transcriptional factor, the Hill function has been widely used as a candidate to represent the binding of transcriptional factors in the form of multimers, though this function has also been commonly used to describe the steep sigmoidal signal response from the input/substrate. In particular, the Michaelis–Menten function is a special case of the Hill function when the Hill coefficient is one. The first attempt at mathematically modelling the interaction was made by Roeder and Glauche (2006) who used the Shea–Ackers formalism, described in the next section, to represent positive and negative regulation. This model successfully realized bistability and an indeterminate state. In mathematical modelling, the indeterminate state means a state which is located in the middle of the two bistable states. After the introduction of an appropriate impulse, the indeterminate state may switch to one of the bistable states. The next model was proposed by Huang et al. (2007) using the Hill function formalism with high cooperativity Hill coefficients. Huang et al.'s model was able to be qualitatively compared to

experimental evidence and showed remarkable agreement, giving support to the idea that lineage choice occurs as a two stage process, first priming and then differentiating. These two models required high cooperativity to display bistable behaviour. However, the release of experimental results by Liew et al. (2006) revealed new knowledge about how mutual regulation of PU.1 and GATA-1 occurs, involving a simple heterodimeric repressive binding between the transcription factors.

In response to this experimental discovery, Chickarmane et al. (2009) published a revised set of equations based on the Shea–Ackers formalism and the assumption that the autoregulation at both PU.1 and GATA-1 occurs through the binding of monomers. Since the resulting dynamical system was not able to attain a bistable state, which is a requirement for modelling differentiation, the authors concluded that an additional mechanism must be involved in the repressive interaction to create a bistable switch. They introduced a third unspecified gene X, and showed that the triple system of differential equations created bistability. Chickarmane et al.'s model also offered insight into the possibility for how an indeterminate state can occur and also gave predictions that feedback from downstream transcription factors can play an important role in irreversibility. However, the introduction of an unknown gene X, simply to produce bistability, is somewhat unsatisfying. An alternative model proposed by Bokes et al. (2009) extended the work of Huang et al. (2007) based on the Hill function formalism, but ensured low cooperativity. This model made some assumptions that saw the mutual antagonism modelled as a competition for free GATA1 and PU.1.

In this paper, these four existing models are reviewed and analysed first. An alternative approach then is proposed based on the Hill function formalism. The proposed model negates the need for any unknown genes to be added as per the Chickarmane et al. (2009) model and has not the assumptions made by Bokes et al. (2009) to achieve both bistability and tristability for various parameter combinations. By separating the strength of cooperativity for autoregulation and repression, we provide a model that enables the effect of various parameters to be more readily explored, and will provide a longer-surviving model as new experimental data comes to hand. The remainder of this paper is organized as follows: Sect. 2 reviews the four existing models by discussing the assumptions made in the model and the resulting network dynamics. Our new model is proposed in Sect. 3. The bifurcation analysis is carried out in Sect. 4. The simulated system dynamics is presented in Sect. 5.

2 Comparative analysis of existing models

2.1 The first model using the Shea–Ackers formalism

Roeder and Glauche (2006) proposed the first mathematical model to study the interactive regulation of genes PU.1 and GATA-1. It was assumed that both PU.1 and GATA-1 are autoregulatory through the binding of homo-dimers and these homodimers activate the expression of genes PU.1 and GATA-1. The concentrations of homodimers are maintained at equilibrium levels via the instantaneous dimer formation and dissolution. In addition, the mutual antagonism between PU.1 and GATA-1 occurs through the formation of heterodimers which not only reduce the availability of

PU.1 and GATA-1 to form homodimers but also decrease the expression of PU.1 and GATA-1. In this model, monomeric bindings were not considered and time delays in transcription, transportation and translation are ignored. Other transcriptional factors in the transcription machinery were excluded from the model for simplicity.

By taking the symmetric system only, and normalising the model variables, Roeder and Glauche proposed a simplified equation model, given by

$$\begin{aligned} \frac{d[G]}{dt} &= \frac{s[G]^2 + uk_u[P]^2}{1 + [G]^2 + k_u[P]^2 + k_r[G][P]} - [G] \\ \frac{d[P]}{dt} &= \frac{s[P]^2 + uk_u[G]^2}{1 + [P]^2 + k_u[G]^2 + k_r[G][P]} - [P], \end{aligned} \tag{1}$$

where $[G]$ and $[P]$ are normalized concentrations of the expression levels of gene GATA-1 and PU.1, respectively.

Depending on the strengths of the specific regulation (s) and unspecific regulation (u), Roeder and Glauche (2006) demonstrated that system (1) has one, two or three stable steady states. Since experiments determined that the heterodimer of GATA-1 and PU.1 inhibits the production of PU.1 directly, but GATA-1 only indirectly (Zhang et al. 2000), Roeder and Glauche introduced an asymmetry into their system by allowing $k_{rP} > 0$. The introduced asymmetry did not change the number of stable steady states but varied the locations of the stable points. In addition, the study of the switch-like behaviour of the system showed that, once the system reaches a steady state at which the expression level of one gene is higher than that of the other, a large change in concentration is required to destabilise the system and move it to the other state. This suggests that it is possible to force the differentiated cells to follow a different lineage if the concentrations of transcription factors are changed sufficiently. In addition, this research also provided interesting insights into the nature of the indeterminate state, a balanced low level co-expression state of transcriptional factors. However, the decrease in the concentration of GATA-1 following the impulse is inconsistent with the experimental results of several research works including Zhang et al. (2000). This may be due to the lack of a continuously modulated set of cooperative lineage-inherent transcriptional factors, which change with the state of differentiation, in the proposed mathematical model (Roeder and Glauche 2006) In summary, this first mathematical model of the PU.1–GATA-1 network offers interesting insights into the nature of bistability, the switch-like behaviour and the possibility of a two-stage priming system.

2.2 Model using summed Hill formalism

Huang et al. (2007) reformulated the interaction between genes PU.1 and GATA-1 using summed Hill functions with the same Hill coefficients. Their description was intended to provide a mathematical model on a coarser level than Roeder and Glauche (2006), ignoring the specific molecular details and exploring the general model. It was assumed that the production of both PU.1 and GATA-1 is autoregulatory and genes PU.1 and GATA-1 inhibit the production of each other. The proposed mathematical

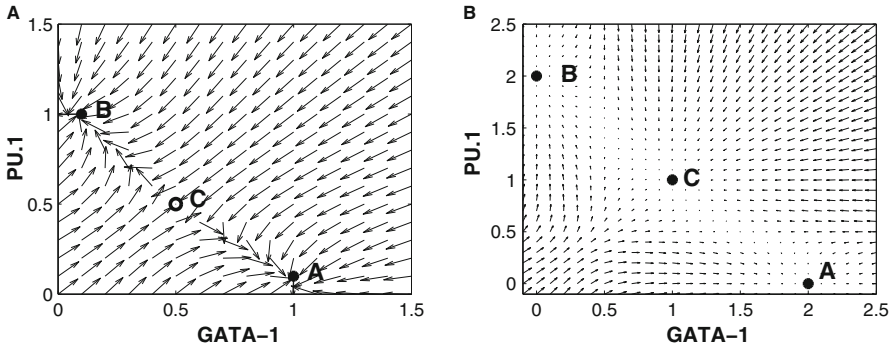

Fig. 1 Typical vector diagrams of system (2). **a** System without autoregulation ($a_1 = a_2 = a = 0$). **b** System with autoregulation ($a = 1$). *Black solid circles* (A, B in **a** and A–C in **b**) are steady states and attractors, and the *empty circle* (C in **a**) is a saddle point. Other parameters of the system are $n = 4, k_1 = k_2 = 1, \theta = 0.5, b_1 = b_2 = 1$

model is given by

$$\begin{aligned}\frac{d[G]}{dt} &= a_1\frac{[G]^n}{\theta_{a1}^n + [G]^n} + b_1\frac{\theta_{b1}^n}{\theta_{b1}^n + [P]^n} - k_1[G]\\ \frac{d[P]}{dt} &= a_2\frac{[P]^n}{\theta_{a2}^n + [P]^n} + b_2\frac{\theta_{b2}^n}{\theta_{b2}^n + [G]^n} - k_2[P].\end{aligned} \tag{2}$$

The system was modelled both with and without the autoregulatory aspect ($a_1 = a_2 = 0$); and the autoregulation was found to confer stability to the progenitor state. The parameter space was explored through a combination of matrices of cell paths and bifurcation diagrams. It was found that the bistability of the system depends on the relative value of b or k to that of a (Huang et al. 2007).

Similar to the model (1) proposed by Roeder and Glauche (2006), the non-diagonal stable points (i.e. $[G] \neq [P]$) of system (2) were associated with the differentiated cells, with point A in Fig. 1 representing the erythrocyte lineage and point B the myeloid lineage. The middle stable point C in Fig. 1b when the system has autoregulation was identified with the progenitor state and two methods of differentiation were investigated. One explanation for differentiation was that an external factor, such as a myeloid-stimulating agent that may inhibit GATA1 activation by lowering a_1 or increasing k_1 to induce myeloid differentiation (Huang et al. 2007), causes the system to be disrupted in a non-symmetric way. In this scenario, the cell is almost certain to go to state B. The second explanation involved an external signal destabilising state C in Fig. 1b allowing the cell to differentiate. In this scenario the cell could go either way but allows external influences to easily guide the cell to a required lineage.

In addition, two types of bifurcation were found to occur as the parameters were changed symmetrically, namely the super-critical bifurcation (Type I) and sub-critical pitchfork bifurcation (Type II). It was found that a type I bifurcation in Fig. 2a which led to a loop in the myeloid path always generated a counter-clockwise loop, while a type II bifurcation in Fig. 2b generated a clockwise loop. This qualitative difference

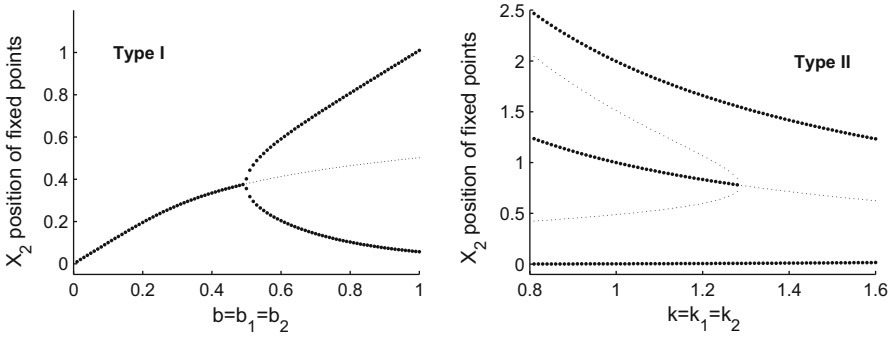

Fig. 2 Types of symmetric bifurcations in the system (2). **a** Super-critical bifurcation (Type I). The system has one single stable steady state when $b \in [0, 0.49]$. When $b \geq 0.5$, it has two stable and one unstable steady states. Model parameters are $n = 4$, $k = 1$, $\theta = 0.5$ and $a = 0.01$. **b** Sub-critical bifurcation (Type II). The system has three stable and two unstable steady states when $k \in [0.8, 1.3]$. When $k \geq 1.3$, it has two stable and one unstable steady states. Model parameters are $n = 4$, $b = 1$, $\theta = 0.5$ and $a = 1$. (*Solid dot line* stable steady states, *dot line* unstable steady states)

allowed the comparison with experimental data without curve fitting, which is difficult in such an open system. The experimental results always showed counterclockwise trajectories in the myeloid loops supporting the type II bifurcation. The concentrations of both PU.1 and GATA-1 were experimentally found to vary together rather than one increasing and the other decreasing as would be expected if differentiation was induced by a non-symmetric change. This lends support to a two stage differentiation whereby the central fixed point is first destabilised, then small external influences can easily alter the fate of a cell. This is the only model whose simulation results were compared with experimental results with good quantitative agreement.

2.3 Model with a master regulator gene

Experimental results showed that the mutual antagonism between PU.1 and GATA-1 seemed to be due to the PU.1–GATA-1 heterodimer (Liew et al. 2006). In addition, there was no experimental evidence to support the autoregulation of PU.1 or GATA-1 by dimers or higher order multimers. Based on these experimental results, it was hypothesized that the assumption in the previous models (1, 2) with high cooperativity might not be correct (Chickarmane et al. 2009). Therefore it was assumed that the production of both PU.1 and GATA-1 is autoregulatory through monomers only and PU.1 and GATA-1 inhibit the production of each other through a PU.1–GATA-1 dimer. However, a mathematical model based on these assumptions failed to realize bistability since the system has only one single stable steady state. To realize bistability, it was assumed the existence of a gene X which is activated by GATA-1 and its product (protein X) inhibits the expression of gene PU.1 (Chickarmane et al. 2009). This assumption led to the following system of differential equations

$$\frac{d[G]}{dt} = \frac{\alpha_1 A + \alpha_2[G]}{1 + \beta_1 A + \beta_2[G] + \beta_3[G][P]} - \gamma_1[G]$$

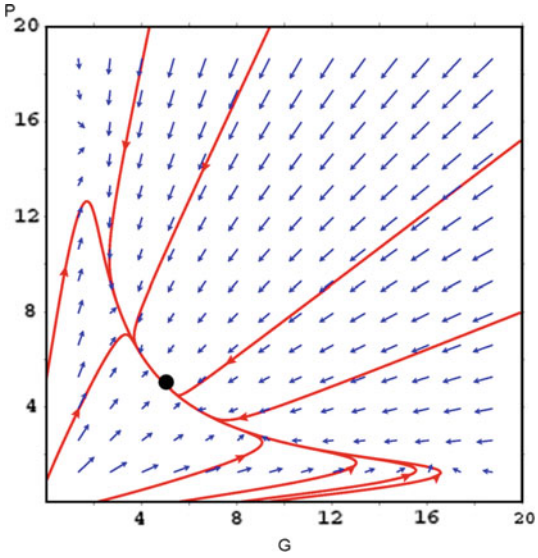

Fig. 3 A typical vector diagram of the PU.1–GATA-1 network model (3) without transcriptional factor X. Parameters are: $\alpha_1 = \beta_1 = \beta_3 = \delta_1 = \epsilon_1 = \epsilon_3 = 1$, $\alpha_2 = \beta_2 = \delta_2 = \epsilon_2 = 0.25$, $\gamma_1 = \gamma_2 = 0.01$, $A = B = 0.01$.

$$\begin{aligned} \frac{d[P]}{dt} &= \frac{\delta_1 B + \delta_2[P]}{1 + \epsilon_1 B + \epsilon_2[P] + \epsilon_3[G][P] + \epsilon_4[G][X]} - \gamma_2[P] \\ \frac{d[X]}{dt} &= \frac{\zeta_1[G]}{1 + \eta_1[G] + \eta_2 C} - \gamma_3[X], \end{aligned} \tag{3}$$

where C is used to account for the environmental factors that may suppress the expression of gene X. The addition of this extra gene gives more effective cooperativity in the interactions and allows the system to become bistable. When at concentrations of about $0.1 < A < 1.1$, there are two stable states, namely one state with high GATA-1 and X as well as low PU.1 expression levels, and the other state with low GATA-1 and X as well as high PU.1 levels (Chickarmane et al. 2009). To test the critical effect of gene X in model (3), we plotted the vector diagram of system (3) when the expression of gene X is always zero. An example shown in Fig. 3 demonstrated that there is clearly only one diagonal stable point, namely $[G] = [P]$.

Simulations using different sets of parameters showed that the bistability is robust and exists for a wide range of values. It was also stated that the inhibition scheme chosen, could equally occur in the opposite direction without changing the overall behaviour, i.e. where PU.1 activates X which suppresses GATA-1. The addition of the transcriptional factor X also has an interesting effect on indeterminate state. By increasing the value of C, all transcription factors will be maintained at intermediate levels, and as the value of C is decreased the system becomes bistable again. Thus it was proposed that the inclusion of the transcriptional factor X stabilised the progenitor state (Chickarmane et al. 2009). The model suggested was that initially GATA-1 and PU.1 are kept at low activation levels by A and B, then by the removal of C the system becomes bistable and the cell differentiates.

Next, the system was expanded to include the downstream transcription factors, C/EBPα and FOG-1. These transcription factors are also mutually antagonistic and

FOG-1 is activated by GATA-1, while C/EBPα is activated by PU.1 (Laiosa et al. 2006). By assuming that C/EBPα activates PU.1, Chickarmane et al. were able to show that the feedback loop resulting from the downstream factors acted to make the switching behaviour non-reversible. FOG-1 was also considered as the transcriptional factor X but it was found that while the system acquired switch-like behaviour, there was no evidence of the indeterminate state.

Chickarmane et al. found that their model of the PU.1–GATA-1 interaction with low cooperativity did not display bistability. Although there are other models that contain low cooperativity and bistability, for example the model in Bokes et al. (2009), they chose instead to introduce another transcriptional factor X into the system. The introduction of this transcriptional factor was found to allow bistable behaviour as well as confer stability to the progenitor state. The hierarchical structure of their model could also be considered in other pluripotential systems (Swiers et al. 2006; Loose et al. 2006). This offers an interesting and innovative method of introducing irreversibility.

2.4 Model using the Michaelis–Menten formalism

An alternative approach was proposed recently to model the PU.1–GATA-1 network with low cooperativity (Bokes et al. 2009). It was assumed that the PU.1 and GATA-1 transcriptional factors inhibit the production of each other, and this regulation was modelled by the production of an inactive heterodimer, that neither activates nor inhibits the production of each directly. In addition, the production of both PU.1 and GATA-1 proteins is autoregulatory through monomers only. Thus the Michaelis–Menten functions were used to describe the autoregulation. After non-dimensionalising and eliminating the equation of the PU.1–GATA-1 heterodimer, the mathematical model was simplified to

$$\begin{aligned} \frac{du}{d\tau} &= \frac{s_1 u}{L_1 + u} - a_1 u + f(u, v) \\ \frac{dv}{d\tau} &= \frac{s_2 v}{L_2 + v} - a_2 v + f(u, v) \\ \text{where } f(u, v) &= \frac{uv}{1 + u + v}\left(-1 + a_1 + a_2 - \frac{s_1 u}{L_1 + u} - \frac{s_2 v}{L_2 + v}\right), \end{aligned} \tag{4}$$

where u and v are dimensionless concentrations of PU.1 and GATA-1, respectively. The term $f(u, v)$ in (4) represents the antagonistic coupling of the two transcriptional factors. If it is neglected in (4), then the model reduced to a coupled system describing gene expression of two independently autoregulating factors which cannot exhibit bistability (Bokes et al. 2009). Significantly, recent models for the PU.1 and GATA-1 interaction (e.g. Roeder and Glauche 2006) do not incorporate a term analogous to $f(u, v)$ in the governing equations (2).

Bokes et al. (2009) depicted four possible situations, depending on the model parameters. The system was seen to exhibit bistability as well as a possible indeterminate state. By examining the steady state conditions of system (4), it was found that the system is bistable provided that it lies in the hatched wedge in Fig. 4 between two

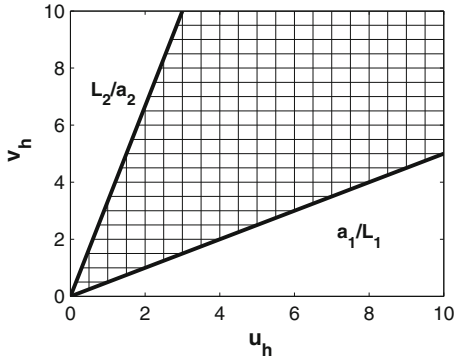

Fig. 4 Requirement for bistability in Bokes et al. system. In this example $a_2/L_2 = 1/3$ and $a_1/L1 = 1/2$

lines $v_h = a_1 u_h / L_1$ and $v_h = L_2 u_h / a_2$, where $u_h = \frac{s_1}{a_1} - L_1$ and $v_h = \frac{s_2}{a_2} - L_2$ (Bokes et al. 2009). The stationary points on the axis were also found to be located at $(u_h, 0)$ and $(0, v_h)$. To create a robust system, L_1/a_1 and L_2/a_2 must be large. To have a robust and bistable switch, the degradation of the heterodimer should be faster than that of the PU.1 and GATA-1 proteins, and/or the two transcription factors should bind together strongly. The bifurcation analysis suggested that a cell in the stable co-expression state, could be considered as an indeterminate state. This state could be left by either through the dimer being transported out of the nucleus or by an increase in an enzyme that breaks down the dimer, leading to differentiation. Differentiation could also be caused by a chemical that decreases the affinity of PU.1 and GATA-1 for each other.

Bokes et al. produced an alternative model that has low cooperativity and exhibits robust bistability and an indeterminate state. They were able to show the range of possible behaviours and propose how a cell transitions from progenitor state to differentiation. The assumption that the mutual antagonism can be correctly modelled as competition with an inactive heterodimer for free transcription factors is interesting, but under the assumption implies that at most one of the stable states can be non-diagonal, namely $u \neq v$, while small amounts of PU.1 and GATA-1 have been found in the opposite lineage (Shivdasani and Orkin 1996; Akashi et al. 2000). Whether this is due to the noisy cell environment or to a non-zero stable value of the inactive factor is unclear.

3 A new mathematical model

Although genes PU.1 and GATA-1 play a key role in determining the development pathways of blood progenitor cells, we still have a poor understanding of the regulatory mechanisms controlling the expression of these two genes. With regarding to GATA-1 molecules, experiments have demonstrated that GATA-1 may exist in the dimeric form (Mackay et al. 1998). However, either monomeric or dimeric GATA-1 molecules may provide a mechanism to regulate the expression of its numerous target genes (Shimizu et al. 2007). Less information is available for the mechanisms regulating the expression of gene PU.1. On the other hand, the introduction of an unknown

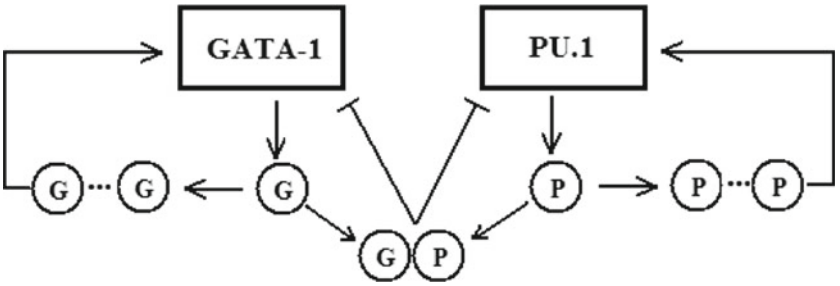

Fig. 5 Network diagram for the PU.1–GATA-1 regulatory network

gene X in Chickarmane et al.'s model is unsatisfying although this model successfully realized bistability using low cooperativity (Chickarmane et al. 2009). One of the predicted mechanisms, namely PU.1 activating gene X which suppresses GATA-1, is not consistent with the most recent experimental discovery showing that PU.1 positively regulates GATA-1 expression in most cells (Takemoto et al. 2010). Therefore an important question regarding the mechanisms regulating the PU.1–GATA-1 network is, after removing the gene X from the system, what is the minimal cooperativity to realize bistability and an indeterminate state. To answer this question, we developed a mathematical model based on the summed Hill functions that have been used in the model in Huang et al. (2007). In the light of new experimental evidence by Liew et al. (2006), the antagonistic factor was changed to the PU.1–GATA-1 heterodimer. But we will test different cooperativities of the antagonistic factor and the antagonistic Hill coefficient that might be 1 or greater than 1.

Since the model proposed by Huang et al. (2007) is the only one whose simulation results were supported by experimental data, we follow this approach using summed Hill functions to represent autoregulation and antagonism. Our proposed model is based on the following assumptions:

- The production of both PU.1 and GATA-1 is autoregulatory;
- Transcriptional factors PU.1 and GATA-1 inhibit the production of each other through a PU.1–GATA-1 heterodimer;
- There are independent binding site for the autoregulation and for the inhibition by the heterodimer;
- It is assumed that the inhibition acts on a background expression term;
- The concentrations of homodimers are assumed at equilibrium levels via the instantaneous dimer formation and dissolution;
- Time delays in transcription, transportation and translation are ignored;
- The Hill coefficient for autoregulation was separated from the antagonism coefficient;
- To simplify the analysis, the system was modelled to be structurally symmetrical, i.e. the Hill coefficients were the same for GATA-1 and PU.1; and
- Monomeric bindings were not considered.

The network diagram is shown in Fig. 5. Hill functions were used to model the system resulting in the following equations:

$$\begin{aligned}\frac{d[G]}{dt} &= a_1\frac{[G]^n}{\theta_{a1}^n+[G]^n}+b_1\frac{\theta_{b1}^m}{\theta_{b1}^m+[G]^m[P]^m}-k_1[G]\\ \frac{d[P]}{dt} &= a_2\frac{[P]^n}{\theta_{a2}^n+[P]^n}+b_2\frac{\theta_{b2}^m}{\theta_{b2}^m+[G]^m[P]^m}-k_2[P],\end{aligned} \tag{5}$$

where [G] and [P] are expression levels of genes GATA-1 and PU.1, respectively. The first term in each equation represents gene expression by auto-stimulation and the second term is the cross-inhibition regulated by the other transcriptional factor in the network. The third term is a first-order degradation of the transcriptional factor. Instead of using the multiplicative inputs from auto-stimulation and cross-inhibition (Chickarmane et al. 2009), the proposed model uses the additive inputs from auto-stimulation and cross-inhibition. A detailed discussion about the assumption of the additive inputs can be found in Huang et al. (2007).

We further constrained our study to the symmetric system in which the system parameters satisfy $a_1 = a_2, b_1 = b_2, \theta_{a1} = \theta_{a2}, \theta_{b1} = \theta_{b2}$ and $k_1 = k_2$. We also removed the degradation rate $k = k_1 = k_2$ by using $x = k[G]$, $y = k[P]$, $a = ka_1$, $b = kb_1, \theta_1 = k\theta_{a1}, \theta_2 = k^2\theta_{b1}$, the system (1) can be written in the following simple form as

$$\begin{aligned}\frac{dx}{dt} &= a\frac{x^n}{\theta_1^n+x^n}+b\frac{\theta_2^m}{\theta_2^m+x^my^m}-x\\ \frac{dy}{dt} &= a\frac{y^n}{\theta_1^n+y^n}+b\frac{\theta_2^m}{\theta_2^m+x^my^m}-y.\end{aligned} \tag{6}$$

and then the steady state (x, y) satisfy

$$\begin{aligned}a\frac{x^n}{\theta_1^n+x^n}+b\frac{\theta_2^m}{\theta_2^m+x^my^m} &= x\\ a\frac{y^n}{\theta_1^n+y^n}+b\frac{\theta_2^m}{\theta_2^m+x^my^m} &= y.\end{aligned} \tag{7}$$

4 Bifurcation analysis

Using bifurcation diagrams, we investigated the existence of bistability of system (5) with different binding cooperativity of n and m. It was found that, when $n \geq 2$, this system could support bifurcation and an indeterminate state, even when the cooperativity in the mutual antagonism is one, namely $m = 1$. Our first question is what is the influence of the Hill coefficient n on the existence of bistability and an indeterminate state of system. To answer this question, we generated 1,000 sets of model parameters $(a_1, b_1, \theta_{a1}, \theta_{b1}, k_1)$ and the value of each parameter is a sample of the uniformly distributed random variable $U(0, 2)$. Together with $m = 1$ and $n = 2$, 3 or 4, we examined each parameter set by determining whether system (5) has the bistability property and an indeterminate state. When $n = 2$, numerical results suggested that only 16 parameter sets could generate system dynamics showing bistable steady states

and an indeterminate state. When $n = 3$, the number of parameter sets was increased to 42; and this number was increased further to 89 if $n = 4$. We have also tested the bistability property of the system (5) when $n = m = 1$. However, we failed to find one set of parameters on which the system exists bistability property, though this test cannot exclude the possibility that this system may have the bistability property with a particular model parameter set when $n = m = 1$. Repeated numerical tests based on other 1000 sets of model parameters suggested the similar conclusions regarding the percentages of model parameter sets showing the bistability property. Thus our tests suggested that the system (5) is more likely to have bistability property when a larger value of the Hill coefficient n is used.

To compare with the bifurcation results in Fig. 2 (Huang et al. 2007, Fig. 6a shows the bifurcation characteristics of system (5) with changing values of a when the cooperative binding coefficient is $n = 4$. It displays type II bifurcation as per Huang et al., which also occurs when k or θ is increased. When $a > 0.63$, the system shows a tristable attractor landscape. If the value of a decreases below the first threshold value $a = 0.63$ but is still above the second threshold value $a = 0.47$, the system locates in a bistable region with two stable and one unstable steady states. However, the decrease of the maximal expression rate could not cross the second threshold value $a = 0.46$. If the expression rate is too low, then the system will enter the monostable mode and any temporary increase of the gene expression will not switch the system into another stable state. However, when we increased the values of parameter $b = b_1 = b_2$, Fig. 6b shows that the system changes from a tristable system to a monostable one. Similar observation can be found in Fig. 6c. When we increased the value of $k = k_1 = k_2$ from 1 to 1.6, the system changes from a tristable system to a bistable one.

Figure 7 shows the phase diagram before a and after b destabilisation of the progenitor state. It is proposed that, similarly to the results in Huang et al. (2007), a cell in an initial state of intermediate co-expression will differentiate if an external signal decreases the value of a or increases k. The change of parameters may represent the biological mechanisms that regulate the decreased expression of GATA-1 (Shimamoto et al. 1997) or the accelerated degradation of GATA-1 in the presence of inhibitors (Hernandez-Hernandez et al. 2006). Thus this model allows bistability even with antagonistic cooperativity coefficient $m = 1$. In addition, the lowest order of multimer is lower than that in the proposed model in Huang et al. (2007). Bistability can be found even when $n = 2$, corresponding to dimer autoregulation. The system was found to exhibit bistability over a range of parameter values though unfortunately, this range could not be concisely described due to the complex nature of the system.

We further analysed the parameter space of the system (6) in which the system exists the bistability property and an indeterminate state. Similar to the stability area in Fig. 4 (Bokes et al. 2009), we considered different values of $u = a - \theta_1$ and $v = b - \theta_2$. Since it is difficult to find any analytic solution of the nonlinear equations (7), we used the Newton-Raphson method to find the numerical solution of (7) and searched the fixed points of the system (6) with different values of the model parameters. Figure 8 gives the tristability area of the system (6) by further assuming that $n = 4, a = b, 0 < \theta_1 \leq a$ and $0 < \theta_2 \leq b$. When $a = b = 1$, the values of θ_1 and θ_2 in the tristability area of the system satisfy $\theta_1 > \theta_2$. However, when the values

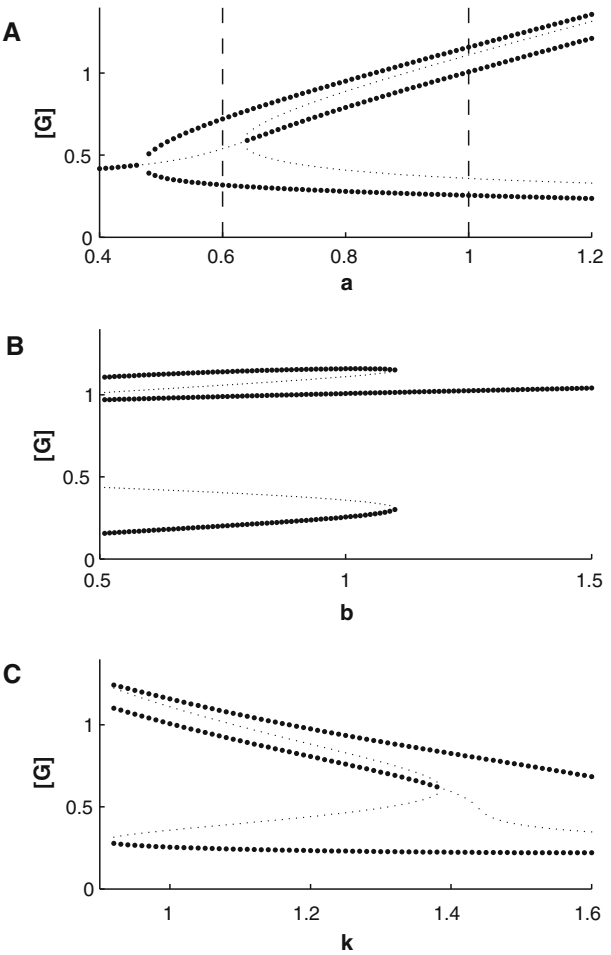

Fig. 6 Bifurcation diagram of the proposed system (5). Stable steady states are indicated by *solid dot lines*, while unstable steady states by *dotted lines*. **a** The Type II bifurcation with decreasing values of $a = a_1 = a_2$, as that in the system in Huang et al. (2007). Model parameters are: $b_1 = b_2 = 1$ and $k_1 = k_2 = 1$. **b** Bifurcation with increasing values of $b = b_1 = b_2$. Parameters: $a_1 = a_2 = 1$ and $k_1 = k_2 = 1$. **c** Bifurcation with increasing values of $k = k_1 = k_2$. Parameters: $a_1 = a_2 = 1$ and $b_1 = b_2 = 1$. Other parameters are $n = 4, m = 1, \theta_{a1} = \theta_{a2} = 0.5$, and $\theta_{b1} = \theta_{b2} = 0.07$

of a and b are larger, more tristable steady states of the system (6) locate in the area $\theta_2 > \theta_1$.

We also examined the parameter space of the diagonal steady states of the system (6). By letting $x = y$, the mathematical model is given by

$$\frac{dx}{dt} = a\frac{x^n}{\theta_1^n + x^n} + b\frac{\theta_2^m}{\theta_2^m + x^{2m}} - x. \tag{8}$$

Since it is difficult to find the analytical solution for the steady states of the above nonlinear differential equation, we again used the numerical methods to search the

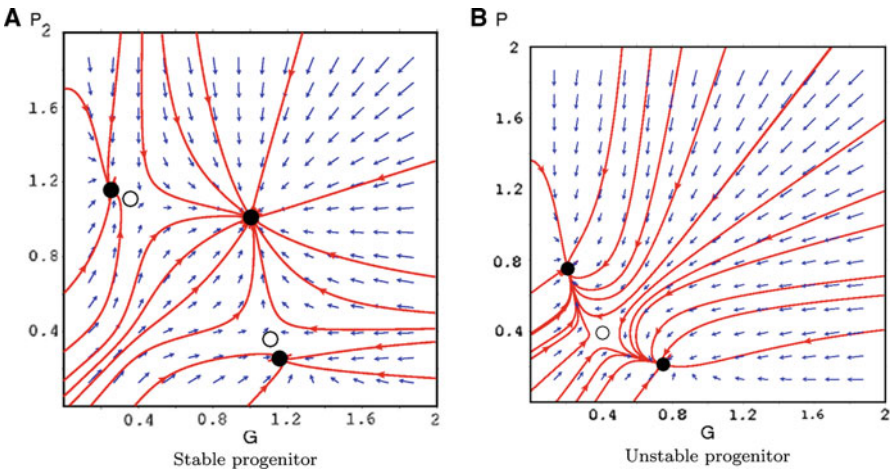

Fig. 7 Vector diagrams of the proposed system (5). **a** Parameters as per Fig. 6, $a = 1$. **b** Parameters as per Fig. 6, $a = 0.6$. Other parameters are $n = 4$, $m = 1$, $b_1 = b_2 = 1$, $\theta_{a1} = \theta_{a2} = 0.5$, $\theta_{b1} = \theta_{b2} = 0.07$, and $k_1 = k_2 = 1$

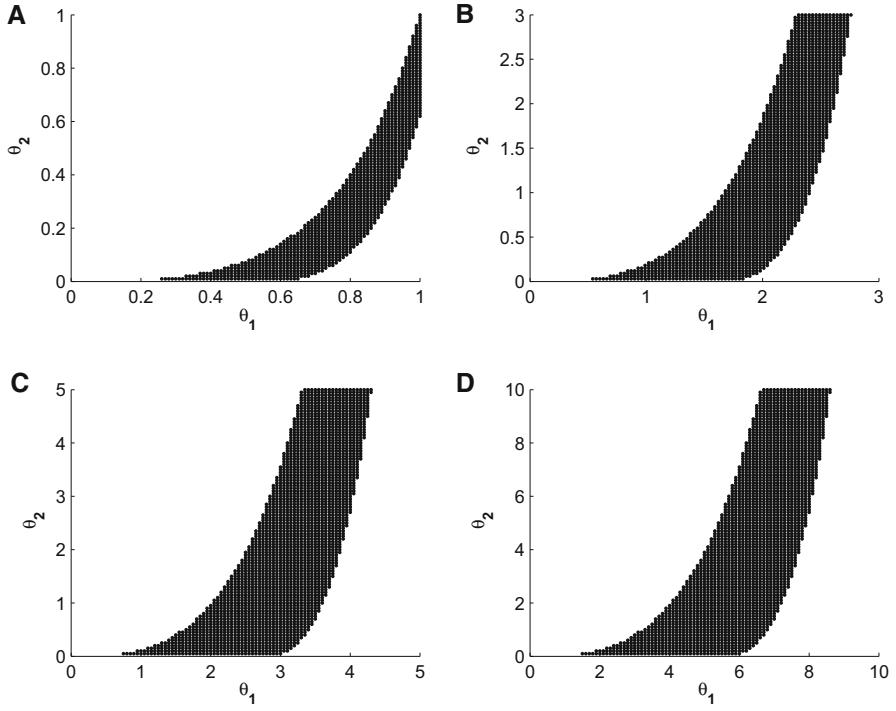

Fig. 8 System (6) is tristable if the model parameters θ_1 and θ_2 are within the *shadowed area*. **a** $a = b = 1$. The *straight line* is $\theta_1 = \theta_2$, **b** $a = b = 3$, **c** $a = b = 5$, **d** $a = b = 10$

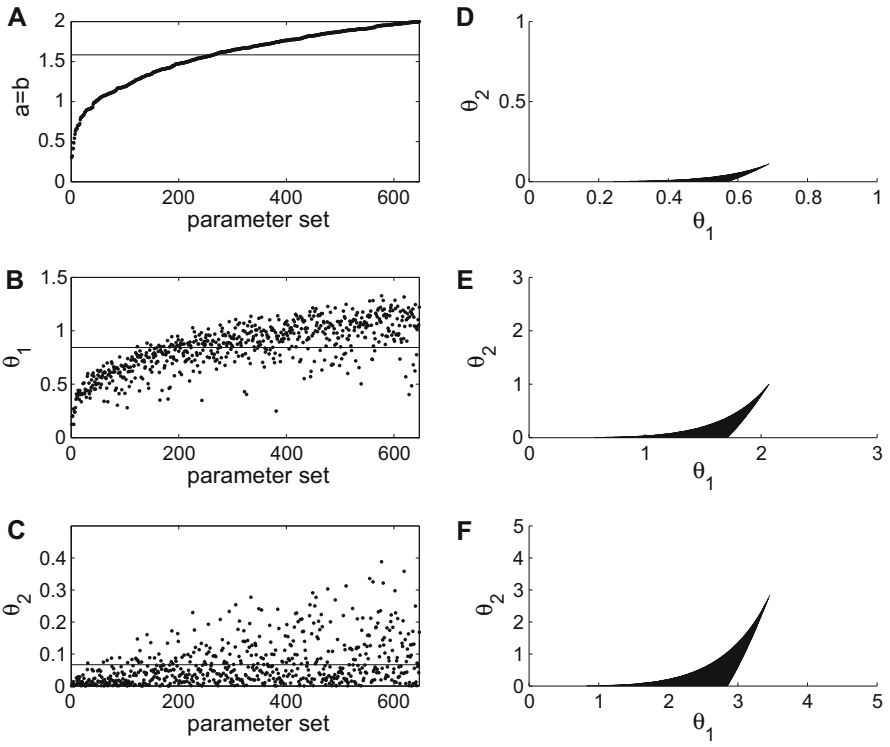

Fig. 9 The bistable property of the diagonal system (8). **a–c** The 647 model parameter sets $(a, b(=a), \theta_1, \theta_2)$ which can generate bistability property. These parameter sets were sorted according to the value of a. The *straight line* in each figure is the averaged value of the parameter. **d** The bistable region of the diagonal system (8) with $a = b = 1$. **e** $a = b = 3$. **f** $a = b = 5$

steady state of the system (8) with different parameters. For the convenience of representing results, we first constrained our study to the special case of $a = b$. By generating 200,000 sets of model parameters from the uniformly distributed random variable $U(0, 2)$, we found that 647 parameter sets of these 200,000 sets can generate two stable steady states and one unstable steady state. However, this diagonal system does not have tristability property anymore. The minimal value of a in these 647 parameter sets in Fig. 9a is 0.3033; and we later confirmed that the system locates in a monostable region when $a < 0.3$. When the value of a increases, the value of θ_1 in Fig. 9b increases proportionally. Similar observations have been found for the maximal values of θ_2 in Fig. 9c, though the value of θ_2 may also remain small. If we removed the assumption $a = b$, simulation results suggested that a wide range of the model parameters $(a, b, \theta_1, \theta_2)$ locate in the bistable region. We further searched the bistable regions of system (8) with $a = 1$, 3, or 5, which are presented in Fig. 9d–f.

5 Genetic switching

Gene overexpression is a widely used experimental method to study the system dynamics under a particular perturbation and explore the critical function of a

specific component of the system. This method has been used to study the regulatory mechanisms in the PU.1–GATA-1 network (Zhang et al. 2000). After carrying out bifurcation analysis and finding the conditions of bistability and an indeterminate state, the next question is whether our model can realize the genetic switching under different over-expression conditions. To answer this question, we simulated the proposed model (5) with different amplitudes of impulse. This impulse is represented by a modified expression rate a_1 of GATA-1, given by

$$a_1 = \begin{cases} a_{10} & t < 10 \text{ or } t > t + t_1 \\ a_{10} + a_1^*, & t \in [10, 10 + t_1] \end{cases} \tag{9}$$

where $a_{10} = 0.6$ is the normal expression rate during the entire simulation time period. The amplitude of impulse a_1^* is non-zero only at the impulse time period $[10, 10 + t_1]$. We also tested the influence of the duration of impulse by setting the duration as $t_1 = 0.5$ h or $t_1 = 5$ h. Simulations in Fig. 10 suggested that the genetic switching is regulated by both the amplitude and duration of impulse. In the subcritical scenarios (Fig. 10a, d), the concentration of PU.1 returned to the original expression level after a temporary increase stimulated by the impulse. However, in the supercritical scenarios (Fig. 10b, c, e, f), the expression level of PU.1 reached the second steady state and stayed at that state after the impulse withdrew. At the same time, the expression level of GATA-1 began to decrease and then reached the steady state with a lower expression level. Compared with the simulations in Fig. 10b, c, simulations in Fig. 10e, f indicated that the amplitude of impulse for realizing the genetic switching can be smaller if the duration of impulse is larger.

6 Conclusions

In this work we proposed a mathematical model to study the interactive regulation of genes PU.1 and GATA-1. Based on the assumption that activation and inhibition of the gene expression in the PU.1–GATA-1 network are independent (Huang et al. 2007), we added the antagonism regulation of the PU.1–GATA-1 heterodimer that inhibits the expression of both genes PU.1 and GATA-1. In particular, we investigated the conditions regarding the order of cooperative binding for realizing bistability and an indeterminate state. Bifurcation analysis indicated that the order of cooperativity in the antagonism ($m = 1$) is adequate to realize bistability. However, a relatively high order of autoregulation, namely $n \geq 2$, is required to realize system dynamics with bistability and an indeterminate state. Here the indeterminate state is that located in the middle of the three stable steady state of the system. After the introduction of impulse with appropriate amplitude and duration, the indeterminate state will switch to one of the bistable states. Using a lower order of autoregulation and antagonism than that in Huang et al.'s model (Huang et al. 2007), our proposed model realized the similar results showing bistability and a possible indeterminate state, as well as similar type II pitchfork bifurcation. In addition, the system was found to be robust, in that it exhibited bistable behaviour over a range of parameter values. Our analysis provided a minimal condition to realize bistability and an indeterminate state in a network

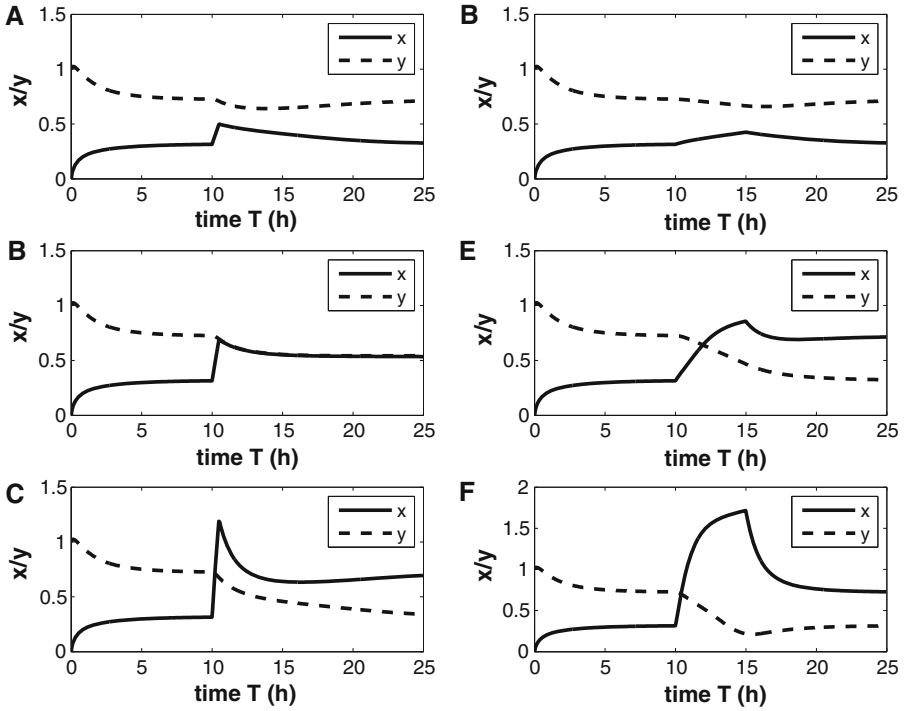

Fig. 10 Scenarios for subcritical and supercritical factor overexpression. In the subcritical scenarios **a** and **d** the concentrations return to the original levels, while in the supercritical scenarios **b**, **c**, **e** and **f** the over expression of GATA-1 leads to a change in cell state and different final concentrations. Over-expression was applied as a long term (**a**–**c**) or short term (**d**–**f**) influence at time $t = 10$ h. Parameters were $a_{10} = 0.6$, $b = 1$, $\theta_a = 0.5$, $\theta_b = 0.07$, $n = 4$, $m = 1$ and $k = 1$. Short term influences were applied from $t = 10$ h to $t = 10.5$ h with amplitude of **a** $a_1^* = 0.002$, **b** 0.004, **c** 0.01. Long term influences were applied from $t = 10$ to $t = 15$ with amplitude **d** $a_1^* = 0.0002$, **e** 0.001, and **f** 0.005

including the regulation between genes PU.1 and GATA-1 only. The proposed model provided a novel platform to analyze bistability and indeterminate state under various cooperativities of autoregulation and repression.

The differentiated states of the PU.1-GATA-1 network are characterised by having a much greater concentration of one of the transcription factors, while in the indeterminate state concentrations are roughly equal. The differentiated states are represented by stable attractors because under normal circumstances cell fates are irreversible. However, we also note that forced lineage switching using GATA-1 was demonstrated by Kulessa et al. (1995) and Heyworth et al. (2002). The current consensus on the way differentiation occurs is that the indeterminate state is initially stable, allowing progenitor cells to self renew and remain uncommitted, but an external signal of some kind destabilises this state and causes the progenitor cell to differentiate (Enver et al. 2009). This model allows for both stochastic (random noise) and external influences to easily change the cell fate at the moment of destabilisation. Thus, successful models must have a method whereby a stable indeterminate state can be destabilised. In

this work we used different amplitudes and durations of the GATA-1 activity impulse to realize genetic switching. Simulation results suggested that the successful switching depended on the total amount of impulse which is the product of the amplitude and duration. In addition, any small perturbation to the initial condition of the unstable state ($P = G = 0.5388$ when $a = 0.6$) leads the system to one of the stable states ($P = 0.7209$ and $P = 0.3184$ or vise versa). However, the stable state ($P = G = 0.6686$ when $a = 0.7$) may withstand quite a large perturbation to the initial condition and stay at this intermediate steady state. Therefore our simulation results supported the conclusions in Huang et al. (2007) which suggested that a stable indeterminate state is more likely.

The Shea–Ackers model established the relationship between the cooperative binding of transcriptional factors, namely the monomer, dimer or a higher order multimer, and the coefficients of the Hill function in the mathematical model for genetic regulation (Shea and Ackers 1985). This principal has been widely used in the modelling approaches of gene networks including the λ-phage pathway, lactose operon, and p53 network (Hasty et al. 2000; Santillán and Mackey 2004; Tian and Burrage 2004; Ma et al. 2005). However, due to the complex structure of the transcriptional machinery and unknown regulatory mechanisms, the Hill function model with a large Hill coefficient has also been successfully used to realize bistable behaviours in gene networks (Gardner et al. 2000; Ozbudak et al. 2004; Kobayashi et al. 2004; Tian and Burrage 2006). This comment can also be applied to the mathematical modelling of the PU.1–GATA-1 network; and this is the key motivation for us to develop a mathematical model to explore the minimal conditions for realizing bistability and an indeterminate state. Our research results suggested that the proposed model is more likely to have bistability property when a larger value of the Hill coefficient n is used. More research work may be needed to explore the functions of the complex transcriptional machinery and/or other transcriptional factors in regulating the expression of genes GATA-1 and PU.1. It is clear that the advances in experimental discoveries will provide more experimental data and additional regulatory mechanisms for the development of more realistic mathematical models.

Our analysis demonstrated that the proposed symmetrical model has the regions of tristability and bistability (see Figs. 6, 8, 9). We also found that by decreasing the value of $a = a_1 = a_2$ below a certain threshold or by increasing the value of either $k = k_1 = k_2$ or $b = b_1 = b_2$ above a certain threshold the system changes from a tristable system to a bistable one. The destabilisation of the indeterminate state causes differentiation, so from our model we would expect that a decrease in self-regulation or increase in the breakdown of the transcription factors would cause differentiation. It is difficult to speculate on the exact biological mechanisms responsible for the change in system behaviour before we know how differentiation is triggered in a progenitor cell. However, our analysis suggested that these could occur through the binding of another transcription factor which inhibits PU.1 and GATA-1 (decreasing a); or though the transport out of the nucleus of the transcription factors (increasing k); or the introduction of an enzyme which creates the heterodimer (increasing b and k).

A growing body of evidence demonstrated that gene expression is a stochastic process. Key species of molecules such as DNA and mRNA may have small copy numbers; and the changes of copy numbers of these key species may cause significant

variations of the system dynamics. In particular, both extrinsic and intrinsic noise have been investigated for the lineage-specific gene networks (Chang et al. 2008; Palani and Sarkar 2009). Stochastic differential equation models derived from the Langevin approach may be used to realize the function of external noise in generating different genetic switching in different simulations. In addition, stochastic models based on detailed chemical reactions may be also very interesting for studying the influence of internal noise on the selection of different developmental pathways. Therefore stochastic modelling will be a topic of the next stage of modelling approaches. In addition, further work is recommended into the bifurcation properties of the proposed systems, such as the systems in (Bokes et al. 2009; Chickarmane et al. 2009), to determine if under an almost symmetrical parameter change they behave similarly to the system proposed by Huang et al. (2007). This will allow a more detailed qualitative analysis of the behaviour of each system. The irreversible behaviour of a system with downstream feedback will also be investigated in the system proposed in this work. This will provide a comparison study to Chickarmane et al.'s work and give an indication whether downstream feedback is a recurring feature that causes irreversibility.

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