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ORIGINAL ARTICLE

Dynamic Stabilization in the PU1-GATA1 Circuit Using a Model with Time-Dependent Kinetic Change

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Abstract The PU.1 and GATA1 genes play an important role in the differentiation of blood stem cells. The protein levels expressed by these genes are thought to be regulated by a self-excitatory feedback loop for each gene and a cross-inhibitory feedback loop between the two genes. A mathematical model that captures the dynamical interaction between these two genes reveals that constant levels of self-excitation and cross-inhibition allow the most self-exciting or cross-inhibiting gene to dominate the system. However, since biological systems rarely exist in an unchanging equilibrium, we modeled this gene circuit using discrete time-dependent changes in the parameters in lieu of steady state parameters. These time-dependent parameters lead to new phenomena, including the development of new limit cycles and basins of attraction. These phenomena are not present in models using constant parameter values. Our findings suggest that even small perturbations in the PU.1 and GATA1 feedback loops may substantially alter the gene expression and therefore the cell phenotype. These time-dependent parameter models may also have implications for other gene systems and provide new ways to understand the mechanisms of cellular differentiation.

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1 Introduction

The set of expression levels of the genes in a cell determines its phenotype and function. How this state of the cell is maintained or varied is essential to understanding the stability of cell states and the differentiation of cells into other states. A common motif in systems biology is a two gene regulatory circuit where the protein expressed by each gene binds to the regulatory region of the other gene and inhibits its expression. The importance of this motif is that it is bistable: the expression of one gene ultimately inhibits the expression of the other gene. For example, the classical shift of the phage lambda that infects E. coli from its lytic state (where it reproduces and kills its host) to its lysogenic state (where it integrates into the host DNA and lies in waiting) is determined by the dynamics of the cross-inhibition between cro and C1 (Ptashne and Gann 2002). The same design principle has been used to construct a bistable "toggle switch" in a synthetic gene regulatory system (Gardner et al. 2000). These motifs play an important role in cellular differentiation where the fate of the cell depends on which gene is expressed. For example, each day approximately five million common myeloid precursor cells in the bone marrow of each person start to differentiate into either erythroid or myelomonocytic lineages. This first differentiation step is established by the cross-inhibition of PU.1 and GATA1. If the expression of GATA1 dominates, the cell becomes erythroid and if PU.1 dominates, the cell becomes myelomonocytic. It is not yet clear whether intrinsic or extrinsic signals to this gene regulatory circuit play the dominant role in deciding the fate of these cells.

Whatever the underlying cause of the switch between different possible cell fates, the question remains as to how the undifferentiated stem cell state is destabilized and how the lineage-committed state is stabilized. In the case of the myeloid precursor cells, Roeder and Glauche (2006) and Huang et al. (2007) proposed that both PU.1 and GATA1 each express a transcription factor protein that stimulates the expression of its own gene. This self-excitatory positive feedback then tips the cross-inhibitory see-saw into stabilizing the gene whose expression is highly elevated. Although there is strong evidence that the expression of PU.1 stimulates its own production (Okuno et al. 2002), evidence is lacking that GATA1 stimulates its own production (Yu et al. 2002). Moreover, Chickarmane et al. (2009) and Bokes et al. (2009) showed that even if this positive feedback exists, it must be sufficiently cooperative to overcome the cross-inhibitory circuit in order to stabilize one of the two attractors that represent the two myeloid lineages. They noted that the observed self-excitation positive feedback is only weakly cooperative and is therefore not sufficient to destabilize the progenitor state to allow for cell lineage commitment. In order to understand how a stable expression state is achieved, Chickarmane et al. (2009) therefore proposed that additional, presently unknown, networks of interactions act collectively to stabilize the new state. In comparison, Bokes et al. (2009) proposed that changes in the rate of dissociation of the PU.1-GATA1 complex, due to enhanced degradation or transport out of the nucleus, act to stabilize the new state. The combination of

kinetic fluctuations and stochastic influence has also been examined for a variety of bistable systems, including the PU.1-GATA1 circuit (Roeder and Loeffler 2002; Losick and Desplan 2008; Lei 2008).

These previous models focused on computing how the gene expression depends on the kinetic parameters which were assumed to remain constant or decay over time. But the hallmark of living things is that both internal states and external environmental conditions vary in time. Such time-dependent variations may be especially important in the PU.1-GATA1 circuit. It has long been proposed that the destabilization of multipotent myeloid cells leading to a committed lineage may be initiated by an event that results in an increase in one of the two gene's expression levels relative to the other (Ravid and Licht 2001). Stochastic effects have also been advocated as a possible trigger of such an increase (Levens and Gupta 2010; To and Maheshri 2010; Kaern et al. 2005). However, the precise role of extrinsic and intrinsic signals as well as stochastic effects on myeloid fate decisions remains unclear (Graf 2002). Oscillatory gene expression is one proposed intrinsic precursor for blood stem cell fate decisions in vivo (Graf and Stadtfeld 2008). Recent work has demonstrated that hematopoietic progenitor clonal cells may possess an intrinsic priming for future differentiation into either the erythrocytic or myelomonocytic lineage via slow transcriptome noise fluctuation (Chang et al. 2008). Activity-level fluctuations are also theorized to play important roles in protein and gene regulation and adaptation (Day 2009; Gerland and Hwa 2009; Henzler-Wildman and Kern 2007). In addition to stochastic fluctuations, GATA1 levels are known to oscillate in response to the cell cycle, and it is well-known that oscillatory behavior is exhibited in a wide range of biomolecular systems (Koga et al. 2007; Goldbeter 1996). In fact, the hematopoietic stem cell activity is linked to the biological rhythms of the circadian cycle (Mendez-Ferrer et al. 2008).

In this paper, we therefore explore how fluctuations in transcription factor activity levels are influenced by time-dependent changes in the kinetic parameters. We show how such changes can destabilize an uncommitted cell state and stabilize expression states. Which expression states become more accessible to an uncommitted cell depends on the amplitude and timing of these parameter changes. Paradoxically, the ever changing environments produced by complex genetic regulatory circuits may actually provide a mechanism to stabilize genes into constant patterns of expression. This new concept of "dynamical stabilization" provides another way to destabilize uncommitted cell states and stabilize expression states. We examine the properties of such dynamical stabilization in a specific model of the PU.1-GATA1 gene interaction system, although the mechanism itself may well have much more general applications. Understanding the response of gene regulatory systems to dynamical changes in the controlling variables may shed light on cellular differentiation and suggest possible new approaches to re-programming cells.

2 The Models

2.1 General Model

We based our model on a bistable toggle switch derived from biological rate equations (Gardner et al. 2000). Toggle switch models capture the influence that two cross-

inhibitory genes have on one another and thus provide an ideal starting point for modeling the antagonistic nature of the PU.1-GATA1 interaction. Building on this model form, we added a term representing each gene's self-excitation, which leads to the differential equations:

$$\begin{cases} \dot{x} = -k_1 x + \frac{a_1 x^n}{1+x^n} + \frac{b_1}{1+y^n}, \\ \dot{y} = -k_2 y + \frac{a_2 y^n}{1+y^n} + \frac{b_2}{1+x^n}. \end{cases} \tag{1}$$

In this model, x represents the GATA1 expression level and y represents the PU.1 expression level, k_1 and k_2 represent activity decay, a_1 and a_2 represent each gene's self-excitation, and b_1 and b_2 represent the cross-inhibition each gene imposes on the other. We assume that all parameter values are real and that all parameter values are non-negative. This formulation allows flexibility in modeling the strength of each gene's self-excitation and cross-inhibition. For the numerical simulations, the parameters were fixed to ranges from 0 to 2 for the a- and b-terms and 4 for n. These values are similar to those chosen by Huang et al. (2007), and were selected as they provide values that allow a host of different phase space configurations to include multiple stabilities which can qualitatively match known PU.1 and GATA1 activity in respect to cell lineage stabilization. The k parameters were fixed at 1, which represents a modest gene activity decay. While this model provides a starting point for our simulations, we further considered that there is little evidence that GATA1 stimulates its own production (Yu et al. 2002). Therefore, we derived a second model that, without the PU.1 and GATA1 self-excitation terms, provides a more minimalist model.

2.2 Minimalist Model

The minimalist model is a simple revision of the original bistable toggle switch model described in (1). We derived this minimalist model for two reasons. Foremost, there is limited evidence that GATA1 is self-excitatory (Yu et al. 2002). Furthermore, we wanted to examine whether behaviors observed in other bistable models of the PU.1-GATA1 circuit could be captured using a simpler model. Our minimalist model omits the PU.1 and GATA1 self-excitation terms, leading to the differential equations:

$$\begin{cases} \dot{x} = -k_1 x + \frac{b_1}{1+y^n}, \\ \dot{y} = -k_2 y + \frac{b_2}{1+x^n}. \end{cases} \tag{2}$$

The dynamical properties of our original and minimalist models are straightforward when the parameters are constants, but more interesting properties emerge when the parameters are time dependent.

2.3 Time-Dependent Parameters

Previous models of the PU.1 and GATA1 interaction determined the dynamics of this system for a range of constant and non-constant parameter values (see, e.g., Huang

et al. 2007; Chickarmane et al. 2009; Bokes et al. 2009). Here we explore the dynamics of both the general and minimalist models when there are time-dependent changes in one or more of the parameters. Our model allows PU.1 and GATA1 to influence the eventual committed lineage through the duration of each gene's expression. It should be noted that such time-dependent parameter changes are applied on the system as diffeomorphisms in the system's vector field. Accordingly, our system introduces time as the third dimension in our phase space configurations, and for this standard 2D approaches to the vector field analysis are not always applicable. In particular, the precise location of stable and unstable manifolds is made especially difficult, and these features are not explicitly plotted in our 2D phase space projections. All numerical solutions were generated using MATLAB (Mathworks, Inc.) using the software's ode45 Runge–Kutta solver. The ode45 solver uses algorithms considered useful and accurate for a majority of applications (Dormand and Prince 1980). We numerically examined a subset of results using Newton's method to check whether the numerical simulation outputs were sensitive to the specific solver. Results from Newton's method replicated what we found using the ode45 Runge–Kutta solver, lending support to the accuracy of the results.

3 Constant Parameters

3.1 General Model

The phase space of this model depends on the values of the constant parameters (Fig. 1). Similar to other investigations (see, e.g., Huang et al. 2007), our model demonstrates a single stability when the self-excitation and cross-inhibition are weakly expressed ($a_{1,2}$, $b_{1,2} \approx 0$). At stronger levels of self-excitation and cross-inhibition, the model's state space is governed by up to four stabilities, provided that the PU.1 and GATA1 self-excitation and cross-inhibition are of similar strength. When one gene is sufficiently stronger in either its self-excitation and cross-inhibition of the other gene, then the system will trend toward a single stability.

These four stabilities illustrated in Fig. 1 are: (1) an attractor close to the origin that represents near zero expression levels of both genes (panel row 1, column 1), (2) the erythorid lineage attractor at the lower right (panel row 1, column 3), (3) the myelomonocytic lineage at the upper left (panel row 3, column 1), and (4) a "central attractor" where PU.1 and GATA1 have balanced self-excitation with low level of cross-inhibition (panel row 3, column 3).

In the previous bistable models of the PU.1-GATA1 circuit, the attractors representing lineage-committed states are separated by either a stable manifold at low gene expression levels or a central attractor, generally at $x = y$ (Huang et al. 2007; Bokes et al. 2009). In the blood stem cell modeling efforts, one concern has been the destabilization of the metastable, central, attractor (Huang et al. 2007). Huang et al. (2007) have examined the destabilization of this central attractor by increasing transcription factor decay rates, which result in the central attractor undergoing a subcritical pitchfork bifurcation. However, there are some limitations to this assignment. Foremost, the central attractor basin is not readily perturbed and is stable provided

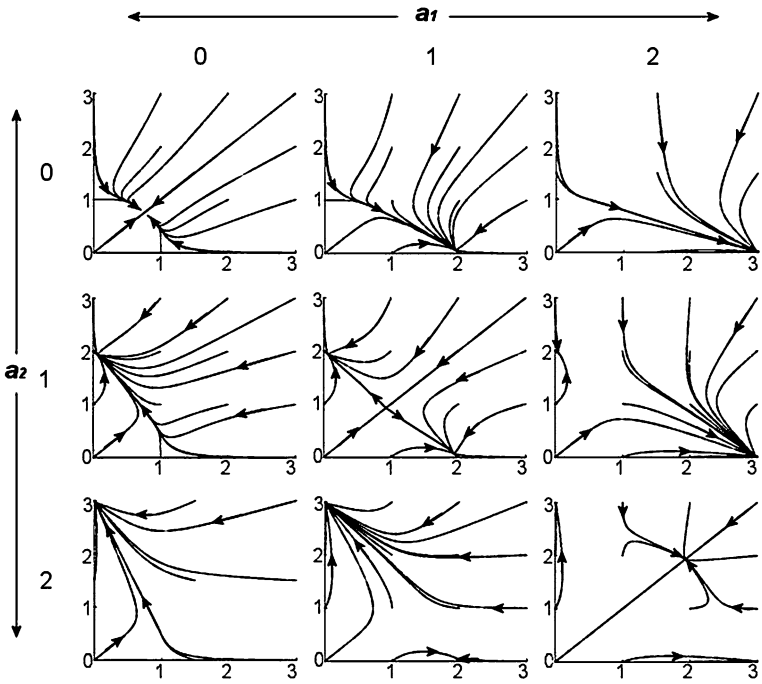

Fig. 1 Phase space configurations of the model (see (1)) given fixed parameter values. Each *panel* represents the y vs. x phase space for trajectories originating from a grid of initial states. The set of parameters for these possible configurations were $k_{1,2} = 1$, $b_{1,2} = 1$, $n = 4$, and $a_{1,2}$ varied according to the values indicated on the *horizontal* and *vertical axes* of the entire 3×3 array. When present in each *panel*, the attractor for the erythorid lineage where GATA1 dominates is in the *lower right* and the attractor for the myelomonocytic lineage where PU.1 dominates is in the *upper left*

the PU.1 and GATA1 expression levels remain highly active and exhibit only modest decay. Accordingly, an uncommitted cell locked in this central basin of attraction is unlikely to be easily perturbed to a committed lineage. With this type of qualitative representation, stochastic effects on cell fate are minimized, as small stochastic perturbation of an uncommitted cell will fail to destabilize the central attractor.

3.2 Minimalist Model

The phase space configurations (Fig. 2) of our minimalist model include stabilities at: (1) the attractor close to the pitchfork bifurcation when $b_1 = b_2 = 1$, (2) the erythorid lineage attractor situated near the x-axis (panel row 1 column 2), (3) the myelomonocytic lineage situated near the y-axis (panel row 2, column 1), or (4) the latter two attractors separated by a saddle (panel row 3, column 2).

One qualitative difference between the general and minimalist models is that the minimalist model does not exhibit a central attractor (Fig. 2, panel row 3, column 3). That is, the central attractor is eliminated from the system. Another difference is that there is a faster convergence to the erythorid lineage and myelomonocytic lineage attractors which are now more prominent with low levels of cross-inhibition (for

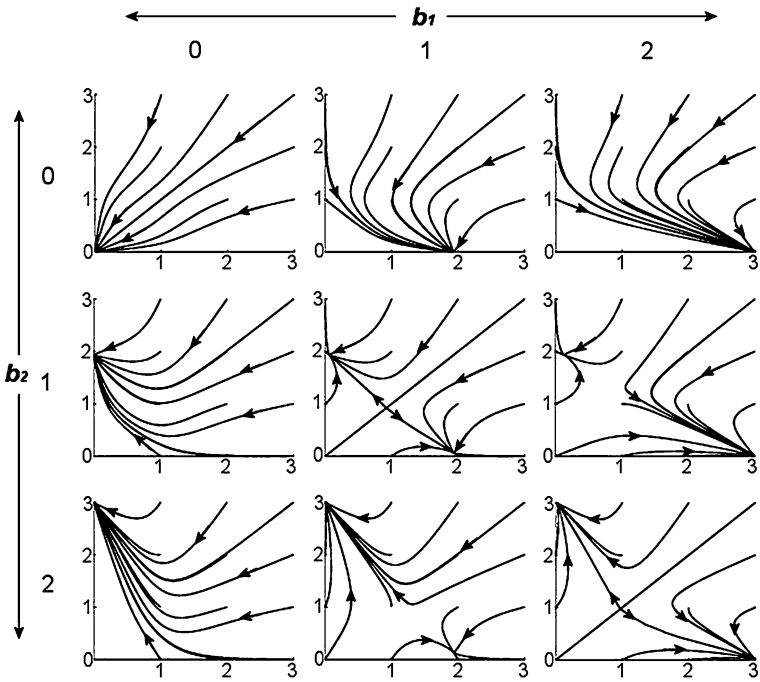

Fig. 2 Phase space configurations of the minimalist model (see (2)) given fixed parameter values. Each *panel* represents the y vs. x phase space for trajectories originating from a grid of initial states. The set of parameters for these possible configurations were $k_{1,2} = 1$, $n = 4$, and $b_{1,2}$ varied according to the values indicated on the *horizontal* and *vertical axes* of the entire 3×3 array. When present in each *panel*, the attractor for the erythorid lineage where GATA1 dominates is in the *lower right* and the attractor for the myelomonocytic lineage where PU.1 dominates is in the *upper left*

example, Fig. 2, panel row 1, column 2 and panel row 2, column 1). Nonetheless, this model is still capable of representing an uncommitted cell state with mutual weak expression of PU.1 and GATA1. If these two genes are expressed at sufficiently low levels, then only a single attractor, representative of an undifferentiated cell, will be present in the phase space (panel row 1, column 1). As the transcription factor expression levels increase, though the cell becomes increasingly pressured to commit to a given lineage. Provided both transcription factors maintain equal expression, the progenitor cell may precariously remain in an uncommitted state, represented by the saddle point (panel row 3, column 3), however any fine difference in the PU.1-GATA1 expression or any stochastic influence will be sufficient to cause cell lineage commitment.

4 Time-Dependent Parameters

4.1 General Model

The final stem cell fate is determined by the PU.1 and GATA1 expression levels, which are dependent on the strength and duration of each gene's self-excitation and

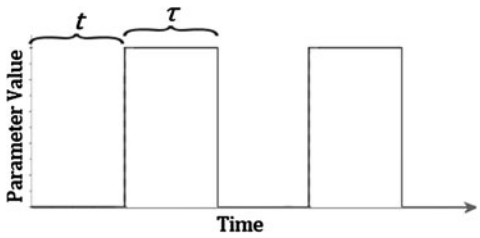

Fig. 3 Time-dependent parameter values. The *horizontal axis* represents time while the *vertical axis* represents the parameter value. These time-dependent changes were applied to one or both self-excitation or cross-inhibition parameters. Initially the parameter value is constant at a given value for a duration t before changing to a new value for a duration τ

cross-inhibition. Our goal in running these simulations was not to identify the full bifurcation diagram, which is well understood for models of a form similar to the one we used (see, e.g., Huang et al. 2007).

Instead, our objective was to explore changes in the global phase space structure in response to time-dependent changes in the parameters. Therefore, we explored how each gene's strength and duration of activity qualitatively affected the phase space by modeling the a-terms and b-terms as step functions. We choose to model gene activity-level changes with step functions as a computational simplification of small stochastic perturbation in the PU.1-GATA1 self-excitation and cross-inhibition levels. We introduced these time-dependent changes by replacing one or more of the self-excitation or cross-inhibition parameters by a square wave function with different on- and off-period cycles. This function is piecewise constant across these two intervals, t and τ (Fig. 3).

The discrete shift in a parameter's value from the end of interval t to start of interval τ acts as a switch. One way to look at this is by means of a time-dependent vector field. However, since this dependence is piecewise trivial, it is better to consider our system as the iteration of two independent and non-commuting flows, say $\mathcal{X}_1$ and $\mathcal{X}_2$. The former corresponds to the ODE introduced above when $a = 1$ and the latter corresponds to the ODE introduced above when $a = 0$. Those two vector fields are non-commuting in the classical Lie bracket sense; that is two vector field commute if and only if their respective flow commute. The system under consideration will be the composition of both vector field, i.e.,

$$g : \mathbb{R}^2 \to \mathbb{R}^2, \quad (x, y) \mapsto X_1^{\{t\}} \circ \mathcal{X}_2^{\{\tau\}}(x, y),$$

where $\mathcal{X}_1^{\{t\}}$ is the time t of $\mathcal{X}_1$ and $\mathcal{X}_2^{\{\tau\}}$ is the time τ of $\mathcal{X}_2$. The resulting map is a diffeomorphism and in general it is not the time 1 of a planar vector field.

Here we have used discontinuous changes in the parameter values. Perhaps, more realistically, sigmoidal functions could have been used. However, a numerical analysis comparing sigmoidal self-excitation or cross-inhibition curves and these discontinuous curves revealed no significant difference in the qualitative results.

4.1.1 Time-Dependent Self-Excitation in One Gene

There exist two factors that influence the potential final states available to the progenitor cell in our model when either PU.1 and GATA1 maintains a constant self-excitation while the other gene undergoes periodic change in its self-excitation. The principle factor in determining whether the stem cell is more likely to develop into an erythrocyte or myelomonocyte is the strength of gene self-excitation over a long duration. In cases where both PU.1 and GATA1 initiate self-excitation at the same intensity, if one of the two genes can periodically achieve a stronger self-excitation, then that gene will dominate the final states available to the progenitor cell. That is, if GATA1 is as active as PU.1 initially, but GATA1 has stronger self-excitation than PU.1 for short periods of time over a long duration, then the progenitor cell is more likely to develop into an erythrocyte. Likewise, given equal initial activation between PU.1 and GATA1, a periodically greater PU.1 self-excitation will influence the stem cell to more likely commit to the myelomonocytic lineage. In these cases, increasing durations of stronger self-excitation lead to the attractor being spread across a larger area of phase space. In addition to the larger basin of attraction, the periodically stronger gene has faster trajectory convergence on the attractor. However, these trajectories do not formally settle on the attractor associated with the periodically stronger gene. Instead, they fall into a cycle of rapid nearly linear oscillations as the gene repetitively cycles between its less active and more active states. The trajectories converging on the non-fluctuating gene exhibit only small oscillations. Qualitatively, the oscillations still represent stabilization on a committed cell lineage, as the oscillations are embedded within a particular lineage's basin of attraction.

The difference in the strength of the two genes during the periodically stronger activation is critical to influencing the final cell fate. When one gene periodically achieves only a slightly stronger self-excitation (e.g. an increase in a of 0.1), even a high frequency of stronger self-excitation is insufficient to significantly alter the potential final cell states. However, modestly stronger periodic self-excitation by one gene allows that gene to have a dominant attractor basin with its associated lineage. For instance, if GATA1 changes the intensity of its self-excitation from an arbitrary value of 1.0 to 2.0 once every 20 units of time (including at $t = 0$) for a duration of 1 unit of time, then the basin of attraction for the erythrocytic lineage is noticeably greater than the basin of attraction for the myelomonocytic lineage (Fig. 4).

The general rule is that the most potently expressed gene over time dictates the final evolved outcome of the progenitor cell. This also holds if either PU.1 or GATA1 periodically lowers its self-excitation to a weaker state for some duration. During even brief moments of weaker self-excitation, the non-fluctuating gene is capable of exerting a dominant influence over the system. For example, if GATA1 lowers its self-excitation very briefly once every 20 units of time, then the basin of attraction for the myelomonocytic lineage will encompass a greater phase space area than the basin of attraction for the erythorid lineage (Fig. 5). The change in self-excitation is especially important early in the system interaction. Due to relatively fast trajectory convergence within the basins of attraction, delaying these fluctuations results in phase space configurations that are qualitatively similar to scenarios without these fluctuations.

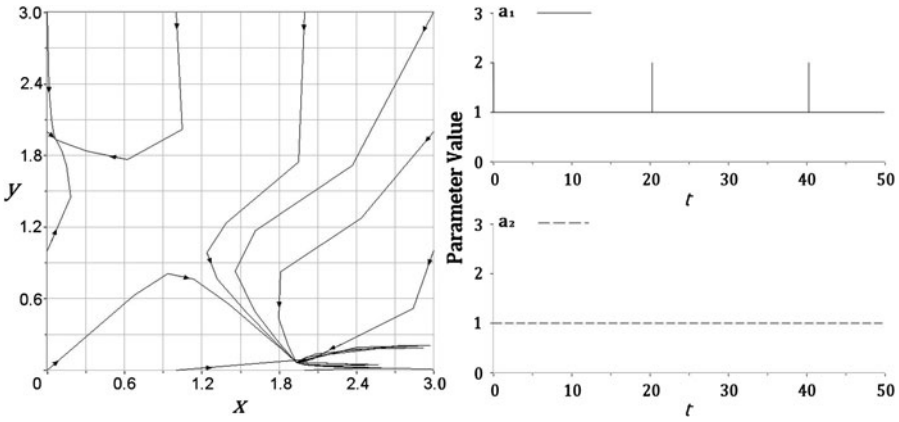

Fig. 4 Brief pulses in a gene's self-excitation allow the pulsing gene to achieve a dominant basin of attraction. In this figure, GATA1 pulses to a parameter value of $a = 2.0$ once every 20-units of t while PU.1 maintains a constant self-excitation at $a = 1.0$. This brief, intermittent pulsing extends the basin for the attractor of the erythorid lineage at the *lower right*

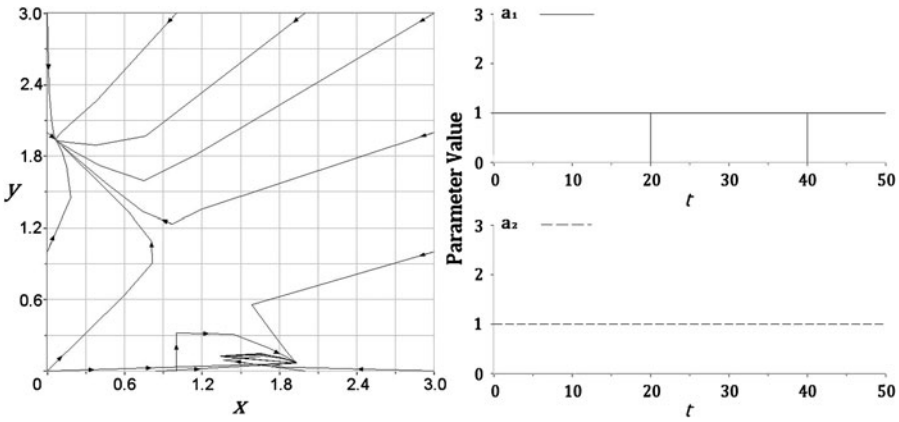

Fig. 5 Brief reductions in self-excitation of one gene can result in the other gene achieving a dominant basin of attraction. Here, GATA1 periodically weakens its self-excitation to $a = 0$ once every 20-units of t while PU.1 maintains a constant self-excitation of $a = 1.0$. The phase space becomes biased toward the myelomonocytic attractor (*upper left*) while the erythorid basin exhibits the formation of a limit cycle (*lower right*)

Interestingly, the presence of a single fluctuating self-excitation term in the PU.1-GATA1 gene system can result in limit cycles in the phase space (Figs. 5, 6). In cases where one gene's self-excitation remains constant while the other gene's self-excitation periodically fluctuates, limit cycles develop in association with the gene having the periodic self-excitation. Close inspection of the individual integration points shows that the trajectories are convergent to these orbit-like features. As long as the periodic changes in self-excitation persist, no fixed point stabilization is achieved by these orbit-trapped trajectories. The non-fluctuating self-excitation term, however, allows for only a single fixed point stability.

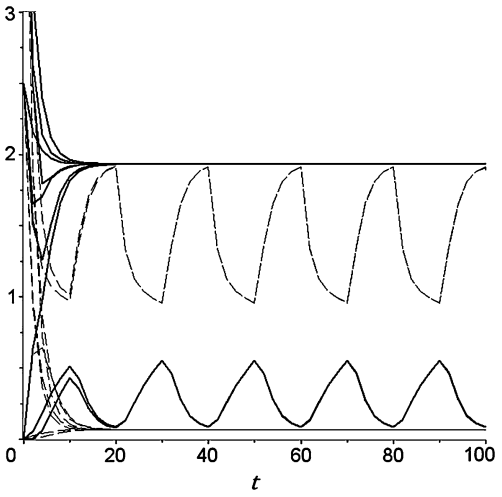

Fig. 6 Plotting the x (*bold lines*) and y (*thin*, *dashed lines*) coordinates of many trajectories over time reveals convergence on a limit cycle. The attractor associated with the non-fluctuating gene can be seen by trajectories converging to a steady value over time

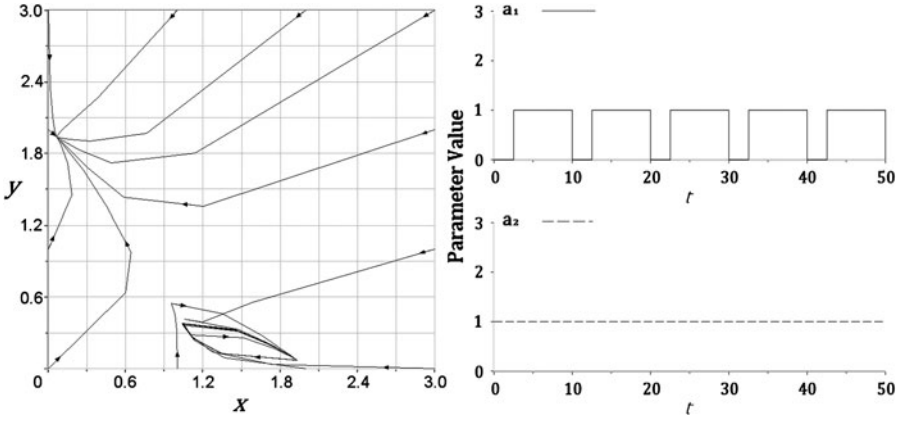

Fig. 7 Briefly weaker self-excitation on the part of one gene allows the other gene to achieve a larger basin of attraction with its associated lineage. In the case illustrated, GATA1 periodically strengthens its self-excitation to $a = 1.0$ starting at $t = 3$ and remains strengthened for 7 units of t. PU.1 remains at constant self-excitation at $a = 1$. Since the duration of the GATA1 self-excitation weak state is longer than in Fig. 5, the size of the limit cycle is larger around the erythroid attractor at the *lower right*

The expanse of these orbit-like features in phase space depends on the duration and period of the changes in self-excitation as well as whether the fluctuating gene periodically becomes dominant or weaker. Longer durations of the weakening of the self-excitation result in large limit cycles (Fig. 7).

When the self-excitation of the fluctuation gene is on average sufficiently weak, then the attractor associated with the non-fluctuating gene term can expand to capture the entire phase space (Fig. 8). In such a case, the fluctuating gene still influences the system. It acts as a slow manifold for a set of trajectories originating far from the non-fluctuating gene's stable node. In phase space, these trajectories exhibit transient limit cycles prior to escape into the stable gene's attractor basin.

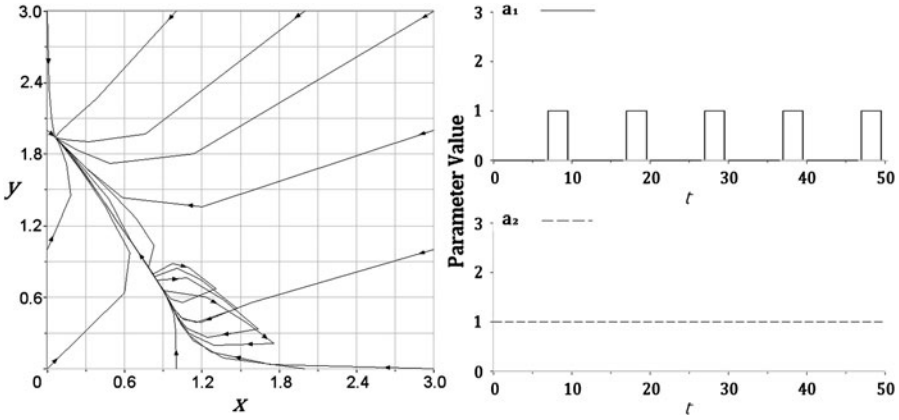

Fig. 8 When the self-excitation of one gene is unable to match the non-fluctuating gene's self-excitation level to a sufficient degree, the limit cycles associated with the fluctuating gene expand to the point that they intersect the saddle point. This results in all the trajectories eventually escaping into the non-fluctuating gene's attractor. In this case, the self-excitation of GATA1 periodically strengthens its self-excitation to $a = 1$ once every 7-units of t and remains strengthened for 3 units of t. PU.1 remains at constant self-excitation at $a = 1$. Since the GATA1 self-excitations matches that of the PU.1 self-activation for such short duration, the phase space is dominated by the myelomonocytic attractor at the *upper left*

A final observation from these simulations is that brief, but intense, bursts of self-excitation can result in a phase space dominated by a single attractor for that gene. This attractor is most potent if the bursting occurs early in the interaction (near $t = 0$) and is thereafter periodically repeated. The gene self-excitatory bursting establishes a fast convergence of initial condition trajectories to the bursting gene's attractor basin. With sufficiently potent gene bursting, the trajectories can be moved far enough from the non-bursting gene's attractor that reestablishment of a more equivalent levels of gene expression allows for only limited, slow reconvergence on the non-bursting gene's attractor. Qualitatively, this finding suggests that a single strong pulse of genetic activity can force the progenitor cell to commit to the lineage associated with the activity pulse and that the complete deactivation of one gene is unnecessary for the eventual evolution of a stable final cell lineage.

These results from numerical simulations suggest that the time-dependent self-excitation of PU.1 and GATA1 significantly affects what committed blood cell lineages are available to a progenitor cell. Importantly, these numerical simulations indicate that complete deactivation of either the PU.1 and GATA1 gene is not necessary for the progenitor cell to stabilize to one lineage. Only temporary changes in one of the two genes self-excitation intensities are needed for alteration of the final stem cell state. Specifically, the progenitor cell's lineage commitment can be directed by either of the two genes expressing stronger self-excitation over a sustained time period or through brief intense bursts.

4.1.2 Synchronous Time-Dependent Self-Excitation in Both Genes

Modeling the PU.1-GATA1 interaction with both genes exhibiting fluctuating self-excitation levels reveals outcomes that are quite similar to those arising from cases

where the self-excitation varies in only one of the two genes. Specifically, whichever gene has the longest and strongest self-excitation generates the dominant attractor basin in phase space. In cases where both self-excitation terms a fluctuate according to a step function, if the maximum of those step functions is at least 2.0, then the central attractor develops in phase space. This central attractor strongly separates the two lineage-committed basins of attraction that are situated near the axes, which allows the central attractor to operate as a stability inducing feature. The central attractor limits trajectory movement from one lineage-committed basin to the other. This behavior effectively stabilizes the cell state. Provided that the two a-terms reach a strength of 2.0 or greater for some time interval, the phase space trajectories will be prevented from crossing from one of the two fate committed attractor basins to the other, unless the a-term of one gene goes to zero.

In cases where the self-excitation $a_{1,2}$ is weaker than 2.0, other phase space configurations are possible depending on the precise time-dependent changes in the self-excitation terms of PU.1 and GATA1.

When the time-dependent fluctuations in self-excitation are synchronized, but opposite in phase, that is, where the self-excitation of one gene is decreased when that of the other gene is increased, the phase space becomes dominated by two separate limit cycles (Fig. 9). Qualitatively, such a phase space configuration is nearly identical to the bistable case that arises with constant self-excitation parameter values.

4.1.3 Asynchronous Time-Dependent Self-Excitation in Both Genes

When the PU.1 and GATA1 self-excitation periods are different, there are highly perturbed trajectories about the boundary region between the attractors (Fig. 10). Trajectories crossing what would be the saddle region if the two genes were expressed at a constant low self-excitation ($a_1 = a_2 = 1.0$) can have sharp changes in direction in time. The net effect of these perturbations is similar to a slow manifold. The amount

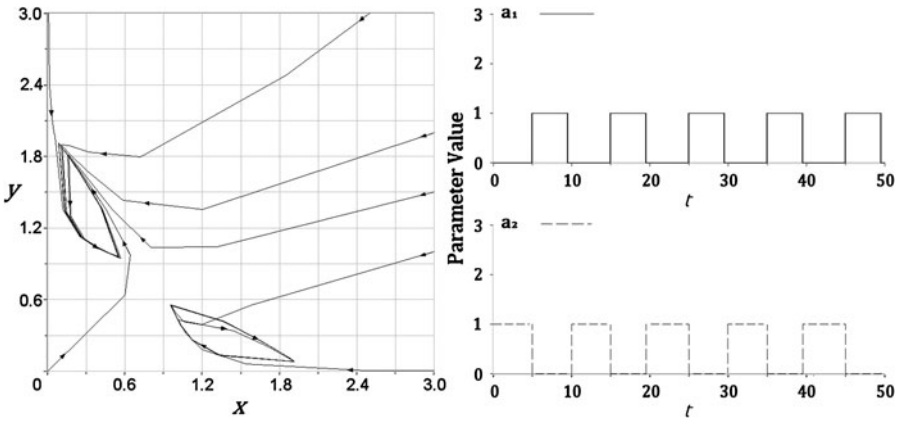

Fig. 9 If the self-excitation of both genes exhibit anti-phase fluctuations of equal intensity, then dual limit cycles emerge in the phase space. These limit cycles remain within the basins of attraction associated with their respective attractors, although the gene with the initially stronger self-excitation level will have a larger overall basin of attraction

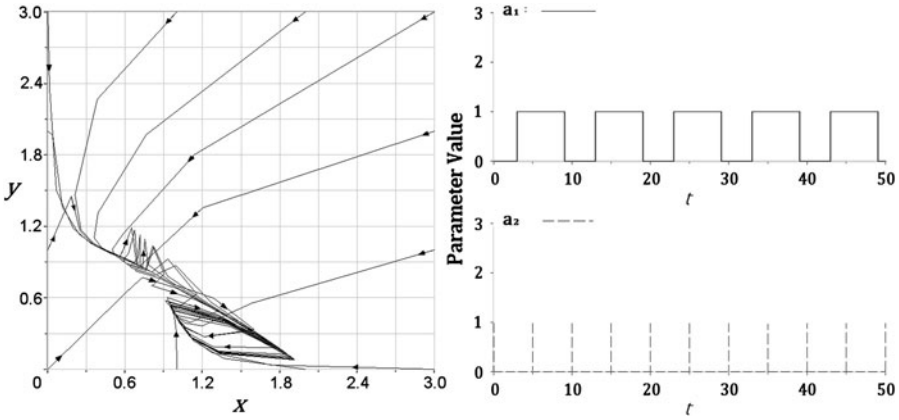

Fig. 10 Highly perturbed trajectories can emerge in phase space if the time-dependent changes in self-excitation allow trajectories from either basin of attraction to converge on the saddle for certain periods. Despite the perturbation, the trajectories are convergent on a single limit cycle associated with the gene expressing the longest self-excitation

of perturbation is associated with the expansion of the limit cycle of the non-dominant gene into the saddle region. The observed trajectory behavior is to be expected. If the boundary of a given limit cycle coincides with the location of the saddle during specific discrete time periods, then the trajectories that converged on the limit cycle fall upon the saddle point. When the trajectories trapped by the limit cycle reach the saddle point, the extreme instability of this point results in trajectories quickly moving in one of two directions along the unstable manifold. If trajectories cycle periodically from the limit cycle to the saddle point, then the instability present with the saddle point works its way into the trajectory behavior, this resulting in the perturbations noted in the phase space. Importantly, stability can still emerge in this unusual case. The perturbed trajectories eventually settle into the limit cycle associated with the gene expressing the longest duration of self-excitation.

Where $a_1 = a_2 = 2.0$ there is a third central attractor which works to channel trajectories toward the basin of attraction of the dominant gene.

4.1.4 Time-Dependent Cross-Inhibition in One Gene

The basins of attraction corresponding to committed lineages are readily perturbed by small changes in the duration and strength of the cross-inhibition of PU.1 and GATA1. Replacing either gene's constant cross-inhibition parameter with a time-dependent step function reveals that periodically weaker cross-inhibition allows the gene with steady cross-inhibitory activity to readily gain dominance over the system. Should both genes retain similar cross-inhibition over a long duration, with one of the two genes briefly weakening, then small limit cycles emerge within the weakening gene's associated lineage basin of attraction. As the weakening is extended across a longer time period, the limit cycles enlarge, becoming more pronounced (Fig. 11). However, once the duration of the weakening exceeds 2 units of time, the limit cycle ceases to exist. This happens as a result of the limit cycle boundaries merging with

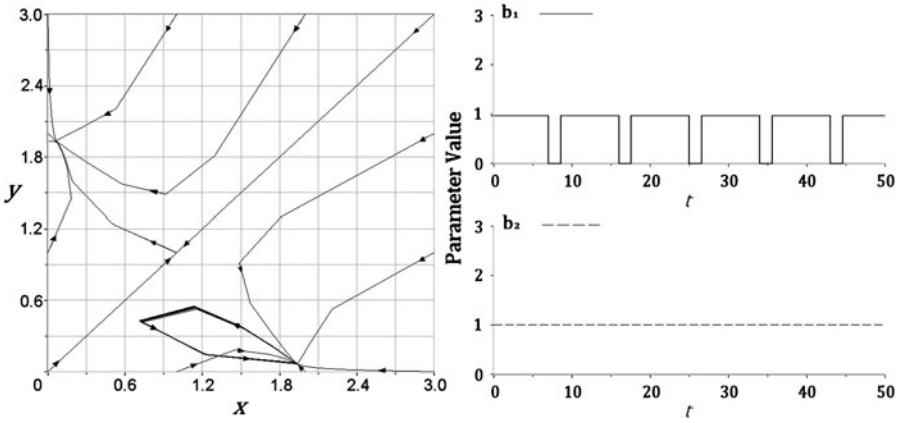

Fig. 11 Briefly weakening cross-inhibition on the part of either gene results in formation of limit cycles in the weakening gene's basin of attraction. In the case illustrated, the cross-inhibition of GATA1 periodically reduces to $b = 0$ every 8-units of t and remains at that level for 2 units of t. PU.1 maintains constant cross-inhibition at $b = 1$. A limit cycle now appears around the erythroid attractor (*lower right*)

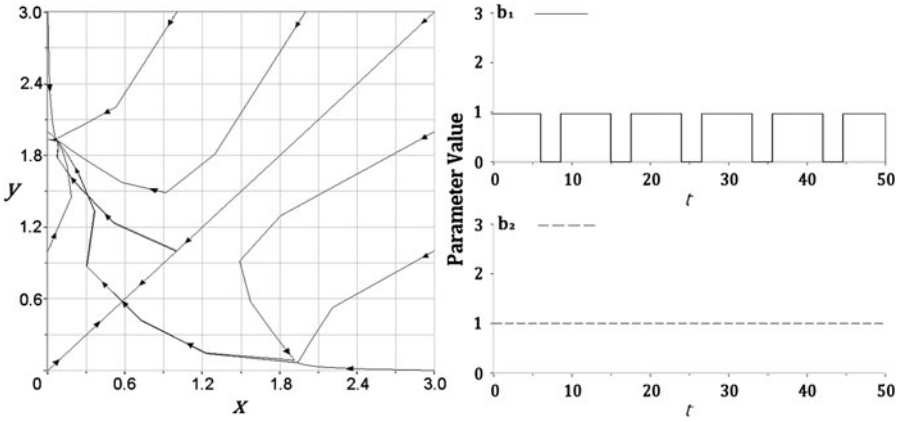

Fig. 12 With longer durations of cross-inhibitory weakening on the part of either gene, the limit cycles associated with the weakening gene merge with the saddle. This results in trajectories escaping into the non-fluctuating gene's basin of attraction, where the trajectories quickly converge on the attractor in that basin. In this case, the cross-inhibition of GATA1 periodically reduces to $b = 0$ every 7-units of t and remains at that level for 3 units of t. PU.1 maintains constant cross-inhibition at $b = 1$

the saddle point that separates the two basins of attraction distinguishing the two lineages. Once the trajectories confined to the limit cycle reach this saddle, they are able to escape the limit cycle and converge to the other attractor (Fig. 12). When this happens, the influence of the weakening gene changes from that of an attractor to a slow manifold.

If either PU.1 or GATA1 has a temporarily stronger cross-inhibition, then its attractor is strengthened and the basin of that attractor is increased. The two basins remain distinct, separated by the stable manifold of the saddle. However, no duration

or strength difference in periodically stronger cross-inhibition is sufficient to allow either gene to gain complete dominance over the system. These results predict that the PU.1-GATA1 interaction should be more sensitive to periodic weakening rather than periodic strengthening of the cross-inhibition of either gene.

4.1.5 Synchronous Time-Dependent Cross-Inhibition in Both Genes

Time-dependent changes in the cross-inhibition parameters of each gene at specific times can alter the fate of the stem cell. Whether or not the trajectories that qualitatively represent the stem cell's fate reach a given attractor basin depends on the duration and strength of the cross-inhibition activity levels.

There is a pitchfork bifurcation in the phase space that depends on the strength of the cross-inhibition. With weak cross-inhibition ($b_{1,2} = 1.0$) the phase space is controlled by a single stability that is close to a pitchfork bifurcation. In this case there is a contraction in the phase space whereby all trajectories converge toward the pre-bifurcating attractor.

With a strong cross-inhibition ($b_{1,2} = 2.0$), this single attractor is replaced by a saddle with a stable manifold separating the erythrocytic and myelomonocytic fate basins of attraction. The only way that trajectories in one of these two basins can reach the other basin is for the saddle-node to again be replaced by an attractor (corresponding to a weakening of both the PU.1 and GATA1 cross-inhibition) or for the cross-inhibition of one gene to become stronger than the other gene, which would result in the more strongly cross-inhibiting gene gaining a larger, dominant, basin of attraction with its associated cell fate.

4.1.6 Asynchronous Time-Dependent Cross-Inhibition in Both Genes

As is found from all the other simulations that employ time-dependent parameter changes, when the cross-inhibition of PU.1 and GATA1 each has different periods then the principle factors in determining the phase space evolution are the duration and strength of the cross-inhibition. The phase space configurations are then qualitatively similar to those configurations permissible with constant cross-inhibition parameters. The limit cycles that arise are confined to each gene's respective attractor basin. In general, the gene with the strongest cross-inhibition and longest duration of sustained cross-inhibition exhibits the larger basin of attraction. A gene that cross-inhibits with strong but short pulses will develop a larger basin of attraction in the phase space than a gene that cross-inhibits with weak but long pulses.

4.2 Minimalist Model without Self-Excitation

The simulations of the minimalist model, which lacks self-excitation, helps us better understand which dynamical properties require self-excitation and which do not. It had been thought that self-excitation is required in order to stabilize the fate of the stem cell. However, a key finding from our simulations of the minimalist model is that time-dependent changes of parameters can also stabilize the fate of the stem cell when self-excitation is not present. If there is time-dependent cross-inhibition of only

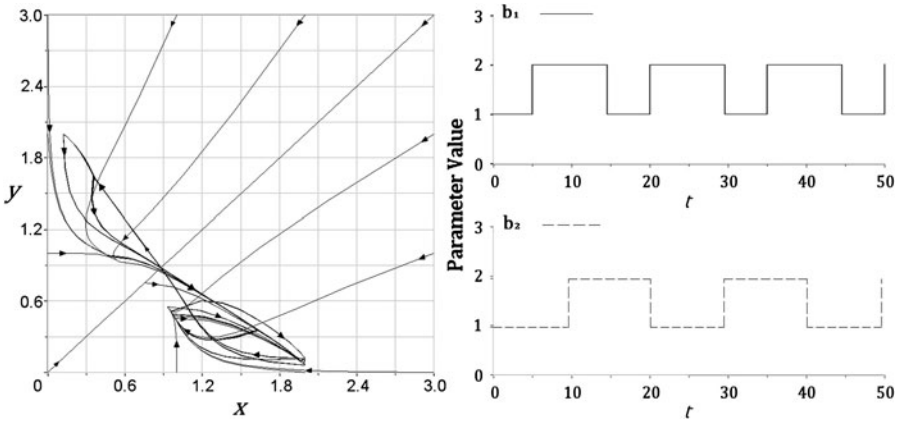

Fig. 13 Dual limit cycles can emerge in phase space if the cross-inhibition of both genes is time-dependent. Such a trajectory behavior arises if the duration that either gene is dominant is insufficient to allow stabilization on that gene's limit cycle. The resultant phase space exhibits fast-slow manifold behavior. The limit cycle associated with each gene is unstable, with trajectories converging into its limit cycle and eventually escaping into the other gene's basin of attraction

one gene, then the gene that possesses the higher average level of cross-inhibition will dominate the phase space. Trajectories falling into the basin of attraction of the gene with average weaker cross-inhibition will escape into the basin associated with the gene with average stronger cross-inhibition. Once the trajectories from the weaker gene cross the stable manifold of the saddle, they remain in the basin of the stronger gene. If the fluctuations in the level of cross-inhibition are small, then a limit cycle can develop in phase space alternating between the two genes (Fig. 13). If the average cross-inhibition of one gene increases, for example, for 1 unit magnitude, 10 percent of the time, then these oscillatory trajectories enter and converge within the basin of attraction associated with the dominant gene. If there are brief but intense pulses in the cross-inhibition of a gene, then the trajectories converge more rapidly on that gene's attractor.

As is the case throughout our simulations, when the cross-inhibition terms are changed from having constant parameter values to having time-dependent parameters, the guiding rule in determining what qualitative results to expect is that whichever gene has the stronger cross-inhibition level over time will have greater influence on the system. Two conclusions may be drawn from these simulations. Foremost, it is advantageous for a gene to steadily cross-inhibit the opposed gene if both genes are mutually cross-inhibiting at similar levels. However, if both genes maintain similar activation levels over a long duration, and one of the genes can periodically emit a stronger burst in cross-inhibition levels, then the bursting gene will benefit with a greater likelihood of the system stabilizing within its attractor.

4.3 Discussion

We explored the dynamical behavior of models of the PU.1-GATA1 gene circuit that include self-excitation and cross-inhibition as well as models with only cross-inhibition since there is limited evidence that GATA1 posses self-excitation (Yu et al.

Table 1 Parameters used to generate the phase space figures. For non-constant parameters, the value corresponding to time t is provided followed by the value corresponding to time τ. The duration of each time interval is also indicated. For all simulations, the k parameters were fixed at 1 while the n parameters were fixed at 4. These parameters are similar to those chosen by Huang et al. (2007)

Figure	Self-excitation		Cross-inhibition	
	GATA1	PU.1	GATA1	PU.1
4	1.0 ($t = 0$ to $t = 20$) 2.0 (at $t = 20$, $t = 40$)	1.0 (constant)	1.0 (constant)	1.0 (constant)
5	1.0 ($t = 0$ to $t = 20$) 0 (at $t = 20$, $t = 40$)	1.0 (constant)	1.0 (constant)	1.0 (constant)
7	0 ($t = 0$ to $t = 3$) 1.0 ($t = 3$ to $t = 10$)	1.0 (constant)	1.0 (constant)	1.0 (constant)
8	0 ($t = 0$ to $t = 7$) 1.0 ($t = 7$ to $t = 10$)	1.0 (constant)	1.0 (constant)	1.0 (constant)
9	0 ($t = 0$ to $t = 5$) 1.0 ($t = 5$ to $t = 10$)	1.0 ($t = 0$ to $t = 5$) 0 ($t = 5$ to $t = 10$)	1.0 (constant)	1.0 (constant)
10	0 ($t = 0$ to $t = 3$) 1.0 ($t = 3$ to $t = 9$)	1.0 at $t = 0$, $t = 5$ 0 ($t = 1$ to $t = 5$)	1.0 (constant)	1.0 (constant)
11	1.0 (constant)	1.0 (constant)	1.0 ($t = 0$ to $t = 8$) 0 ($t = 8$ to $t = 10$)	1.0 (constant)
12	1.0 (constant)	1.0 (constant)	1.0 ($t = 0$ to $t = 7$) 0 ($t = 7$ to $t = 10$)	1.0 (constant)
13	0.0 (minimalist model)	0.0 (minimalist model)	1.0 ($t = 0$ to $t = 5$) 2.0 ($t = 5$ to $t = 10$)	1.0 ($t = 0$ to $t = 10$) 2.0 ($t = 10$ to $t = 20$)

2002). Numerical simulations using constant parameter values in our models reveal phase space configurations similar to those from other recently devised models of blood stem cell differentiation (see, e.g., Huang et al. 2007). Specifically, our model successfully captures the uncommitted cell fate with low levels of PU.1 and GATA1 activity while demonstrating bistability with mutually higher levels of expression of these genes. Our work extends those models by determining the potential phase space configurations where the self-excitatory and cross-inhibitory parameters are time-dependent step functions (parameter choices given in Table 1).

Such a time dependency may well represent biological activity such as gene bursting or the influence of noise, both of which are known to have significant consequences for biological systems (Lei 2008). New phase space patterns arise when one or more of the model parameters fluctuate in time. From a classical dynamical investigation of these novel phase space configurations, we identify a number of novel outcomes with the PU.1-GATA1 circuit.

First, as already known, in models with constant levels of self-excitation and cross-inhibition with these two genes, if one gene is strongly active while the other is weakly active, then the phase space will exhibit a single attractor associated with the strongly active gene.

Second, should both gene's self-excitatory feedback loops fluctuate in time, where neither PU.1 or GATA1 are simultaneously active, then the phase space will exhibit two limit cycles, each one associated with the myelomonocytic or erythrocytic lineage basins of attraction.

Third, if one of the two genes is periodically weaker in its self-excitation, then the bistable phase space configuration does not necessarily exist. Instead, the gene that is intermittently weaker does not constitute an attractor in the phase space. This periodically weaker gene causes the emergence of limit cycles within its traditional basin of attraction. With sufficient gene weakening, these limit cycles act to form a slow manifold connected with the attractor of the stronger gene. The trajectories from the weaker gene slowly evolve into the basin of the attractor of the stronger gene.

Fourth, our minimalist model without self-excitation can lead to stable states and thus stable cell fates when the cross-inhibition is time dependent. In cases where either PU.1 or GATA1 periodically lessens its cross-inhibition of the other gene, then the steadily active gene can achieve a dominant attractor within the phase space. The gene that is associated with the periodic weakened inhibition will be unable to develop a stable attractor in the phase space. We also find that if both genes cross-inhibit each another at similar levels, but one of the genes briefly intensifies its cross-inhibition, then those brief intensifications bursts are capable of preventing any stabilization on the attractor associated with the constant gene's lineage. The bursting gene forms limit cycles within its own basin of attraction, and trajectories from all initial conditions in the phase space converge to these limit cycles. These findings reveal that the proposed self-excitatory feedback loops with the PU.1-GATA1 circuit are not necessarily required for the stabilization of a single cell fate.

Fifth, previous studies have not emphasized the evolution of phase space trajectories from one attractor landscape to another in continuous time. Importantly, our work demonstrates that the phase space configurations arising from time-dependent gene activity in the GATA1-PU.1 circuit may lead to elaborate phase space landscapes that exhibit unanticipated trajectory behaviors.

Collectively these results suggest that small time-dependent changes in self-excitation and cross-inhibition in the GATA1-PU.1 circuit may be sufficient to induce significant changes in what fates are available to the progenitor cell. The dynamics that we have observed with time-dependent parameters should heighten the awareness that time varying deterministic and stochastic factors may play an important role in determining the fate of blood stem cells. These findings may inform additional modeling efforts as well as experimental research and may be applicable to a wide range of such binary toggle switch systems.

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