Mathematical Analysis of a Multistable Switch Model of Cell Differentiation

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Abstract

Non-binary simultaneous decision network of gene regulation represents a cell differentiation process that involves more than two possible cell lineages. The simultaneous decision network is an alternative to the hierarchical models of gene regulation and it exhibits possible presence of multistable master switches. To investigate the qualitative behavior of the dynamics of the simultaneous decision network, we employ geometric techniques in the analysis of the network's corresponding system of ordinary differential equations (ODE). We determine the location and the maximum number of equilibrium points given a set of parameter values. Our analysis shows that the solution to the ODE model always converge to a stable equilibrium point. Varying the values of some parameters, such as the degradation rate and the amount of exogenous stimulus, can decrease the size of the basin of attraction of an

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undesirable steady state as well as increase the size of the basin of attraction of a desirable steady state. A sufficient change in some parameter values can silence or reactivate gene transcription that results to cell fate switching without the aid of stochastic noise. We further show that increasing the amount of exogenous stimulus can shutdown multistability of the system such that only one stable equilibrium point remains.

Keywords: cellular programming, deterministic reprogramming, gene regulatory network, hill function, ordinary differential equation, multistability

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1. Introduction

The field of Biomathematics has proven to be useful and essential for understanding the behavior and control of dynamic biological interactions. These interactions span a wide spectrum of spatio-temporal scales — from interacting molecules in a cell to individual organisms in a community, and from fast interactions occurring within seconds to those that slowly progress in years. Mathematical and *in silico* models enable scientists to generate quantitative predictions that may serve as initial input for testing biological hypotheses to minimize trial and error, as well as to investigate complex biological systems that are impractical or infeasible to study through *in situ* and *in vitro* experiments [1].

One classic question that scientists want to answer is how simple cells generate complex organisms. In this study, we are interested in the analysis of gene interaction networks that orchestrate the differentiation of stem cells

to various cell lineages that make up an organism [2, 3, 4, 5, 6]. Cellular reprogramming can induce cells to switch cell lineages (transdifferentiation) [7, 8, 9, 10] or switch back to a pluripotent state (dedifferentiation) [11, 12, 13, 14, 15]. We are motivated by the prospects of utilizing stem cells in regenerative medicine, in revolutionizing drug discovery, and in the control of cancer stem cells that had been hypothesized to maintain the growth of tumors [16, 17, 18, 19, 20].

According to Waddington's model [21], cell differentiation is similar to a ball rolling down a landscape of hills and valleys. The ridges of the hills can be regarded as the unstable equilibrium points while the parts of the valleys where the ball can stay without rolling further (i.e., at relative minima of the landscape) can be regarded as stable equilibrium points or attractors. An attractor represents a specific cell type. The theory that some cells can differentiate into many different cell types gives the idea that the mathematical model representing the dynamics of such cells may exhibit multistability [22, 23, 24]. Cinquin and Demongeot [25] formulated a gene regulatory network (GRN) model that can represent cellular differentiation with more than two possible outcomes (multistability) obtained through different developmental pathways. The simultaneous decision network (see Figure (1)) is one of the possible representations of Waddington's illustration where there are possibly many cell lineages involved. This representation is an alternative model to the binary or boolean hierarchic decision network [26, 25, 27, 28]. Moreover, the Cinquin-Demongeot ODE model can represent not only molecular processes but also other similar biological interactions, such as interaction among species in a community.

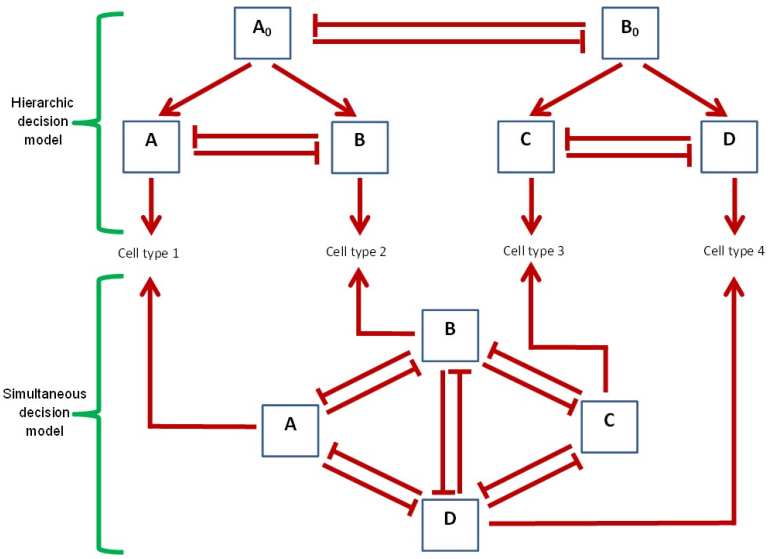

Figure 1: Hierarchic decision model and simultaneous decision model. Bars represent repression or inhibition, while arrows represent activation. [25, 26].

Cinquin and Demongeot translated the simultaneous decision network with autocatalysis (autoactivation) and mutual inhibition into an ODE model [25]. All elements in the original Cinquin-Demongeot ODE model are symmetric, that is, each node has the same relationship with all other nodes, and all equations in the system of ODEs have equal parameter values. In this paper, we further investigate a generalized Cinquin-Demongeot ODE model with more adjustable parameters to represent a wider range of situations. The state variables of the ODE model represent the concentration of the transcription factors (TFs) involved in gene expression towards a certain cell lineage.

Stability and bifurcation analysis of the generalized Cinquin-Demongeot ODE model can help in understanding the dynamics of cellular differentia-

tion. We determine the biologically feasible (nonnegative real-valued) coexisting stable equilibrium points of the ODE model for a given set of parameters. We then identify if varying the values of some parameters, such as those associated with the exogenous stimuli, can steer the system toward a desired state.

Furthermore, we present a case where the generalized Cinquin-Demongeot ODE model can be used. We represent a phenomenological gene regulatory network of a mesenchymal cell differentiation system [29] using the simultaneous decision model. This GRN is composed of four nodes consisting of pluripotency and differentiation modules. The differentiation module represents a circuit of transcription factors that activate osteogenesis, chondrogenesis, and adipogenesis.

2. ODE model representing GRN dynamics

Models of GRN often use the function H^+ (or H^-) which is bounded monotone increasing (or decreasing) with values between zero and one. Examples of such function are the sigmoidal H^+ and H^- called the classical *Hill functions*, which are defined as

$$H^+([X]) := \frac{[X]^c}{K^c + [X]^c},\ c > 1 \tag{1}$$

for activation of gene expression and

$$H^-([X]) = 1 - H^+([X], K, c) = \frac{K^c}{K^c + [X]^c},\ c > 1 \tag{2}$$

for repression [30, 28, 31]. The variable $[X]$ is the concentration of the molecule involved. The parameter K is the threshold or dissociation constant and is equal to the value of X at which the Hill function is equal

to 1/2. The parameter c is called the Hill constant or Hill coefficient and describes the steepness of the Hill curve. The Hill constant often denotes multimerization-induced cooperativity and may represent the number of cooperative binding sites. However, in some cases, the Hill constant can be a positive real number (not necessarily integer-valued) [30]. If $c = 1$, then there is no cooperativity [25] and the Hill function becomes the Michaelis-Menten function which is hyperbolic. If data are available, we can estimate the value of c by inference.

2.1. The original Cinquin and Demongeot ODE model

A state $X = ([X_1], [X_2], ..., [X_n])$ represents a temporal stage in the cellular differentiation or programming process. We define $[X_i]$ as a *component* (coordinate) of a state which represents the concentration of the corresponding TF protein. A stable state (stable equilibrium point) $X^* = ([X_1]^*, [X_2]^*, ..., [X_n]^*)$ represents a certain cell type, e.g., pluripotent, tripotent, bipotent, unipotent or fully (terminally) differentiated cell.

Let us suppose we have n antagonistic transcription factors such that each TF expression is subject to a first-order degradation (exponential decay). The parameters β, c and g represent the relative speed of transcription (or strength of the unrepressed TF expression relative to the first-order degradation), cooperativity and "leak", respectively [25]. The parameter g is a basal expression of the corresponding TF and a constant production term that enhances the value of $[X_i]$, which is possibly affected by an exogenous

stimulus. The original Cinquin-Demongeot ODE model [25] is

$$\frac{d[X_i]}{dt} = \frac{\beta[X_i]^c}{1 + \sum_{j=1}^{n} [X_j]^c} - [X_i] + g, \ i = 1, 2, ..., n. \tag{3}$$

The function formed by the term

$$\frac{\beta[X_i]^c}{1 + \sum_{j=1}^{n} [X_j]^c} \tag{4}$$

represents a multivariate Hill-like curve.

In this study, we consider Cinquin-Demongeot model with autocatalysis because autocatalysis is a common property of cell fate-determining factors known as "master" switches [25]. For simplification, only the transcription regulation process is considered in modeling cell differentiation. The model is also assumed to be intracellular and cell-autonomous (i.e., we only consider processes inside a single cell without the influence of other cells).

By using an ODE model, we assume that the time-dependent macroscopic dynamics of the GRN are continuous in both time and state space. We assume continuous dynamics because the process of lineage determination involves a temporal extension, that is, cells pass through intermediate stages [32]. ODEs are primarily used to represent the average dynamics of phenomenological (coarse-grained) regulatory networks [32].

2.2. The generalized Cinquin-Demongeot ODE model

In [25], Cinquin and Demongeot suggested to extend their model to include combinatorial interactions and non-symmetrical networks (i.e., each node does not have the same relationship with other nodes and all equations

in the system of ODEs do not have equal parameter values). We include more adjustable parameters to their model to represent a wider range of situations. In this generalized model, some cell differentiation factors can be stronger than others. We generalize the Cinquin-Demongeot (2005) ODE model as follows:

$$\frac{d[X_i]}{dt} = F_i(X) = \frac{\beta_i [X_i]^{c_i}}{\overline{K_i}^{c_i} + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}}} + \alpha_i s_i - \rho_i [X_i] \qquad (5)$$

where $i = 1, 2, ..., n$ and n is the number of nodes. To have biological significance, we restrict the parameters to be nonnegative real numbers.

The parameter β_i is the relative speed of transcription, ρ_i is the assumed first-order degradation rate associated with X_i, and γ_{ij} is the differentiation stimulus that affects the inhibition of X_i by X_j. If $\gamma_{ij} = 0$ then X_j does not inhibit the growth of $[X_i]$. Let $g_i = \alpha_i s_i$, which represents basal or constitutive expression of the corresponding TF that is affected by the exogenous stimulus with concentration s_i and rate α_i. In other words, g_i is a constant production term that enhances the concentration of X_i.

We define the multivariate function H_i by

$$H_i([X_i], [X_2], ..., [X_n]) = \frac{\beta_i [X_i]^{c_i}}{\overline{K_i}^{c_i} + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}}} \qquad (6)$$

which comes from the classical Hill equation. The terms $\sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}}$ in the denominator reflects the inhibitory influence of other TFs on the change of concentration of X_i. For simplicity, let $K_i = \overline{K_i}^{c_i} > 0$, which is related to the threshold or dissociation constant.

The parameter $c_i \geq 1$ represents the Hill constant and affects the steepness of the Hill curve associated with $[X_i]$, and denotes autocatalysis (homomultimerization-induced positive cooperativity). The parameter c_{ij}, $j \neq i$ denotes mutual inhibition (heteromultimerization-induced negative cooperativity). Cooperativity describes the interactions among binding sites where the affinity or relationship of a binding site positively or negatively changes depending on itself or on the other binding sites. If $1 < c_i \leq n$ then X_i has autocatalytic cooperativity, and if $1 < c_{ij} \leq n$ then the affinity of X_j to X_i has negative cooperativity. In addition, cooperativity requires more than one binding site. The state variable X_i has no autocatalytic cooperativity if $c_i = 1$, while the affinity of X_j to X_i has no negative cooperativity if $c_{ij} = 1$.

Notice that the lower bound of H_i (6) is zero and its upper bound is β_i. Thus, the parameter β_i can also be interpreted as the maximal expression rate of the corresponding TF.

We only consider the biologically feasible points — those that are real-valued and nonnegative. The initial value $X_0 = ([X_1]_0, [X_2]_0, ..., [X_n]_0)$ should always be biologically feasible.

Proposition 1. *The flow of the ODE model (where $X_0 \in \mathbb{R}^{\oplus n}$ can be any initial condition) is always in $\mathbb{R}^{\oplus n}$ (that is, always nonnegative).*

Proof. Since we are considering only the biologically feasible points, then either $d[X_i]/dt|_{[X_i]=0} = 0$ or $d[X_i]/dt|_{[X_i]=0} > 0$ but $d[X_i]/dt|_{[X_i]=0} \not< 0$. That is, if a component of a state variable is zero then the component will either stay zero or become positive but never negative (Note that the instantaneous rate of change $d[X_i]/dt|_{[X_i]=0} > 0$ happens only when $g_i > 0$). Hence, we are sure that the values of the state variables of the generalized Cinquin-

Demongeot ODE model (5) with non-negative initial condition are always non-negative. □

2.3. Geometry of the Hill function

The Hill function defined by Equation (6) is a multivariate sigmoidal function when $c_i > 1$ and a multivariate hyperbolic-like function when $c_i = 1$. We can investigate the multivariate Hill function by looking at the univariate function defined by

$$H_i([X_i]) = \frac{\beta_i [X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}}} \tag{7}$$

where each $[X_j]$, $j \neq i$ is taken as a parameter. This means that we project the high-dimensional space onto a two-dimensional plane. If $c_i = 1$, the graph of the univariate Hill function in the first quadrant of the Cartesian plane is hyperbolic (for any value of $[X_j]$, $j \neq i$). If $c_i > 1$, the graph of the univariate Hill function in the first quadrant is sigmoidal or "S"-shaped (for any value of $[X_j]$, $j \neq i$).

It is always true that

$$\frac{\beta_i [X_i]^{c_i}}{K_i + [X_i]^{c_i}} \geq \frac{\beta_i [X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}}} \tag{8}$$

for any value of $[X_j]$ $\forall j$. In other words, when the value of

$$K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}} \tag{9}$$

in the denominator of $H_i([X_i])$ increases, the graph of the Hill curve shrinks. Moreover, when the value of c_i increases, the graph of $Y = H_i([X_i])$ gets

steeper. If we add a term g_i to $H_i([X_i])$ then the graph of $Y = H_i([X_i])$ in the Cartesian plane is translated upwards by g_i units.

3. Equilibrium points

Definition 1. *Stable component* and *stable equilibrium point.* If $[X_i]$ converges to $[X_i]^*$ for all initial conditions $[X_i]_0$ near $[X_i]^*$, then we say that the i-th component $[X_i]^*$ of an equilibrium point X^* is stable; otherwise, $[X_i]^*$ is unstable. The equilibrium point $X^* = ([X_1]^*, [X_2]^*, ..., [X_n]^*)$ of the system (5) is stable if and only if all its components are stable.

To find the equilibrium points, we need to solve the multivariate equation $F_i(X) = 0$ by solving the intersections of the $(n + 1)$-dimensional curve induced by $H_i([X_1], [X_2], ..., [X_n]) + g_i$ and the $(n+1)$-dimensional hyperplane induced by $\rho_i[X_i]$. That is, we find the real solutions to

$$\frac{\beta_i[X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} + \alpha_i s_i = \rho_i[X_i]. \tag{10}$$

For easier analysis, we observe the intersections of the univariate functions defined by $Y = H_i([X_i]) + g_i$ and $Y = \rho_i[X_i]$ while varying the value of $K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ in the denominator of the univariate Hill function $H_i([X_i])$ (see Figure (2) for illustration). In the univariate case, we can look at $Y = \rho_i[X_i]$ as a line in the Cartesian plane passing through the origin with slope equal to ρ.

Theorem 1. *Suppose $\rho_i > 0$ for all i. Then the generalized Cinquin-Demongeot ODE model (5) with $X_0 \in \mathbb{R}^{\oplus n}$ always has a stable equilibrium*

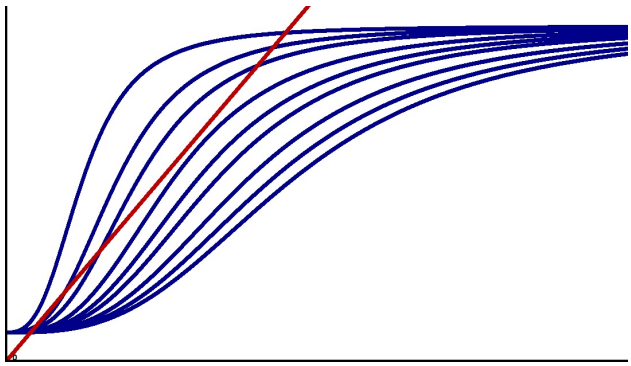

Figure 2: The intersections of $Y = \rho_i[X_i]$ and $Y = H_i([X_i]) + g_i$ with varying values of $K_i + \sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$, an example.

point. Moreover, any trajectory of the model will converge to a stable equilibrium point.

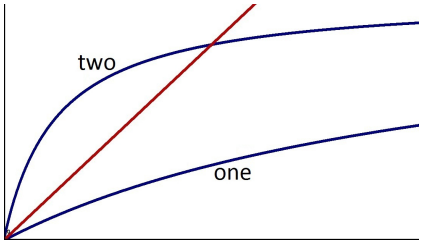

Figure 3: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i([X_i]) + g_i$ where $c_i = 1$ and $g_i = 0$. The value of $K_i + \sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is fixed.

Proof. Figures (3) to (6) illustrate all possible cases showing the topologies of the intersections of $Y = \rho_i[X_i]$ and $Y = H_i([X_i]) + g_i$. We employ the geometric analysis shown in Figure (7) (where we rotate the graph of the curves, making $Y = \rho_i[X_i]$ the horizontal axis) to each topology of the intersections

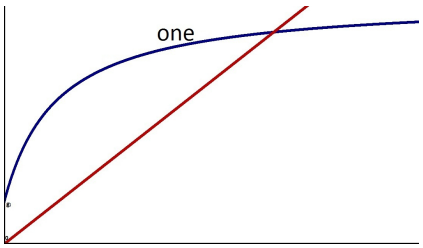

Figure 4: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i([X_i]) + g_i$ where $c_i = 1$ and $g_i > 0$. The value of $K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is fixed.

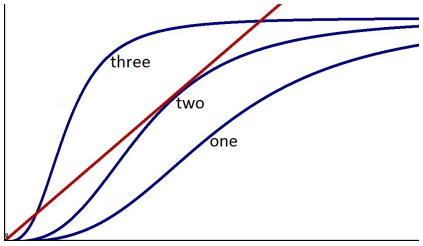

Figure 5: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i([X_i]) + g_i$ where $c_i > 1$ and $g_i = 0$. The value of $K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is fixed.

of $Y = \rho_i[X_i]$ and $Y = H_i([X_i]) + g_i$. Given specific values of $[X_j]$, $j \neq i$, the univariate Hill curve $Y = H_i([X_i])$ and $Y = \rho_i[X_i]$ have the following possible number of intersections (see Figures (3) to (6)):

- two intersections (where one is stable);
- one intersection (which is stable); or
- three intersections (where two are stable).

We can see that there always exists a stable intersection located in the first quadrant (including the axes) of the Cartesian plane. We can also

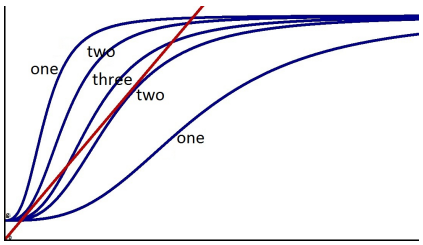

Figure 6: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i([X_i]) + g_i$ where $c_i > 1$ and $g_i > 0$. The value of $K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is fixed.

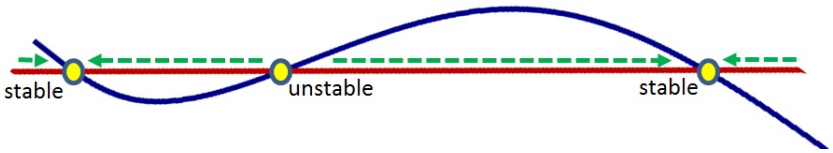

Figure 7: The curves are rotated making the line $Y = \rho_i[X_i]$ as the horizontal axis. Positive gradient means instability, negative gradient means stability. If the gradient is zero, we look at the left and right neighboring gradients.

observe that when there are two or more intersections, the value of one stable intersection is always greater than the value of the unstable intersection — implying that any solution to the ODE is bounded.

By inspecting each component of all possible equilibrium points, we can conclude that there is always an equilibrium point that attracts the trajectory of our ODE model for any initial condition. □

Remark 1. Given nonnegative state variables and parameters in (5), if $g_i > 0$ then $\rho_i > 0$ is a necessary and sufficient condition for the existence of an equilibrium point. Moreover, if $g_i = 0$ and $\rho_i = 0$ then we have an equilibrium point with zero i-th component (i.e., $(..., \overset{i}{0}, ...)$), but this equilibrium point is

obviously unstable.

3.1. Location of equilibrium points

Proposition 2. *Suppose $\rho_i > 0$. If both $\beta_i > 0$ and $g_i > 0$ then g_i/ρ_i cannot be an i-th component of an equilibrium point.*

Remark 2. If $g_i, \rho_i > 0$ then $[X_i] = g_i/\rho_i$ can only be an i-th component of an equilibrium point if $\beta_i = 0$.

Theorem 2. *Suppose $\rho_i > 0$. The value $\frac{g_i+\beta_i}{\rho_i}$ is the upper bound of, but will never be equal to, $[X_i]^*$ (where $[X_i]^*$ is the i-th component of an equilibrium point). The equilibrium points of our system lie in the hyperspace*

$$\left[\frac{g_1}{\rho_1}, \frac{g_1+\beta_1}{\rho_1}\right) \times \left[\frac{g_2}{\rho_2}, \frac{g_2+\beta_2}{\rho_2}\right) \times ... \times \left[\frac{g_n}{\rho_n}, \frac{g_n+\beta_n}{\rho_n}\right). \tag{11}$$

Proof. The minimum value of H_i is zero which happens when $\beta_i = 0$ or when $[X_i] = 0$. Hence, if $H_i([X_1], [X_2], ..., [X_n]) = 0$ then $F_i(X) = g_i - \rho_i[X_i] = 0$, implying $[X_i] = g_i/\rho_i$.

Note that $[X_i]^* < \infty \; \forall i$ because $[X_i]^* = \infty$ cannot be a component of an equilibrium point. The upper bound of H_i is β_i which will only happen when $[X_i] = \infty$. If $H_i([X_1], [X_2], ..., [X_n]) = \beta_i$ then $F_i(X) = \beta_i - \rho_i[X_i] + g_i = 0$, implying $[X_i] = \frac{g_i+\beta_i}{\rho_i}$. □

Remark 3. The Hill curve and $\rho[X_i]$ intersect at infinity when $g_i \to \infty$, $\beta_i \to \infty$ or $\rho_i \to 0$. Moreover, if we have multiple stable equilibrium points lying on the hyperspace (11) then one strategy for increasing the basin of attraction of a stable equilibrium point is by increasing the value of g_i, β_i or

ρ_i (however, the number of stable equilibrium points may change by doing this strategy).

Proposition 3. *The generalized Cinquin-Demongeot ODE model (5) has an equilibrium point with i-th component equal to zero (i.e.,* $[X_i]^* = 0$*) if and only if* $g_i = 0$.

The following corollary is very important because the case where the trajectory converges to the origin $(0, 0, ..., 0)$ is trivial. The zero state neither represents a pluripotent cell nor a cell differentiating into the cell lineages considered in the scope of the given GRN. Zero state may also represent a cell in quiescent stage.

Corollary 1. *The zero state* $(0, 0, ..., 0)$ *can only be an equilibrium point if and only if* $g_i = 0$ *for all* i.

3.2. Cardinality of equilibrium points

In this section, we use the Bézout Theorem [33] to determine the possible maximum number of equilibrium points. It is also important to note that when at least two polynomials in our polynomial system have a non-constant common factor then the polynomial system has infinitely many complex solutions.

Suppose c_i and c_{ij} are integers for all i and j. The corresponding polynomial equation to

$$F_i(X) = \frac{\beta_i [X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}}} - \rho_i [X_i] + g_i = 0 \qquad (12)$$

is

$$\begin{aligned} P_i(X) =& \beta_i[X_i]^{c_i} + (g_i - \rho_i[X_i]) \left(K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} \right) = 0 \\ =& -\rho_i[X_i]^{c_i+1} + (\beta_i + g_i)\,[X_i]^{c_i} - \left(K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} \right) (\rho_i[X_i]) \\ & + g_i \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} + g_i K_i = 0. \end{aligned} \tag{13}$$

Proposition 4. *Assume that there is only a finite number of equilibrium points. Then, by Bezout Theorem, the number of equilibrium points of the generalized Cinquin-Demongeot ODE model (5) (where c_i and c_{ij} are integers) is at most*

$$max\{c_1+1, c_{1j}+1\ \forall j\} \times max\{c_2+1, c_{2j}+1\ \forall j\} \times ... \times max\{c_n+1, c_{nj}+1\ \forall j\}.$$

Bézout Theorem does not give the exact number of equilibrium points but only the upper bound. In addition, Proposition (4) is dependent on the value of c_i and c_{ij} as well as on n. According to Cinquin and Demongeot, manipulating the strength of cooperativity (c_i and c_{ij}) is of minimal biological relevance [25]. Nevertheless, the possible dependence of the number of equilibrium points on n (dimension of our state space) has a biological implication. The dependence on n may be due to the potency of the cell.

It is necessary to check if all equations in the polynomial system have no common factor of degree greater than zero, because if they do then there will be infinitely many complex solutions. We determine the set of parameter values (where the strengths of cooperativity are integer-valued) that would

give rise to a system of equations having a non-constant common factor. We have found one case (which is a Michaelis-Menten-like symmetric system) where such common factor exists.

Lemma 1. *Suppose* $c_i = c_{ij} = 1$, $g_i = 0$, $\gamma_{ij} = 1$, $\beta_i = \beta_j = \beta > 0$, $\rho_i = \rho_j = \rho > 0$ *and* $K_i = K_j = K > 0$, *for all* i *and* j. *Then the ODE model (5) has infinitely many non-isolated equilibrium points if* $\beta > \rho K$. *Moreover, if* $\beta \leq \rho K$ *then there is exactly one equilibrium point which is the origin.*

Corollary 2. *Suppose* $c_i = c_{ij} = 1$, $g_i = 0$, $\gamma_{ij} = 1$, $\beta_i = \beta_j = \beta > 0$, $\rho_i = \rho_j = \rho > 0$ *and* $K_i = K_j = K > 0$, *for all* i *and* j. *If* $\beta > \rho K$ *then the equilibrium points of the ODE system (5) are the origin and the non-isolated points lying on the hyperplane with equation*

$$\sum_{j=1}^{n} [X_j] = \frac{\beta}{\rho} - K, \ [X_j] \geq 0 \ \forall j. \tag{14}$$

When all parameters are equal to 1 except for $c_i = c_{ij} = 2$ and $g_i = 0$ for all i, j, then the only equilibrium point is the origin. Actually, this kind of system is the original Cinquin-Demongeot ODE model [25] without "leak" where $\beta = 1$ and $c = 2$ (refer to system (3)). In the following discussion, we present theorems stating sufficient (but not necessary) conditions for the origin to become the sole equilibrium point. Recall that zero state represents a trivial case.

Theorem 3. *If* $c_i > 1$, $g_i = 0$ *and*

$$\rho_i({K_i}^{1/c_i}) \geq \beta_i \tag{15}$$

for all i, then our system has only one equilibrium point which is the origin.

For $c_i = 1$ and $g_i = 0$, we state the following theorem:

Theorem 4. *Suppose $c_i = 1$, $g_i = 0$ and $\beta_i/K_i \leq \rho_i$ for all i. Then our system has only one equilibrium point which is the origin.*

Suppose $c_i \geq 1$ and $g_i = 0$ for all i. In general, the origin is the only equilibrium point of our ODE model (5) if and only if the univariate curve $Y = H_i([X_i])$ lies below the decay line $Y = \rho_i[X_i]$ (i.e., $H_i([X_i]) < \rho_i[X_i]$, $\forall[X_i] > 0$) for all i. This phenomenon indicates that exponential decay is faster than the activation of the TFs. We expect that the associated gene expression will be silenced.

Remark 4. When $[X_i] = 0$ and $g_i = 0$, the n-dimensional system reduces to an $(n-1)$-dimensional system. For example, the equilibrium points $([X_1]^*[X_2]^*, [X_3]^*, 0)$ of a system with $n = 4$ and $g_4 = 0$ are exactly the equilibrium points of the corresponding system with $n = 3$.

In the next subsection, we determine the stability of the equilibrium points of the generalized Cinquin-Demongeot (2005) ODE model (5) for a given set of parameters.

3.3. Stability of equilibrium points

Recall Theorem (1). This theorem assures us that if the ODE system (5) has exactly one equilibrium point then this point is stable. Moreover, suppose $\rho_i > 0$ for all i, then any trajectory of our system (5) never converges to a neutrally stable center, to a limit cycle, or to a strange attractor because the

trajectory of the ODE model converges to a stable equilibrium point for any nonnegative initial condition.

The following Theorems (5) and (6) present cases where the solution of our ODE system may converge to the zero state (depending on the initial condition), which is biologically trivial.

Theorem 5. *In our system (5), suppose* $g_i = 0$ *and* $c_i = 1\ \forall i$*. Then the origin is a stable equilibrium point when* $\rho_i > \beta_i/K_i\ \forall i$*, or an unstable equilibrium point when* $\rho_i < \beta_i/K_i$ *for at least one* i*. When* $\rho_i = \beta_i/K_i$ *for at least one* i*, then we have a nonhyperbolic equilibrium point, which is an attractor only when* $[X_i]$ *is restricted to be nonnegative and* $\rho_j \geq \beta_j/K_j\ \forall j \neq i$*.*

Theorem 6. *Suppose* $\rho_i > 0$*,* $g_i = 0$ *and* $c_i > 1\ \forall i$*, then the origin is a stable equilibrium point of the system (5).*

Theorem 7. *Suppose* $c_i > 1$*. If* $[X_i]^* = 0$ *(i.e., the* i*-th component of an equilibrium point is zero), then it is always a stable component.*

Theorem (7) is very important because this proves that when the i-th TF (where $g_i = 0$) is switched-off then it can never be switched-on again, unless we introduce an exogenous stimulus or we introduce some stochastic noise. Dedifferentiation, such as activating silenced TFs that induce pluripotency, has been shown to be possible through deterministic [34, 35] and stochastic [36, 37, 38, 39, 40] cellular reprogramming.

Theorem 8. *Suppose* $c_i = c_{ij} = 1$*,* $g_i = 0$*,* $\gamma_{ij} = 1$*,* $\beta_i = \beta_j = \beta > 0$*,* $\rho_i = \rho_j = \rho > 0$*,* $K_i = K_j = K > 0$ *and* $\beta > \rho K$*, for all* i *and* j*. Then the origin is an unstable equilibrium point of the system (5) while the points*

lying on the hyperplane

$$\sum_{j=1}^{n} [X_j] = \frac{\beta}{\rho} - K. \tag{16}$$

are stable equilibrium points.

Proof. From Corollary (2), the origin and the points lying on the hyperplane are equilibrium points of the system (5). Moreover, recall that the graph of the Hill function with $c_i = 1$ is hyperbolic.

Suppose $\sum_{j=1, j\neq i}^{n} [X_j] = 0$ in the denominator of H_i (6). At $[X_i] = 0$, the slope of the Hill curve $Y = H_i([X_i])$ is

$$\frac{\partial H_i}{\partial [X_i]} = \frac{\beta}{K}. \tag{17}$$

Since $\beta > \rho K$ then $\beta/K > \rho$. This implies that the slope of $Y = H_i([X_i])$ at $[X_i] = 0$ is greater than the slope of the decay line $Y = \rho[X_i]$. Therefore, when $\sum_{j=1, j\neq i}^{n} [X_j] = 0$ in the denominator of H_i (6), there are two possible intersections of $Y = H_i([X_i])$ and $Y = \rho[X_i]$. The intersection is at the origin (which is unstable) and at $[X_i] = \beta/\rho - K$ (which is stable).

Now, suppose $\sum_{j=1, j\neq i}^{n} [X_j]$ in the denominator of H_i varies. Then the intersection of $Y = H_i([X_i])$ and $Y = \rho[X_i]$ is at the origin (which is unstable) and at $[X_i] = \beta/\rho - K - \sum_{j=1, j\neq i}^{n} [X_j]$ (which is stable). Hence, the hyperplane $[X_i] = \beta/\rho - K - \sum_{j=1, j\neq i}^{n} [X_j]$ is a set of stable equilibrium points. See Figure (8) for illustration.

□

In GRNs, the existence of infinitely many non-isolated equilibrium points can be biologically volatile. A small perturbation in the initial value of the system may lead the trajectory of the system to converge to a different attractor. The basin of attraction of each stable non-isolated equilibrium point

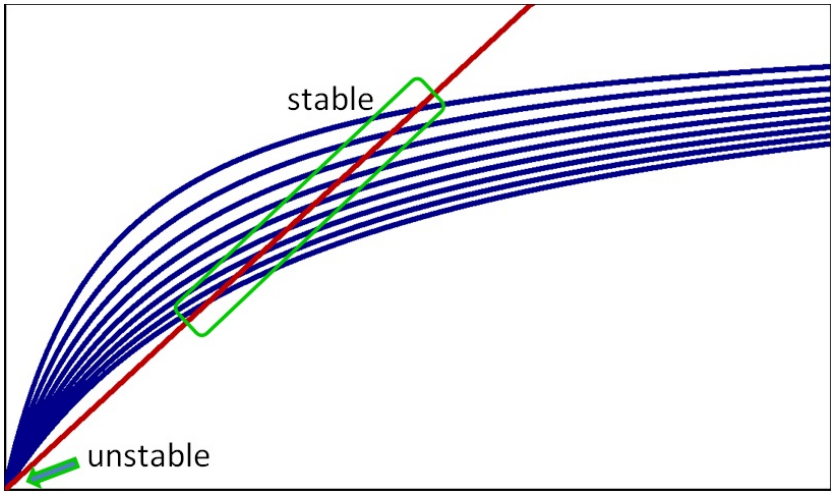

Figure 8: The origin is unstable while the points where $[X_i]^* = \beta/\rho - K - \sum_{j=1, j\neq i}^{n} [X_j]^*$ are stable.

may not be as large compared to the basin of attraction of a stable isolated equilibrium point. This special phenomenon represents competition where the co-expression, extinction and domination of the TFs depend on the value of each TF, and the dependence among TFs is a continuum. The existence of an attracting hyperplane is also discovered by Cinquin and Demongeot in [25].

The size of the basin of attraction of an equilibrium point depends on the number of existing equilibrium points and on the size of the hyperspace (11). Note that the hyperspace (11) is fixed for a given set of parameter values, and the basin of attraction of each existing equilibrium point is distributed in this hyperspace. If there are multiple stable equilibrium points then there are multiple basins of attraction that share the region of the hyperspace.

4. Bifurcation of parameters

Varying the values of some parameters can decrease the size of the basin of attraction of an undesirable equilibrium point as well as increase the size of the basin of attraction of a desirable equilibrium point. We can mathematically manipulate the parameter values to ensure that the initial condition is in the basin of attraction of our desired attractor.

Intuitively, we can make the i-th component of an equilibrium point dominate other components by increasing β_i or g_i or, in some instances, by decreasing ρ_i. Decreasing the value of K_i or sometimes increasing the value of c_i minimizes the size of the basin of attraction of the lower-valued stable intersection of $Y = H_i([X_i]) + g_i$ and $Y = \rho_i[X_i]$, thus, the chance of converging to an equilibrium point with $[X_i]^* > [X_j]^*$ $j \neq i$ may increase. However, the effect of K_i and c_i in increasing the value of $[X_i]^*$ is not as drastic compared to β_i, g_i and ρ_i, since K_i and c_i do not affect the upper bound of the hyperspace (11). In addition, increasing the value of c_i or of c_{ij} may result in an increased number of equilibrium points, and probably in multistability (by Proposition (4)).

In this section, we determine how to obtain an equilibrium point that has an i-th component sufficiently dominating other components, especially by introducing an exogenous stimulus. We focus on the parameter g_i because the introduction of an exogenous stimulus is experimentally feasible, and manipulating the values of the other parameters may not have biological relevance.

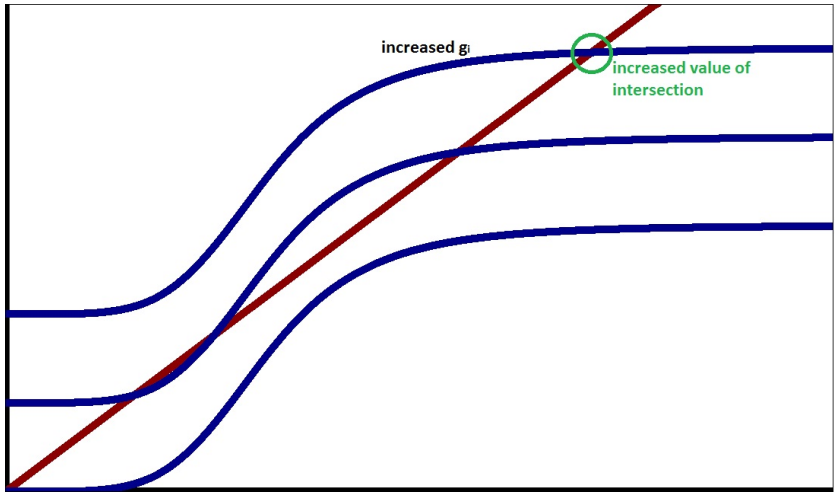

Figure 9: Increasing the value of g_i can result in an increased value of $[X_i]$ where $Y = H_i([X_i]) + g_i$ and $Y = \rho_i([X_i])$ intersects.

4.1. Increasing the effect of exogenous stimuli

If we increase the value of g_i up to a sufficient level, then we can increase the value of $[X_i]$ where $Y = H_i([X_i]) + g_i$ and $Y = \rho_i([X_i])$ intersect. We can also make such increased value of $[X_i]$ the only intersection. See Figure (9) for illustration.

Moreover, as we increase the value of g_i up to a sufficient level, we increase the possible value of $[X_i]^*$. Since $[X_i]$ inhibits $[X_j]$, then as we increase the value of $[X_i]^*$, we can decrease the value of $[X_j]$, $j \neq i$ where $Y = H_j([X_j]) + g_j$ and $Y = \rho_j([X_j])$ intersect. We can also make such decreased value of $[X_j]$ the only intersection. If $g_j = 0$, we can make $[X_j] = 0$ the only intersection of $Y = H_j([X_j])$ and $Y = \rho_j([X_j])$.

Therefore, by sufficiently changing the value of g_i we can have a sole stable equilibrium point where the i-th component dominates the others. For any

initial condition, the trajectory of the ODE model (5) will converge to this sole equilibrium point. By varying the value of g_i, we can manipulate the potency and fate of a stem cell.

Example 1. Consider that all parameters in the generalized Cinquin- Demongeot ODE model (5) are equal to 1 except for $c_i = c_{ij} = 2$, $\gamma_{ij} = 1/8$, $\rho_i = 1/21$ and $g_i = 0$, where $i, j = 1, 2$. The nonlinear system is of the form:

$$\begin{aligned} \frac{[X_1]^2}{1 + [X_1]^2 + \frac{1}{8}[X_2]^2} - \frac{1}{21}[X_1] = 0 \\ \frac{[X_2]^2}{1 + [X_2]^2 + \frac{1}{8}[X_1]^2} - \frac{1}{21}[X_2] = 0. \end{aligned} \tag{18}$$

This system has 9 equilibrium points which is equal to the Bezout upper bound of the number of possible equilibrium points. There are only 4 stable equilibrium points out of the 9. The four attractors represent a bipotent cell, two fully differentiated cells and a trivial case.

Now, suppose we introduce $g_1 = 0.5$, then there will be exactly one attractor which represents a fully differentiated cell. The fully differentiated cell expresses the gene associated with $[X_1]$.

5. The MacArthur et al. GRN

The current -omics (genomics, transcriptomics, proteomics, etc.) and systems biology revolution [41, 42, 43, 44, 45] are continually providing details about gene networks. In this section, we present a GRN (originally illustrated by MacArthur et al. as Figures 1 and 2 in [29]) where the generalized Cinquin-Demongeot ODE model can be employed. This gene network shows the coupled interaction among stem-cell-specific transcription factors and

lineage-specifying transcription factors induced by exogenous stimuli. The interaction depicted in the GRN involves the differentiation of multipotent stem cells to three mesenchymal stromal stem cells, namely, cells that form bones (osteoblasts), cartilages (chondrocytes), and fats (adipocytes).

The MacArthur et al. GRN [29] is composed of a pluripotency module (a circuit consisting of OCT4, SOX2, NANOG and their heterodimer and heterotrimer) and a differentiation module (a circuit consisting of RUNX2, SOX9 and PPAR-γ) [29, 46]. The transcription factors RUNX2, SOX9 and PPAR-γ activate the formation of bone cells, cartilage cells and fat cells, respectively. In mouse ES cells, RUNX2 is stimulated by retinoic acid (RA) and BMP4; SOX9 by RA and TGF-β; and PPAR-γ by RA and Insulin.

The TF proteins OCT4, SOX2, NANOG, OCT4-SOX2, OCT4-SOX2-NANOG, SOX9, RUNX2 and PPAR-γ are the nodes in the original MacArthur et al. GRN [29]. The path NANOG $\rightarrow$ OCT4-SOX2-NANOG $\rightarrow$ OCT4 $\rightarrow$ OCT4-SOX2 $\rightarrow$ SOX2 $\rightarrow$ OCT4-SOX2-NANOG $\rightarrow$ NANOG is one of the positive feedback loops of the gene network. A positive feedback loop that contains OCT4, SOX2, NANOG and their multimers can be regarded as an autoactivation loop of the pluripotency module.

Furthermore, both the OCT4-SOX2-NANOG and OCT4-SOX2 multimers inhibit SOX9, RUNX2 and PPAR-γ. However, SOX9, RUNX2 and PPAR-γ inhibit OCT4, SOX2 and NANOG. This implies that the pluripotency module and the differentiation module mutually inhibit each other.

Since the pluripotent module exhibits autoactivation and mutual inhibition with all the TFs in the differentiation circuit, then we can simplify the pluripotency module as one node while preserving the essential qualita-

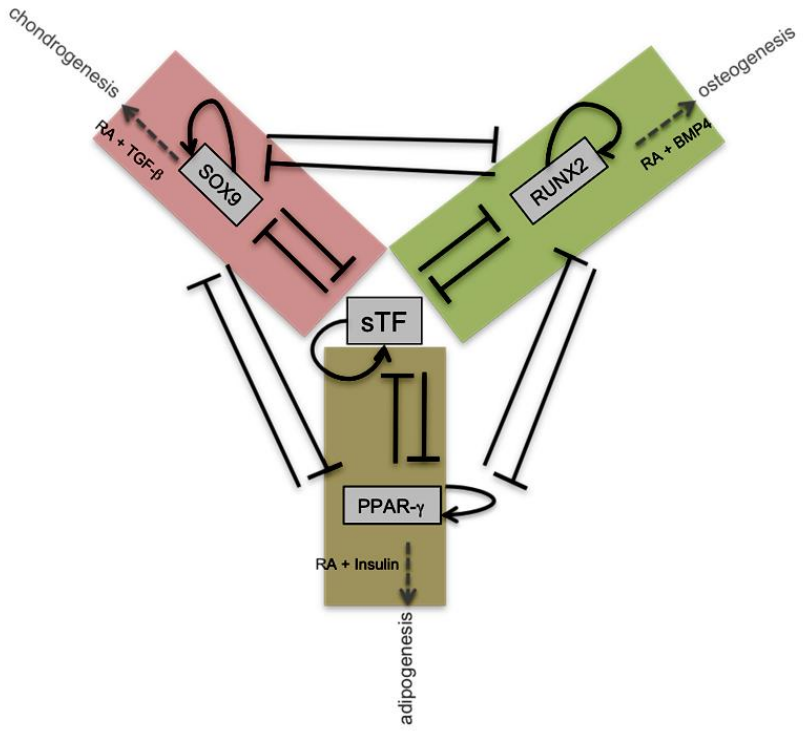

Figure 10: The simplified MacArthur et al. GRN representing the mesenchymal cell differentiation system. Bars represent repression or inhibition, while arrows represent activation

tive dynamics. We denote the pluripotency module as the sTF (stemness transcription factor). From eight nodes, we only have four nodes as represented by the coarse-grained biological network in Figure (10). Since each node undergoes autocatalysis (autoactivation) and inhibition by the other nodes (as shown by the arrows and bars) then the simplified GRN is in the simultaneous-decision-model form that can be translated into a Cinquin-Demongeot ODE model.

One limitation of a phenomenological model is that it excludes time-

delays that may arise from the deleted molecular details. However, a phenomenological model is sufficient to address the general principles of cellular differentiation and cellular programming, such as the temporal behavior of the dynamics of the GRN [32].

In our simplified network, we have four nodes and thus, $n = 4$. Let $[X_1] = [RUNX2]$, $[X_2] = [SOX9]$, $[X_3] = [PPAR{-}\gamma]$ and $[X_4] = [sTF]$. The parameter s_i represents the effect of the growth factors stimulating the differentiation towards the i-th cell lineage, specifically, $s_1 = [RA + BMP4]$, $s_2 = [RA + TGF{-}\beta]$, $s_3 = [RA + Insulin]$ and $s_4 = 0$.

MacArthur et al. [29] conducted numerical simulations to investigate the behavior of the system and tried to analytically analyze the system but only for a specific case — when the pluripotency module is switched-off. The ODE model that they analyzed when the pluripotency module is switched-off follows the original Cinquin-Demongeot [25] formalism with $c = 2$.

MacArthur et al. [29] analytically proved that the three cell types (tripotent, bipotent and terminal states) are simultaneously stable for some parameter values. Based on their deterministic computational analysis, the pluripotency module cannot be reactivated once silenced, that is, it becomes resistant to reprogramming. They argued that the pluripotency module can only be reactivated by introducing stochastic noise to the system [29]. However, using the generalized Cinquin-Demongeot ODE model, we can show that dedifferentiation is possible even without the aid of stochasticity. We can introduce sufficient amount of exogenous stimulus to the TF that can silence the expression of genes and can induce pluripotency.

5.1. Biological interpretation of equilibrium points

A TF is switched-off or inactive if its concentration is approximately zero, and switched-on otherwise. Moreover, we say that $[X_i] \neq 0$ sufficiently dominates $[X_j]$ if $[X_j]/[X_i] < \epsilon \leq 1$, where ϵ is an acceptable tolerance constant.

If no component representing a node from the differentiation module sufficiently dominates $[sTF]$ (e.g., $[sTF] \geq [OCT4]$, $[sTF] \geq [SOX2]$ and $[sTF] \geq [PPAR - \gamma]$) and sTF is switched-on, then the state represents a pluripotent cell. If all the components of a state are approximately equal and all TFs are switched-on (i.e., genes are equally expressed), then the state represents a primed stem cell.

If at least one component from the differentiation module sufficiently dominates $[sTF]$, then the state represents either a partially differentiated or a fully differentiated cell. If exactly three components from the differentiation module are approximately equal, then the state represents a tripotent cell. If exactly two components from the differentiation module are approximately equal and sufficiently dominate all other components (possibly including $[sTF]$), then the state represents a bipotent cell. If exactly one component from the differentiation module sufficiently dominates all other components (possibly including $[sTF]$) but sTF is still switched-on, then the state represents a unipotent cell.

If sTF is switched-off, then the cell had lost its ability to self-renew. If exactly one TF from the differentiation module remains switched-on and all other TFs including sTF are switched-off, then the state represents a fully differentiated cell.

A trajectory converging to the zero state is a trivial case because the zero state does not represent a cell differentiating into bone, cartilage or fat. The trivial case may either represent a cell differentiating towards other cell lineages (e.g., towards becoming a neural cell) which are not in the domain of our GRN or a cell that is in quiescent stage.

6. Conclusions

We are able to show the qualitative dynamics of the non-binary simultaneous decision network by investigating the mathematical properties of the generalized Cinquin-Demongeot ODE model. The simultaneous decision network can represent multistability that may give rise to co-expression or to domination by some transcription factors. Manipulating the values of some parameters can influence the expression of genes and the potency of stem cells. The introduction of an exogenous stimulus is a possible deterministic strategy for controlling cell fate towards a chosen lineage or for reprogramming cells back to pluripotency. Deterministic cellular reprogramming can result to a system with a sole attractor, which can probably regulate the effect of moderate stochastic noise in gene expression.

Suppose the solution to our system tends to an equilibrium point with silenced transcription factor. If we want to reactivate this transcription factor then one strategy is to add an exogenous stimulus. The idea of introducing a sufficient amount of stimulus is to make the solution of our system escape a certain equilibrium point. However, it is sometimes impractical or infeasible to continuously add such a constant amount of inducement to control cell fates. Consequently, we may rather consider an exogenous stimulus that

degrades through time. Introducing a depleting amount of stimulus can affect cell fate when there are multiple stable equilibrium points and when the convergence of trajectories is dependent on the initial condition.

Random noise can be introduced to the ODE model. Stochasticity can induce cells to switch lineages or to switch back to a pluripotent state; however, this technique is not always efficient, especially in the absence of multistability. When deterministic cellular reprogramming is not possible, combining deterministic and stochastic techniques could be done, such as by supplementing a flexible amount of stimulus to complement the effect of stochastic noise.

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APPENDIX A: Proofs

Proof of Proposition (2)

Proof. Suppose $\beta_i > 0$, $g_i > 0$, and g_i/ρ_i is an i-th component of an equilibrium point. Then

$$F_i\left([X_1], ..., \frac{g_i}{\rho_i}, ..., [X_n]\right) = \frac{\beta_i \left(\frac{g_i}{\rho_i}\right)^{c_i}}{K_i + \left(\frac{g_i}{\rho_i}\right)^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} - \rho_i \frac{g_i}{\rho_i} + g_i = 0$$

$$= \frac{\beta_i \left(\frac{g_i}{\rho_i}\right)^{c_i}}{K_i + \left(\frac{g_i}{\rho_i}\right)^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} = 0$$

implying that $\beta_i \left(g_i/\rho_i\right)^{c_i} = 0$. Thus $\beta_i = 0$ or $g_i = 0$, a contradiction. □

Proof of Proposition (3)

Proof. If $g_i = 0$ then

$$F_i(X) = \frac{\beta_i [X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} - \rho_i [X_i] + 0 = 0,$$

implying $[X_i] = 0$ is a root of $F_i(X) = 0$. Furthermore, if $[X_i] = 0$ is a root of $F_i(X) = 0$ then by substitution,

$$\frac{\beta_i [0]^{c_i}}{K_i + [0]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} - \rho_i [0] + g_i = 0,$$

g_i must be zero. □

Proof of Lemma (1)

Proof. Recall Equation (13), we have the corresponding polynomial system $P_i(X) = 0$ $(i = 1, 2, ..., n)$:

$$\beta_i[X_i]^{c_i} - \rho_i K_i[X_i] - \rho_i[X_i]^{c_i+1} - \rho_i[X_i] \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$$
$$+ g_i K_i + g_i[X_i]^{c_i} + g_i \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} = 0.$$

Suppose $c_i = c_{ij} = 1$, $g_i = 0$, $\gamma_{ij} = 1$, $\beta_i = \beta_j = \beta > 0$, $\rho_i = \rho_j = \rho > 0$ and $K_i = K_j = K > 0$. Then the polynomial system can be written as $(i = 1, 2, ..., n)$

$$\beta[X_i] - \rho K[X_i] - \rho[X_i]^2 - \rho[X_i] \sum_{j=1, j\neq i}^{n} [X_j] = 0$$
$$\Rightarrow [X_i] \left(\beta - \rho K - \rho[X_i] - \rho \sum_{j=1, j\neq i}^{n} [X_j] \right) = 0$$
$$\Rightarrow [X_i] = 0 \text{ or } \left(\beta - \rho K - \rho[X_i] - \rho \sum_{j=1, j\neq i}^{n} [X_j] \right) = 0. \tag{19}$$

Notice that the factor

$$\beta - \rho K - \rho[X_i] - \rho \sum_{j=1, j\neq i}^{n} [X_j]$$
$$= \beta - \rho K - \rho \sum_{j=1}^{n} [X_j]$$

is common to all equations in the polynomial system. Thus, there are infinitely many complex-valued solutions. However, note that we have restricted the state variables to be nonnegative, so we do further investigation to determine the conditions for the existence of an infinite number of solutions given strictly nonnegative variables. We focus our investigation on real-valued solutions.

Suppose $B = \beta - \rho K$.

Case 1: If $\beta = \rho K$ then $B = 0$. Thus, $B - \rho \sum_{j=1}^{n} [X_j]$ will never be zero except when $[X_j] = 0 \ \forall j = 1, 2, ..., n$ (since $[X_j]$ can take only nonnegative values). Hence, the only equilibrium point to the system is the origin.

Case 2: If $\beta < \rho K$ then $B < 0$. Thus, $B - \rho \sum_{j=1}^{n} [X_j]$ will always be negative and will not have any zero for any nonnegative value of $[X_j]$. Hence, the only equilibrium point is the origin (that is, $[X_i] = 0 \ \forall i = 1, 2, ..., n$, see Equation (19)).

Case 3: If $\beta > \rho K$ then $B > 0$. Thus, there exist solutions to the equation $B - \rho \sum_{j=1}^{n} [X_j] = 0$. Notice that the set of nonnegative real-valued solutions to $B - \rho \sum_{j=1}^{n} [X_j] = 0$ is a hyperplane (e.g., it is a line for $n = 2$ and it is a plane for $n = 3$). Hence, there are infinitely many non-isolated equilibrium points when $\beta > \rho K$. □

Proof of Theorem (3)

Proof. Let us first consider the case where $[X_j] = 0$, for all $j \neq i$. Recall that the upper bound of $H_i([X_i])$ is β_i. Moreover, recall that when $[X_i] = K_i^{1/c_i}$ then $H_i([X_i]) = \beta_i/2$. Note that $(K_i^{1/c_i}, \beta_i/2)$ is the inflection point of our univariate Hill curve. We substitute $[X_i] = K_i^{1/c_i}$ in the decay function $Y = \rho_i[X_i]$, and if the value of $\rho_i({K_i}^{1/c_i})$ is larger or equal to the value of the upper bound β_i then $Y = H_i([X_i])$ and $Y = \rho_i[X_i]$ only intersect at the origin.

Now, as the values of $\gamma_{ij}[X_j]$ for all $j \neq i$ increase then the univariate Hill curve $Y = H_i([X_i])$ will just shrink and will definitely not intersect the decay line $Y = [X_i]$ except at the origin. □

Proof of Theorem (4)

Proof. Let us first consider the case where $[X_j] = 0$, for all $j \neq i$. Recall that $Y = H_i([X_i])$ where $c_i = 1$ is a hyperbolic curve. The partial derivative

$$\frac{\partial H_i}{\partial [X_i]} = \frac{\partial}{\partial [X_i]} \left(\frac{\beta_i [X_i]}{K_i + [X_i]} \right) = \frac{K_i \beta_i}{(K_i + [X_i])^2}$$

means that the slope of the hyperbolic curve is monotonically decreasing as $[X_i]$ increases. The partial derivative at $[X_i] = 0$ is

$$\frac{\partial H_i}{\partial [X_i]} = \frac{\beta_i}{K_i} \leq \rho_i,$$

which means that the slope of $Y = H_i([X_i])$ at $[X_i] = 0$ is less than the slope of the decay line $Y = \rho_i [X_i]$ at $[X_i] = 0$. Hence, the Hill curve $Y = H_i([X_i])$ lies below the decay line for all $[X_i] > 0$. □

Proof of Theorem (5)

Proof. The characteristic polynomial associated with the Jacobian of our system when $X = (0, 0, ..., 0)$ is

$$\begin{aligned} |\mathbf{J}F(\mathbf{0}) - \lambda \mathbf{I}| &= \begin{vmatrix} \frac{\beta_1}{K_1} - \rho_1 - \lambda & 0 & \cdots & 0 \\ 0 & \frac{\beta_2}{K_2} - \rho_2 - \lambda & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & \frac{\beta_n}{K_n} - \rho_n - \lambda \end{vmatrix} \\ &= \left(\frac{\beta_1}{K_1} - \rho_1 - \lambda \right) \left(\frac{\beta_2}{K_2} - \rho_2 - \lambda \right) ... \left(\frac{\beta_n}{K_n} - \rho_n - \lambda \right). \end{aligned}$$

The eigenvalues (λ) are $\beta_1/K_1 - \rho_1, \beta_2/K_2 - \rho_2, ..., \beta_n/K_n - \rho_n$. Therefore, the zero vector is a stable equilibrium point when $\rho_i > \beta_i/K_i \ \forall i$. The zero vector is an unstable equilibrium point when $\rho_i < \beta_i/K_i$ for at least one i.

If $\rho_i = \beta_i/K_i$ for at least one i then we have a nonhyperbolic equilibrium point. Geometrically, we can see that this is a saddle — stable at the right and unstable at the left of $[X_i]^* = 0$. Hence, if we restrict $[X_i] \geq 0$ and if $\rho_j \geq \beta_j/K_j \ \forall j \neq i$, then this nonhyperbolic equilibrium point is an attractor. □

Proof of Theorem (6)

Proof. By Corollary (1), if $g_i = 0$ for all i then the origin is an equilibrium point. The characteristic polynomial associated with the Jacobian of our system when $X = (0, 0, ..., 0)$ is

$$|\mathbf{J}F(\mathbf{0}) - \lambda\mathbf{I}| = \begin{vmatrix} -\rho_1 - \lambda & 0 & \cdots & 0 \\ 0 & -\rho_2 - \lambda & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & -\rho_n - \lambda \end{vmatrix}$$
$$= (-\rho_1 - \lambda)(-\rho_2 - \lambda)...(-\rho_n - \lambda).$$

The eigenvalues (λ) are $-\rho_1, -\rho_2, ..., -\rho_n$ which are all negative. Therefore, the zero state is a stable equilibrium point. □

Proof of Theorem (7)

Proof. Recall from Theorem (3) that our system has an equilibrium point with i-th component equal to zero if and only if $g_i = 0$. The only possible topologies of the intersections of $Y = H_i([X_i])$ and $Y = \rho_i[X_i]$ are shown in Figure (11). Notice that zero i-th component is always stable. □

APPENDIX B: Numerical results for Example (1)

The approximate values of the equilibrium points of the ODE system (18) are:

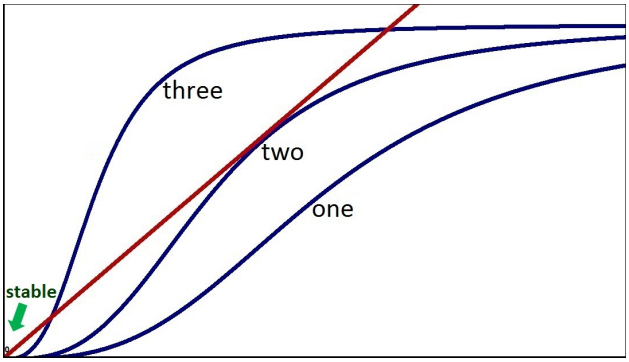

Figure 11: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i([X_i]) + g_i$ where $c > 1$ and $g = 0$. The value of $K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is taken as a parameter.

$([X_1]^* = 18.62, [X_2]^* = 18.62)$ — stable (bipotent),

$([X_1]^* = 20.89, [X_2]^* = 3.11)$ — unstable,

$([X_1]^* = 3.11, [X_2]^* = 20.89)$ — unstable,

$([X_1]^* = 0.05, [X_2]^* = 0.05)$ — unstable,

$([X_1]^* = 0, [X_2]^* = 0.05)$ — unstable,

$([X_1]^* = 0.05, [X_2]^* = 0)$ — unstable,

$([X_1]^* = 0, [X_2]^* = 20.95)$ — stable (terminal state),

$([X_1]^* = 20.95, [X_2]^* = 0)$ — stable (terminal state),

$([X_1]^* = 0, [X_2]^* = 0)$ — stable (trivial case).

When $g_1 = 0.5$ is introduced, the sole equilibrium is $([X_1]^* = 31.48, [X_2]^* = 0)$.