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Control of Directed Differentiation by Parameter Perturbations

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Abstract

Directed differentiation is the process which selectively induce differentiation of stem cells toward definitive cells, but how directed differentiation occurs when stem cells confront the external cues has not been established theoretically. We demonstrate here directed differentiation of a simplified two-component network by using systematic parameter perturbations. These methods and results will provide theoretically directive significance for the study of molecular mechanisms underlying cell differentiation, the flexible control of directed differentiation, and the development of novel therapies.

Keywords Stem cells; directed differentiation; perturbation; bifurcation

1 Introduction

Cell differentiation is a developmental process in which cell structures and functions become increasingly specialized. It is a selective expression of genes and the performance of biological adaptation. All stem cells share the ability to self-renew and multipotentially differentiate into various different lineages [1, 2]. Cellular differentiation is an intrinsically complex process requiring coordinated dynamical expression of many genes and proteins response precisely to external or internal cues [1, 2, 3, 4]. The control of cell differentiation has both deterministic and stochastic elements. The deterministic regulatory circuits define stable steady states towards which individual cells are drawn over time, whereas stochastic fluctuations can initiate transitions between alternate steady states [2, 5].

In the context of cell differentiation, the aim of systems biology approaches is to characterize the adaption of selective regulation so as to decide the differentiation process by suitably manipulating the genetic applets and environmental cues [1, 2, 3, 4]. The ultimate goal is to understand dynamical behavior of the underlying molecular circuits and elucidate how these circuits control cell fate decision and even cellular reprogramming.

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To do so, given a deterministic regulatory network, we need to systematically investigate the effects of various genetic and environmental cues on cell differentiation.

The mechanism of cell differentiation can be interpreted as a sequence of cell fate decisions. It has been shown that a positive feedback topology with some nonlinearity on its constituent interactions can generate distinct epigenetic states [6, 7, 8]. Here we study the directed differentiation of a simplified two-component network by systematic parameter perturbations representing combinatorial genetic and environmental cues. The methods and results in this paper will provide a theoretically directive significance for the study of molecular mechanisms underlying cell differentiation, the flexible control of directed differentiation, and the development of novel therapies.

2 Methods and results

Two-component mutual activation and inhibition positive feedback loops are common motifs that occur in many biological systems. To describe the dynamics of these feedback loops, here we consider a simple motif in which two transcriptional factors activate their own expression and mutually activate or repress each other's expression [9] (see Fig.1). We represent the dynamics of the motif with a plausible mathematical model and a minimal set of parameters of biological significance. The following simplified system with

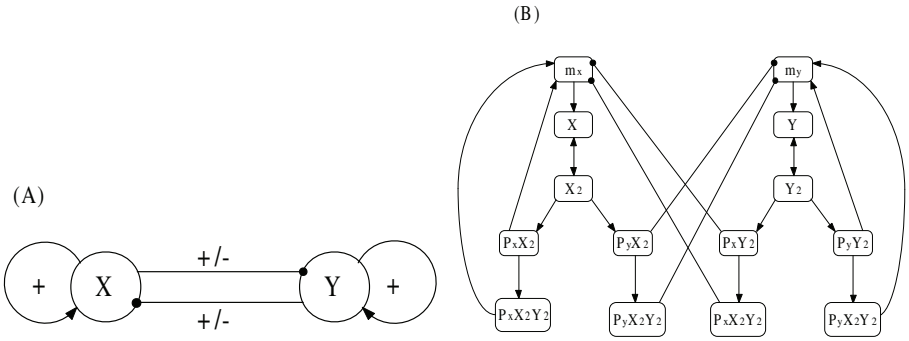

Figure 1: Schematic representation of the simple network. (A) The simple motif in which two transcriptional factors activate their own expression and mutually activate or repress each other's expression. (B) The biochemical reaction network, where m_X and m_Y are messenger RNAs of X and Y, and X_2 and Y_2 are homodimers and $P_iX_iY_i$ ($i \in \{x,y\}$) are heterotetramer of X and Y.

rescaled parameters are analyzed:

$$\frac{dx}{dt} = \alpha_x \frac{1+\rho_x x^2+\nu_x\sigma_x y^2+\mu_x\sigma_{xy}x^2y^2}{1+x^2+\sigma_x y^2+\sigma_{xy}x^2y^2} - \delta_x x, \tag{1}$$

$$\frac{dy}{dt} = \alpha_y \frac{1+\rho_y y^2+\nu_y\sigma_y x^2+\mu_y\sigma_{yx}x^2y^2}{1+y^2+\sigma_y x^2+\sigma_{yx}x^2y^2} - \delta_y y, \tag{2}$$

where x and y are the concentration of the two regulators. We choose the case $\mu_i = 0$ and $\nu_i = 0$, which corresponds to the case of the mutual inhibition, to illustrate our approach.

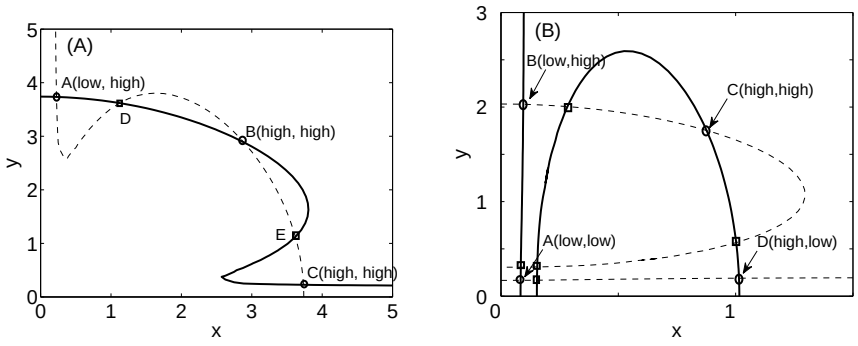

Figure 2: Plots of the nullclines of Eqs.(1)-(2). The solid and dashed curves represent the nullclines of x and y, respectively. (A) The parameter values are $\alpha_x = \alpha_y = 0.1$, $\rho_x = \rho_y = 40$, $\nu_x = \nu_y = 0$, $\sigma_x = \sigma_y = 0.2$, $\mu_x = \mu_y = 0$, $\sigma_{xy} = 0.01$, and $\delta_x = \delta_y = 1$. (B) The parameter values are $\rho_x = \rho_y = 25$ and $\sigma_x = \sigma_y = 0.1$. The other parameter values are the same as those in (A).

Similar simplified models have been used to describe the coexistence of several expression states in specific cellular systems such as those involved in hematopoiesis or embryonic stem cells differentiation [2, 4, 5, 10].

To clarify the mechanism of cell directed differentiation, the multistability and the directed transition between alternate stable steady states can be obtained through bifurcation analysis of the deterministic differential Eqs.(1)-(2). We illustrate multistability by plotting the nullclines, whose intersections identify the steady states of the system and two situations are shown in Fig.2. We choose the situation shown in Fig.2(A) to illustrate our analysis. Any other situation can be similarly discussed. Among the five intersection points in Fig. 2(A), the three points A, B, and C are stable steady states, while the points D and E are unstable. Such a multistability plays a significantly role in cell differentiation and decision making. Without confusion, we can qualitatively describe the three states as A (low, high), B (high, high), and C (high, low) with low/ high denoting the concentrations of the two proteins despite of the quantitative relationship $\bar{x}_A < \bar{x}_B < \bar{x}_C$ and $\bar{y}_A > \bar{y}_B > \bar{y}_C$. The four steady states shown in Fig.2(B) can be qualitatively described as A (low, low), B (low, high), C (high, high), and D (high, low), which are labeled with circles respectively.

α_x	ρ_x	ν_x	σ_x	μ_x	δ_{xy}	δ_x	α_y	ρ_y	ν_y	σ_y	μ_y	δ_{yx}	δ_y
+	+	+	−	+	−	−	−	−	−	+	−	+	+

Table 1: Signs of partial derivatives $\partial\bar{x}/\partial p$ and $\partial\bar{y}/\partial p$ over different parameters.

Differentiation can be progression or decision, depending on the number of differentiated states [9]. Different differentiated states can be quantitatively discriminated. For example, that macrophage differentiation is favored when the level of PU.1 is higher than

Parameters	α_x	ρ_x	ν_x	σ_x	μ_x	δ_{xy}	δ_x	α_y	ρ_y	ν_y	σ_y	μ_y	δ_{yx}	δ_y
$B \to A$	$-$	$-$	$-$	$+$	$-$	$+$	$+$	$+$	$+$	$+$	$-$	$+$	$-$	$-$
$B \to C$	$+$	$+$	$+$	$-$	$+$	$-$	$-$	$-$	$-$	$-$	$+$	$-$	$+$	$+$

Table 2: Classification of parameter perturbations for directed differentiation.

Parameters	α_x	ρ_x	ν_x	σ_x	μ_x	δ_{xy}	δ_x	α_y	ρ_y	ν_y	σ_y	μ_y	δ_{yx}	δ_y
$A \to C$	$+$	$+$	$+$	$-$	$+$	$-$	$-$	$-$	$-$	$-$	$+$	$-$	$+$	$+$
$C \to A$	$-$	$-$	$-$	$+$	$-$	$+$	$+$	$+$	$+$	$+$	$-$	$+$	$-$	$-$

Table 3: Classification of parameters for directed switching between different steady states.

that of C/EBPα, whereas neutrophil differentiation is favored when the level of C/EBPα is higher than that of PU.1 [11]. In the terms of bifurcation, the undifferentiated stem cells states can be viewed as the monostable (sometimes multistable) states and the initiation of differentiation corresponds to a bifurcation. The multiple specialized differentiated cells correspond to multiple coexisting stable steady states. Perturbation of different parameters may lead to different bifurcation diagrams and system behaviors. Furthermore, specific parameter perturbation may affect certain system states such as production, transcription, translation and degradation rates and thus may induce directed differentiation. Therefore, the directed differentiation of stem cells by systematic parameter perturbations need to be fully clarified.

Taking $\sigma = \sigma_x = \sigma_y$ as the control parameter, the bifurcation diagrams of Eqs.(1)-(2) are shown in Fig.3. Due to the expression $\sigma_i = K_j^i / K_i^i$ $(i, j \in \{x, y\})$, increasing or decreasing the value of σ can be obtained through regulating the corresponding biochemical reactions, e.g. the DNA-bing or dimerization reactions. Similarly, other parameters can also be changed by accelerating or slowing down the corresponding reactions. For the given parameter values, the system can be monostable, bistable, or multistable, depending on the control parameter values, for example, as shown in Fig.3 (A). The codimension 2 bifurcation diagram with ρ and σ as control parameters is shown in Fig.3(B). The dynamics can be monostable, bistable, or multistable, depending on the values of the two control parameters. We use $\mathrm{I_H}$, $\mathrm{II_A}$, and $\mathrm{III_H}$ to represent the parameter regimes where the system is monostable, bistable and tristable respectively. We consider how to obtain directed differentiation by systematic parameter perturbations. In terms of bifurcation, the directed differentiation can be predicted based on (a) the initial condition in which the system is present; and (b) parameters chosen to systematically perturb. For the case shown in Fig.3(A), the initial condition is the expression state located in the intermediate equilibrium locus corresponding to the parameter value $\sigma \simeq \sigma_2$ and $\sigma < \sigma_2$ because differentiation initiates at $\sigma = \sigma_2$. Taking the undifferentiated state B in Fig.3 as initial condition, the differentiated state can be A or C, depending on the parameters chosen to perturb. Directed differentiation means that the system must make a decision to choose a specific transition from the two choices. Take the initial condition which is the steady state for the parameter value closing to a bifurcation point, e.g. B in Fig.3(A). By specific

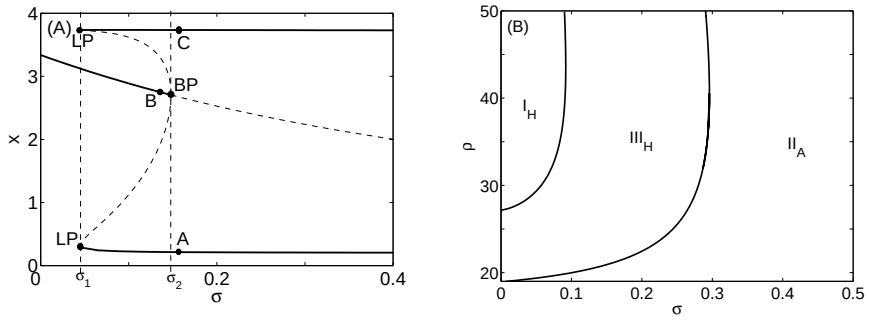

Figure 3: Bifurcation diagrams of Eqs.(1)-(2). (A) The bifurcation diagram showing the steady state values of x as a function of σ. The solid and dashed lines represent stable and unstable steady states, respectively. The parameter values are the same as those in Fig.2(A). (B) The codimension two bifurcation diagram.

combinations of parameter perturbations, directed differentiation, i.e. selective differentiation toward definitive cell fate A or C, can be realized. From the point of derivatives, $\partial\bar{x}/\partial p > 0$ (< 0) means increasing the value of parameter p has positive (or negative) effects on the steady state value $\bar{x}$. According to the signs of partial derivatives $\partial\bar{x}/\partial p$ and $\partial\bar{y}/\partial p$, we can categorize all the parameters into different groups. The signs of the two derivatives over different parameters are shown in the two columns in Table 1.

According to the signs of the derivatives, we can know the tendency of the differentiation by perturbing individual parameters. For example, to induce the directed differentiation from B to A, we can increase or decrease the parameters respectively, or combinatorially, as shown in Table 2. When $\nu_i \in [0,1)$ and $\mu_i \in [0,1)$, $i = \{x,y\}$, the motif has the mutual inhibition topology. Due to the relationship $\bar{x}_B > \bar{x}_A$ and $\bar{y}_B < \bar{y}_A$, directed differentiation from B to A means decreasing the $\bar{x}$ value and increasing the $\bar{y}$ value. Note that the system is a monotone system due to its special topology [6, 7], therefore, increasing the value of ρ_y will induce the increase of $\bar{x}$ and decease of $\bar{y}$ simultaneously. Therefore, such a consistency can simplify the analysis. With the increase of ρ_y until some threshold is exceeded, the directed differentiation will be realized. The directed differentiation from B to C can be obtained with the opposite change of parameters as the directed differentiation from B to A. The effects of each parameter on the potential to induce directed differentiation can be similarly discussed according the results shown in Table 2. Thus, the effects of systematic parameter perturbations by the combinatorial effects of perturbing some parameters simultaneously can be obtained. When multiple parameters are chosen to perturb, perturbing which has opposite effects on directed differentiation, e.g. increase σ_x and μ_x or ρ_x and ρ_y simultaneously, the final potential on directed differentiation depends on their cumulative effects and further fine tuning is needed. The directed differentiation from B into C or A by perturbing ρ_x or ρ_y independently is shown in Figs.4(A)-(B). Note that the differentiated states are qualitatively equivalent to C and A shown in Fig.3(A) although some quantitative differences do exist. When complex networks with more components are discussed, the effects of parameter variations on steady

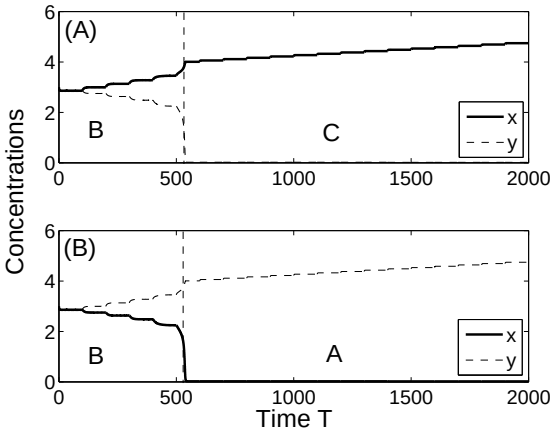

Figure 4: Directed differentiation induced by gradual parameter perturbations. (A) The directed differentiation from B state into C state, where B and C are shown in Fig.3(A). The parameter ρ_x is chosen to increase gradually with step size $\Delta\rho_x = 0.5$. (B) The directed differentiation from B state into A state by increasing ρ_y gradually with step size $\Delta\rho_y = 0.5$. The total steps are 10 and the time span is 200 long enough for the system evolving to steady states in each step.

states are not trivial. In this case, we can analyze the potential for directed differentiation by combining the components which are directly affected by the chosen parameters to perturb and the sign of paths from these directly affected components to other components. See [13] for more details.

Besides the directed differentiation which are realized through bifurcation and systematic parameter perturbations, the switching between different stable steady states, which need not exceed a bifurcation point, can be also studied similarly. Stochastic switching, or sometimes transient differentiation [16, 17], between two different states can take place due to the epigenetic alternation induced by transcriptional regulators. Besides the stochastic switching, deterministic switching can also occur. Here we study deterministic switching by using the proposed technique. For the given parameter values, the system two stable steady states corresponding to two distinct phenotypes, as shown in Fig.2(A). In the bistable regime, the system can evolve to either the upper steady states 'C'or the lower steady states 'A', depending on the initial conditions. Similarly, the switching between C and A can also be predicted based on (a) the initial conditions in which the system is present; and (b) the parameters chosen to perturb. According to the results shown in Table 3 and the relationship $\bar{x}_A < \bar{x}_C$ and $\bar{y}_A > \bar{y}_C$, the switching between A and C can be easily realized because x and y are mutually inhibitory, i.e. increase of x will induce decrease of y simultaneously. For the situation shown in Fig.3(A), two types of differentiation, i.e. from B to A or to C, have opposite tendency.

Systematically perturbing parameters represent combinatorial external cues of directed differentiation. Therefore, any genetic or stochastic mechanism of directed dif-

ferentiation can be attributed to the systematic parameter perturbations. The proposed approach provides a framework for theoretical analysis of directed differentiation and artificial control strategies. The analytical method proposed provides a flexible control technique which can be used to therapies related to stem cells.

3 Dissussion

Based on a thorough study of a simple illustrative example, the present work aimed at defining directed differentiation of stem cells by performing bifurcation analysis and systematic parameter perturbations. The proposed method provides directive significance not only for study of molecular mechanisms underlying directed differentiation but also for its flexible control for clinical application. Directed differentiation and switching can be realized through bifurcation analysis and systematic parameter perturbation. Parameter variations in real biological systems can be realized easily. For example, if the parameter chosen is related to an enzyme reaction, we can add an enzyme repressor so as to change the enzyme activity. If an enzyme activator is needed theoretically, because of the difficulty in realization, we can choose other parameters, perturbing which has similar effects on the potential to induce the directed differentiation. Similarly, if the parameter chosen is related to a gene, we can use polymerase chain reaction (PCR) silencing technique to up- or down-regulate its expression. Such an idea has been used to find multiple drug targets [18].

A simple motif with mutual activation or inhibition topologies is used to illustrate the proposed approach. Actually, the technique can be applied to more general networks, which can be monotone and non-monotone, because the technique just depends on the effects of parameter variations on the steady state values. When more complex networks are considered, the decomposition technique [19] can be used to decompose a complex network into some simple modules. By combining the technique proposed here and the decomposition technique, directed differentiation in more complex networks can be similarly analyzed.

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