# Fate decisions of $CD4^+$ T cells

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## Introduction

In vertebrates, the immune response against pathogens is mounted by the innate immune system and the adaptive immune system [1-3]. The latter form of immune response involves immunological memory which allows the system to recognize subsequent invasions of the pathogens following their initial response and activate enhanced responses. This memory endows long-term protection from pathogens and serves as the basis for vaccination. T cells, or T lymphocytes, play key roles in the adaptive immune system. They are named based on their developmental origin: their T cell lineage is determined in the thymus gland [4]. The antigen receptors on the surface of T cells, known as T cell receptors (TCR), are highly specific to the type of pathogen that they are combating. The diversity of these receptors is achieved by somatic hypermutation [5,6] and genetic recombination, which generate a large number of antigen receptors. Specific recognition of antigens by TCR initiates the activation of the T cell and subsequent responses, including direct or indirect elimination of the infected cells. In addition to the receptors that recognize antigens from pathogens, T cells also have co-receptors that facilitate the antigen-specific activation. Some of these co-receptors serve as surface markers that distinguish two major types of T cells. T cells that express co-receptor CD8 on their surfaces ($CD8^+$ T cells) are cytotoxic T cells which are responsible for killing infected cells directly. $CD4^+$ T cells are helper T cells that eliminates pathogens indirectly by activating other parts of the immune system, including $CD8^+$ T cells and innate immune cells such as macrophages and natural killer cells [7-9]. Within the class of $CD4^+$ T cells, there exists several types of T helper cells, e.g. T helper 1 cells (Th1), T helper 2 cells (Th2), and T helper 17 (Th17) cells [10]. The differentiation (i.e. lineage specification) of these T helper cell types marks the final maturation of these T cells upon which they become functional. The differentiation of $CD4^+$ T cells is triggered by TCR activation, but the specification towards specific lineages is also determined by other signals such as cytokines [11], which are small immunomodulating molecules released from immune cells. For example, the differentiation of Th2 cells requires activations of TCR (antigen-specific receptor), CD3 (co-receptor), CD28 (secondary stimulatory receptor) and interleukin-4 (IL-4, a cytokine) receptor, whereas IL-12 and interferon-γ (IFN-γ) bias the differentiation toward Th1 cells in the absence of IL-4 [12].

The goal of this chapter is to use the differentiation of $CD4^+$ T cells as a paradigm to illustrate how gene regulatory networks (GRNs) control the dynamics of cell differentiation. We will address the following questions in terms of the differentiation using simple mathematical models: 1) how

robust fate decisions of T cells are made with the help of GRN; 2) how a population of undifferentiated T cells differentiate to a population of cells with diverse molecular signatures and functions; and 3) how strengths of the TCR and cytokine signals influence the differentiated population of T cells. Insights into these questions will not only help to elucidate the understanding of T cell biology, but also shed light on the fundamental mechanisms underlying the complex immune responses.

## Physiology and Molecular Mechanisms

$CD4^+$ T cells coordinate the immune systems by communicating with a wide range of immune cells. These T cells perform this function primarily by releasing cytokines. Each type of $CD4^+$ T cells produce and release a unique set of cytokines, known as signature cytokines, which specifically activate or inhibit the activities of certain groups of immune cells. There are at least four types of $CD4^+$ T cells. Th1 cells are primarily responsible for clearing intracellular infection. Th2 cells lead to response against extracellular parasites. Th17 cells are used to eliminate extracellular pathogens and fungi. There is a distinct group of $CD4^+$ T cells called regulatory T cells (Treg cells) that serve as immunosuppressors, which modulate the immune system and prevent autoimmune diseases. More recently, other types of $CD4^+$ T cells, such as follicular T cells (Tfh cells), Th9 cells and Th22 cells, were also discovered [13,14]. In addition to their unique functions and cytokine profiles, different types of $CD4^+$ T cells also exhibit distinct gene expression patterns in general, indicating that large gene regulatory programs are required to achieve cell-type specification. It has been shown that the differentiation of each type of $CD4^+$ T cell is governed by the upregulation of at least one transcription factor (TF) that is considered the master regulator of the cell type. Well-known master regulators include T-bet (Th1), Gata3 (Th2), RORγt (Th17) and Foxp3 (Treg) [12]. Upon the stimulation of TCR and other receptors on an undifferentiated $CD4^+$ T cell, known as the naïve T cell, a specific master regulator is upregulated, and this activation leads to changes of the transcriptional profile in the T cell, including the upregulation of the genes that encode the signature cytokines of a T cell type (Figure 1A). The differentiation is irreversible with respect to the withdrawal of the stimulants, suggesting the robustness of the cell type specification [15,16]. The microenvironment of the naïve T cell, which includes the types and amounts of cytokines available during the differentiation, is critical for determining the generated cell type. These cytokines are known as the polarizing signals (as opposed to TCR signals which are common to all cell types) that guide the differentiation toward specific cell types [13].

Although irreversibility of the $CD4^+$ T cell differentiation was observed [16], the deterministic view of this system was challenged by three characteristics of the differentiation. First, a population of naïve T cells can generate a diverse group of functional $CD4^+$ T cells in terms of the expression patterns of signature cytokines and master regulators in the same microenvironment (Figure 1B). These observations were made with *in vitro* differentiation experiments in which experimentalists precisely control the signals that the naïve T cells receive [17-20]. Secondly, the composition of this heterogeneous cell population can be changed by altering the polarizing signals before the differentiation (Figure 1B), or even after the differentiation is completed if the signals are sufficiently strong. This indicates the plasticity of the $CD4^+$ T cells, and it also provides evidence against the possibility that the heterogeneous differentiation is due to the predetermined

diversity within the naïve T cell population. Finally, it has been observed that cells that co-express master regulators of multiple cell types (e.g. RORγt and Foxp3) stably exist *in vitro* and *in vivo* [21-23]. This further suggests the diversity of the CD4$^+$ T cells is beyond the model of lineage specification toward cells that stably express a single lineage-defining master regulator (Figure 1B).

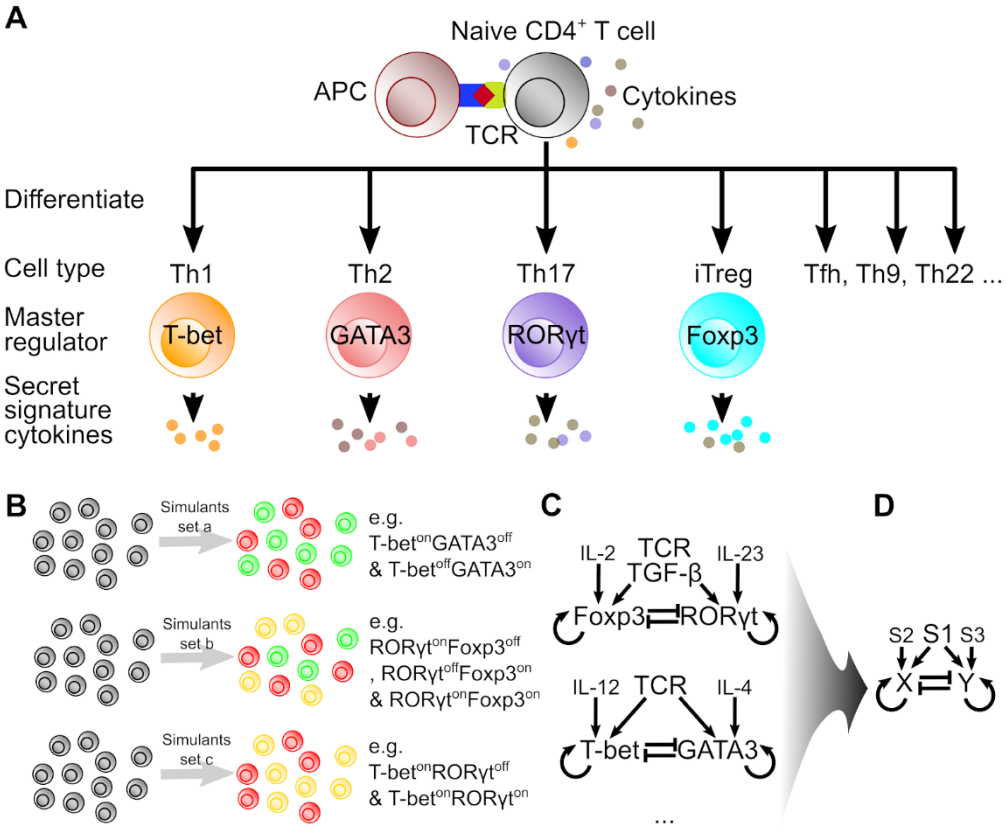


**Figure 1. Overview of CD4$^+$ T cell differentiation and network diagram for modeling. A**. Illustration of cell fate choice of a naïve CD4$^+$ T cell upon the simulation of TCR by an antigen-presenting cell (APC) and the influence of surrounding cytokines. Four examples of differentiated cell types and their master regulators are shown. **B**. Examples of scenarios in which a population of naïve CD4$^+$ T cells are differentiated into a heterogeneous population. Red and green cells represent single-positive cells where one master regulator is upregulated. Yellow cells represent double-positive cells where two master regulators are upregulated. **C**. Examples of regulatory networks controlling dual-master-regulator differentiation systems. **D**. A generic network summarizing gene networks for CD4$^+$ T cell differentiation. X and Y represent two master regulators. S1 represents the common activator (e.g. TCR). S2 and S3 are polarizing signals representing two sets of cytokines.

The goal of our models for CD4$^+$ T cells is to capture the intriguing and perplexing observations mentioned above and provide a mechanistic and theoretical basis for the phenomena. Our idea is simply that the gene regulatory networks in these T cells, which primarily involve interactions

among the master regulators as well as their responsiveness to signals from extracellular factors, govern the dynamics of the differentiation, including heterogeneity of the derived cell population, the tunability of the population composition based on the signal types and strengths, and the existence of stably co-expressed master regulators.

How do different master regulators of different $CD4^+$ T cell types interact with each other? It was found that two master regulators typically have a cross-repressive relationship: they inhibit the expression of each other. For example, it was shown that T-bet and Gata3 mutually inhibit each other and form a double-negative (i.e. positive) feedback loop. In addition to this feedback loop, the master regulators often involve other forms of positive feedback, including self-activation through transcriptional upregulation or autocrine regulation (master regulator activated by cytokine released from the same cells). These gene regulations form a cross-repression-autoactivation network motif (Figure 1C and D) that is common in $CD4^+$ T cells as well as other systems involving binary cell fate decisions. In addition to this network motif involving master regulators, $CD4^+$ T cells have a recurring signaling structure connecting extracellular simulants to master regulators: the TCR stimulation, which serves as the main activation signal for differentiation, activates all master regulators, whereas each cytokine in the microenvironment of the cells polarizes the differentiation toward specific lineages by activating a unique master regulator. This network structure is illustrated in Figure 1D in the dual-master-regulator paradigm. A common activator TCR signal upregulates two master regulators, and two representative polarizing signals (cytokines) activate the two master regulators respectively. In the next section, we build simple mathematical models based on this recurring network structure in $CD4^+$ T cells and examine how this regulatory network controls the dynamics of differentiation in general.

## Mathematical Models and Results

As shown in the influence diagram (Figure 1D), we start our analysis with the generic differentiation network motif which serves as a unifying framework for understanding $CD4^+$ T cell differentiation. Once we have obtained a basic understanding on this motif, we can connect the framework to specific models and simulations that can be compared with experiments.

### *Master regulator cross-repression with TCR signal*

We first consider a core subnetwork within the proposed cell differentiation motif: a mutual inhibition, double-negative feedback loop between master regulators X and Y, both of which are activated by TCR signal. This simplified system can be described by the following ODEs:

$$\frac{dX}{dt} = \gamma_X(F(\omega_X^o + \omega_{XY}Y + s_1 + s_2) - X)$$

$$\frac{dY}{dt} = \gamma_Y(F(\omega_Y^o + \omega_{YX}X + s_1 + s_3) - Y)$$

$$F(W) = \frac{1}{(1 + e^{-\sigma W})} \quad (1)$$

Here, $X$ and $Y$ represent the dimensionless activities of factors X and Y respectively. The nonlinear function $F(W)$ is a sigmoidal function that describes the activation of a factor under the influence of other factors, and the ODE indicates that this function also describes the steady state activity of the factor. Parameter $\gamma$ is the timescale of the change of a factor. $\sigma$ describes the nonlinearity of the function $F$. $W$ denotes the overall influence (weight) of other factors on a factor, which has taken an offset parameter $\omega^o$ into account. $\omega^o$ also determines the basal activity of the factor in the absence of any other factors. Importantly, $\omega_{XY}$ is a weight parameter describing the strength of the inhibition of X by Y, and $\omega_{XY}Y$ is the effective weight with given activity of Y. $\omega_{YX}$ represents the strength of the other inhibitory regulation in the feedback loop. $s_1$ is a parameter representing the strength of the TCR signal. In this particular model, the overall weight $W$ for $X$ is the summation of $\omega_X^o$, $\omega_{XY}Y$ and $s_1$, reflecting the assumption that gene X is only influenced by Y and signal $S_1$. We provide a set of generic parameter values to this model, and they are symmetrical with respect to X and Y: $\gamma_X = \gamma_Y = 1$, $\omega_X^o = \omega_Y^o = -1$, $\omega_{YX} = \omega_{XY} = -2$, $\sigma_X = \sigma_Y = 5$. We set the initial conditions to be a state where $X$ and $Y$ are equally low ($X = Y = 0.01$), and we set the strength of the signal S1 to 2 ($s_1 = 2$), mimicking the stimulation of the TCR upon antigen binding. Note that this form of equations is similar to those with Hill functions, and it has been used to describe complex gene regulatory networks for processes such as epithelial-mesenchymal transition [24].

Solving the ODEs numerically with these parameter values and initial conditions gives rise to trajectories of X and Y as functions of time, but it is helpful to visualize the trajectories in the space of X and Y (Figure 2A). Under the conditions described above, the trajectory of the simulation starts from an $X^{off}Y^{off}$ state and stays in the region before S1 stimulation. When the S1 signal is raised to 1, the cell state starts to change with increasing X and Y, and it is stabilized at a state with intermediate levels of X and Y at the end of the simulation (Figure 2A). We simply define that a cell is differentiated when either X or Y is above 0.5. In this simulation, we have obtained a differentiated cell that co-express X and Y. Does this cellular phenotype match any experimental observation? Before we make a conclusion, we examine the robustness of the steady state phenotype with respect to slight changes of the initial conditions. To perform this analysis in XPPAUT, we use the '`mice`' option under '`Initialconds`'. By repeatedly clicking in the bottom-left region in the X-Y space, we simulate a small population of cells, each of which express low levels of X and Y with a slight cell-to-cell variability in terms of the initial states, reflecting a biologically plausible scenario. With this procedure, we observe that the simulated cells are stabilized at either the $X^{on}Y^{off}$ state or the $X^{off}Y^{on}$ state, representing two canonical types of $CD4^+$ T cell, at the end of the simulations (Figure 2A). This is an interesting and biologically meaningful observation, because it shows that with the same simulant, a population of naïve $CD4^+$ T cells that are essentially identical can be differentiated into a population with remarkable diversity in terms of the expression of master regulators. This scenario was observed experimentally: activating TCR without supplying additional cytokine led to a heterogeneous population containing both Th1 and Th2 cells [25].

What is the mechanistic basis of this phenomenon from the viewpoint of dynamical systems? To answer this question, we take the advantage of the model's simplicity, specifically the amenable features of the 2-dimensional system, and use phase plane analysis to identify key elements in this dynamical system, including vector field, nullclines, steady states and their stabilities. To find the nullclines in XPPAUT, we first erase the existing trajectories by clicking on '`Erase`'. Suppose we would like to understand the features of the system in the presence of the TCR signal. We set the S1 signal to 1. We then click on '`Nullcline`' and '`New`' buttons, and two nonlinear curves are shown in the X-Y plane (Figure 2B). These two curves represent the positions where dX/dt=0, and dY/dt=0, respectively, and their intersections represent the steady states of the system. In this case, three steady states are governed by the structure of the nullclines. To view the vector field of the system, we first click on the '`Dir.field/flow`' and the '`Direct field`' buttons, and then we choose a suitable number of elements in the grid (e.g. 20). As a result, a vector field is shown, and each arrow in the field represents the direction of the solution (i.e. the trajectory as the system moves forward with time) at a particular state in the X-Y space (Figure 2B). The stabilities of the three steady states can be inferred from the vector field: most of the vectors close to the two 'polarized' $X^{on}Y^{off}$ and $X^{off}Y^{on}$ states are pointing toward them, whereas most of the vectors close to the co-expressing state are pointing away from it. Therefore, $X^{on}Y^{off}$ and $X^{off}Y^{on}$ states are stable, representing long term behaviors of cellular phenotype, whereas the $X^{on}Y^{on}$ state is unstable and therefore only represents a transient state of cells. We can confirm the inferred stabilities in a more rigorous manner by using '`Sing pts`' and '`Mouse`' buttons in XPPAUT to examine the interactions one at a time. The output will show us the stability of each steady state by calculating the eigenvalues of the Jacobian matrix at the state. The two stable steady states are known as 'attractors' of the system, and their basins of attractions are separated by a curve called separatrix which happens to pass the $X^{low}Y^{low}$ region in this model (Figure 2B, yellow). As such, slight cell-to-cell variability in the naïve cells tips them into distinct basins of attractors, which leads the cell differentiation to two stable 'polarized' phenotypes.

Our initial model successfully explains the basis of heterogeneous differentiation, but how can we explain the observed stable hybrid cell states in $CD4^+$ cells (e.g. cells co-expressing T-bet and Gata3 and those co-expressing RORγt and Foxp3)? To address this question, we vary the TCR signal in a wide range of strengths (e.g. 0 - 3). Unsurprisingly, lowering the TCR signal leads to the disappearance of the $X^{on}Y^{off}$ and $X^{off}Y^{on}$ states and a stable $X^{off}Y^{off}$ state, representing the naïve cell, is generated (Figure 2C). In contrast, increasing the TCR signal generates a system with a single stable state with a $X^{on}Y^{on}$ phenotype, which may correspond to the 'double-positive' cells observed experimentally [17,23] (Figure 2D). However, this model would predict that the double-positive cells only appear in the presence of strong TCR signals, which do not allow the formation of single-positive cells. This prediction is inconsistent with the observations that the double-positive $CD4^+$ cells often coexist with the single-positive ones under uniform culturing conditions [17]. To capture this phenomenon, we need to consider a model that contains additional elements included in the network motif (Figure 1D), namely the autoactivation of the master regulators.

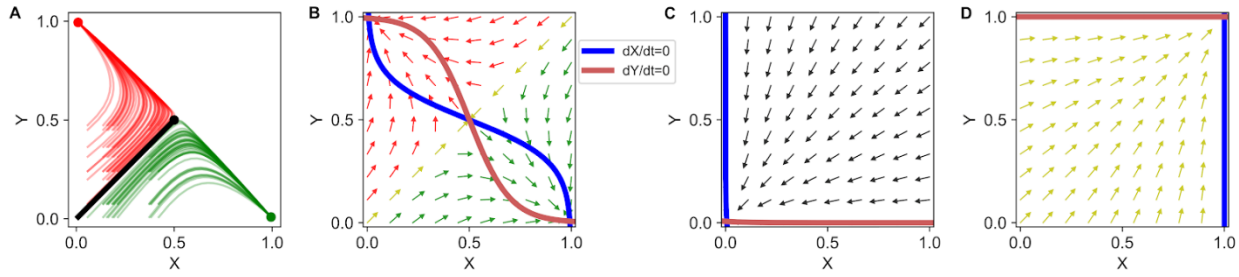


**Figure 2. Simulation and analysis of the cross-repression model. A.** Model was simulated with TCR signal $s_1$=2. Black: simulation trajectory under a perfectly symmetrical (X=0.01, Y=0.01) initial condition. Red and green: simulation trajectories under randomly chosen initial conditions in the $X^{low}Y^{low}$ region representing naïve T cells. Dots show the steady states of the simulations. **B**. Phase plane of the system. Red and green arrows represent vectors in two basins of attractions. Yellow arrows show vectors at the separatrix. Blue and indian red curves show nullclines of X and Y respectively. **C**. Phase plane without TCR signal ($s_1$=0). **D**. Phase plane with strong TCR signal ($s_1$=5).

*Master regulator cross-repression and autoactivation with TCR signal and polarizing cytokines*

To add the autoactivation to our initial model, we modify the weight W in both ODEs:

$$W_X = \omega_X^o + \omega_{XY}Y + \omega_{XX}X + s_1$$
$$W_Y = \omega_Y^o + \omega_{YX}X + \omega_{YY}Y + s_1 \quad (2)$$

Here, $\omega_{XX}X$ describes the influence of factor X by itself. To examine the effect of the autoactivation, we set the weight parameters $\omega_{XX}$ and $\omega_{YY}$ to 1.5, and we perform the analysis of the nullclines of this new system. By varying the S1 signal strength ($s_1$), the two nullclines may have 1, 3, or 5 intersections, depending on the choice of $s_1$. While the scenarios of 1 and 3 intersections (i.e. 1 or 2 stable steady states) are similar to what we observed with our first model, there are two ranges of $s_1$ that allows for 5 interactions of the nullclines, an observation unique to this model (e.g. Figure 3A). By examining the stability of the steady states, we find that certain low levels of $s_1$ allow the coexistence of $X^{off}Y^{off}$, $X^{on}Y^{off}$ and $X^{off}Y^{on}$ stable states (see Exercises), whereas certain high levels of $s_1$ allow the coexistence of $X^{on}Y^{on}$, $X^{on}Y^{off}$ and $X^{off}Y^{on}$ stable states (Figure 3A) (i.e. two types of 'tri-stable' systems). The tri-stable system with low levels of $s_1$ may be important for the irreversibility of T cell differentiation (see Exercises), and the tri-stable system with high levels of $s_1$ may provide a mechanistic basis of for the observations that double-positive $CD4^+$ cells coexist with the single-positive ones. The latter statement is supported by the fact that the separatrices (note that we have three basins of attractors in this scenario) are still adjacent to the $X^{low}Y^{low}$ region. Therefore, when we simulate a cell population with slight cell-to-cell variability in terms of initial conditions, we obtain a diverse population of differentiated cells containing three phenotypes, due to the small initial variations which tip the cells to different basins of attractions (Figure 3B).

The changes of the steady states of the system with respect to S1 signal can be succinctly delineated by a one-parameter bifurcation diagram. In XPPAUT, bifurcation diagrams can be generated in the 'AUTO' window under the 'File' option. Note that to get these bifurcation diagrams we need to use an equilibrium point that has been calculated from your solution of ODEs. In the 'AUTO' window, click on 'Run' 'Steady state' buttons. In Figure 3C for example, steady state levels of $X$ (i.e. intersections of the nullclines) are plotted with respect to $s_1$. Thick curves in the diagram represent stable steady states, whereas thin curves represent unstable ones. With the increase of $s_1$, the system makes four switches in terms of the number of stable steady states: 1) 1-to-3 ($s_1$=0.07164); 2) 3-to-2 ($s_1$=0.4831); 3) 2-to-3 ($s_1$=2.017); and 4) 3-to-1 ($s_1$=2.428). This is consistent with our phase-plane analysis, and this diagram captures all qualitative changes of the system without the need of changing $s_1$ manually.

What is the role of the polarizing signals (cytokines) in shaping the dynamical system and the cell differentiation? This question can be addressed by simplify making another modification to the weight W in the ODEs

$$\begin{aligned} W_X &= \omega_X^0 + \omega_{XY}Y + \omega_{XX}X + s_1 + s_2 \\ W_Y &= \omega_Y^0 + \omega_{YX}X + \omega_{YY}Y + s_1 + s_3 \end{aligned} \quad (3)$$

Here parameters $s_2$ and $s_3$ represent polarizing signals that bias differentiation toward X expression and Y expression respectively. We use phase-plane and simulations again to show the effect of adding a polarizing signal, e.g. $s_2$, to the system. Raising $s_2$ from 0 to 0.05 results in a slight shift of vector field and basins of attractions which favor X expression (Figure 3D). This symmetry breaking effect gives rise to a biased cell population if we randomly choose the initial conditions for the naïve in the $X^{low}Y^{low}$ region (Figure 3E). Increasing $s_3$ while holding $s_2$ constant has the opposite effect (Figure 3F and G). This analysis shows that the polarizing signals (cytokines) can change the compositions of the heterogeneous cell population by breaking the symmetry of the system.

Our model parameters are assumed to be symmetrical with respect to X and Y, except for the polarizing signals. Perfectly symmetrical gene regulation is an unlikely scenario in biology. In fact, any changes to the internal gene regulatory parameters (e.g. $\omega_{YX}$) will give rise to an asymmetrical system in a manner similar to the effect of polarizing signals. This explains the observation that the double-positive cells often coexist with only one of the two types of single-positive cells.

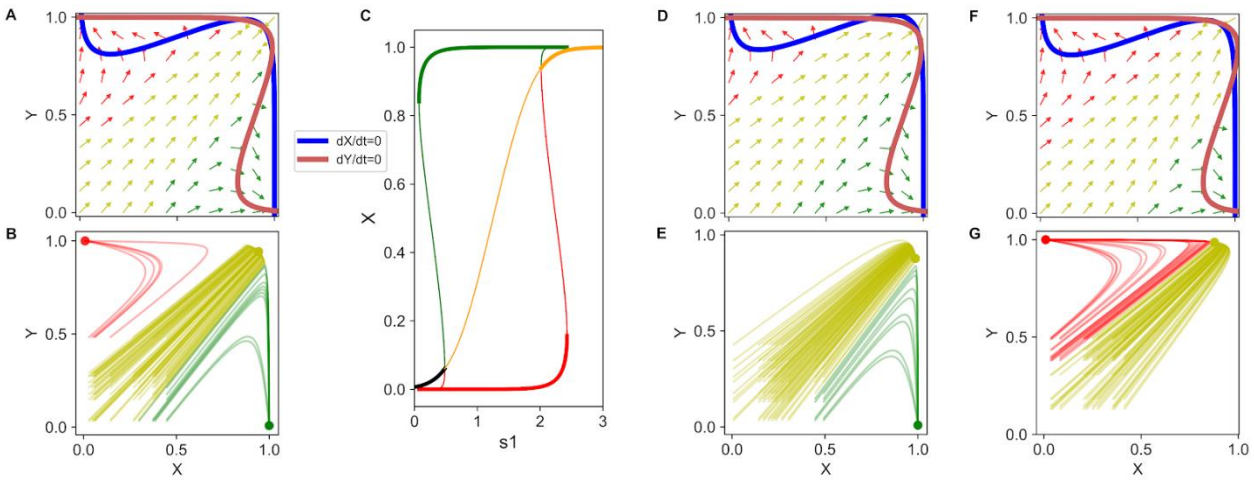


**Figure 3. Simulation and analysis of the cross-repression and autoactivation model. A**. Phase plane under strong TCR signal condition ($s_1$=2.05). B. Simulation trajectories under strong TCR signal condition. Initial conditions were randomly chosen in the $X^{low}Y^{low}$ region. **C**. Bifurcation diagram showing the steady state of X with respect to s1. Thin curves represent unstable steady states. Thick curves represent unstable steady states. **D**. Phase plane under strong TCR signal condition in the presence of polarizing signal S2 ($s_2$=0.05). **E**. Simulation trajectories under strong TCR signal condition in the presence of polarizing signal s2. Initial conditions were randomly chosen in the $X^{low}Y^{low}$ region. **F**. Phase plane under strong TCR signal condition in the presence of polarizing signal S3 (s3=0.05). **G**. Simulation trajectories under strong TCR signal condition in the presence of polarizing signal $s_3$. Initial conditions were randomly chosen in the $X^{low}Y^{low}$ region.

## Discussion

In this case study, we have used mathematical models to show how $CD4^+$ T cells differentiate to functional cell types. Specifically, we show that a simple gene regulatory network containing cross-repression and autoactivation of two lineage defining master regulators can govern differentiation of naive $CD4^+$ T cells into a heterogeneous population with distinct subsets of cells in terms of the master regulator expression. In addition, the model captures the existence of the double-positive cell phenotype which has high expression levels of more than one master regulator [26,27]. Although different types of $CD4^+$ T cells have specific roles in eliminating different pathogens, it has been proposed that the diversity and the flexibility of the $CD4^+$ T cell populations may be important for the 'agility' of the immune system [28]. Therefore, the robustness formation of the heterogeneous, functional $CD4^+$ T cells, as well as the double-positive phenotype, may serve as the cellular basis of this feature of the immune system. In terms of the extracellular signals, the models show that T cell receptors trigger the heterogeneous differentiation, but the polarizing cytokines can bias the differentiation toward specific types. Therefore, the system has a remarkable capacity of generating either diverse or uniform cell populations depending on the microenvironment of the cells.

We use a generic description of master regulators and signals in our models. This is because the network motif represents several sub-systems of $CD4^+$ T cells with similar structures of gene regulatory network (Figure 1C). If one needs to obtain a more quantitative model for a specific sub-system, then this generic model can be optimized through, for example, fitting parameters to quantitative measurements. In addition, the cross-repression-autoactivation network motif has been found in several other developmental systems in which binary cell fate decisions are made in a robust manner [29]. Understanding this motif will help to reveal the principles underlying a wide range of biological systems.

In these simple models, only two master regulators are considered. A more realistic description of the $CD4^+$ T cells must include the interactions of more than two master regulators. Previous studies have provided this type of holistic views of $CD4^+$ T cells [30,31], and these models capture many phenotypes that the simple dual-regulator models cannot describe, including the possible existence of triple-positive phenotypes. In terms of modeling strategy, we have used ODE based models. As an alternative approach, Boolean-network based models have been applied to $CD4^+$ T cell differentiation, with a more comprehensive description of players but less detailed kinetics in the system [32].

More recent studies of $CD4^+$ T cells have shown the possibility that these cells are in the continuum of the space of master regulator expression rather than discrete states [33]. The model presented in this chapter cannot describe the expression continuum due to the simplicity of the gene networks. Taking more genes and the stochasticity of their expression into account would provide a more accurate and quantitative model of the $CD4^+$ T cells. Nonetheless, our models show that the interactions between master regulators can shape the attractors of the system, and these attractors guide the differentiation of these T cells, which may be diverse at the population level but robust in terms of decisions of individual cells. This may serve as a unifying principle governing differentiation of $CD4^+$ T cells and other systems.

## Methods

The XPPAUT code to analyze the models in this case study is shown below.

Code 1

```
dX/dt=1/(1+exp(-sigmaX*(w0X+wXY*Y+s1+wXX*X+s2)))-X
dY/dt=1/(1+exp(-sigmaY*(w0Y+wYX*X+s1+wYY*Y+s3)))-Y

init X=0.001
init Y=0.001

#par s1=2.05
par s1=0
#par s1=2
#par s1=5

par s2=0.00, s3=0.00
```

```
par sigmaX=5
par sigmaY=5
par w0X=-1.0
par w0Y=-1.0
par wXY=-2
par wYX=-2

par wXX=1.5
#par wXX=0
# set to 0 for the 1st model

par wYY=1.5
#par wYY=0
# set to 0 for the 1st model

@ dt=0.01, total=100, method=stiff, maxstore=10000000,
bound=100000000
@ XP=X, YP=Y, XLO=-0.1, YLO=-0.1, XHI=1.1, YHI=1.1, NMESH=500
@ AUTOYMIN=-0.1, AUTOYMAX=1.1, PARMIN=0, PARMAX=3, AUTOXMIN=0,
AUTOXMAX=3
@ DSMIN=0.0001, DS=0.002, DSMAX=0.1, NMAX=500, NPR=200

done
```

**Exercises**

1. Reversibility of cell differentiation.

(1) Generate a one-parameter bifurcation diagram for the first model (the one without autoactivation). This diagram should show the steady state of X with respect to the strength of signal S1 (similar to Figure 3C). Simply modify the code above by changing the $\omega_{XX}$ and $\omega_{YY}$ to 0, and increase the range of the parameter for bifurcation analysis to 0-4 (`PARMIN=4, AUTOMAX=4`) for a better view of the diagram. Run the system to the steady state, and then run bifurcation analysis as described in the Results.

(2) Compare the diagram generated in (1) with Figure 3C in the low-middle range of $s_1$. Predict the roles of the autoactivation on the reversibility for the differentiation of $X^{on}Y^{off}$ and $X^{off}Y^{on}$ phenotypes when the signal first increases and then decreases.

(3) Add the following lines of code to the existing code:

```
global 1 {ta-10} {s1=0.1}
ta=t
```

The two lines describe the scenario mentioned in (2): the signal is reduced to 0.1 at Time 10. Run simulations under the conditions $\omega_{XX} = \omega_{YY} = 0$, and $\omega_{XX} = \omega_{YY} = 1.5$ separately, and validate

the prediction you make in (2). To run the simulations, use the '`mouse`' option and click on an initial condition in the lower-left corner of the X-Y space.

2. An alternative description of gene expression variability.

In this chapter we have used different initial conditions to describe cell-to-cell variability. This variability can be described by other methods as well. For example, there exist random fluctuations in gene expression, which can tip the cells into different basins of attractions. Implement this alternative way to describe variability by replacing the two equations in Code 1 with the following lines:

```
dX/dt=1/(1+exp(-sigmaX*(w0X+wXY*Y+s1+wXX*X+s2)))-X+wX*eX
dY/dt=1/(1+exp(-sigmaY*(w0Y+wYX*X+s1+wYY*Y+s3)))-Y+wY*eY
wiener wX, wY
```

Next, add the following parameter values to the bottom of the parameter value list:

`par eX=0.02, eY=0.02`. The two parameters describe the amplitude of the fluctuations in gene expression. Simulate multiple cells again with the 'Master regulator cross-repression and autoactivation' model. Do you observe heterogeneity in the differentiated population if you start the simulations and the same initial condition? What is the effect of increasing or decreasing the amplitude of the noise on the population composition?

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