Behavior Analysis on Boolean and ODE models for Extension of Genetic Toggle Switch from Bi-stable to Tri-stable

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Abstract

The artificial genetic circuit (AGC) is a gene network in which its expression timing, period and function are designed by computational and biological techniques. The AGCs for the functions of electronic circuits like a toggle switch and an oscillation circuit were realized in \textit{E.coli} by Gardner and Elowitz in 2000\textsuperscript{[1],[2]}, respectively. We visualized and verified the structure and behavior of Boolean network (BN) and ordinary differential equation (ODE) models for 2-variable genetic toggle switch in the phase plane by using GINsim and Scilab. ODE is the most widely used formalism for biological processes, while BN model allows us to easily understand mathematical expression of biological processes. Then, we extended the models to 3-variable genetic toggle switch from the 2-variable ones demonstrating a mathematical formalization by showing correspondence between state transitions of BN and trajectory paths of ODEs in the phase space.

Keywords: synthetic biology, artificial genetic circuit,

1. Introduction

Currently, artificial genetic circuits can be construct using genetic engineering, which is called synthetic biology. We investigated the behavior and mathematical formalization about genetic toggle switch as artificial genetic circuits. The genetic toggle switch works as an electronic toggle switch so that the state of the gene expression between two genes can be switched by the regulation of related factors. For the bi-stable genetic toggle switch, Gardner et al. showed the constructing method of the bi-stable genetic toggle switch circuit in 2000\textsuperscript{[1]}. They specified and confirmed the conditions for the bi-stable behavior, the logical model of mutual suppression and the ordinary differential equations (ODEs).

In this paper, we obtained the state transition graph(STG) from the Boolean Network (BN) model based on the logical model for the bi-stable genetic toggle switch, and associated the STG with the ODE model to investigate the correspondence between the state transitions in STG and trajectories of the ODEs. After that, we extended the bi-stable genetic toggle switch to tri-stable one, and analyzed the dynamic behavior of the ODE model comparing with the state transitions in the STG. In the case of a bi-stable genetic toggle switch, nullcline could be shown in a 2-dimensional diagram. On the other hand, nullcline of the ODEs for the tri-stable genetic toggle switch is drawn as a 3-dimensional graph. A graph of each nullcline can be associated with its STG. The phase plain can be divided into 4 areas centered on the unstable point based on the correspondence between the state transitions in STG and trajectories of the ODEs for the bi-stable genetic toggle switch. The tri-stable genetic toggle switch has 8 state transitions in STG and the phase space can be divided into 8 spaces centered on unstable point. By extending to tri-stable genetic toggle switch, the stable point increased from 2 to 3, nullcline is represented as planes from lines. As a result, the genetic toggle switch has been extended from bi-stable to tri-stable, and we demonstrated a mathematical formalization by the correspondence between state transitions of BN and trajectory paths of ODEs in the phase space.

2. Dynamic behavior, Boolean network and Gene structure of bi-stable genetic toggle switch

The bi-stable genetic toggle switch gets steady state when either repressor 1 gene or repressor 2 gene is expressed in Figure1. Thus, it has 2 stable steady states. Figure 2 shows the logical model and Figure 3 shows the state transition diagram for bi-stable genetic toggle

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switch. The gene structure of the bi-stable genetic toggle switch produced by Gardner[1] is shown in Figure 1, and ODEs is shown in Figure 4.

When the value of ODEs do not change with time, the set of \( \frac{dx}{dt} = 0 = \frac{dy}{dt} \) is called nullcline (Figure 4, 5). The intersection of the trajectories of the ODEs is called the stationary point, and the bi-stable genetic toggle switch has 2 stable steady state points \((X_A, X_B)\) and 1 unstable point \((X_U)\). The line drawn diagonally from the origin is called separatrix. The convergent stable steady state point is determined depending on the initial value of the ODEs on the phase plane which is divided into the 2 regions \((S_A, S_B)\) by the separatrix (Figure 5-T1, T2).

The state transition graph of bi-stable genetic toggle switch shown in Figure 2 has 4 states. In Figure 5, let \( \theta \) be the value of \( x \) and \( y \) of the unstable point, and let it be the \((x_0, \theta, \theta)\) threshold. Then the phase space can be divided into 4 regions. Each region with each state in the STG are defined in the following conditional formula (Figure 6). \( R \) denotes the set of real numbers.

\[
P_{00} = \{(x, y) \in \mathbb{R}^2 | x \leq \theta \text{ and } y \leq \theta \},
\]

\[
P_{01} = \{(x, y) \in \mathbb{R}^2 | x \leq \theta \text{ and } y > \theta \},
\]

\[
P_{10} = \{(x, y) \in \mathbb{R}^2 | x > \theta \text{ and } y \leq \theta \},
\]

\[
P_{11} = \{(x, y) \in \mathbb{R}^2 | x > \theta \text{ and } y > \theta \},
\]

The stable steady state points \((X_A, X_B)\) are in the regions \(P_{01}\) and \(P_{10}\) respectively in Figure 6. Because the trajectories never go across the separatrix, the state \(P_{00}\) and \(P_{11}\) do not transit between the two regions. There is no transition path between \([0,1]\) and \([1,0]\) in the STG. Thus, the four states in the STG of the bi-stable genetic toggle switch can be associated with 4 regions obtained by dividing the phase plane.

\[
\begin{align*}
\frac{dx}{dt} &= \frac{a}{1 + y^n} - x, \\
\frac{dy}{dt} &= \frac{b}{1 + x^n} - y.
\end{align*}
\]
3. Extension of tri-stable genetic toggle switch

We extend the STG as a BN model and the correspondence between state transitions of BN and trajectory paths of ODEs in the phase plane representing the bi-stable genetic toggle switch to the tri-stable ones. The tri-stable genetic toggle switch gets stable steady states when one of the repressor genes (A, B and C) is expressing. Thus, it has 3 stable steady states. Figure 7 shows a logical model, and Figure 8 shows a STG of tri-stable genetic toggle switch. Figure 9 shows the ODEs for tri-stable genetic toggle switch obtained by extending the ODEs for the bi-stable ones.

The nullcline of the ODEs for tri-stable genetic toggle switch has 4 intersections, and these intersections are consisted of 3 stable steady state points (Xα, Xβ, Xγ) and 1 unstable point (X0). In the case of tri-stable genetic toggle switch, it is also possible to control the gene expression by giving various initial value for each parameter (x, y and z)(Figure 10-T1,T2,T3).

In Chapter 2, we distributed the states in the STG and nullcline of the bi-stable genetic toggle switch. There are 8 states in the STG. Let \( \theta \) be the value of \( x, y, z \) of the unstable point, and let it be the \( (X_0 = (0, \theta, \theta)) \) threshold. Then the graph of nullclines can be divided into 8 regions. Each region with each state in the STG are defined as shown in the following conditional formula (Figure 11).

\[
\begin{align*}
P_{000} & = \{(x,y,z) \in \mathbb{R}^3| \ x \leq 0 \ & \land \ y \leq 0 \ & \land \ z \leq 0 \}, \\
P_{001} & = \{(x,y,z) \in \mathbb{R}^3| \ x \leq 0 \ & \land \ y \leq 0 \ & \land \ z > \theta \}, \\
P_{010} & = \{(x,y,z) \in \mathbb{R}^3| \ x \leq 0 \ & \land \ y > \theta \ & \land \ z \leq \theta \}, \\
P_{011} & = \{(x,y,z) \in \mathbb{R}^3| \ x \leq 0 \ & \land \ y > \theta \ & \land \ z > \theta \}, \\
P_{100} & = \{(x,y,z) \in \mathbb{R}^3| \ x > \theta \ & \land \ y \leq 0 \ & \land \ z \leq \theta \}, \\
P_{101} & = \{(x,y,z) \in \mathbb{R}^3| \ x > \theta \ & \land \ y \leq 0 \ & \land \ z > \theta \}, \\
P_{110} & = \{(x,y,z) \in \mathbb{R}^3| \ x > \theta \ & \land \ y > \theta \ & \land \ z \leq \theta \}, \\
P_{111} & = \{(x,y,z) \in \mathbb{R}^3| \ x > \theta \ & \land \ y > \theta \ & \land \ z > \theta \}.
\end{align*}
\]

The stable steady state points are in the regions [001], [010] and [100] respectively in Figure 11. Trajectories T1, T2 and T3 transit to one of the stable steady state points through the spaces which correspond to the state transition path of STG. In other words, there is no trajectories in the phase space not according to the state transition paths in STG. For example,T1 is the trajectory of the ODEs for the tri-stable genetic toggle switch when \( (x, y, z) = (1.8, 1.1, 1.5) \) is given as an initial state.

Trajectory start from the region \([1,1,1]\) which is associated with the STG. After that, it goes to the region \([1,0,1]\)-\([1,0,0]\) so as to correspond to the state transition path in the STG and reaches to the stable steady state point \( X_3 \) (Figure 12). Therefore, the state transition in the STG based on the logical model corresponds to the behavior of the ODE model in the phase space for the tri-stable genetic toggle switch after the extension from bi-stable to tri-stable.

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4. Separatrix of tri-stable genetic toggle switch

The separatrix of the ODE model for the tri-stable genetic toggle switch in the phase space is shown in Figure 16. The trajectories transition to one of the stable steady state points without crossing the separatrix (Figure 13, 14, 15). In other words, there are three regions divided by the separatrix which decide the destination point of the trajectory as a stable steady state point. The separatrix of the ODEs for the bi-stable genetic toggle switch is represented as a line. After the extension from bi-stable to tri-stable, the separatrix is also extended from a line to a plane.

**Movement of Figure 11 from P₀₀₀ to any one of P₀₀₁, P₀₁₀ and P₁₀₀ is determined by the starting point. Also from P₁₁₁ to P₀₀₁, P₀₁₀, P₁₀₀ are similar. We considered how to transit from P₁₁₁ to which regions (P₀₀₁, P₀₁₀, P₁₀₀) and stabilize. We divided the region P₁₁₁ in detail as in the following formula. (Similarly for P₀₀₀, P₀₀₁, P₀₁₀, P₁₀₀, P₀₁₁, P₁₁₀)**

\[ P_{111} = P_{111A} \cup P_{111B} \cup P_{111C} \]

where

\[ P_{111A} = \{(x, y, z) \in P_{111} | x > y \& x > z \& x > \theta\} \]

\[ P_{111B} = \{(x, y, z) \in P_{111} | y > x \& y > z \& y > \theta\} \]

\[ P_{111C} = \{(x, y, z) \in P_{111} | z > x \& z > y \& z > \theta\} \]

We divided the areas further on P₁₁₁A as in the following formula. (Similarly for P₁₁₁B, P₁₁₁C)

\[ P_{111A} = P_{111A_A} \cup P_{111A_B} \cup P_{111A_C} \]

where

\[ P_{111A_A} = \{(x, y, z) \in P_{111} | x > y \& x > z \& y > z \& x > \theta\} \]

\[ P_{111A_B} = \{(x, y, z) \in P_{111} | x > y \& x > z \& y < z \& x = \theta\} \]

\[ P_{111A_C} = \{(x, y, z) \in P_{111} | x > y \& x > z \& y = z \& x > \theta\} \]

We can understand how the trajectory is drawn from the starting point value.
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Figure 18. Trajectories started from $P_{111A}$ ($P_{111B}, P_{111C}$) and $P_{111A}$ ($P_{111B}, P_{111C}$) approach to $X_0(X_0,X_0)$ via different regions, $P_{110}$ and $P_{11n}$, respectively.

Figure 17 $P_{100}$ and $P_{111}$ are divided into three regions.

5. Conclusion

In this paper, we confirmed the state transition of the logical model of the bi-stable genetic toggle switch conducted by Gardner, and found the correspondence between the BN model and the ODE model. By setting the value of the unstable point of the ODEs as 0, the phase plane can be divided into 4 regions. We showed the correspondence between the STG and the behavior of the trajectories. Then, we extended a genetic toggle switch from bi-stable to tri-stable. The nullcline of the ODEs for bi-stable one is represented as 2-dimensional curve in the phase plane but the nullcline of ODEs for tri-stable one is represented as a 3-dimensional diagram. The stable steady state point indicated by the intersection of nullclines increased from 2 to 3. Likewise, we distributed the state transitions based on the logical model to the phase space based on the ODE model. We confirmed the correspondence between state transitions of BN and trajectory paths of ODEs in the phase space. Finally, we sought the separatrix of the ODEs for the tri-stable genetic toggle switch. The separatrix is also extended from a line to planes after the extension from bi-stable to tri-stable.

By extending from bi-stable to tri-stable, we can generalize the extension to multi-stable genetic toggle switch. The ODEs for multi-stable genetic toggle switch are shown below.

$$\frac{d}{dt}u_i = \frac{a_i}{1+\sum_{j=1}^{n}u_j} - u_i \quad (i \neq j)$$

6. References
