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Effects of Time Delay on Multistability of Genetic Toggle Switch

De-qing Zhu, Hui-jun Jiang, Zhong-huai Hou*

Department of Chemical Physics & Hefei National Laboratory for Physical Sciences at the Microscales, iChEM, University of Science and Technology of China, Hefei 230026, China

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The effect of time delay on a genetic toggle switch, whose undelayed dynamics shows low protein expression states (L-states), high expression states (H-states) and coexistence of them different transcription-factor binding rates α, is investigated by using the delayed stochastic simulation method. Interestingly, we find that the delay induces a transition from the coexistence state to L-state or H-state by suppressing the other state. Moreover, the phase diagram on the α-τ plane is obtained by extensive simulations. It is observed that, the coexistence state is remarkably narrowed by increasing delay time, and completely disappears above a triple-point-like point where direct transitions between H-state and L-state are possible.

Key words: Genetic toggle switch, Delay, Multistability

I. INTRODUCTION

Genetic toggle switch is one of the essential elements in gene regulatory networks realizing cell differentiation where cells with the same genotype can present different phenotypes [1–3]. So far, various genetic toggle switches have been revealed and constructed [3–5], which usually exhibit robust bistability. One of the typical examples, as shown in Fig.1, is a toggle switch composed of two genes X and Y, where expression of gene X is repressed by protein B which is encoded by gene Y, while the expression of gene Y is repressed by protein A encoded by gene X. Depending on different conditions, this genetic toggle switch may exhibit two exclusive states A or B. Usually, genetic toggle switches should be bistable, but multistability [7–10] can also be presented in some special cases. For instance, Lipshtat *et al.* found that the fluctuation of molecule numbers would lead to new kinds of stability in a genetic toggle switch without cooperation binding [11]. They predicted that monostability would appear under conditions of weak repression, whereas tristability could occur under strong repression. In a recent experimental study, we have constructed an artificial genetic toggle switch [12]. Different from the findings in Lipshtat's work, we found an additional kinetic stable state, where all the genes are expressed very low, can coexist stably with the other two known stable states. Further theoretical analysis revealed that discreteness and fluctuation in small systems are the reasons behind the emergence of the third state.

On the other hand, time-delayed interactions are unavoidable in gene expression process [13–15], which generally consists of many sub-processes such as dimerization, protein-DNA binding/unbinding, transcription, translation, degradation, and so on. Analysis of gene regulatory networks shows that there is a vast separation of time scales during the expression. For example, dimerization, protein-DNA binding/unbinding are typically fast, while transcription, translation, and degradation are relatively slow. It is important to note that the transcriptional and translational processes are not just slow but also composed of multistage reactions involving sequential assembly of long molecules. These multistage processes should be treated as delayed reactions, in which the initiating events are separated from the appearance of products by certain interval of time delay. Since the presence of time delay is a strong non-Markovian property, various studies have shown that they can remarkably affect dynamic behaviors of complex systems. It has been found that time delay in gene

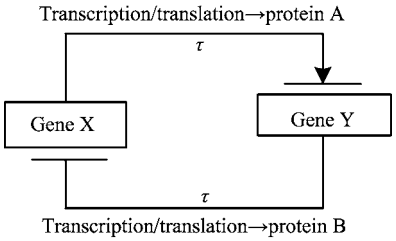

FIG. 1 Schematic of the genetic toggle switch. Two genes X and Y mutually repress each other via their encoding proteins A and B. Among the multistage reactions of transcriptional and translational processes, some of them may be very slow, hence the repressions should involve delayed interactions.

*Author to whom correspondence should be addressed. E-mail: hzhlj@ustc.edu.cn

expression can cause a system to be oscillatory even when its counterpart without delay exhibits no oscillations, and such delay-induced instabilities can compromise the ability of a negative feedback loop to reduce the deleterious effects of noise [16–19]. Other examples include delay-induced extra entropy production [20], delay-induced bifurcation of dominant transition pathways [21], delay-induced excitability [22], delay-induced oscillation [16], to list just a few. It is thus very interesting for us to investigate how time delay would affect the multistability of genetic toggle switches.

In this work, we study the effect of time delay in a genetic toggle switch as depicted in Fig.1 by using the delayed stochastic simulation method. Without delay, the system can present low protein expression states (L-states), high expression states (H-states) and coexistence of them, depending on the binding rate α of the transcription factor. Starting from the coexistence state occurring for a moderate α, transitions to H-state or L-state are observed with increasing delay τ for relative small or large α, respectively. In addition, a phase diagram of the system is obtained on the α-τ parameter plane. Interestingly, there is a triple-point-like point located at (α_c,τ_c) where the two transition points, one from the coexistence-state to H-state and the other from coexistence-state to L-state, are merged. For a fixed delay time $\tau \geq \tau_c$, the coexistence-state disappears for all values of α, and direct transitions between H-state and L-state are possible, indicating a cooperative effect of delay τ and binding rate α on the multistability dynamics of the genetic toggle switch.

II. MODEL AND METHOD

In a gene regulatory network, the expression process consists of dimerization, protein-DNA binding/unbinding, transcription, translation, and degradation. The key steps of the expression process are transcription and translation, where DNA is firstly transcribed into a complete messenger RNA and then translated into the target protein via a series of chemical reactions, for example RNA polymerase binds to a gene's promoter. For simplicity, a generic model has been proposed to describe the dynamics of the genetic toggle switch, where transcription and translation are combined into one step [23]. In this model, gene X(Y) can express protein A(B), and be repressed when the produced protein B(A) binds on its promoter P_X (P_Y), respectively. The set of biochemical reactions can be described by 6 elementary reactions as follows,

$$X + P_X \xrightarrow{\kappa} X + P_X + A \tag{1}$$

$$Y + P_Y \xrightarrow{\kappa} Y + P_Y + B \tag{2}$$

$$P_X + B \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} P_X B \tag{3}$$

$$P_Y + A \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} P_Y + A \tag{4}$$

$$A \xrightarrow{\gamma} \phi \tag{5}$$

$$B \xrightarrow{\gamma} \phi \tag{6}$$

where κ denotes the rate of gene expression, $\alpha(\beta)$ are respectively the binding(unbinding) rate of the transcription-factor, ϕ is the degradation product of proteins and γ is the corresponding rate. Note that all the reactions given by Eq.(1) to Eq.(6) are instantaneous. As mentioned above, transcriptional and translational processes are compound multistage reactions where the sequential assembly of long molecules may be very slow. As a result, a change of the current state of the system may affect the dynamics after a time interval. Thus, delayed interaction needs to be included in the process of gene encoding protein. Here, we modify the original model by considering Eq.(1) and Eq.(2) as delayed reactions with a delay time τ. That is, the current number of A and B are determined by the number of $\{X,P_X\}$ and$\{Y,P_Y\}$ at time $t-\tau$. Therefore, Eq.(1) and Eq.(2) should be replaced by

$$X^{(\tau)} + P_X^{(\tau)} \xrightarrow{\kappa} X + P_X + A \tag{7}$$

$$Y^{(\tau)} + P_Y^{(\tau)} \xrightarrow{\kappa} Y + P_Y + B \tag{8}$$

where the variables with superscript (τ) denote the number of corresponding species at time τ before. A schematic of the delayed toggle switch is shown in Fig.1. In the present study, we use Eq.(3) to Eq.(8) to investigate the effects of delay on the system's dynamics.

To study the dynamics of above toggle switch, one must take into account the internal molecular fluctuations unavoidable in such small reaction systems. A widely used method has been the exact stochastic simulation algorithm (SSA) proposed by Gillespie [24]. Generally, to follow a chemical reaction, one only needs to know which reaction μ would take place in the next step and how long the waiting time Δt should be before it happens. At the beginning of each SSA step, the propensity function a_ν for each reaction ν and the total propensity $a_0=\sum_\nu a_\nu$ are calculated. Then two random numbers r_1 and r_2 with uniform distribution between 0 and 1 are generated. Based on these, Δt and μ are determined by $\Delta t=(1/a_0)\ln(1/r_1)$ and $\sum_{\nu=1}^{\mu-1} a_\nu < r_2 a_0 \leq \sum_{\nu=1}^{\mu} a_\nu$. After Δt and μ are taken, the numbers of molecules in the system and the time of the reaction are updated accordingly. For systems with delayed reactions, however, one must perform a little bit change to the algorithm [16, 25, 26]. Specifically, when the next reaction μ is a delayed one, no reaction takes place at present, and a waiting time list is built to store the delayed event which will occur at time $t_d=t+\Delta t$, where t is the current time. If reaction μ is not delayed, its reaction time t_μ should be compared with the time in the waiting list of scheduled delayed reactions. If there is a delayed reaction that occurs sooner than

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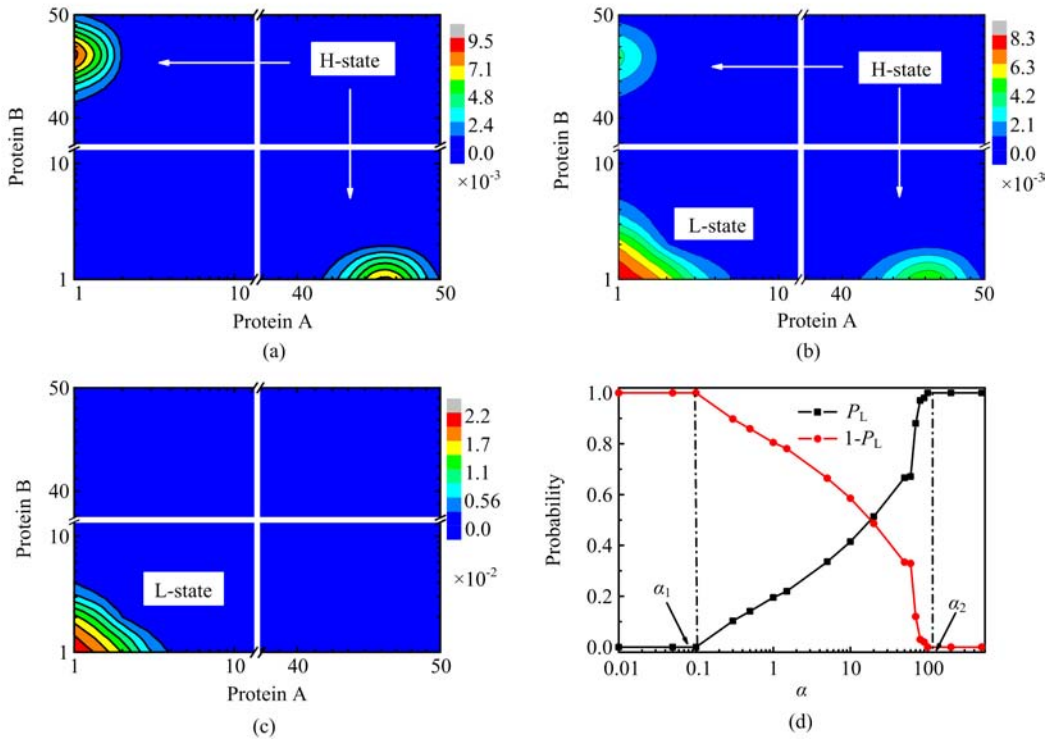

FIG. 2 Multistability of the genetic toggle switch without delay. Probability distributions in the x-y plane are shown in (a) for α=0.1, (b) for α=10, and (c) for α=100, respectively. The H-state where at least one of the protein concentrations is high and the L-state where both protein concentrations are low are indicated by arrows. (d) The distribution probability $P_{\rm L}$ of the L-state as a function of reaction rate α.

t_μ, this delayed reaction will be carried out instead of μ and the time is updated to t_d which is the finished time for the delayed event, otherwise, the molecule numbers of all species are updated according to reaction μ and the time is advanced to t_μ. We will adopt this latter delay-SSA in our present work to investigate the effects of delay.

In simulations, we take the initial molecular numbers as X=Y=50, P_X=P_Y=1 and A=B=0. The multistability of the system is described by the probability distribution in the X-Y plane obtained from a long time simulation with totally N=10^8 steps. Other parameters are fixed as κ=1, γ=0.1 and α/β=10. We choose α as the control parameter.

III. RESULTS AND DISCUSSION

As shown in Fig.2, for different reaction rate α, multistability can exist in the genetic toggle switch without delay. For α=0.1 (Fig.2(a)), the system stays in the H-state, where one of the proteins is highly expressed and the other is not. There are two peaks in the figure, indicating the system is bistable for this parameter. When α is increased to α=10 (Fig.2(b)), beside the high-expression states, the switch can also present a low-expression state, where both proteins are expressed rarely. Further increasing α to 100, as shown in Fig.2(c), H-state disappears and the system exhibits only L-state. These results are consistent with the findings reported by Andrecut *et al.* [23]. In order to quantitatively describe how the multistability of genetic toggle switch depends on α, we define an order parameter $P_{\rm L}$ which is the distribution probability of the L-state. The calculated $P_{\rm L}$ as a function of α is presented in Fig.2(d). Clearly, the genetic toggle switch shows H-state for small α. When α increases to a threshold α_1, a transition from H-state to a coexistence of H-state and L-state occurs. The coexistence state can further develop to L-state while α bypasses another threshold α_2. Findings in Fig.2 illustrate that transcription-factor binding rate α can dramatically affect the multistability of genetic toggle switches.

We now turn to investigate the effects of time delay. Since the coexistence state occurs for mediate α, we are interested in how delay affects the dynamics in this regime. Firstly, α=1.2 is set to be close to the transition point α_1, where both H-state and L-state can be observed in the absence of delay as shown in Fig.3(a). For moderate delay time such as τ=0.03 (Fig.3(b)), two interesting observations can be concluded. On one hand, the single peak of L-state splits into two separated peaks where one of the proteins A or B is expressed slightly higher than the other. On the other hand, both of the two new peaks shift to positions with relative higher

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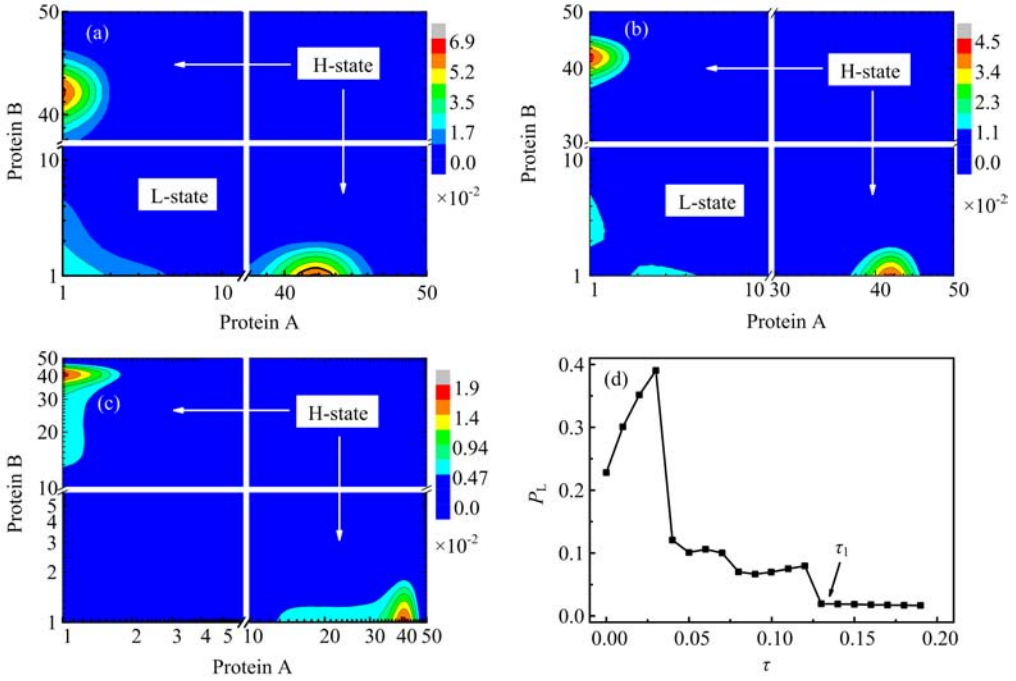

FIG. 3 Multistability of delayed genetic toggle switch for α=1.2, and (a) τ=0.0, (b) τ=0.03, (c) τ=1.0. (d) Dependence of the distribution probability $P_{\rm L}$ of the L-state on delay time τ.

protein concentrations compared to the one without delay. Keeping increasing τ, the two peaks will further move towards their corresponding peaks of H-state. Finally, these peaks are merged into H-state and the L-state disappears, as shown in Fig.3(c) for τ=1.0. In order to show a complete dependence of this process on τ, the order parameter $P_{\rm L}$ is calculated as a function of τ, which is plotted in Fig.3(d). Clearly, when τ is relative small, $P_{\rm L}$ is obviously nonzero, indicating the existence of both H-state and L-state. While τ is relative large, L-state disappears and only H-state exists where $P_{\rm L}$=0. A transition point τ_1 can then be identified where the coexistence state starts to completely turn into H-state. It is noted that decreasing of $P_{\rm L}$ is not monotonous for τ less than $\tau_1\approx$1.2, which implies a complicated effect of delay and more studies are deserved to understand its underlying mechanism. Findings in Fig.3 illustrate that, near the transition boundary α_1 between H-state and coexistence state, time delay can suppress the L-state and induces a transition from coexistence of L-state and H-state to H-state only.

The above results seem to indicate that time delay tends to repress the L-state. Nevertheless, this is not the case if α is close to the other transition point α_2. In Fig.4, multistability behavior for the toggle switch with α=20 is shown for different values of delay time τ. Figure 4(a) gives the coexistence state for τ=0. With increasing delay time to a moderate value such as τ=0.03 (Fig.4(b)), the peak position of the L-state shifts to relative high protein concentration compared to the one without delay. Different from the case with α=1.2, the L-state keeps and does not split. Instead, the probability of H-state decreases when compared to the one without delay. With further increasing τ, the H-state disappears and only L-state is left, whose peak moves to a position with higher protein concentration as depicted in Fig.4(c) for τ=0.15 for instance. The dependence of $P_{\rm L}$ on τ is presented in Fig.4(d). Similarly, there is again a transition point at $\tau_2\approx$1.5 where the coexistence state turns into a monostable state with only L-state left ($P_{\rm L}$=1). Note that $P_{\rm L}$ increases monotonously with τ, which is also different from the case α=1.2 as shown in Fig.3. Therefore, for control parameter close to the transition point α_2, delay can suppress the H-state which is quite on contrary to the role it plays for α close to α_1.

Above findings clearly demonstrate that how time delay affects the multistability dynamics depends strongly on the binding rate α of the transcription factor. To provide a complete picture, we have calculated the phase diagram of the delayed toggle switch in τ-α plane with extensive simulations, which is shown in Fig.5. Several interesting remarks can be made for this phase diagram. When α is relative small, the coexistence state turns into H-state and the L-state is suppressed with the increasing of delay time τ. While for a relatively large α, which is slightly smaller than α_2 for τ=0, the coexistence state can change into the L-state and H-state is suppressed with increasing τ. For a certain value of τ that is not too large, there always exists three regimes

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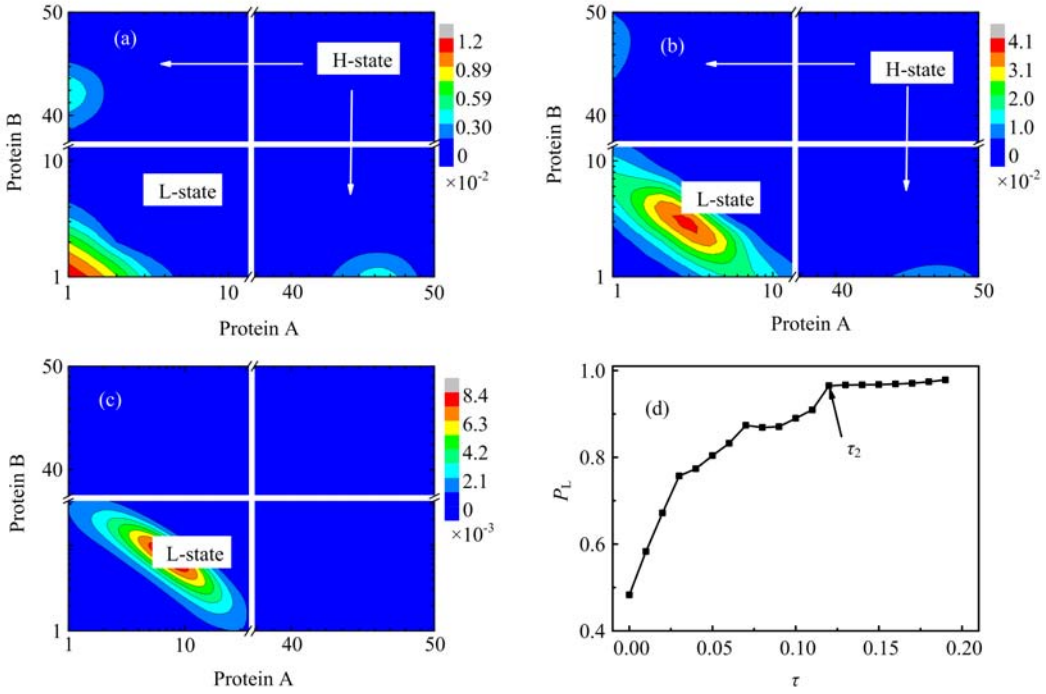

FIG. 4 Multistability of delayed genetic toggle switch for α=20, and (a) τ=0.0, (b) τ=0.03, (c) τ=1.5. (d) Dependence of the distribution probability $P_{\rm L}$ of the L-state on delay time τ.

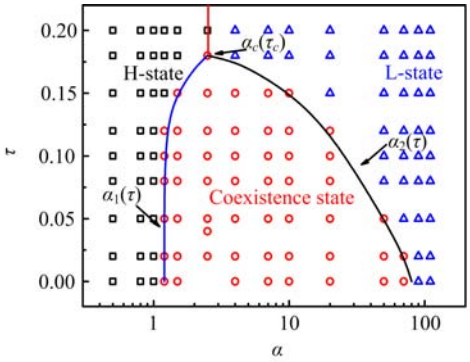

FIG. 5 Phase diagram of the delayed toggle switch in the τ-α plane. The solid line indicate the locations of α_1 and α_2 as functions of τ. For large enough τ larger than τ_c, the difference between the H-state and L-state disappears.

for different α values, namely, the system can exhibit H-state, coexistence state and L-state sequentially as α increases [11, 12]. Note that the transition points α_1 and α_2 both depend on τ. With the increment of τ, $\alpha_1(\tau)$ gradually increases while $\alpha_2(\tau)$ gradually decreases, and they are merged into a triple-point-like point located at α=α_c and τ=τ_c. Thus when the delay time τ>τ_c, the coexistence state disappears for all values of α, and direct transitions between H-state and L-state are possible. In addition, for α<$\alpha_1(0)$ (or α>$\alpha_2(0)$), the genetic toggle switch presents only H-state (or L-state) no matter what the value of τ is.

IV. CONCLUSION

In conclusion, we have investigated the effects of delay on the dynamic behavior of a typical genetic toggle switch by using the delayed stochastic simulation method. Three regimes (H-state, coexistence state, and L-state) are present in the system, depending on the values of the transcription-factor binding rate α. Time delay can considerably change the transition boundary between these regimes, resulting in a narrowing of the coexistence regime for moderate values of α. Consequently, time delay may suppress the H-state or the L-state depending on the values of α. In addition, we have obtained the complete phase diagram in the $\alpha-\tau$ parameter plane, and found that there exists a critical point denoted by (α_c,τ_c). While for τ<τ_c the system can bypass sequential transitions from H-state to coexistence state and further to L-state with increasing α, only direct transition from H-state to L-state can be observed for $\tau\geq\tau_c$. Since transcriptional and translational delay is ubiquitous in gene regulatory networks, this work may provide new insights about the stability dynamics of genetic toggle switches.

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