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A theoretical study on activation of transcription factor modulated by intracellular Ca^{2+} oscillations

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Abstract

This work presents both deterministic and stochastic models of genetic expression modulated by intracellular calcium (Ca^{2+}) oscillations, based on macroscopic differential equations and chemical Langevin equations, respectively. In deterministic case, the oscillations of intracellular Ca^{2+} decrease the effective Ca^{2+} threshold for the activation of transcriptional activator (TF-A). The average activation of TF-A increases with the increase of the average amplitude of intracellular Ca^{2+} oscillations, but decreases with the increase of the period of intracellular Ca^{2+} oscillations, which are qualitatively consistent with the experimental results on the gene expression in lymphocytes. In stochastic case, it is found that a large internal fluctuation of the biochemical reaction can enhance gene expression efficiency specifically at a low level of external stimulations or at a small rate of TF-A dimer phosphorylation activated by Ca^{2+}, which reduces the threshold of the average intracellular Ca^{2+} concentration for gene expression.

Keywords: Transcription factor; Intracellular calcium oscillation; Kinetic model

1. Introduction

It is well known that DNA contains the complete genetic information that defines the structure and function of an organism. Proteins are formed using the genetic code of the DNA. Three different processes are responsible for the inheritance of genetic information and for its conversion from one form to another. Replication: a double stranded nucleic acid is duplicated to give identical copies. This process perpetuates the genetic information. Transcription: a DNA segment that constitutes a gene is read and transcribed into a single stranded sequence of RNA. The RNA moves from the nucleus into the cytoplasm. Translation: the RNA sequence is translated into a sequence of amino acids when a protein is formed. During translation, the ribosome reads three bases (a codon) at a time from the RNA and translates them into one amino acid. In eukaryotic cells, the second step (transcription) is necessary because genetic material in the nucleus is physically separated from the site of protein synthesis in the cytoplasm in the cell. Therefore, a DNA does not directly create a protein; instead, it produces an intermediary to pass the genetic information.

Regulation of gene expression by signals from both outside and inside the cell plays an important role in many biological processes. It has become increasingly evident that genetic regulation is a complex process involving nonlinear interactions, positive and negative feedback within signaling pathways, time delays, protein oligomerization, crosstalk between different pathways, and fluctuation in biochemical reactions [1–12]. To investigate the capability of genetic regulatory systems for complex dynamic activity, Smolen et al. [1] proposed simple kinetic models that incorporate autoregulation and stimulus-dependent phosphorylation of transcription factors (TFs), dimerization of TFs, crosstalk, and feedback.

The oscillation of intracellular calcium (Ca^{2+}) plays an important role in the control of many cellular processes. For instance, Ca^{2+} oscillation modulates the secretion and egg activation at the time of fertilization [13,14]. Ca^{2+} spike frequency can optimize gene expression [15,16], and Ca^{2+} oscillation increases the activation of the glycogen phosphorylase in the phosphorylation–dephosphorylation cycle in [17–19]. In order to

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study how intracellular Ca^{2+} oscillations contribute to the efficiency and specificity of signaling, Dolmetsch et al. [20] developed a Ca^{2+} clamp technique to investigate the role of oscillation amplitude and frequency of Ca^{2+} in regulating gene expression driven by the proinflammatory transcription factors NF-AT, Oct/OAP and NF-κB. Their experimental results demonstrated two important features of nuclear signaling by Ca^{2+} oscillations. First, oscillations enhance signaling efficiency and specificity at low levels of stimulation. This effect arises from highly nonlinear dependence of transcription on Ca^{2+} oscillations which periodically exceed the threshold for activation. In contrast, a small constant increase in Ca^{2+} concentration does not affect signaling efficiency and specificity. Since Ca^{2+} has a tendency to oscillate at low receptor occupancy in many cells, the system is very sensitive to weak external stimuli. Second, oscillations give rise to specificity on an otherwise highly pleiotropic Ca^{2+} signal. By differentially controlling the activation of different genes, oscillation frequency may direct cells along specific developmental pathways.

Although it was experimentally shown that intracellular Ca^{2+} oscillations increase the efficiency and specificity of gene expression, little theoretical study on the activation of transcription factor modulated by intracellular Ca^{2+} oscillations [21,22] has been carried out. Specially, it is not clear how the intracellular Ca^{2+} oscillations mediate the efficiency and specificity of gene expression within the framework of kinetics. On the other hand, the cellular biochemical reactions usually occurred in a finite system, i.e., the total number of biochemical reaction molecules in a cell is often limited. The occurrence of each biochemical reaction event in cellular systems is random, which is called as the intrinsic fluctuations. In particular, noise or random fluctuations in gene expression may produce variability cellular behavior [4,7,8,10,24,25]. An important fact about this internal fluctuation is that it scales with the system size, and vanishes in the thermodynamic limit [23]. Now a question is how does the intrinsic fluctuation affect the activation of transcription factor modulated by intracellular Ca^{2+} oscillations in gene expression process?

Based on the model of genetic regulation [1] and the minimal model of cytosolic Ca^{2+} oscillations [26], the mechanisms of gene expression mediated by intracellular Ca^{2+} oscillations and the effects of finite size (which reflects the intensity of intrinsic fluctuation) on the transcriptional regulation process have been studied by kinetics in this paper. First, the macroscopic differential equations of a genetic expression system modulated by intracellular Ca^{2+} oscillations are presented. The theoretical results are qualitatively consistent with the experimental findings: oscillations reduce the effective Ca^{2+} threshold for activating transcription factor; the average activation of gene expression increases with the increase of average amplitude of intracellular Ca^{2+} oscillation, but decreases with the increase of period of intracellular Ca^{2+} oscillation. Second, stochastic model of genetic expression system modulated by intracellular Ca^{2+} oscillations is presented by virtue of chemical Langevin equation [27]. The effects of intrinsic fluctuation on the activation of gene expression modulated by intracellular Ca^{2+} oscillations are theoretically investigated. Here, we explore theoretically the possible role of internal fluctuation in genetic regulation mediated by cytosolic Ca^{2+}. This paper ends with some conclusions.

2. Deterministic model

The kinetic model of genetic regulation used here is based on that proposed by Smolen et al. [1] (see Fig. 1). A single transcriptional activator (TF-A) is considered as part of a pathway mediating a cellular response to a stimulus. The TF forms a homodimer that can bind to responsive elements (TF-REs). The *tf-a* gene incorporates a TF-RE, and when homodimers bind to this element, TF-A transcription is increased. Binding to the TF-REs is independent of dimer phosphorylation. Only phosphorylated dimers can activate transcription. The fraction of phosphorylated dimers depends on the activity of kinases and phosphatases whose activity can be regulated by external signals. Thus, this model incorporates both signal-activated transcription and positive feedback on the rate of TF synthesis. It is assumed that the transcription rate saturates with TF-A dimer concentration to maximal rate k_f, which is proportional to TF-A phosphorylation. At negligible dimer concentration, the synthesis rate is R_{bas}. TF-A is eliminated with a rate constant k_d. Binding processes are considered comparatively rapid, so the concentration of dimer is proportional to the square of TF-A monomer activation (X). This simplification gives a model with a single ordinary differential equation for the concentration of TF-A

$$\frac{dX}{dt} = \frac{k_f X^2}{X^2 + K_d} - k_d X + R_{bas}, \quad (1)$$

where K_d is the dissociation concentration of TF-A dimer from TF-REs. The theoretical model for the genetic regulatory systems should manifest multiple stable steady states, and brief perturbations could switch the model between these states

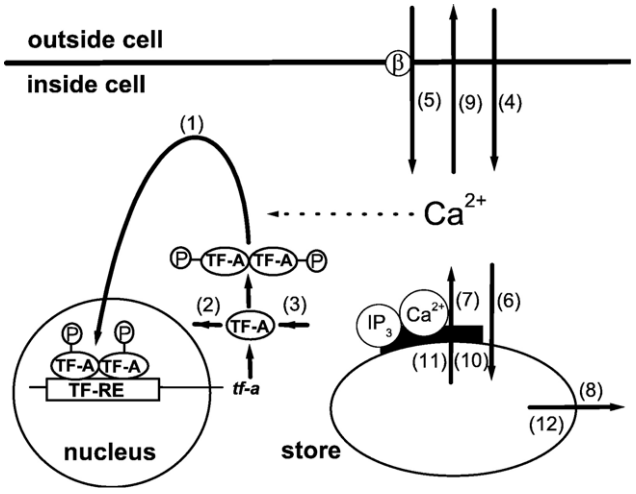

Fig. 1. A schematic description of the model. Transcription factor actives transcription with a maximal rate k_f when phosphorylated (P) and binds as a dimer to specific responsive-element DNA sequences (TF-REs). There are twelve reaction channels marked with (1)–(12), and the detailed description of each biochemical reaction channel is listed in Table 2.

[1,6,28]. Such transitions might explain, for example, how a brief pulse of hormone or neurotransmitter could elicit a long-lasting cellular response.

Intracellular Ca^{2+} oscillations have been intensively studied in different theoretical models, for comprehensive reviews see Refs. [29] and [30]. In a simple periodic Ca^{2+} oscillation, oscillations are either based on Ca^{2+}-induced Ca^{2+} release (CICR) [31], or require the periodic variation of inositol 1,4,5-trisphosphate (IP_3) [32,33], or rely on the detailed dynamics of IP_3 receptor [34–37]. The minimal cytosolic Ca^{2+} oscillation is caused by the interplay between two releasable pools of Ca^{2+}, one sensitive to the IP_3 and the other activated by Ca^{2+}. There are two variables in this model [26], the first is the concentration of free Ca^{2+} in the cytosol, and the second is the concentration of Ca^{2+} in the IP_3-insensitive pool. These variables are denoted by Z and Y, respectively. When assuming that buffering is linear with respect to Ca^{2+} concentration, the time evolution of the system is governed by

$$\frac{dZ}{dt} = v_0 + v_1\beta - v_2 + v_3 + k_1 Y - kZ, \tag{2}$$

$$\frac{dY}{dt} = v_2 - v_3 - k_1 Y, \tag{3}$$

with

$$v_2 \frac{V_{M2} Z^n}{K_2^n + Z^n}, \quad v_3 = \frac{V_{M3} Y^m}{K_R^m + Y^m} \frac{Z^p}{K_A^p + Z^p}, \tag{4}$$

where β is the external control parameter that denotes the degree of extracellular stimulation and $0<\beta<1$. The external control parameter β has two supercritical Hopf bifurcation points (as shown Fig. 2), $H_{\beta 1}$ and $H_{\beta 2}$, and the simple and regular intracellular Ca^{2+} oscillations will occur in the range of $H_{\beta 1} \le \beta \le H_{\beta 2}$. The mean Ca^{2+} level and the frequency of Ca^{2+} oscillation increase as the level of external simulation increases in the range of Ca^{2+} oscillation. Fig. 2 also shows the period of cytosolic Ca^{2+} oscillation with respect to the external control parameter. Below the lower critical value, a low steady-state level of cytosolic Ca^{2+} is established, above the large critical value, the system evolves toward a higher stable steady-state level of cytosolic Ca^{2+}.

Table 1
Parameter values

Parameter	Value	Parameter	Value
K_a	0.5 μM	K_b	0.5 μM
γ	9	K_{d0}	10
k_d	1 min^{-1}	R_{bas}	0.1 min^{-1}
k_1	0.7 min^{-1}	k	10 min^{-1}
v_0	1 μM min^{-1}	v_1	5.7 μM min^{-1}
V_{M2}	30 min^{-1}	K_2	0.5 μM
V_{M3}	325 μM min^{-1}	K_R	1.7 μM
K_A	0.46 μM	n	2
m	2	p	4

Taking into account the activation of TF-A modulated by intracellular Ca^{2+} concentration in transcriptional regulatory process, we assume that Ca^{2+}activates the TF-A dimer phosphorylation (with a maximal rate k_{f0}) and the dissociation concentration (with a normalized Michaelis constant K_{d0}) of TF-A dimer from TF-REs. Thus, k_f and K_d in Eq. (1) are given by:

$$k_f = k_{f0}\left(1 + \frac{\gamma Z^4}{K_a^4 + Z^4}\right), \quad K_d = \frac{K_{d0}}{1 + Z^4/K_b^4}, \tag{5}$$

where γ is a multiplicative factor, K_a and K_b are the activation constants. In the following computation, the parameter values of Eqs. (1)–(5) are listed in Table 1.

The effect of intracellular Ca^{2+} oscillations on the activation of gene expression can be deduced from Eqs. (1)–(5). For different external stimulations β, the time evolutions of TF-A activation and Ca^{2+} concentration are shown in Fig. 3. In the range of intracellular Ca^{2+} oscillations (e.g. β=0.15 and 0.3 as shown in Fig. 3), it can be found that the average level of TF-A

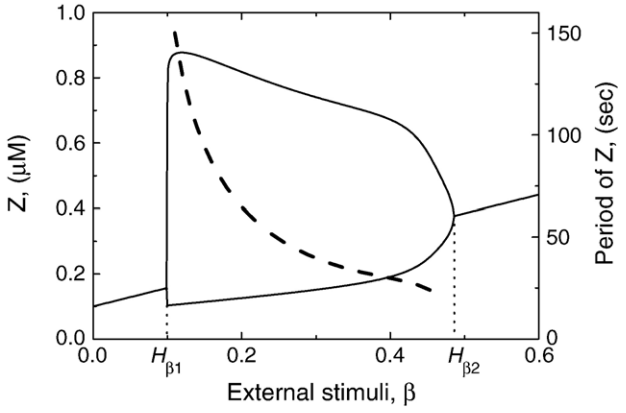

Fig. 2. The bifurcation diagram (solid line) and the period (dashed line) of intracellular Ca^{2+} oscillations with respect to the external control parameter β. The other parameter values are given in Table 1.

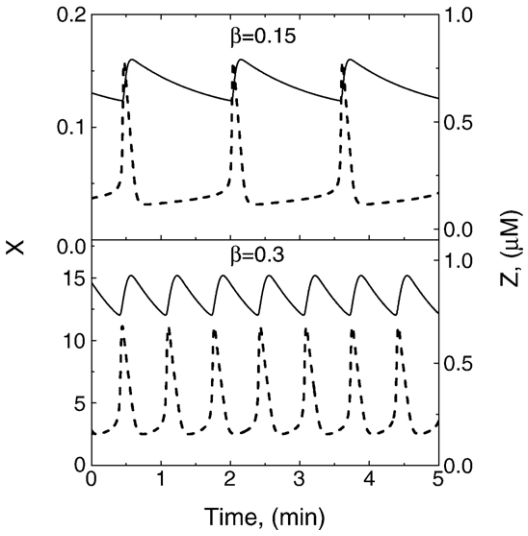

Fig. 3. Time course of TF-A (solid line) and Ca^{2+} concentration (dotted line) for different external stimulation β. k_{f0}=6 min^{-1}. The other parameter values are given in Table 1.

for large β is much higher than that for small β (note the change in the vertical scales for the TF-A in Fig. 3, but no change for the Ca^{2+}). It means that intracellular Ca^{2+} oscillations increase the activation of gene expression. With the increase of external stimuli β, the frequency of TF-A oscillations is increased.

Fig. 4 plots the average activation of TF-A with an increased mean Ca^{2+} level and with a stimulation by an equivalent sustained cytosolic Ca^{2+} concentration. It is clear that the average activation of gene expression is increased with the increase of mean intracellular Ca^{2+} concentration. In the range of Ca^{2+} oscillations, it should be noted that the average activation of gene expression modulated by intracellular Ca^{2+} oscillations is larger than that stimulated by an equivalent sustained cytosolic Ca^{2+} concentration (as shown by the inset in Fig. 4). The result shows that the intracellular Ca^{2+} oscillations enhance efficiency of gene expression specifically. It is known that [26], in the range of Ca^{2+} oscillations, the period of intracellular calcium oscillations is increased with the increase of external stimuli β. Fig. 5 shows the average activation of TF-A is varied with the increase of period of intracellular Ca^{2+} oscillations. It can be seen that the average activation of TF-A is decreased with the increase of period of intracellular Ca^{2+} oscillations.

The above results show that, even in a simple genetic transcriptional regulation process (including autoregulation and stimulus-dependent phosphorylation of transcription factor, dimerization of transcription factor, and positive feedback), the oscillation of intracellular Ca^{2+} concentration can decrease the effective Ca^{2+} threshold for the activation of gene expression. The average activation of transcription factor is increased with the increase of the average intracellular Ca^{2+} concentration, but decreased with the increase of the period of intracellular Ca^{2+} oscillations. Our theoretical results are qualitatively in agreement with the experimental observation obtained by Dolmetsch et al. [20] about the expression of transcription factors in lymphocytes.

3. Stochastic model

The kinetics for both transcriptional regulatory process and intracellular Ca^{2+} oscillations are described by macroscopic

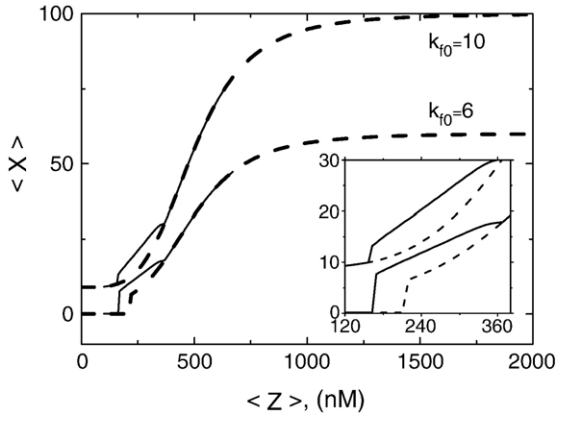

Fig. 4. Average activation of TF-A with an increased mean level cytosolic Ca^{2+} oscillations (solid line), and with a stimulation by an equivalent sustained cytosolic Ca^{2+} concentration (dashed line). The other parameter values are given in Table 1.

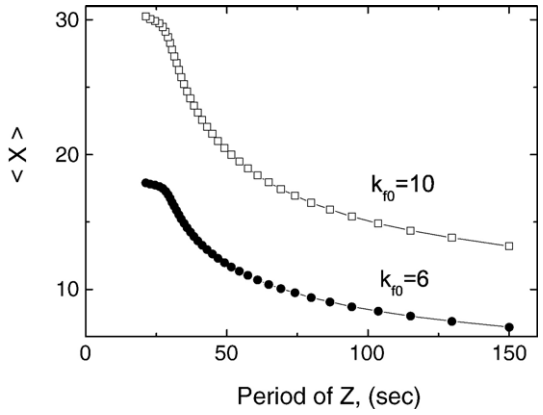

Fig. 5. Average activation of TF-A vs. period of intracellular Ca^{2+} oscillations. The other parameter values are given in Table 1.

differential equations in the last section. In realistic cell, however, the number of intracellular molecules is not large, and intracellular milieu becomes noisy. Internal noises are caused by the discrete nature of matter [23], for example, internal fluctuations in a biochemical reaction arise because the reaction consists of individual reactive collisions. On the other hand, the macroscopic features are determined altogether by molecules. Thus one expects the importance of internal fluctuations to be relatively small when the system is large. This led to the rule of thumb that in a collection of N molecules the internal fluctuations are of order $N^{1/2}$. Their effect on the macroscopic properties will therefore be of order $N^{-1/2}$, and the size of cell is a parameter that measures the relative importance of the internal noises. Recently, there has been an increasing interest in the finite size effect on some biological systems [38–40] by virtue of the chemical Langevin equation [27].

The key species in the model of transcription factor modulated by intracellular Ca^{2+} oscillations discussed here

Table 2
Reaction channel and corresponding transition rate

Reaction channel	Description	Transition rate
(1) $x \to x+1$	Signal-activated transcription and TF-A modulated by intracellular Ca^{2+} oscillations	$r_1 = \Omega \frac{k_f(Z)X^2}{X^2 + K_d(Z)}$
(2) $x \to x-1$	Dissociation of TF-A	$r_2 = \Omega k_d X$
(3) $x \to x+1$	Synthesis of TF-A	$r_3 = \Omega R_{bas}$
(4) $z \to z+1$	Constant input of cytosolic Ca^{2+} from extracellular medium	$r_1 = \Omega v_0$
(5) $z \to z+1$	Stimulus-induced influx of cytosolic Ca^{2+} from extracellular medium	$r_2 = \Omega v_1 \beta$
(6) $z \to z-1$	Pumping of cytosolic Ca^{2+} into internal store	$r_3 = \Omega v_2$
(7) $z \to z+1$	Input of cytosolic Ca^{2+} from internal store	$r_4 = \Omega v_3$
(8) $z \to z+1$	Input of cytosolic Ca^{2+} from internal store	$r_5 = \Omega k_1 Y$
(9) $z \to z-1$	Leakage of cytosolic Ca^{2+} into extracellular medium	$r_6 = \Omega k Z$
(10) $y \to y+1$	Pumping of cytosolic Ca^{2+} into internal store	$r_7 = \Omega v_2$
(11) $y \to y-1$	Release of Ca^{2+} from internal store	$r_8 = \Omega v_3$
(12) $y \to y-1$	Leakage of internal pool Ca^{2+} from internal store	$r_9 = \Omega k_1 Y$

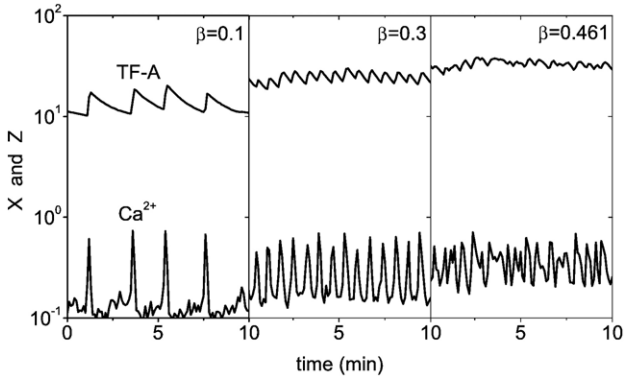

Fig. 6. Time course of TF-A and Ca^{2+} under different external stimulation β. $k_{f0}=10$ and $\Omega=10^3$. The other parameter values are given in Table 1.

are the TF-A monomer activation with its concentration being represented by X, the cytosolic Ca^{2+} with its concentration being represented by Z, and the Ca^{2+} sequester in an internal store with its concentration being represented by Y. When the number of intracellular reaction molecules is finite, we denote the number of TF-A molecules in cytosol as x, the number of calcium ions in cytosol as z, and the number of calcium ions in internal store as y. Then, the relationship between the concentration and the number of molecules is $X=x/\Omega$, $Z=z/\Omega$, and $Y=y/\Omega$, where Ω is the total cell volume. Note that there are twelve reaction channels R_j ($j=1, \ldots, 12$) as shown in Fig. 1 for the three species S_i ($i=1, 2, 3$).

Following Gillespie's argument [27], let a system's state at the current time t be $(x(t), z(t), y(t))$ and a random variable $K_j(x(t), z(t), y(t), \tau)$ for any $\tau>0$ be the number of R_j reactions that occur in the subsequent time interval $[t, t+\tau]$. Since each of these reactions will increase the S_i population by $v_{j,i}$, thus, the number of S_i molecules in the system at time $t+\tau$ will be

$$\begin{aligned} x(t+\tau) = {} & x(t) + K_1(x(t),z(t),y(t),\tau) \\ & - K_2(x(t),z(t),y(t),\tau) \\ & + K_3(x(t),z(t),y(t),\tau), \end{aligned} \tag{6}$$

$$\begin{aligned} z(t+\tau) = {} & z(t) + K_4(x(t),z(t),y(t),\tau) \\ & + K_5(x(t),z(t),y(t),\tau) - K_6(x(t),z(t),y(t),\tau) \\ & + K_7(x(t),z(t),y(t),\tau) + K_8(x(t),z(t),y(t),\tau) \\ & - K_9(x(t),z(t),y(t),\tau), \end{aligned} \tag{7}$$

$$\begin{aligned} y(t+\tau) = {} & y(t) + K_{10}(x(t),z(t),y(t),\tau) \\ & - K_{11}(x(t),z(t),y(t),\tau) \\ & - K_{12}(x(t),z(t),y(t),\tau). \end{aligned} \tag{8}$$

An excellent approximation to $K_j(x(t), z(t), y(t), \tau)$ in Eqs. (6)–(8) can be obtained if the following two conditions are imposed [27]: (i) τ is small enough so that K_j varies little during the period t, $t+\tau$. Each $K_j(x(t), z(t), y(t), \tau)$ will be a statistically independent Poisson random variable, $\mathcal{P}_j(r_j(x(t),z(t),y(t)),\tau)$:

$$K_j(x(t),z(t),y(t),\tau) = \mathcal{P}_j(r_j(x(t),z(t),y(t)),\tau). \tag{9}$$

(ii) τ is large enough so that the expected number of occurrences of each reaction channel R_j in $[t, t+\tau]$ be much larger than 1, which allows us to approximate each statistically independent Poisson random variable $\mathcal{P}_j(r_j(x(t),z(t),y(t)),\tau)$ by a normal random variable $\mathcal{N}_j(m_j, \sigma_j^2)$ with the same mean m_j and variance σ_j^2:

$$\begin{aligned} & \mathcal{P}_j(r_j(x(t),z(t),y(t)),\tau) \\ & \quad = \mathcal{N}_j(r_j(x(t),z(t),y(t))\tau, r_j(x(t),z(t),y(t))\tau). \end{aligned} \tag{10}$$

The linear combination theorem for $\mathcal{N}_j(m_j, \sigma_j^2)$ is $\mathcal{N}_j(m_j, \sigma_j^2) = m_j + \sigma_j\mathcal{N}_j(0,1)$, and $\mathcal{N}_j(0,1)$ is the unit normal random variable. Thus, Eqs. (6)–(8) have the canonical form of standard Langevin equations for multivariate continuous Markov processes. By using the two conditions and the relationship between the concentration and the molecular number of each species are satisfied, the chemical Langevin equations corresponding to the macroscopic differential equations Eqs. (1)–(3) can be obtained from Eqs. (6)–(8)

$$\frac{dX}{dt} = r_1 - r_2 + r_3 + \frac{1}{\sqrt{\Omega}}\left[\sqrt{r_1}\eta_1(t) - \sqrt{r_2}\eta_2(t) + \sqrt{r_3}\eta_3(t)\right], \tag{11}$$

$$\begin{aligned} \frac{dZ}{dt} = {} & r_4 + r_5 - r_6 + r_7 + r_8 - r_9 \\ & + \frac{1}{\sqrt{\Omega}}\left[\sqrt{r_4}\eta_4(t) + \sqrt{r_5}\eta_5(t) - \sqrt{r_6}\eta_6(t)\right. \\ & \left. + \sqrt{r_7}\eta_7(t) + \sqrt{r_8}\eta_8(t) - \sqrt{r_9}\eta_9(t)\right], \end{aligned} \tag{12}$$

$$\begin{aligned} \frac{dY}{dt} = {} & r_{10} - r_{11} - r_{12} \\ & + \frac{1}{\sqrt{\Omega}}\left[\sqrt{r_{10}}\eta_{10}(t) - \sqrt{r_{11}}\eta_{11}(t) - \sqrt{r_{12}}\eta_{12}(t)\right], \end{aligned} \tag{13}$$

where $r_{i=1, \text{ sub } \cdots, 12}$ are the transition rates as shown in Table 2, $\eta_{i=1, \cdots, 12}(t)$ are Gaussian white noises with $\langle\eta_i(t)\rangle=0$ and $\langle\eta_i(t)\eta_j(s)\rangle=\delta_{ij}\delta(t-s)$. It can be noted that the internal fluctuation item is proportional to $1/\sqrt{\Omega}$ in the mesoscopic stochastic differential

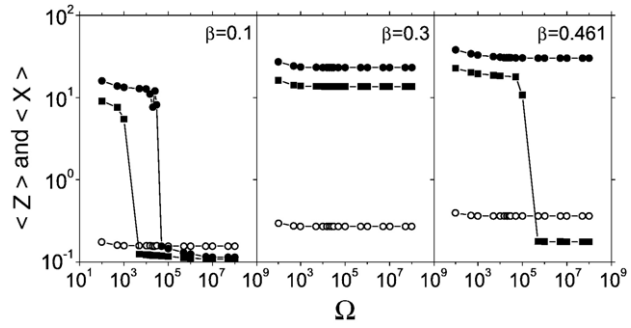

Fig. 7. Average activation of TF-A (filled square for $k_{f0}=6$ and filled circle for $k_{f0}=10$) and average intracellular Ca^{2+} concentration (empty circle) vs. volume size Ω under the different external control parameter β. The other parameter values are given in Table 1.

Eqs. (11)–(13) when the other parameters are fixed, and vanishes in the thermodynamic limit (i.e. $\Omega \to \infty$).

The effects of intrinsic fluctuations on the activation of transcription factor modulated by intracellular Ca^{2+} oscillations in gene expression process can be drawn from Eqs. (11)–(13) through numerical simulations. When the volume size is fixed at $\Omega = 10^3$, the time evolution of TF-A and Ca^{2+} concentration are given in Fig. 6 under different external stimulation β. The cytosolic Ca^{2+} concentration and the TF-A are randomly oscillated due to the intrinsic fluctuation. The average level of TF-A and the average intracellular Ca^{2+} concentration are increased with the increase of the external stimulation β.

Fig. 7 shows the average TF-A and the average intracellular Ca^{2+} concentration as a function of volume Ω under the different external control parameter β. (i) When the external stimuli β is small (e.g. $\beta = 0.1$), there exists a critical volume at which the average TF-A is jumped down from the high level to a lower one for both $k_{f0} = 6$ and $k_{f0} = 10$. Thus, large internal noise (or small volume) of the cell can enhance gene expression efficiency specifically at small external stimulation. (ii) When β takes a medium value (e.g. $\beta = 0.3$, which lies in the region of intracellular Ca^{2+} oscillations), the average level of TF-A is hardly varied with the increase of the volume Ω. (iii) When the external stimuli β is large (e.g. $\beta = 0.461$), the average level of TF-A shows a very different variation with the increase of volume Ω for different k_{f0}. For the small k_{f0} (e.g. $k_{f0} = 6$), there is a critical volume size at which the average TF-A is jumped down from a high level to a lower one. For the large k_{f0} (e.g. $k_{f0} = 10$), however, the average level of TF-A is hardly varied with the increase of volume Ω. Thus, even when a small rate k_{f0} of the TF-A dimer phosphorylation is activated by Ca^{2+}, large internal noise (or small volume) of cell can enhance gene expression efficiency specifically at large external stimulation β.

At a given $k_{f0} = 10$, Fig. 8 shows the average level of TF-A as a function of average intracellular Ca^{2+} concentration under the different volume size Ω. With the increase of average intracellular Ca^{2+} concentration, the average TF-A jumps mutationally from a low level to a high value at a critical average intracellular Ca^{2+} concentration (as shown by the inset in Fig. 8). The relation between the average TF-A and average

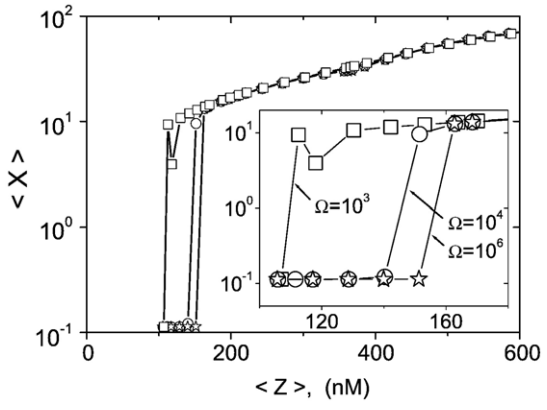

Fig. 8. Average activation of TF-A vs. average intracellular Ca^{2+} concentration under the different volume size Ω for varying extracellular stimulations. $k_{f0} = 10$. The other parameter values are given in Table 1.

Ca^{2+} concentration is of a steep sigmoidal nature. This result is due to the cooperativity in the gene expression modulated by intracellular Ca^{2+} random oscillations, and leads to a phenomenon known as "zero-order ultra-sensitivity" [31,17,18]. The critical average intracellular Ca^{2+} concentration depends on the size of cell volume Ω. The smaller the value of Ω, the lower the threshold of the average Ca^{2+} concentration will be. Therefore, large intrinsic fluctuation reduces the threshold of the average intracellular Ca^{2+} concentration for gene expression.

4. Discussions and conclusions

Based on the model of genetic regulation proposed by Smolen et al. [1] and the minimal model of cytosolic Ca^{2+} oscillations [26], both the deterministic and the stochastic models of a genetic expression system modulated by intracellular Ca^{2+} oscillations are presented in this paper. The genetic regulation discussed here includes autoregulation and stimulus-dependent phosphorylation of transcription factor, dimerization of transcription factor, and positive feedback. Taking into account the activation of TF-A modulated by intracellular Ca^{2+} concentration in transcriptional regulatory process, a modulation mechanism of intracellular calcium oscillations on the TF-A dimer phosphorylation (with a maximal rate k_{f0}) and the dissociation concentration (with a normalized Michaelis constant K_{d0}) of TF-A dimer from TF-REs is proposed through Eq. (5). The effects of intracellular Ca^{2+} oscillations on the activation of gene expression are theoretically investigated.

It should be pointed out that a stochastic mathematical model was proposed by Pirone and Elaton [21] to explain the graded and binary responses observed in inducible gene expression. IL-2 is involved in the immune response and is transcribed following activation of T-cells. Such activation requires, in turn, the activation of specific T-cell receptors by antigen-presenting cells and binding of lymphokines produced by activated macrophages. These two signals lead to Ca^{2+} potentiation and their model, the rate for the nuclear factor of activated T-cells (NF-AT) binding depends on the ionomycin concentration I, and was assumed as a Hill functional response such that $\hat{k}_{ON} = \hat{k}_{ON} I^n / (K_M^n + I^n)$, where K_M is the Michaelis constant and denotes the ionomycin concentration at which $k_{ON}(I)$ is half its maximum value $\hat{k}_{ON}$, and n is the Hill coefficient. Their modeling results are in good qualitative agreement with experimental data of Fiering et al. [41].

For the determinative case, we demonstrate that intracellular Ca^{2+} oscillations decrease the effective Ca^{2+} threshold for the activation of gene expression. The average activation of gene expression increases as the average oscillation amplitude of intracellular Ca^{2+} increases, but decreases with the increase of the oscillation period of intracellular Ca^{2+}. These results are in qualitative agreement with the experimental results observed by Dolmetsch et al. [20].

For the stochastic case, by means of chemical Langevin equation, the mesoscopic stochastic differential equations are given to describe the intracellular random biochemical reactions in the gene expression processes modulated by cytosolic Ca^{2+}. The intensity of intrinsic fluctuation is inversely proportional to

the size of the biochemical system. The relation between the average TF-A and the average concentration of Ca^{2+} shows a steep sigmoidal nature. When the average intracellular Ca^{2+} concentration increases, the average TF-A jumps mutationally from a low level to a high value at a critical average intracellular Ca^{2+} concentration. It is found that a large internal fluctuation of the biochemical system can enhance gene expression efficiency specifically at a low level of external stimulation or at a small rate of the TF-A dimer phosphorylation activated by intracellular Ca^{2+}, which reduces the threshold of the average intracellular Ca^{2+} concentration for gene expression. These theoretical predictions are expected to be verified by using the similar experimental techniques concerning the expression of transcription factors in lymphocytes.

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