Internal Noise-Sustained Circadian Rhythms in a *Drosophila* Model

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ABSTRACT Circadian rhythmic processes, mainly regulated by gene expression at the molecular level, have inherent stochasticity. Their robustness or resistance to internal noise has been extensively investigated by most of the previous studies. This work focuses on the constructive roles of internal noise in a reduced *Drosophila* model, which incorporates negative and positive feedback loops, each with a time delay. It is shown that internal noise sustains reliable oscillations with periods close to 24 h in a region of parameter space, where the deterministic kinetics would evolve to a stable steady state. The amplitudes of noise-sustained oscillations are significantly affected by the variation of internal noise level, and the best performance of the oscillations could be found at an optimal noise intensity, indicating the occurrence of intrinsic coherence resonance. In the oscillatory region of the deterministic model, the coherence of noisy circadian oscillations is suppressed by internal noise, while the period remains nearly constant over a large range of noise intensity, demonstrating robustness of the *Drosophila* model for circadian rhythms to intrinsic noise. In addition, the effects of time delay in the positive feedback on the oscillations are also investigated. It is found that the time delay could efficiently tune the performance of the noise-sustained oscillations. These results might aid understanding of the exploitation of intracellular noise in biochemical and genetic regulatory systems.

INTRODUCTION

Circadian rhythms provide internal daily periodicity, which is used by a wide range of organisms to anticipate daily changes in the environment. (1). The molecular mechanism of these rhythms relies on negative feedback exerted by a protein on the expression of its gene (2–4). A number of genes and their protein products involved in such a regulatory mechanism have been identified. For example, in *Drosophila*, the proteins PER and TIM form a complex that indirectly represses the activation of the *per* and *tim* genes; whereas in *Neurospora*, it is the FRQ protein that represses the expression of its gene *frq* (2,3). Many theoretical models (5–8) have been proposed for circadian rhythms based on such a control mechanism, and these models successfully predict that in a certain range of parameter values, the genetic regulatory network undergoes sustained oscillations of the limit cycle type corresponding to circadian rhythmic behavior, whereas outside this range, the network operates in a stable steady state.

It is widely recognized that the assumption of the deterministic description of genetic regulatory networks may be questionable because of the stochasticity of gene expression (9,10). Accordingly, the origin and roles of intrinsic noise in these networks have received considerable interest (11–15). Circadian rhythms, as a paradigm of genetic regulatory networks, are mainly regulated by gene expression at the molecular level (2). Because the molecules of mRNA and protein involved in the regulatory mechanism act at rather low concentrations (7), internal noise, resulting from the stochastic nature of the biochemical reaction events, is remarkable and has been studied in some postulated mechanisms of circadian rhythms (15,16). For example, it is reported that internal noise could destroy the periods and amplitudes of circadian oscillations, appearing in a deterministic model so that the ability to function reliably in the presence of internal noise might impose a constraint on the oscillation mechanism (15). Furthermore, many studies have investigated robustness or resistance of circadian clock systems to internal noise in the context of viewing noise as a nuisance (16–19). In recent years, however, complementary work has reported that, instead of controlling or eliminating noise, cellular processes could amplify or exploit the noise in some sense (14). For instance, in the cellular regulatory processes, intrinsic fluctuations may enhance the sensitivity of intracellular regulation (20), induce bifurcations that have no counterpart in the deterministic description (21), facilitate the control of cellular functions (22), or induce oscillations not present in the deterministic model (23). With respect to circadian rhythms, it has been reported that internal noise sustains reliable oscillations in a circadian clock model with certain parameter values, which give rise to a stable steady state in the deterministic limit, and the regularity of such oscillations becomes the best at a finite system size (i.e., a certain amount of noise) (18,24). This phenomenon resembles the constructive and nontrivial effects of external noise: stochastic resonance (25) and coherence resonance (26), both of which have been extensively investigated in a variety of science communities. Based on the aforementioned findings, it was argued that some of the cellular regulatory systems might not only be resistant or robust to the cellular noise but also could utilize it to perform their functions under conditions in which these functions would not be possible solely by deterministic means (14,18).

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Despite the success and general applicability of the *Drosophila* models based on only a negative feedback loop, recent studies have indicated that a positive feedback could also characterize the *Drosophila* oscillator, and it is interlocked to the negative feedback by virtue of a common core gene product dCLOCK (27–29). Several detailed models, incorporating both two loops and time delays, have been developed to investigate the mechanism for circadian oscillation and the functional roles of positive feedback and time delays (30–32). Because of the complexity of these detailed models, it is difficult to understand intuitively the oscillation mechanism and the positive or negative effects of internal noise on circadian oscillations. Very recently, Smolen et al. (33) constructed a reduced *Drosophila* model from their earlier, detailed model (30) and studied the mechanism for circadian oscillation and its robustness to molecular fluctuations in the reduced model. However, to the best of our knowledge, few studies have investigated the nontrivial influences of internal noise in these models. In this article, the constructive roles of internal noise have been investigated in the reduced model. It is found that internal noise could sustain circadian oscillations in the steady-state region (SS region) of the deterministic model. The effective signal/noise ratio (SNR), measuring performance of the noise-sustained oscillations, undergoes a maximum with variation of the internal noise intensity, which demonstrates the occurrence of intrinsic coherence resonance (ICR). However, in the oscillatory (OSC) region of the deterministic model, coherence of circadian oscillations monotonically decreases with increase of the internal noise intensity, whereas the period of the oscillations hardly changes over a wide range of internal noise intensity, indicating that the deterministic oscillations are robust to the internal noise.

MODEL AND METHODS

The model used here is a minimal representation of the transcriptional regulation essential for circadian rhythms in *Drosophila* (33). The reduced model contains both a negative feedback loop, in which PER binds dCLOCK and thereby deactivates *per* transcription, and a positive feedback loop, in which activation of *per* transcription by dCLOCK results in binding of dCLOCK by PER and derepression of *dclock* transcription. The differential equations for PER concentration p and dCLOCK concentration l have two terms, one for synthesis and the other for degradation. Because regulation of degradation is not included, simple first-order degradation rate constants are assumed. The differential equations for PER and dCLOCK are introduced as follows:

$$\frac{dp(t)}{dt} = v_{sp}R_{sp} - k_{dp}p(t) \tag{1}$$

$$\frac{dl(t)}{dt} = v_{sc}R_{sc} - k_{dc}l(t) \tag{2}$$

with

$$R_{sp} = \frac{l_{free}(t-\tau_1)}{K_1 + l_{free}(t-\tau_1)} \tag{3}$$

$$R_{sc} = \frac{K_2}{K_2 + l_{free}(t-\tau_2)}, \tag{4}$$

where $l_{free} = (l - p)$ or zero, whichever is greater. This model has consistently used the assumption that the dynamics of PER and TIM was represented by a ''lumped'' variable, ''PER'' (30), which is based on the findings that in *Drosophila*, TIM alone does not appear to regulate transcription, and the time courses of PER and TIM proteins are similar in shape and largely overlap (34). It has been reported that light enhances the degradation of phosphorylated TIM (35), the removal of TIM from the complex of PER and TIM leads to the enhancement of phosphorylation of PER (36), and multiple phosphorylations of PER could trigger degradation of PER (37). These observations suggest that light, by accelerating TIM degradation, will accelerate PER phosphorylation and accordingly improve the degradation of PER. Based on these findings, it is assumed that the effect of light on the model was simulated by enhancing PER degradation, and therefore, the first-order degradation rate constant for PER, k_{dp} in Eq. 1, as a light-controlled parameter, increases with light in this model. τ_1 and τ_2 define the time delay between *per* transcription and the synthesis of new PER protein and that between *dclock* transcription and the synthesis of new dCLOCK protein, respectively. τ_1 has been constrained by some experimental data (28,38,39), whereas τ_2 has not been experimentally determined. The discrete time delays are implemented as follows: at each time step of a simulation, the values of Eqs. 3 and 4 are calculated and stored. The stored values are used τ_1 h and τ_2 h later to compute the rates of PER synthesis and dCLOCK synthesis, respectively. For most simulations with the *Drosophila* model (Eqs. 1–4), a standard set of parameter values was used as follows: $\tau_1 = 10$ h, $\tau_2 = 10$ h, $v_{sp} = 0.5$ nMh^{-1}, $v_{sc} = 0.25$ nMh^{-1}, $k_{dc} = 0.5$ h^{-1}, $k_{dp} = 0.5$ h^{-1}, $K_1 = 0.3$ nM, and $K_2 = 0.1$ nM. Using these parameters' values, the deterministic model readily simulated circadian oscillations, the robustness of which, with respect to molecular fluctuations, was also investigated (33). In this article, the above algorithm to execute time delays is adopted, and the parameter k_{dp} is chosen as the control parameter.

To account for the internal noise, the aforementioned deterministic description in the *Drosophila* model is no longer valid. Generally, one can describe the reaction system as a birth-death stochastic process governed by a chemical master equation, which describes the time evolution of the probability of having a given number of molecules of reaction species (40). Although there is no procedure to solve this master equation analytically, it provides the starting point for numerical simulations. The exact stochastic simulation (ESS), introduced by Gillespie (41), implements such a master equation approach to stochastic chemical dynamics. It associates a probability with each reaction and, at each time step, stochastically determines the reaction that takes place according to its probability as well as the time interval to the next reaction. The numbers of molecules of different reacting species as well as the probabilities are updated at each time step. According to the ESS method, we denote the number of PER protein as P and the number of dClOCK protein as L for the current model. It should be emphasized that for simplicity, separate nuclear and cytoplasmic compartments are not taken into consideration, and the concentrations p and l are referenced to the total cell volume (33). Then, the relation between the concentration and the number of molecules could be expressed as: $p = P/V$ and $l = L/V$. Herein, similar to the interpretation by Gonze and Goldbeter (17), parameter V, which has a dimension of volume, is sometimes referred to as the system size and used to control the number of molecules of species present in the system. As shown in Table 1, the biochemical reactions in the *Drosophila* model can be decomposed into four elementary reaction steps. Note that all the transition rates $W_{i\ =\ 1,\ldots,4}$ of the reaction steps are proportional to the system size V.

The ESS method exactly accounts for the stochastic nature of the reaction events and has been widely used to study the properties and effects of internal noise in a variety of systems (17,19,24), but it is very time consuming and hardly applicable if the system size is large. In addition, it cannot afford us a clear perspective on the origin and magnitude of the internal noise in the system. To solve these problems, Gillespie developed a chemical Langevin

TABLE 1 Reaction steps and corresponding transition rates involved the *Drosophila* model

Transition processes	Description	Transition rate
(1) $P \rightarrow P+1$	The synthesis of PER activated directly by dCLOCK and repressed indirectly by itself	$W_1 = a_1 V = V v_{sp}(l_{free}(t-\tau_1)/(K_1+l_{free}(t-\tau_1)))$
(2) $P \rightarrow P-1$	The degradation of PER	$W_2 = a_2 V = V k_{dp} p(t)$
(3) $L \rightarrow L+1$	The synthesis of dCLOCK activated indirectly by PER and repressed directly by itself	$W_3 = a_3 V = V v_{sc}(K_2/(K_2+l_{free}(t-\tau_2)))$
(4) $L \rightarrow L-1$	The degradation of dCLOCK	$W_4 = a_4 V = V k_{dc} l(t)$

method (42), which means that if a macroinfinitesimal time scale exists in a system, stochastic dynamics of the system can be well approximated by a chemical Langevin equation (CLE). From the form of the CLE, one can clearly find how the internal noise involved in the chemical reactions is related to the parameter values, the system size, and the state variables that evolve with time. The CLE for this model reads:

$$\frac{dp(t)}{dt} = (a_1 - a_2) + \frac{1}{\sqrt{V}}(\sqrt{a_1}\xi_1(t) - \sqrt{a_2}\xi_2(t)) \quad (5)$$

$$\frac{dl(t)}{dt} = (a_3 - a_4) + \frac{1}{\sqrt{V}}(\sqrt{a_3}\xi_3(t) - \sqrt{a_4}\xi_4(t)), \quad (6)$$

where $a_{i=1,\ldots,4}$ are the transition rates per volume, as shown in Table 1, and $\xi_{i=1,\ldots,4}$ are Gaussian white noises with $\langle\xi_i(t)\rangle = 0$ and $\langle\xi_i(t)\xi_j(s)\rangle = \delta_{ij}\delta(t-s)$. When the second terms in the brackets at the right side of Eqs. 5 and 6 are removed, these equations are equivalent with the deterministic Eqs. 1 and 2, respectively. Therefore, the internal noise is actually denoted by the second terms, from which it is clearly seen that the magnitude of internal noise scales as $1/\sqrt{V}$ and also depends on the control parameters and the concentrations of PER and dCLOCK.

Here, we use stochastic methods (ESS and CLE methods) to study the influences of internal noise in the *Drosophila* model. For this purpose, the magnitude of internal noise over a relatively wide range should be scanned. Furthermore, it is necessary to keep the corresponding deterministic kinetics unchanged to ensure that the investigation is purely of the effect of internal noise. The deterministic kinetics does not depend on the system size V, so the magnitude of the internal noise could be tuned by changing V. Note that the CLE are mainly used for numerical simulation throughout the work, and the ESS method is also used to show consistency if necessary.

RESULTS AND DISCUSSION

To investigate the influences of internal noise, it is necessary to study the corresponding deterministic kinetics of the *Drosophila* model for comparison. For the purpose, Eqs. 1 and 2 are integrated numerically using the Euler method with time step of 0.01 h. It should be noted that the initial conditions $x(t=0) = 0.5$ nM, $y(t=0) = 0.1$ nM, $l_{free}(t-\tau_1) = 0$ in $(0, \tau_1)$ and $l_{free}(t-\tau_2) = 0$ in $(0,\tau_2)$, except when stated otherwise, are used throughout this work. Fig. 1 *a* plots the maximum and minimum values of [PER], measuring amplitude of the circadian oscillations, and Fig. 1 *b* displays the corresponding period of the oscillations. With variation of the control parameter k_{dp}, the system undergoes a Hopf bifurcation (HB) point at $k_{dp} = 2.80\ h^{-1}$, as shown in Fig. 1 *a*. The HB point divides the parameter space into two regions: the OSC region to the left side and the SS region to the right.

Internal noise-sustained circadian oscillations in the SS region of the *Drosophila* model

It is well known that external noise or internal noise often has constructive effects in the nonequilibrium SS region near the bifurcation point (26,43–45). For instance, the phenomena of intrinsic noise-induced oscillation and ICR have been observed for the parameter values that give rise to a stable steady state in a deterministic model (24). Here, influences of internal noise in the *Drosophila* oscillator, within the SS region near the HB point, are investigated first. Equations 5

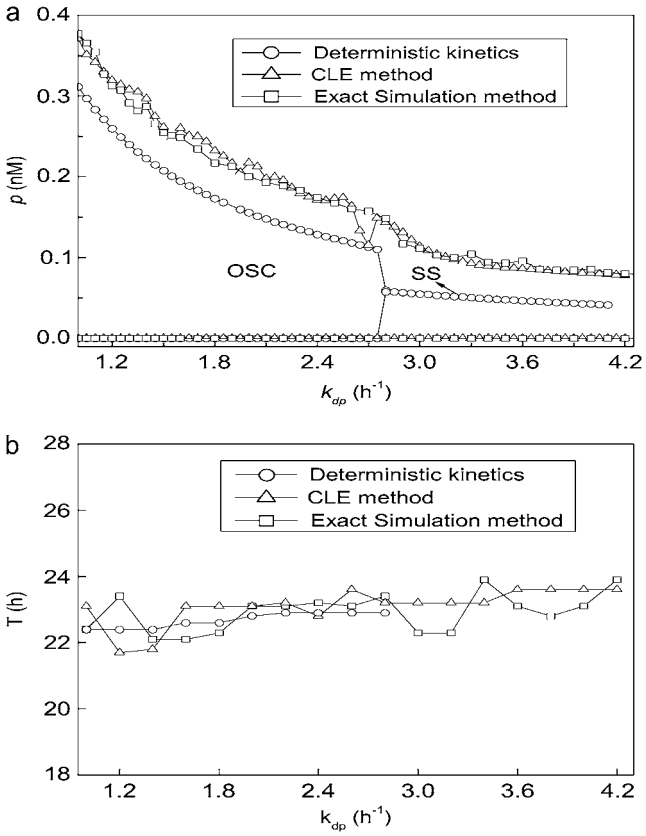

FIGURE 1 (*a*) The bifurcation diagram of the stochastic *Drosophila* model with respect to the control parameter k_{dp}, obtained with ESS method (*squares*) and CLE method (*triangles*), and the diagram of the deterministic model (*circles*). (*b*) The corresponding periods of the stochastic and deterministic oscillations as functions of the k_{dp}. Here, the stochastic simulation is carried out for $V = 700$ and the HB point in the deterministic limit is $k_{dp} \approx 2.80\ h^{-1}$.

and 6 are integrated numerically using the Euler-Maruyama method (46) with time steps of 0.01 h. Stochastic simulation by the ESS or CLE method demonstrates that internal noise sustains oscillation in the SS region and that the oscillatory pattern is stable over time. Furthermore, under different initial conditions (i.e., different values of $x(t = 0)$, $y(t = 0)$, $l_{\text{free}}(t - \tau_1)$ in $(0,\tau_1)$ and $l_{\text{free}}(t - \tau_2)$ in $(0,\tau_2)$), the period and amplitude of stochastic oscillations hardly change for the same parameter values, which indicates that the oscillations are very robust to variations on the initial conditions. Fig. 1 *a* presents the maximum and minimum values of the oscillations in the stochastic model with system size $V = 700$, which shows good qualitative agreement between the ESS and CLE methods. In Fig. 1 *a*, it is clearly seen that in the stochastic case, the bifurcation point defined in the deterministic kinetics disappears, and ''stochastic'' oscillations clearly appear in the SS region. Such oscillations are distinct from random noise in that there is a clear peak in the power spectrum (see Fig. 4, discussed below), which means that the oscillations have periodic information. Fig. 1 *b* displays the period of the stochastic oscillations, which are obtained from the inverse of the frequency of the highest peak in the corresponding power spectrum. In the figure, the periods of stochastic oscillations fluctuate slightly around periods of circadian oscillations in the OSC region of the deterministic model. These results indicate that the phenomenon of noise-sustained oscillation is in agreement with the recent report that, for the dynamics of an irreversible biochemical reaction system in the mesoscopic world, circular motion is a necessary characteristic of nonequilibrium steady state even when the corresponding macroscopic system shows no sign of oscillation (47). To exhibit this point concretely, Fig. 2 displays the time series of [PER] and [dCLOCK] in the stochastic model with $k_{\text{dp}} = 2.85\ \text{h}^{-1}$ and $V = 700$, obtained by the ESS method and CLE method, respectively. The time series for the deterministic model with $k_{\text{dp}} = 2.70\ \text{h}^{-1}$ are also plotted in Fig. 2 *c* for comparison. As shown in Fig. 2, *a* and *b*, all the curves demonstrate that the [PER] and [dCLOCK] show reliable oscillations with periods close to 24 h, and the profiles of stochastic oscillations in Fig. 2, *a* and *b*, are similar to those of the deterministic oscillations in Fig. 2 *c*. It should be emphasized that the amplitude of the oscillations decreases slightly when the control parameter k_{dp} is away from the bifurcation point, as shown in Fig. 1 *a*, and numerical simulation demonstrates that the oscillations finally disappear in the system with $k_{\text{dp}} = 17\ \text{h}^{-1}$ and $V = 700$. In this article, the oscillation supported by intrinsic noise is denoted as noise-sustained circadian oscillation (NSCO).

How can the system produce a reliable oscillation even when the deterministic kinetics predicts a stable steady state? As shown in Fig. 1 *a*, numerical simulation demonstrates that for the deterministic *Drosophila* system, fixed points and limit cycles are the only possible attractors in the phase space of [PER] and [dCLOCK]. When the system evolves toward a fixed point in the deterministic limit, large molecular

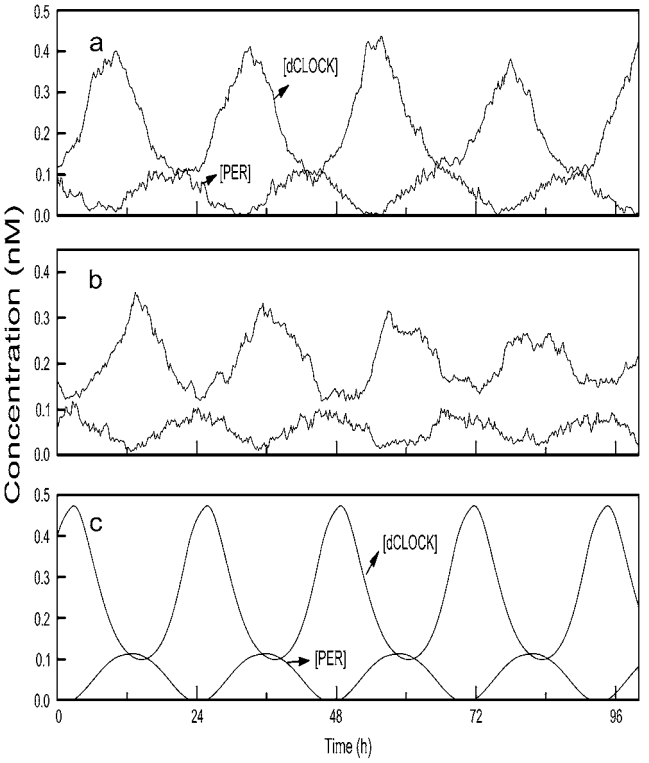

FIGURE 2 The time series of the oscillations of the proteins PER and dCLOCK in the stochastic model with $k_{\text{dp}} = 2.85\ \text{h}^{-1}$ and $V = 700$, simulated by the ESS method (*a*) and CLE method (*b*), and in the deterministic model with $k_{\text{dp}} = 2.70\ \text{h}^{-1}$ (*c*).

fluctuation, arising from small system size, could produce a perturbation of sufficient magnitude near the fixed point, which drives the system far away from the point and sends the system into a new cycle. Furthermore, as shown in Fig. 2, the new cycle might approach a limit cycle attractor, which appears in the vicinity of the HB point. This situation is similar to that observed in a two-dimensional autonomous system, where external noise stimulates coherent motion of the system, initially located in a fixed point (43).

It is well known that most cellular oscillations are readily simulated by adjusting the parameters to be within the oscillatory region (24). However, it has also been reported that a sensory system in vivo may work in the very vicinity of supercritical HBs through a mechanism of self-tuned criticality to amplify the weak signal (48), and a genetic relaxation oscillator could naturally poise very near the oscillatory regime when the native degradation rate is chosen (49). These findings imply that some cellular systems might live in the stable SS region near the bifurcation point. For the biological clock system, to the best of our knowledge, it is not clear whether it would chose to live in the stable SS region near the bifurcation point or the oscillatory region. As mentioned above, circadian rhythms have been readily simulated by many theoretical models, which produce sustained oscillations of limit cycle type, corresponding to circadian

rhythmic behavior, in a certain range of parameter values and, outside this range, give rise to a steady state (50). Despite these results, it is worth considering whether these circadian oscillators could operate reliably within the cellular context. For example, based on the possibility that global changes in transcription and translation rates may arise from variations in nutrition, growth conditions, or temperature and affect the period of transcription or translation-based oscillators, Barkai and Leibler (15) have suggested that the ability to maintain constant circadian periodicity despite global changes in the state of the cell is probably necessary for the circadian clock to be embedded successfully within the cell. In *Drosophila*, it has been observed that light pulses of appropriate duration and magnitude, when applied at the appropriate phase, can permanently or transiently suppress circadian oscillations by driving the light-controlled parameter out of the domain of existence of sustained oscillations (51). In these cases, the stable limit cycle oscillators might not produce sustained circadian oscillations, and noise-driven oscillators obtained here might be preferred by the organisms to maintain constant circadian periodicity in the context of NSCO (Fig. 1). Very recently, it has been shown that intrinsic noise arising from a small number of protein molecules could induce stochastic oscillations (52,53) that are possibly precise oscillations of high quality although the fluctuations of amplitude are large. Thus, it is possible that the phenomenon of stochastic oscillations might provide another explanation for a circadian clock being embedded successfully within the cell and coordinate an organism's activity to the day-night cycle of its environment.

''Robustness'' is used commonly to denote the persistence of a certain type of dynamic behavior over a significant range of parameter values (19). Herein, as shown in Fig. 1 *a*, the deterministic model sustains circadian oscillations in a precise region of the parameter space, whereas the stochastic model could sustain the oscillations with similar periods in a wider region by virtue of NSCO. This implies that the *Drosophila* might take advantage of internal noise to enhance the robustness of circadian oscillations to variations of the light-controlled parameter k_{dp}, which might provide another point of view to explain the experimental phenomenon that, in constant darkness, researchers generally observed individual *Drosophila* with different biochemical parameters able to sustain circadian rhythms with a very similar period (54). In addition, further investigations on the power spectrum of the NSCO have demonstrated that the fundamental frequency of the peak could basically remain unchanged for various system sizes (see Fig. 4, discussed below), which means that the period of the oscillations also remains constant over a range of internal noise. The phenomenon suffices to provide a crucial feature of circadian clocks, i.e., the ability to maintain a constant period over a wide range of internal and external fluctuations (1). All these findings confirm that the reduced model could be adopted to explain noise effects on the mechanism for circadian rhythms in cellular regulatory systems.

ICR in the SS region of the *Drosophila* model and its robustness to time delay

Figs. 3 and 4 display time series of the [PER] and the corresponding power spectrum at $k_{dp} = 2.85\ h^{-1}$ for $V =$ 50,000, 700, and 20, respectively. The smoothed curves in Fig. 4 are obtained by nearest averaging over 25 points from the original ones. The time series used to calculate the power spectrum contains 16,384 data points with an average time interval 0.2 h. During the estimation of the power spectrum, we used a Welch window function, which is adapted to modify the relation between the spectral estimate P_k at a discrete frequency k and the actual underlying continuous spectrum $P(f)$ at nearby frequencies and is expressed as follows:

$$w_j = 1 - \left(\frac{j - \frac{1}{2}(N-1)}{\frac{1}{2}(N+1)}\right)^2, \quad (7)$$

where N is sampled points, and j ranges from 0 to N (55). As mentioned above, changing the system size V could modulate the absolute molecular numbers of proteins PER and dCLOCK, leading to various magnitudes of molecular fluctuations. For example, the second terms at the right side of Eqs. 5 and 6 represent the internal noises, so the magnitude of internal noise in the concentration of protein PER is calculated as ~ -0.01–0.01 for $V = 50{,}000$, ~ -0.09–0.09 for $V = 700$, and ~ -0.6–0.6 for $V = 20$; the range of internal noise in the concentration of protein dCLOCK is

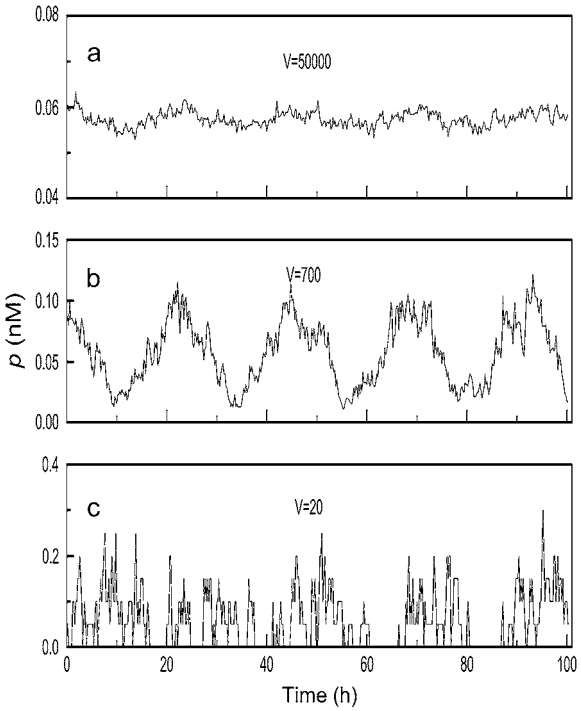

FIGURE 3 The time series for stochastic oscillations of the protein PER for $k_{dp} = 2.85\ h^{-1}$ and three different system sizes, $V = 50{,}000$ (*a*), 700 (*b*), and 20 (*c*), respectively. The curve for $V = 50{,}000$ is obtained from the CLE method, whereas the other two curves are obtained by the ESS method.

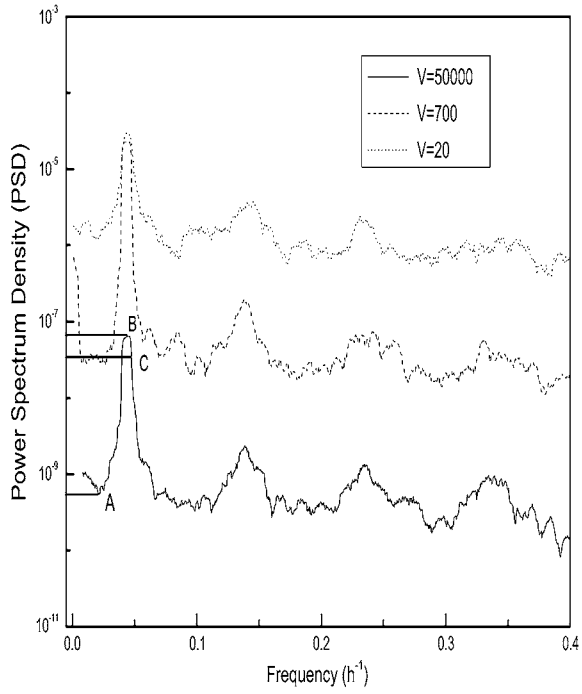

FIGURE 4 The power spectra for the oscillations of the protein PER for three cell sizes, $V = 50{,}000$, 700, and 20, respectively. The curve for $V = 50{,}000$ is obtained from the CLE method, whereas the other curves are obtained by the ESS method. The points A, B, and C in the PSD curve for $V = 50{,}000$ describe how to calculate the effective SNR, i.e., $\beta = [P(B)/P(A)] \times w_B/(w_C - w_B)$, where point C is located by the condition $P(C) = P(B)/e$. Note that an arbitrary unit is used for the PSD.

~ -0.008–0.008 for $V = 50{,}000$, ~ -0.06–0.06 for $V = 700$, and ~ -0.6–0.6 for $V = 20$. Therefore, variation of the system size could affect the amplitude or period of the NSCO. In a very large system (i.e., a system with a large number of proteins PER and dCLOCK), the internal noise could be neglected, and the deterministic equations are applied so that the oscillator with $k_{dp} = 2.85\ h^{-1}$ is in the steady state. As shown in Fig. 3, for large or small system size (e.g., $V = 50{,}000$ or 20), NSCO are very weak or overwhelmed by the internal noise, so that the rhythmicity of the oscillations are inconspicuous, whereas for moderate system size (e.g., $V = 700$), the oscillations are the most pronounced, and rhythmicity could be intuitively observed. Furthermore, in Fig. 4, both signal level and noise background increase at the peak with the decrease of the system size from 50,000 to 20, whereas the peak for the intermediate system size is the most pronounced among the three. Thus, the results in Figs. 3 and 4 both imply that the performance of the NSCO might be best at an appropriate system size (i.e., an intermediate internal noise intensity), characterizing a resonance behavior. In addition, it should be emphasized that, although the time series in Fig. 3, *a* and *c* do not discern clear rhythmicity, the power spectra in Fig. 4 show that there are clear peaks at similar fundamental frequencies for the three system sizes. Moreover, numerical simulations have shown that the period of the oscillations is $\sim$23.5 h for the three system sizes, and the standard deviations (SD) of the distribution of the period are SD = 8.2 for $V = 50{,}000$, SD = 7.6 for $V = 700$, and SD = 9.4 for $V = 20$, respectively (see histograms in Fig. 10 of the Appendix). Therefore, as the system size V decreases, the amplitude of the NSCO evidently increases, whereas the period nearly remains constant.

To measure the performance of the NSCO quantitatively, an effective SNR is computed as follows: $\beta = R/(\Delta w/w_p)$, where w_p is the frequency at the peak; $R = P(w_p)/P(w_2)$, and $P(\cdot)$ is the power spectrum density (PSD) for a given frequency and $P(w_2)$ the smallest PSD value between $P(0)$ and $P(w_p)$; Δw is the width between w_p and the frequency w_1 satisfying $w_1 > w_p$ and $P(w_1) = P(w_p)/e$. One has reported that if the effective SNR is used, stochastic simulations by the CLE and ESS methods both show internal noise stochastic resonance in a circadian clock model (56). It should be noted that the values of β are calculated by averaging the results of 20 independent runs throughout this article. Fig. 5 *a* displays the plot of the effective SNR β versus the system size V at $k_{dp} = 2.85\ h^{-1}$. In Fig. 5 *a*, simulation result by the ESS or CLE method demonstrates that an obvious peak appears at an optimal system size $V \sim 700$, which exhibits a kind of system size resonance (57,58). Because magnitude of the internal noise is changed by variation of the system size, the presence of the maximum of β in Fig. 5 *a* also indicates the occurrence of ICR, indicating that the NSCO plays the best performance at an optimal internal noise intensity. Because explicit time delays for the negative feedback loop of the model seem to provide a noise-independent mechanism, which may not be the case in real physiology of the clock, the effects of internal noise are also investigated in another simple two-variable model without explicit time delays, proposed by Tyson et al. (32). It is found that the behaviors of the NSCO and ICR also appear in the model, where the deterministic model is in a stable steady state, when the control parameters are set at $k_{p1} = 26\ C_p\ h^{-1}$, $K_{eq} = 50\ C_p$, and adopting the values of other parameters shown in Table 1 of Tyson et al. (32). In addition, to our knowledge, there are relatively few simulations of stochastic models with time delays and rather few analytical results. As a consequence, it is an open question which algorithms are better for simulating such models. Recently, Yi and Jia (59) demonstrated the excellent quantitative agreement between the CLE method and the fixed time-step algorithm in studying the interplay between the external noise and intrinsic noise in the *Drosophila* model. Here, one can also easily observe good qualitative agreement between the CLE method and ESS method by comparing the results in Figs. 1, 2, and 5 *a*. Therefore, the CLE method is reliable and convenient to study systematically the effects of internal noise in this model.

Recent findings have demonstrated that the role of time delays in circadian rhythms should be taken into account because the delays in the corresponding reactions are par-

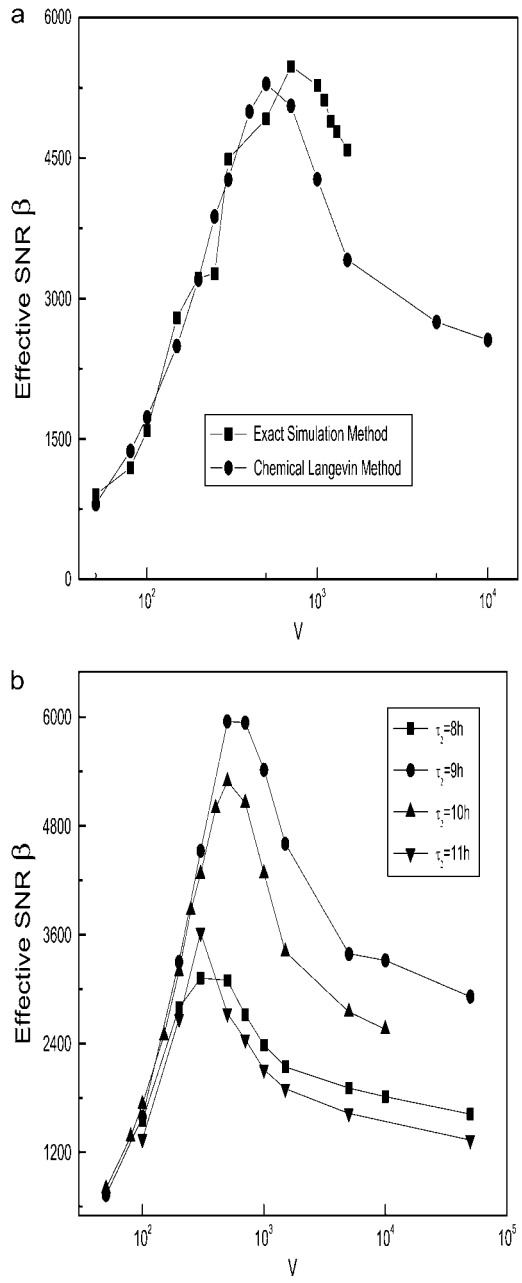

FIGURE 5 (*a*) Dependence of the effective SNR β on the system size V for $k_{dp} = 2.85\ h^{-1}$ and $\tau_2 = 10$ h. (*Solid squares*) Results obtained by the ESS method. (*Solid circles*) Results obtained from the CLE method. (*b*) Dependence of SNR on the system size at $\tau_2 = 8$ h, 9 h, 10 h, and 11 h. The results are all obtained from the CLE method.

ticularly long (several hours) in comparison with other characteristic times of the system (30,60–62). Furthermore, it has been demonstrated that nonlinear negative feedback of mature protein on its own gene expression and time delay are critical to determine the free-running periodicity in some delay models of the circadian pacemaker (60,62). However, Ruoff et al. (63) have demonstrated that when a delay equation approximates a simple transcription-translation process without feedback, it could lead to oscillations for certain delay times despite the fact that the consecutive processes are not oscillatory at all. This finding suggests that the issue related to the use of delay equations leading to possible artifacts needs a more careful analysis. Therefore, the roles of time delays τ_1 and τ_2 in the current model need to be investigated extensively in the deterministic and stochastic cases.

Smolen et al. (33) have demonstrated that, in the deterministic model, both the negative feedback loop and the time delay τ_1 are essential for the production of stable circadian oscillations and have found that decreasing τ_1 diminishes the oscillation period and that eliminating τ_1 abolishes the circadian oscillations. Here, we investigate the roles of the time delays τ_1 and τ_2 in the deterministic and stochastic cases. The deterministic system initially lies in a steady state when $k_{dp} = 2.85\ h^{-1}$, $\tau_1 = 10$ h, and $\tau_2 = 10$ h. When τ_2 is changed moderately, the deterministic system is always in the same steady state, whereas when τ_1 is changed, except for the above steady state, the system could oscillate for a small range of τ_1 (i.e., $10.4 < \tau_1 < 13.4$; see Fig. 7 *a* in the Appendix for details). In the stochastic case, stochastic oscillations could exist in the system with $V = 700$ and $k_{dp} = 2.85\ h^{-1}$ for all τ_1 and τ_2 that have been adopted to study the dynamics of the deterministic system (see Figs. 7 *b* and 8 *a* in the Appendix). It is found that with the increase of τ_1, the period of the oscillations monotonously increases, and the amplitude significantly increases for appropriate values of τ_1 (see Fig. 7, *b* and *c*, in the Appendix); with the increase of τ_2, the period of the stochastic oscillations nearly linearly increases, and the amplitude first increases and then decreases, showing two maximal values for $\tau_2 = 9$ h and $\tau_2 = 10$ h (see Fig. 8 in the Appendix). In addition, when τ_1 is removed (i.e., $\tau_1 = 0$), different steady states appear in the deterministic system for various values of the k_{dp} (see Fig. 9 *a* in the Appendix for details). However, in the presence of internal noise, stochastic oscillations, having same periods close to 20.6 h and nearly identical amplitudes, appear for the various k_{dp} (see Fig. 9 *b* in the Appendix). Furthermore, for the parameters $V = 700$ and $k_{dp} = 2.85\ h^{-1}$, the period and amplitude of such oscillations in the absence of τ_1 are smaller than those in the presence of τ_1.

How could the time delays influence the performance of the internal NSCO? Because τ_1 are constrained by some experimental data, only the effects of τ_2 are investigated in detail here. The values of time delay $\tau_2 = 8$ h, 9 h, 10 h, and 11 h are taken for example. Fig. 5 *b* shows the dependence of β for the [PER] on V for $\tau_2 = 8$ h, 9 h, 10 h, and 11 h. It is shown that each curve in the figure has a maximum, which indicates the appearance of the ICR for each value and demonstrates the robustness of the resonance phenomenon to the time delay τ_2. Meanwhile, the resonance peak increases or decreases depending on τ_2 and reaches a maximum at $\tau_2 = 9$ h, which means that the NSCO might show the best performance in the *Drosophila* with an optimal system size

and an optimal value of time delay τ_2. Interestingly, as shown in Fig. 5 *b*, for $\tau_2 = 9$ h or 10 h, the best performance of the NSCO obviously appears at $V \approx 10^3$, which is of the same order of real living cells in vivo (64,65). Therefore, it is expected that the kinetic parameters of *Drosophila* involved in the mechanism for circadian rhythms might be evolved to be optimal for the size of a cell. In addition, to explain the effects of stochastic variations of the time delay on the performance of the noise-induced oscillations, time delays $\tau_1(t)$ and $\tau_2(t)$ are stochastically chosen uniformly from an interval $(0,\delta)$ at an increment of 0.01 h in simulation. The two time delays are statistically independent. It is found that for various δ (i.e., $\delta = 8, 9, 10, 11$, or 12), the system with $k_{dp} = 2.85\ h^{-1}$ always lies in a stable steady state in the absence of internal noise. In the presence of internal noise, the stochastic system displays oscillations. But the values of the effective SNR for such oscillations are very small for various system sizes (e.g., $V = 20$, 700, and 10,000), and the oscillations are overwhelmed even for $V = 700$. In addition, when only τ_1 or τ_2 is varied in simulation, pronounced oscillations could be found for $V = 700$. However, the values of the effective SNR are still much smaller than those shown in Fig. 5 *b*. It should be noted that the data for these results are not shown here. Thus, based on these findings, it is possible that stochastic variations in time delays destroy the coherence of the NSCO and play mainly a negative role in the occurrence of the oscillations.

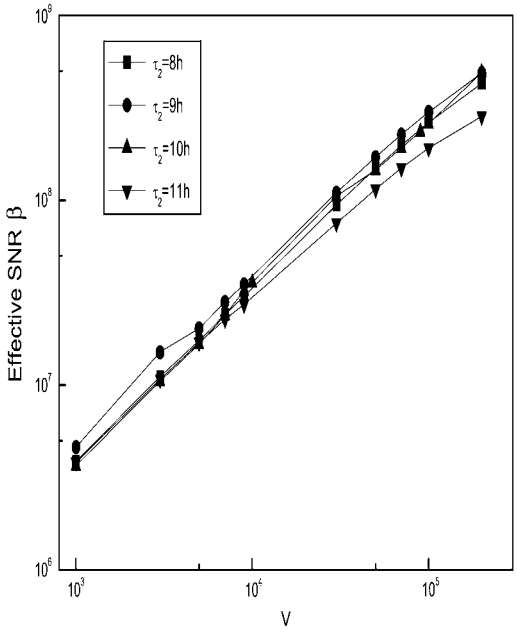

FIGURE 6 The effective SNR β as a function of the system size V for $\tau_2 = 8$ h, 9 h, 10 h, or 11 h and $k_{dp} = 1.5\ h^{-1}$. The results are obtained by the CLE method.

Robustness of the *Drosophila* model within the OSC region

It is important to realize that noise effects in nonlinear systems are usually difficult to predict and rather paradoxical (66). Some previous reports about circadian rhythms have concluded that internal noise often played a destructive role in the oscillatory region of a circadian system because of the phenomenon of phase diffusion (17,67,68), but recently it has been demonstrated that regularity of the oscillations in the oscillatory domain of a deterministic gene regulatory system could be enhanced for appropriate system sizes (24). In the context of these findings and the report about molecular fluctuations (33), it is necessary to investigate intensively what specific roles the internal noise plays in the OSC region of the deterministic *Drosophila* model. As shown in Fig. 1, in relation to properties of the deterministic oscillations, the amplitudes of the noisy oscillations in the OSC region are significantly enhanced by the internal noise, whereas the periods fluctuate just slightly around the ones of the deterministic oscillations. To explain further the effects of internal noise on performance of the noisy oscillations, Fig. 6 displays the plot of the effective SNR β versus system size V for different values of τ_2, obtained using the CLE method. From the figure, it is clearly seen that all curves decrease monotonically with the decrease of the system size, which indicates that internal noise destroys the coherence of circadian oscillations and plays a destructive role in the OSC region. This phenomenon could be also attributed to the aforementioned noise-induced phase diffusion of the oscillations. Despite the decrease of the coherence, the period of the oscillations hardly changes with the decrease of the system size for each τ_2, displaying a crucial ability of circadian clocks to maintain a constant period over a wide range of internal and external fluctuations (1). It should be noted that the data for the period of the noisy circadian oscillations are not shown here. Similar phenomena have been observed in a core molecular model for circadian oscillations using the method of autocorrelation function (19). These results demonstrate that the deterministic circadian oscillations in the model are robust to the internal noise such that the reduced model could provide a reliable picture of the working of circadian clocks in *Drosophila* and can be used to study the molecular mechanism of circadian rhythms. In addition, it is found that the four curves shown in Fig. 6 are very close for various system sizes, which indicates that the time delay in the positive feedback has little effect on the coherence of the noisy oscillations in the OSC region, even though it can greatly change the period of those oscillations, as mentioned above. Recently, some reports have addressed the question of whether or not the positive feedback increases the robustness of the circadian clock to stochastic fluctuations (27,33). These results might provide another dynamic point of view to understand the function of the positive feedback in the mechanism for circadian rhythms.

Hänggi (69) suggested ''It would indeed seem strange to me that nature would not have taken advantage of the

benefits of noise for nonlinear transmission and amplification of feeble information rather than ignoring it.'' Now, such advantageous roles of noise have been expanded to circadian rhythms processes. This work has found that internal noise could help the *Drosophila* model to sustain circadian oscillations in a range of the control parameter k_{dp}, where the oscillations do not exist in the deterministic limit. Moreover, such oscillation could play the best performance at an optimal system size. These results may be of relevance to circadian rhythmic processes in two ways. On one hand, because circadian rhythmic processes are often regulated at the level of single cells, where the internal noise is unavoidable, circadian oscillations existing in circadian clock systems are essentially stochastic oscillations. Therefore, by virtue of the occurrence of NSCO, circadian oscillations with a similar period can be quite robust to the variation of system parameter. On the other hand, instead of trying to resist the internal noise, the systems may exploit it to sustain circadian oscillations and make the performance of the oscillations best at an optimal system size. Actually, similar results have also been obtained in other biological systems (70–74). For example, ion-channel clusters of optimal sizes can improve the encoding of a subthreshold stimulus in neurons (44,70). Optimal intracellular calcium signaling occurs at an optimal size or distribution of the ion-channel clusters (72,73). Such results imply that the optimal system size might be the universal characteristic for mesoscopic biological systems and system size resonance (i.e., ICR) might be a widely used mechanism for living organisms to adapt and function.

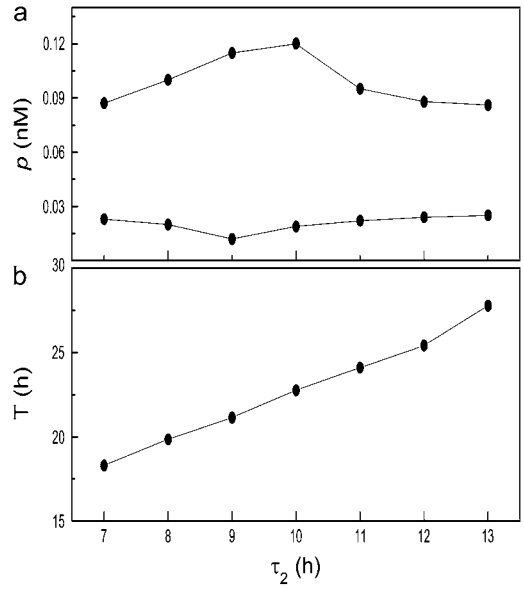

FIGURE 8 The bifurcation diagram (*a*) and the corresponding period (*b*) of the stochastic model with $k_{dp} = 2.85\ h^{-1}$, $\tau_1 = 10$ h, and $V = 700$.

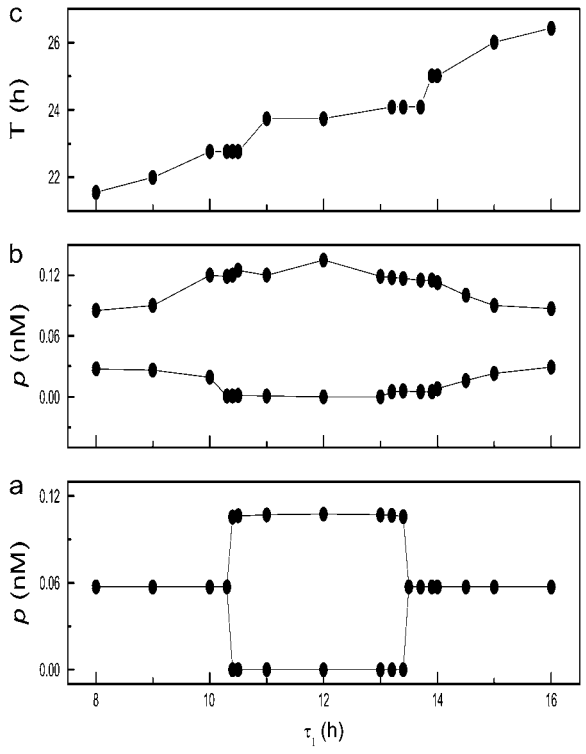

FIGURE 7 (*a*) The bifurcation diagram of the deterministic *Drosophila* model. (*b*) The bifurcation diagram of the stochastic model with $V = 700$. (*c*) The corresponding period of the oscillations in the stochastic model with $V = 700$. The parameters k_{dp} and τ_2 are equal to 2.85 h^{-1} and 10 h, respectively.

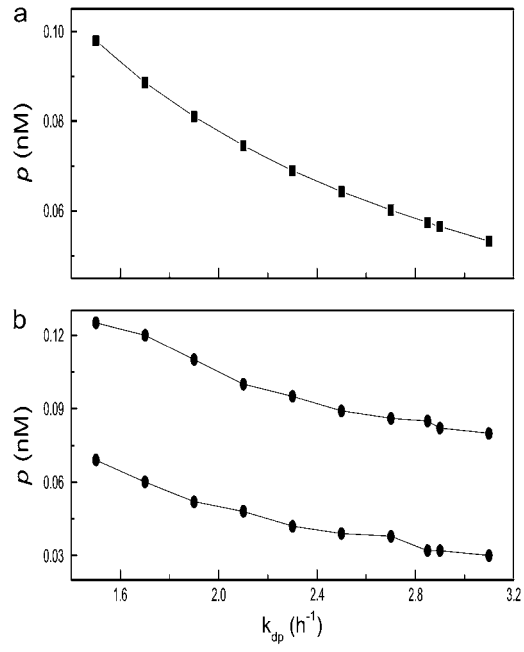

FIGURE 9 The concentration of the protein PER in the deterministic model (*a*) and the stochastic model with $V = 700$ (*b*) as functions of the control parameter k_{dp}. Here the time delays τ_1 and τ_2 are $\tau_1 = 0$ and $\tau_2 = 10$, respectively.

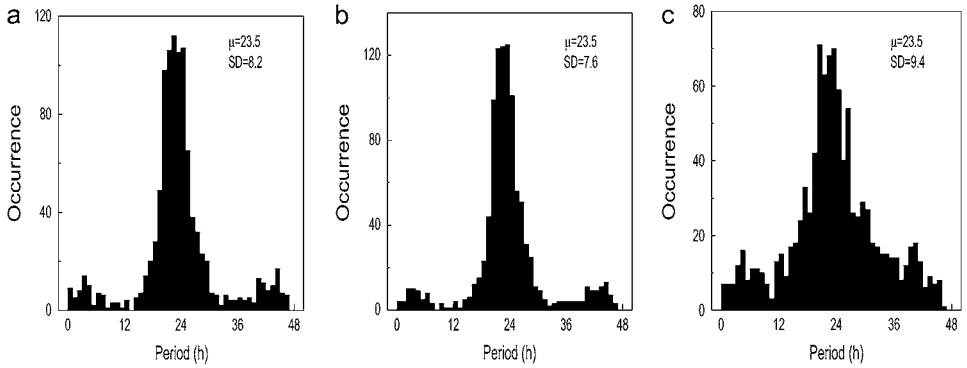

FIGURE 10 The period distribution of the oscillations for (*a*) $V = 50{,}000$, (*b*) $V = 700$, and (*c*) $V = 20$. Other parameters are $k_{dp} = 2.85$, $\tau_1 = 10$ h, and $\tau_2 = 10$ h. It should be noted that here the period histograms have been calculated on a time series of 25,000 h, i.e., more than 1000 cycles, of which the mean value (μ) and standard deviation (SD) are also shown in the figure.

SUMMARY

The constructive influences of internal noise in a reduced model for *Drosophila* have been investigated using the ESS and the CLE. It is found that internal noise could sustain circadian oscillation in appropriate regions of the light-controlled parameter, where the deterministic kinetics cannot produce the oscillations. The performance of noise-sustained oscillations undergoes a maximum with variation of the internal noise intensity, demonstrating occurrence of the ICR. In the oscillatory region of the deterministic model, the coherence of circadian oscillations decreases with the increase of internal noise strength because of the phase diffusion of the oscillations, whereas the period hardly changes for various noise intensities, validating the deterministic model to study the molecular mechanism of circadian rhythms in *Drosophila*. In addition, it is found that the ICR behavior of noise-sustained oscillations in the SS region of the deterministic model always appears for various values of time delay in the positive feedback, and furthermore, the resonance peak reaches a maximum at a specific value of the time delay. But the coherence of the noisy circadian oscillations in the oscillatory region hardly changes for various time delays in the positive feedback. Previous models of circadian rhythm generation in *Drosophila* and *Neurospora* have involved protein phosphorylation and negative feedback but generally have not relied on positive feedback and time delays (5,19,51). However, several models have been set up based on positive feedback loop (32) or time delays (60,62). Thus, it is an important motivation for the research of circadian rhythms that a somewhat complex model is needed to explain further the dynamics of negative and positive feedback at the level of transcriptional regulation as well as the time delays characterizing these processes (30). The present reduced model incorporates both positive feedback and negative feedback, each with a time delay. Therefore, the results obtained in the present article might aid intuitive understanding of stochastic dynamics of feedbacks and time delays in more complex models, which take intrinsic noise into consideration. In addition, our findings seem to yield new insights into the roles of intrinsic noise on circadian rhythm processes and may provide another point of view to understand the constraint on the mechanism of circadian rhythms, imposed by the ability to function reliably in the presence of internal noise (15).

APPENDIX

The effects of variation in time delays τ_1 and τ_2 on the period and amplitude of the deterministic and stochastic oscillations in the system are investigated, as shown in Figs. 7 and 8. Furthermore, the dynamic behavior of the deterministic and stochastic model with $\tau_1 = 0$ is studied for various k_{dp}, as plotted in Fig. 9. In addition, Fig. 10 displays period distribution of stochastic oscillations for $V = 50{,}000$, 700, and 20 to intuitively show the standard deviations of the period for these system sizes.

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