

Circadian rhythms and molecular noise

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(Received 20 April 2006; accepted 17 May 2006; published online 30 June 2006)

Circadian rhythms, characterized by a period of about 24 h, are the most widespread biological rhythms generated autonomously at the molecular level. The core molecular mechanism responsible for circadian oscillations relies on the negative regulation exerted by a protein on the expression of its own gene. Deterministic models account for the occurrence of autonomous circadian oscillations, for their entrainment by light-dark cycles, and for their phase shifting by light pulses. Stochastic versions of these models take into consideration the molecular fluctuations that arise when the number of molecules involved in the regulatory mechanism is low. Numerical simulations of the stochastic models show that robust circadian oscillations can already occur with a limited number of mRNA and protein molecules, in the range of a few tens and hundreds, respectively. Various factors affect the robustness of circadian oscillations with respect to molecular noise. Besides an increase in the number of molecules, entrainment by light-dark cycles, and cooperativity in repression enhance robustness, whereas the proximity of a bifurcation point leads to less robust oscillations. Another parameter that appears to be crucial for the coherence of circadian rhythms is the binding/unbinding rate of the inhibitory protein to the promoter of the clock gene. Intercellular coupling further increases the robustness of circadian oscillations. © 2006 American Institute of Physics. [DOI: 10.1063/1.2211767]

Most living organisms have developed the capability of generating autonomously sustained oscillations with a period close to 24 h. These oscillations, known as circadian rhythms, are said to be endogenous because they can occur in constant environmental conditions.^{1,2} These rhythms originate from the negative autoregulation of gene expression.^{3,4} Deterministic models based on such genetic regulatory processes account for the occurrence of circadian rhythms in constant environmental conditions (e.g., constant darkness), for entrainment of these rhythms by light-dark cycles, and for their phase-shifting by light pulses.⁵⁻⁷ When the numbers of protein and mRNA molecules involved in the oscillations are small, as may occur in cellular conditions, it becomes necessary to resort to stochastic simulations to assess the influence of molecular noise on circadian oscillations.^{8,9}

I. INTRODUCTION

The molecular basis of circadian rhythms represents a topic of key importance for comprehending the dynamics of cellular processes. Experimental studies during the last two decades have unraveled the molecular mechanism of circadian rhythms.¹⁻⁴ Initial studies pertained to the fly *Drosophila* and the fungus *Neurospora*. Molecular studies of circadian rhythms have since been extended to cyanobacteria, plants and mammals. In all cases investigated, the molecular mechanism of circadian oscillations relies on the negative autoregulation exerted by a protein on the expression of its gene.^{3,4} In *Neurospora* the FRQ protein represses the expression of its gene *frq*, while in *Drosophila* the proteins PER and TIM repress, through the formation of a heterodimer, the activation of the *per* and *tim* genes. The situation in mam-

mals resembles that observed in *Drosophila*, but the CRY proteins, instead of TIM, form a regulatory complex with a PER protein to inhibit the expression of the *Per* and *Cry* genes. Light can entrain circadian rhythms by enhancing the expression of the *frq* and *Per* genes in *Neurospora* and mammals, respectively, and by inducing the degradation of the TIM protein in *Drosophila*.

A number of mathematical models for circadian rhythms have been proposed^{5-7,10-15} on the basis of these experimental observations. These models are of a deterministic nature and take the form of a system of coupled ordinary differential equations. The models predict that in a certain range of parameter values the genetic control network undergoes sustained oscillations of the limit cycle type corresponding to the circadian rhythm, whereas outside this range the gene network operates in a stable steady state. These models can be used to study dynamical features of circadian clocks, such as entrainment, phase-shifts induced by light pulses, or temperature compensation.

The question arises as to whether deterministic models are always appropriate for the description of circadian clocks.⁸ Indeed, the number of molecules involved in the regulatory mechanism producing circadian rhythms at the cellular level may be small. This number could vary from a few thousands down to hundreds and even a few tens of protein or messenger RNA molecules in each rhythm-producing cell. At such low concentrations it becomes necessary to resort to a stochastic approach to identify the factors that contribute to the robustness of circadian oscillations.

In this paper, we present the stochastic version of a simple core model for circadian rhythms and discuss the role of several factors that influence the robustness of the circa-

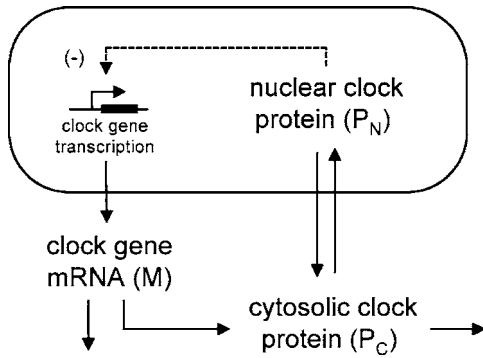


FIG. 1. Scheme of the core model for circadian oscillations. The model is based on the effect exerted by a clock protein (P_C) which can enter the nucleus (P_N) to repress the transcription of its gene into mRNA (M).

dian clock. These factors include cooperativity in repression, distance from a bifurcation point, periodic forcing by a light-dark cycle, and coupling between circadian oscillators.

II. A SIMPLE CORE MODEL

The model, schematized in Fig. 1, is based on the negative feedback exerted by a protein (which will be referred to below as clock protein) on the expression of its gene. The deterministic version of this model, previously proposed for circadian oscillations of the FRQ protein and *frq* mRNA in *Neurospora*, accounts for the occurrence of sustained oscillations in continuous darkness, phase-shifting by light pulses, and entrainment by light-dark cycles.⁷ Similar results have been obtained in more detailed models proposed for *Drosophila* and mammals.^{6,7} The more complex models proposed for *Drosophila* contain between 5 and 10 variables,^{5,6} whereas the mammalian models contain from 16 to 19 variables;¹⁴ an even more complex model for the mammalian clock has been proposed.¹⁵ For the sake of simplicity, we will focus here on the simpler model proposed for *Neurospora* circadian rhythms. The model, based on the regulation exerted by FRQ alone, contains only three variables because no phosphorylation of the clock protein is considered. This is in contrast to the core model used in previous stochastic studies.^{9,16,17} The three-variable model for the *Neurospora* clock can serve as a core model for circadian oscillations, and does not aim at representing the current, more complex view of the molecular mechanism of circadian clocks.

The deterministic model schematized in Fig. 1 is based on the repression exerted by the nuclear form of a clock protein (P_N) on the transcription of its gene into mRNA (M). mRNA is synthesized in the nucleus and transferred to the cytosol where it accumulates and undergoes enzymatic degradation. In the cytosol the clock protein (P_C) is synthesized at a rate proportional to M . The protein enters into the nucleus in a reversible manner. In the nucleus the negative feedback exerted by the nuclear clock protein on gene transcription is described by an equation of the Hill-type, in which n denotes the degree of cooperativity. In *Neurospora* and in mammals, light is known to enhance the transcription rate. In our simulation, we take into account the effect of light by increasing during the light phase the maximum rate of transcription, measured by parameter v_s . The time evolu-

TABLE I. Stochastic version of the single cell model. There are six reaction steps. Each of them is described by a probability, which is directly related to the parameters used in the deterministic equations. Parameter Ω , which has the unit of a volume, is sometimes referred to as the system size and is used to control the number of molecules present in the system.

No.	Reaction	Transition rate
1	$\rightarrow M$	$w_1 = v_s \Omega \frac{(K_I \Omega)^n}{(K_I \Omega)^n + P_N^n}$
2	$M \rightarrow$	$w_2 = v_m \Omega \frac{M}{K_m \Omega + M}$
3	$\rightarrow P_C$	$w_3 = k_s M$
4	$P_C \rightarrow$	$w_4 = v_d \Omega \frac{P_C}{K_d \Omega + P_C}$
5	$P_C \rightarrow P_N$	$w_5 = k_1 P_C$
6	$P_N \rightarrow P_C$	$w_6 = k_2 P_N$

tion of the concentration of the clock gene mRNA (M), and of cytosolic and nuclear clock protein (P_C and P_N , respectively) in a single cell is governed by the following set of ordinary differential equations:

$$\begin{aligned} \frac{dM}{dt} &= v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M}{K_m + M}, \\ \frac{dP_C}{dt} &= k_s M - v_d \frac{P_C}{K_d + P_C} - k_1 P_C + k_2 P_N, \\ \frac{dP_N}{dt} &= k_1 P_C - k_2 P_N. \end{aligned} \quad (1)$$

III. STOCHASTIC VERSION OF THE MODEL

The fluctuations arising from the limited number of molecules participating in the molecular mechanism can be taken into account by describing the chemical reaction system as a birth-and-death stochastic process governed by a master equation.¹⁸ In a given reaction step, molecules of participating species are either produced (birth) or consumed (death). At each step is associated a transition probability, which depends on the numbers of molecules of involved chemical species and on the chemical rate constant. To implement such a master equation approach to stochastic chemical dynamics, we used the Gillespie algorithm.¹⁹ The Gillespie method associates a probability with each reaction; at each time step the algorithm stochastically determines, according to the probability of each of the reactions, the one that takes place as well as the time interval to the next reaction. The numbers of molecules of the different reacting species as well as the probabilities are updated at each time step. In this approach a parameter denoted Ω is used to convert concentrations into numbers of molecules. This parameter has the unit of a volume and is sometimes referred to as the system size.²⁰ Varying Ω allows us to change the number of molecules present in the system. The stochastic version of the deterministic model is presented in Table I. Note that in the approach used here, we did not fully decompose the mo-

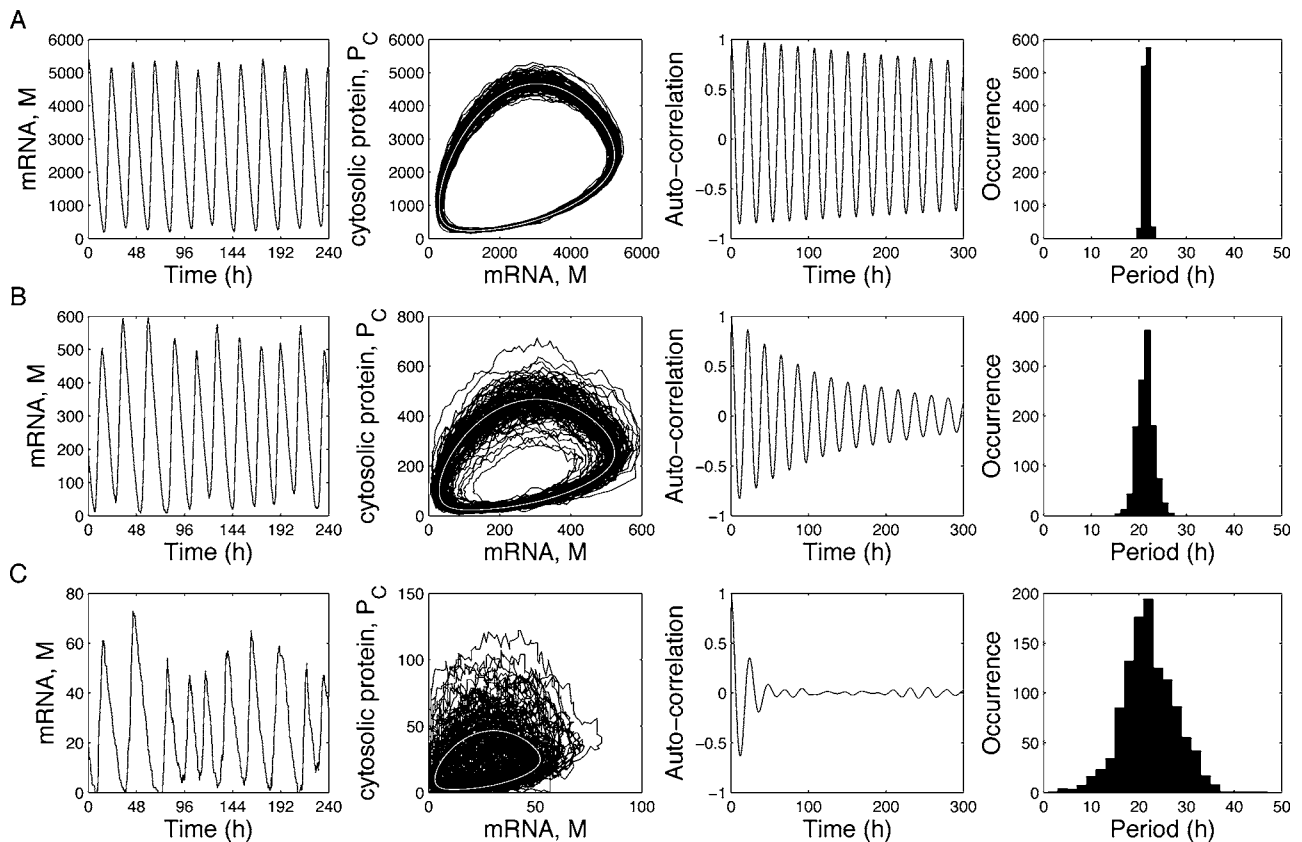


FIG. 2. Oscillations obtained by numerical simulation of the stochastic model for circadian rhythms with the Gillespie algorithm. The panels show oscillations of mRNA concentration, M (left column), limit cycles (second column), autocorrelation function (third column), and the period distribution (fourth column), for (A) $\Omega=1000$, (B) $\Omega=100$, and (C) $\Omega=10$. The autocorrelation and period histograms have been calculated on a time series of 25 000 h, i.e., more than 1000 cycles. The white curve on the phase plane corresponds to the deterministic limit cycle. The deterministic oscillations have a period of 22 h. Parameter values are: $v_s=1.6 \text{ nM h}^{-1}$, $K_I=1 \text{ nM}$, $n=4$, $v_m=0.505 \text{ nM h}^{-1}$, $K_m=0.5 \text{ nM}$, $k_s=0.5 \text{ h}^{-1}$, $v_d=1.4 \text{ nM h}^{-1}$, $K_d=0.13 \text{ nM}$, $k_1=0.5 \text{ nM h}^{-1}$, $k_2=0.6 \text{ nM h}^{-1}$.

lecular mechanism into elementary reaction steps. We previously showed for a five variable deterministic version of the core model for circadian rhythms that a full decomposition of each reaction into elementary steps yields largely similar results to those obtained when the reactions are not decomposed.¹⁶ This means, for example, that instead of decomposing the cooperative repression process into a succession of bimolecular reactions describing the successive binding of n repressor protein molecules to the gene promoter, we will treat cooperative repression as a single step and will use for the probability of its occurrence an equation of the Hill form similar to the one used in the deterministic kinetics. Note that a perfect correspondence between the two approaches would be obtained if the repressor binding rate were infinitely large. The effect of this binding rate on the robustness of the oscillations will be discussed in more detail below.

IV. INFLUENCE OF THE NUMBER OF MOLECULES

Circadian oscillations obtained in the presence of molecular noise are shown in Fig. 2. Stochastic simulations were performed for decreasing values of the parameter Ω , characterizing the number of molecules: (A) $\Omega=1000$, (B) $\Omega=100$, and (C) $\Omega=10$. In the phase plane the mere effect of molecular noise is to transform the deterministic limit cycle, indicated by the white closed curve (second column), into a

cloud of points surrounding the deterministic trajectory. The thickness of the stochastic limit cycle increases as the number of molecules decreases, indicating that the effect of molecular noise becomes more significant. The results further indicate that robust circadian oscillations are still produced by the stochastic model when the maximum numbers of mRNA and protein molecules are in the order of hundreds. It is only when these numbers decrease down to a few tens that noise begins to overcome rhythmicity, even though oscillations still subsist in these conditions [see panel (C)]. In the latter case, the period histogram is much wider but still presents a mean close to a circadian value.

The robustness of circadian oscillations with respect to molecular noise can be quantified further by the autocorrelation function that measures the phase diffusion of the oscillations (Fig. 2, third column). In the presence of noise, the more periodic the evolution, the more slowly the autocorrelation function goes to zero. The envelope of the autocorrelation decreases exponentially, reflecting diffusion of the phase over the whole circadian period in the absence of entrainment.⁹ The half-life of the autocorrelation, corresponding to 50% decrease, provides a measure of the robustness of periodic oscillations with respect to noise. In Fig. 3(A), we plotted the half-life of the autocorrelation as a function of parameter Ω . The results show that the half-life increases in a linear manner with Ω , which is in agreement

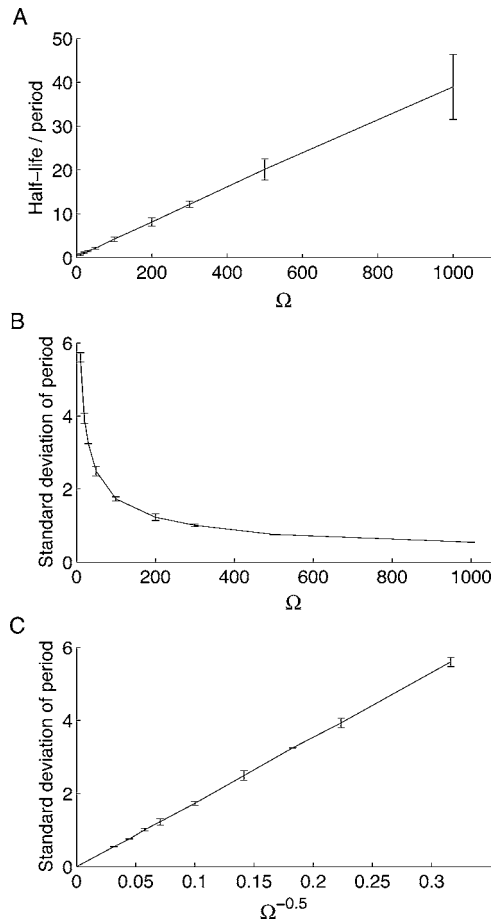


FIG. 3. (A) Half-life of the autocorrelation function and (B) standard deviation of the period distribution as a function of the number of molecules, Ω . The standard deviation of the period distribution shows a linear relationship as a function of $1/\sqrt{\Omega}$ (C). Each point is the averaged measure over five runs. Each run corresponds to a time series of 25 000 h. The vertical bars indicate the standard deviation of the five measurements.

with analytical predictions on the effect of noise on limit cycle oscillations.²¹ The half-life becomes smaller than the mean period for values of Ω below 40, reflecting the take-over of periodicity by noise even though some remnant of circadian behavior is still noticeable for values of Ω as low as 10. Another measure of the effect of noise is provided by the standard deviation of the distribution of the periods (see histograms in the fourth column in Fig. 2). This standard deviation decreases with Ω [Fig. 3(B)] and increases linearly with $1/\sqrt{\Omega}$ [Fig. 3(C)].

In the above simulations the number of mRNA molecules is of the order of the number of protein molecules. The question arises as to how a relative decrease in the number of mRNA molecules, at a given number of protein molecules affects the robustness of circadian oscillations. To assess the role of the relative amounts of mRNA and protein molecules on the robustness of the oscillations, we define a parameter ε which controls the ratio of protein to mRNA. Thus, without changing P_C and P_N , we divide M by ε without changing the dynamics of the system, by dividing by ε parameters v_s and v_m , as well as K_m , and by multiplying k_s by ε . The effect depends on the value of Ω , i.e., on the number of molecules present in the system. Shown in Fig. 4

is the effect of a progressive decrease in M with respect to P_C at a given value $\Omega=100$. The effect of noise increases as the ratio M/P_C decreases, but this appears to be due primarily to a decrease in the absolute level of M . This conclusion is corroborated by the observation that the decrease in robustness when ε increases [Fig. 5(A)] is stronger for $\Omega=100$ as compared to $\Omega=1000$ [Fig. 5(B)].

V. ROLE OF COOPERATIVITY

In the above simulations, we considered a degree of cooperativity of four. This assumption corresponds to a scheme in which four molecules of nuclear protein bind successively to the gene promoter to repress its transcription, with the additional condition that the affinity of binding increases with the number of protein molecules already bound. Cooperativity, however, is not required for oscillatory behavior. Indeed circadian oscillations can already occur if repression involves the binding of a single molecule of nuclear protein, but the domain of sustained oscillations in parameter space becomes larger when the degree of cooperativity increases. In previous studies where the reaction scheme was fully developed into elementary steps, the successive binding of multiple repressor molecules was described explicitly, and several degrees of cooperativity n were considered. The stochastic simulations of the different cases showed that the robustness of the oscillations decreases in absence of cooperativity ($n=1$), and significantly increases as soon as $n>1$. It does not vary much when n increases above the value of 2, but passes through a maximum around $n=3$.⁹ Similar results were obtained with the undeveloped stochastic version of the core model considered here, both analytically and by means of numerical simulations.²² The variation in half-life as well as changes in standard deviation of the period thus indicate that cooperative repression enhances the robustness of circadian oscillations with respect to molecular noise.

VI. DISTANCE FROM A BIFURCATION POINT

The question arises as to whether the proximity from a bifurcation point may influence the robustness of circadian oscillations with respect to molecular noise. To address this issue we first constructed in Fig. 6 a bifurcation diagram showing the onset of limit cycle oscillations as a function of the maximum transcription rate, v_s , for the deterministic model described by Eqs. (1). Below $v_s=0.6$ nM h⁻¹ the system evolves toward a stable steady state. Above this critical bifurcation value, limit cycle oscillations occur. The diagram shows the envelope of the oscillations: the upper and lower branches correspond to the maximum and minimum values of the clock gene mRNA concentration (M) as a function of v_s in the course of sustained oscillations.

The results of stochastic simulations performed for increasing values of parameter v_s are shown in Fig. 7 in the form of time series (left panels) or trajectories in the phase plane (middle panels), together with time course of autocorrelations (right panels). The steady state or limit cycle predicted by the deterministic model for the corresponding parameter values is depicted in the phase plane by a white dot or a white solid curve, respectively. The four rows in Fig. 7

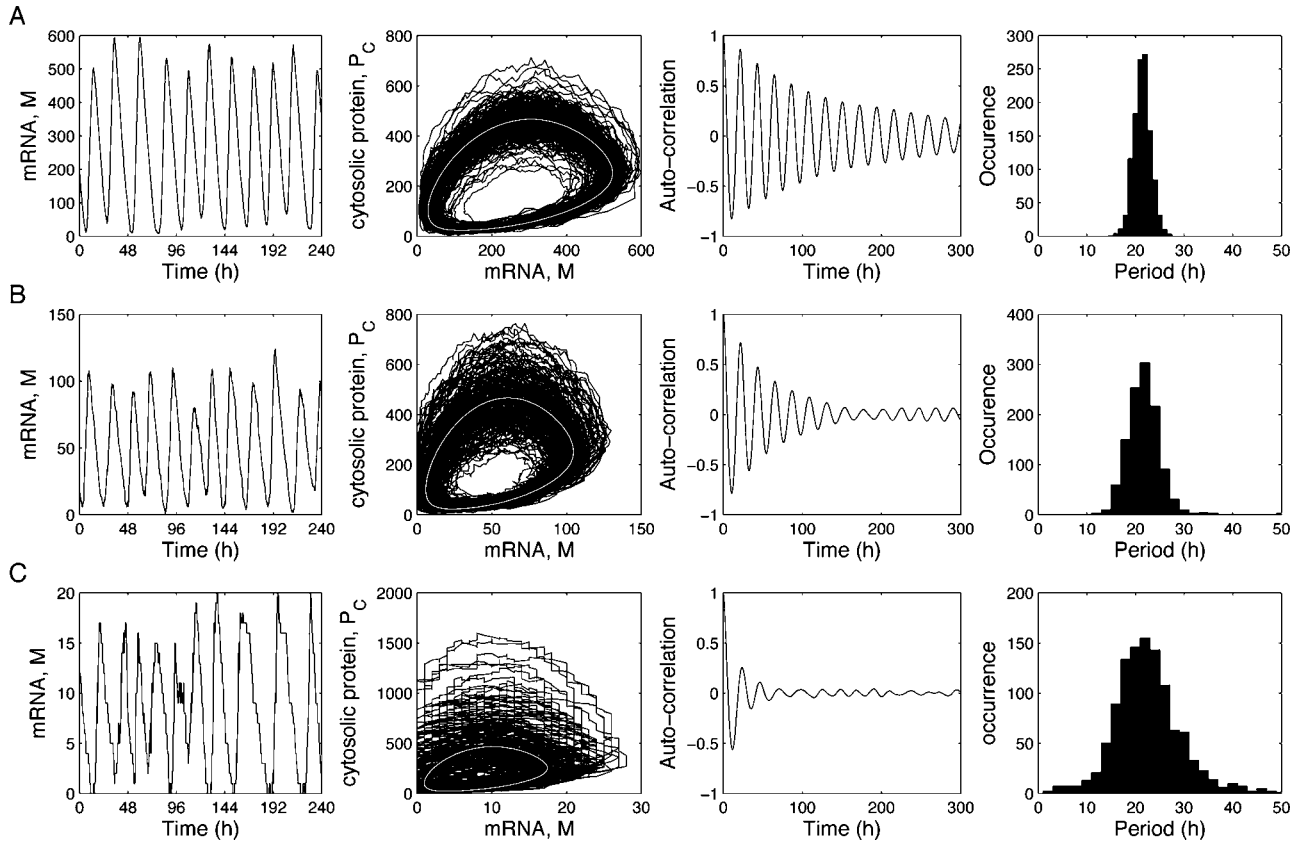


FIG. 4. Effect of the ratio $\varepsilon = \text{protein}/\text{mRNA}$ on the robustness of the oscillations. Simulations have been performed for $\Omega=100$ and (A) $\varepsilon=1$, (B) $\varepsilon=5$, and (C) $\varepsilon=30$. In Eq. (1), in order to incorporate the ratio ε , the following changes have been made: $v_s \rightarrow v_s/\varepsilon$, $v_m \rightarrow v_m/\varepsilon$, $K_m \rightarrow K_m/\varepsilon$, and $k_s \rightarrow \varepsilon k_s$. This transformation leads to a value of M rescaled by ε . $M \rightarrow M/\varepsilon$ while P_C and P_N are not rescaled. Parameter values are as in Fig. 2.

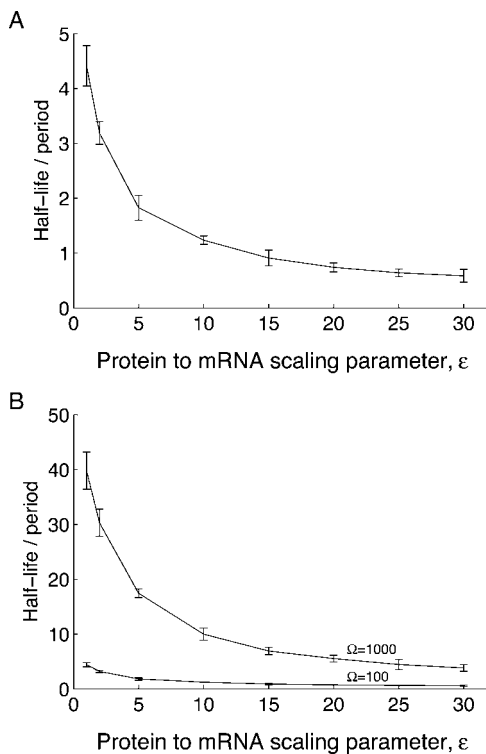


FIG. 5. Half-life of the autocorrelation function as a function of the ratio $\varepsilon = \text{protein}/\text{mRNA}$ level, for (A) $\Omega=100$, and (B) $\Omega=100$ and $\Omega=1000$.

correspond to the four v_s values indicated by dashed vertical lines in Fig. 6. For $v_s=0.1 \text{ nM h}^{-1}$ the deterministic system evolves to a stable steady state far from the bifurcation point. Stochastic simulations then show low-amplitude fluctuations around the deterministic steady state [Fig. 7(A)]. For $v_s=0.55 \text{ nM h}^{-1}$ the deterministic system evolves to a stable steady state close to the bifurcation point, and stochastic simulations show fluctuations of larger amplitude around the

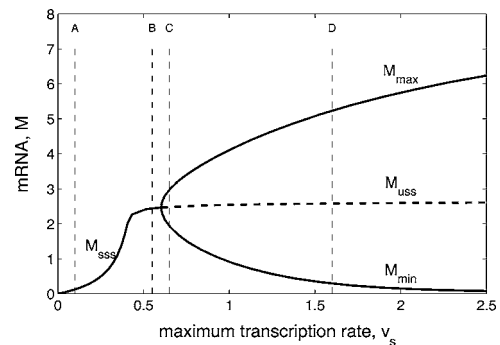


FIG. 6. Bifurcation diagram showing the onset of circadian oscillations in the deterministic model as a function of parameter v_s which measures the maximum transcription rate. The curve shows the steady-state level of the clock gene mRNA, stable (solid line, denoted by M_{SS}) or unstable (dashed line, M_{USS}), as well as the maximum and minimum concentration of mRNA in the course of sustained circadian oscillations (M_{max} and M_{min}). The vertical dashed lines refer to the four values considered for v_s in Fig. 7. Parameter values are as in Fig. 2.

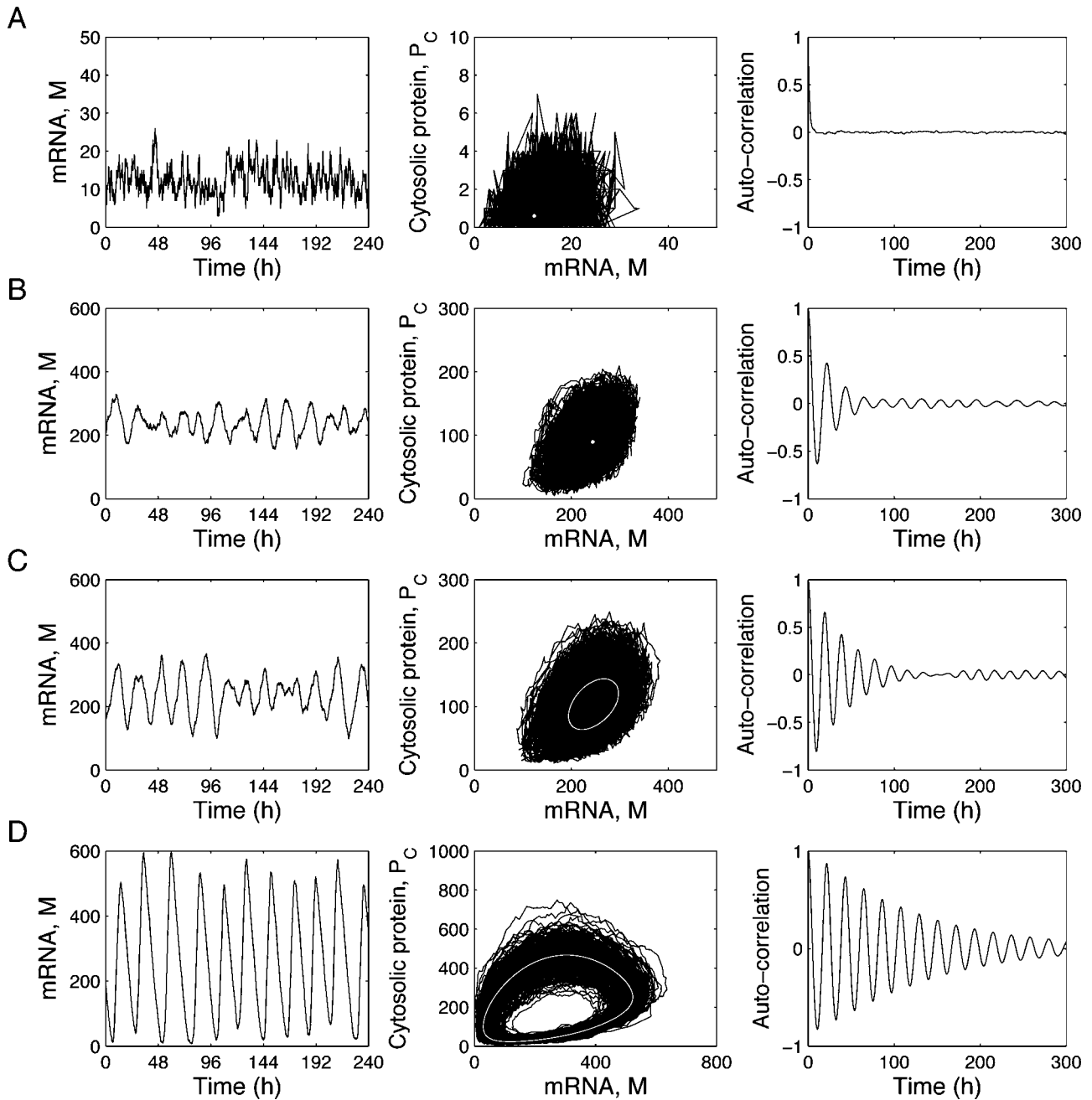


FIG. 7. Effect of the proximity from a bifurcation point on the effect of molecular noise in the stochastic model for circadian rhythms. The different panels are established for the four increasing values of parameter v_s shown in Fig. 6: (A) $v_s=0.1$, (B) 0.55, (C) 0.65, and (D) 1.6 nM h^{-1} (D). These results have been obtained for $\Omega=100$. In the middle panels in (A) and (B) the white dot represents the stable steady state predicted by the deterministic version of the model in corresponding conditions. In (C) and (D) the thick white curve represents the limit cycle predicted by the deterministic version of the model.

deterministic steady state [Fig. 7(B)]. For $v_s=0.65 \text{ nM h}^{-1}$ the deterministic system undergoes limit cycle oscillations of reduced amplitude just beyond the bifurcation point: stochastic simulations show small amplitude, noisy oscillations around the deterministic limit cycle [Fig. 7(C)], which resemble the fluctuations shown in Fig. 7(B). Finally, for $v_s=1.6 \text{ nM h}^{-1}$ the deterministic system undergoes limit cycle oscillations of large amplitude far from the bifurcation point. Stochastic simulations then show large-amplitude, relatively less noisy oscillations around the deterministic limit cycle [Fig. 7(D)].

These results corroborate those obtained^{9,16,17} for the fully developed stochastic version of the five-variable core model for circadian oscillations in showing that below a critical parameter value the system displays low-amplitude fluctuations around a stable steady state, while above this value sustained oscillations occur around an unstable steady state. The effect of fluctuations due to molecular noise decreases as the system moves further away from the bifurcation point into the domain of sustained oscillations.

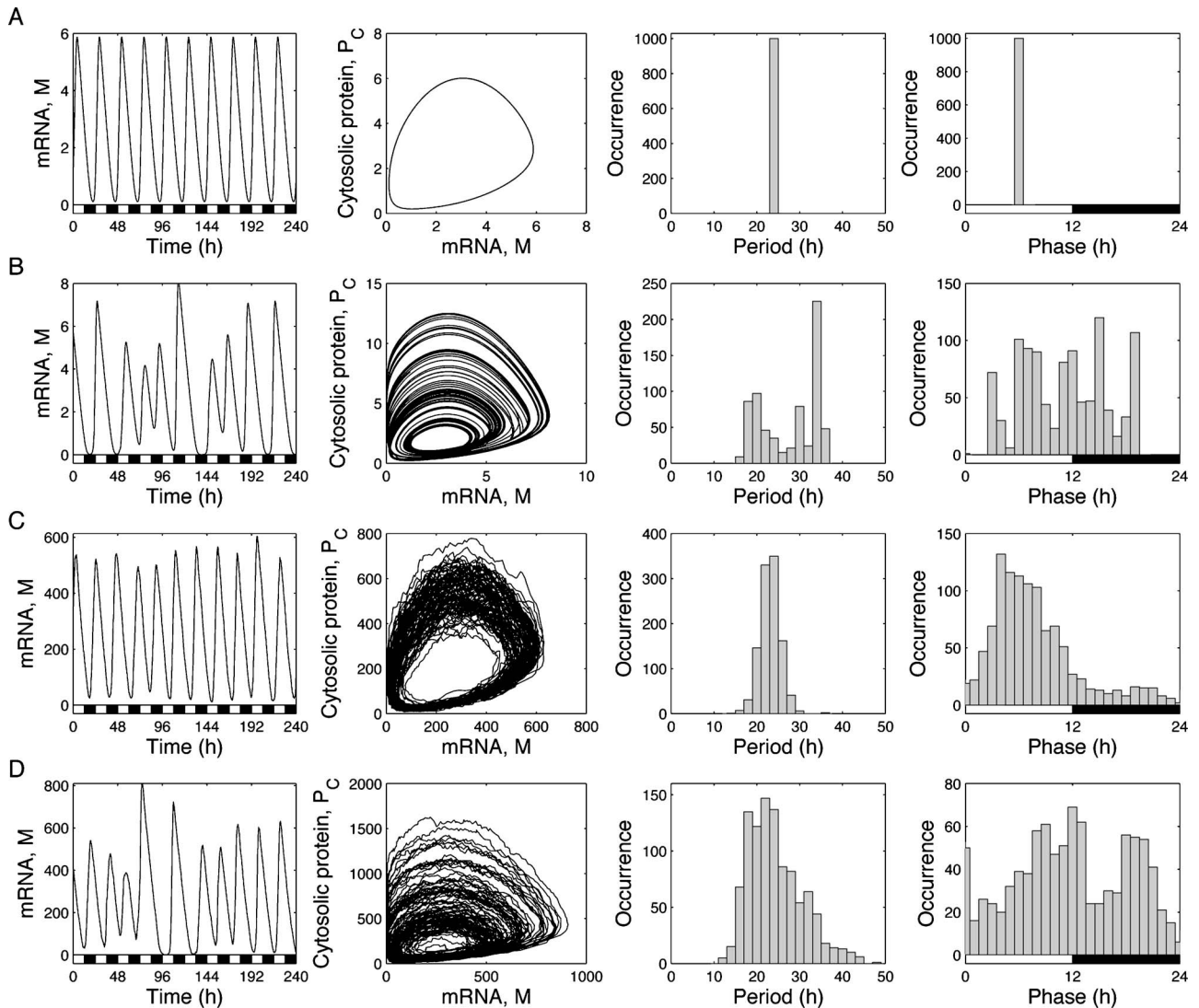


FIG. 8. Effect of molecular noise on circadian oscillations under conditions of periodic forcing by a light-dark cycle. Shown are the circadian oscillations in the number of mRNA molecules (left column), the corresponding limit cycle (second column), the histogram of periods (third column), and the histogram of phases (last column). The phase is defined as the maximum of mRNA (M) over a given LD cycle. The white and black rectangles denote the light and dark phases, respectively. Periodic forcing is achieved by setting during each light phase the parameter measuring the maximum transcription rate, v_s , to 2.2 nM h⁻¹ (A) and (C), or to 4.2 nM h⁻¹ (B) and (D). (A), (B) Deterministic results, (C), (D) stochastic results, obtained for $\Omega=100$. In darkness, v_s is maintained at its basal value, 1.6 nM h⁻¹. Other parameters values are as in Fig. 2. Histograms have been determined for about 1000 successive cycles. These results have to be compared to those obtained in absence of periodic forcing (Fig. 2).

VII. ENTRAINMENT BY A LIGHT-DARK CYCLE

Circadian rhythms are permanently subjected to periodic forcing by the external light-dark (LD) cycle. Therefore it is of interest to check whether entrainment by the LD cycle can occur in the presence of low numbers of molecules. What is the effect of periodic forcing on the robustness of circadian oscillations with respect to molecular noise? By means of stochastic simulations, we determined the characteristics of oscillations after entrainment by a 12:12 h LD cycle (Fig. 8). The effect of light is incorporated by considering that it enhances the rate of transcription of the clock gene, v_s , as observed in *Neurospora* and mammals.^{3,4}

The results indicate that as in the deterministic case [Fig. 8(A)] the circadian clock mechanism can be entrained by the 24 h LD cycle in the presence of molecular noise [Fig. 8(C)]. The mean period indeed is shifted from about 22 h before

entrainment to 24 h upon entrainment. The standard deviation of the period (third column) is not affected significantly by entrainment. The most striking effect of periodic forcing is to stabilize the phase of the oscillations (fourth column) as shown in Fig. 8(C) for the distribution of the phase defined by the time at which mRNA reaches its maximum over a LD cycle.

Deterministic simulations of the model previously showed that periodic forcing could lead to nonautonomous chaos.²³ Such aperiodic behavior occurs when the strength of the forcing, measured by the value of v_s in the light phase, $v_{s,max}$, is sufficiently large, as illustrated by the evolution to a strange attractor in Fig. 8(B) (second column). Stochastic simulations performed in corresponding conditions show the evolution toward an oscillatory regime characterized by increased variability in period, amplitude and phase [Fig. 8(D)]

as compared to the results of stochastic simulations of periodic oscillations [Fig. 8(C)]. Similar results are obtained when entrainment occurs, as in *Drosophila*, via the enhancement by light of the degradation of a clock protein (data not shown).

VIII. RATE OF BINDING TO THE GENE PROMOTER

In the case of the fully developed model, the binding of multiple repressor molecules to the gene promoter of the clock protein is decomposed into elementary steps and the rate constants for binding and unbinding of the repressor therefore appear explicitly in the kinetic treatment. Thus in the case of cooperative binding of four repressor molecules, we have considered four successive steps of association and dissociation characterized by the rate constants a_i and d_i ($i = 1, \dots, 4$). These kinetic parameters have a significant influence on the robustness of circadian oscillations.¹⁷ When the values of these rate constants decrease below a threshold, the system evolves toward a stable steady state, but this steady state is excitable: a small perturbation bringing the system slightly away from the steady state triggers a large excursion in the phase space, which corresponds to a burst of transcriptional activity, before the system returns to the stable steady state. Stochastic simulations performed in these conditions yield largely irregular oscillations. Such irregular oscillations reflect repetitive, noise-induced large excursions away from a stable, excitable steady state.¹⁷ The values of the bimolecular rate constants a_i used by other authors⁸ for simulating the circadian models based on negative autoregulation of gene expression were likely below the critical value corresponding to sustained oscillations.^{9,17} This may explain the lack of robustness observed in their stochastic simulations of circadian oscillations. Our findings that robust oscillations require high binding/unbinding rates are in agreement with the results obtained by other authors for a detailed model of the mammalian circadian clock.²⁴

IX. COUPLING CIRCADIAN OSCILLATORS

Although the oscillations are already produced at the level of a single cell, the generation of a global circadian output is possible only if individual rhythms within a tissue or an organism are synchronized. In the absence of an external signal, this synchronization requires a coupling between cells. In mammals, the central pacemaker is located in the suprachiasmatic nuclei (SCN) of the hypothalamus. The neurons of the SCN are coupled through neurotransmitters. Coupling between circadian oscillators is also manifest in the fungus *Neurospora*. A peculiar property of this fungus is its syncytial morphology: several nuclei are present in a single cell. This suggests that the *Neurospora* clock involves the coupling of multiple oscillators through a set of common variables, the cytosolic forms of the clock gene mRNA and of the clock protein. The latter can enter into any nucleus present in the syncytium.

The question arises as to whether intercellular coupling within a population of cells also contributes to increasing the robustness of circadian oscillations with respect to molecular noise. To test this hypothesis, we will describe the coupling

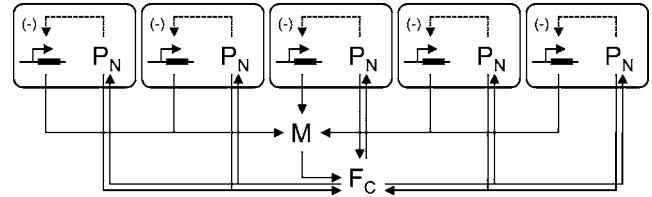


FIG. 9. Scheme of the coupled oscillators model. mRNA (M) and cytosolic protein (P_C) are treated as global variables, while nuclear protein (P_N) is treated as a local variable.

in *Neurospora* in a simple manner. Since several nuclei are present in a given cell, we treat the cytosolic clock gene mRNA (M) and clock protein (P_C) as global variables, while the nuclear form of the protein (P_N) is treated as a local variable, i.e., its concentration is defined in each nucleus [denoted by the superscript (i)] (Fig. 9). We refer to this type of coupling as global coupling. $P_N^{(i)}$ then refers to the concentration of P_N in nucleus i . If N nuclei are considered, the $N+2$ deterministic equations for such a globally coupled model are:

$$\begin{aligned} \frac{dM}{dt} &= v_s \left(\sum_{i=1}^N \frac{K_I^n}{K_I^n + P_N^{(i)n}} \right) - v_m \frac{M}{K_m + M}, \\ \frac{dP_C}{dt} &= k_s M - v_d \frac{P_C}{K_d + P_C} - k_1 P_C + k_2 \left(\sum_{i=1}^N P_N^{(i)} \right), \\ \frac{dP_N^{(i)}}{dt} &= \frac{1}{N} k_1 P_C - k_2 P_N^{(i)}. \end{aligned} \tag{2}$$

The term $1/N$ in the third equation expresses the fact that when N nuclei are present, on average only a fraction ($1/N$) of cytosolic protein can enter a given nucleus. The stochastic version of the coupled model (2) is given in Table II.

In Fig. 10, we show the time series of cytosolic mRNA, M (left column) and nuclear clock protein in each nucleus, $P_N^{(i)}$ (right column) for a system of five coupled oscillators. Three different values of Ω are considered here, (A) $\Omega = 500$, (B) $\Omega = 100$, and (C) $\Omega = 10$. At first sight, we see that the global coupling leads to more robust oscillations compared to the single cell model (Fig. 2). To quantify the ro-

TABLE II. Stochastic version of the globally coupled model. There are $4 + 2N$ reaction steps.

No.	Reaction	Transition rate
1	$\rightarrow M$	$w_1 = v_s \Omega \sum_{i=1}^N \frac{(K_I \Omega)^n}{(K_I \Omega)^n + P_N^{(i)n}}$
2	$M \rightarrow$	$w_2 = v_m \Omega \frac{M}{K_m \Omega + M}$
3	$\rightarrow P_C$	$w_3 = k_s M$
4	$P_C \rightarrow$	$w_4 = v_d \Omega \frac{P_C}{K_d \Omega + P_C}$
5	$P_C \rightarrow P_N^{(i)}$	$w_5^{(i)} = \frac{1}{N} k_1 P_C$
6	$P_N^{(i)} \rightarrow P_C$	$w_6^{(i)} = k_2 P_N^{(i)}$

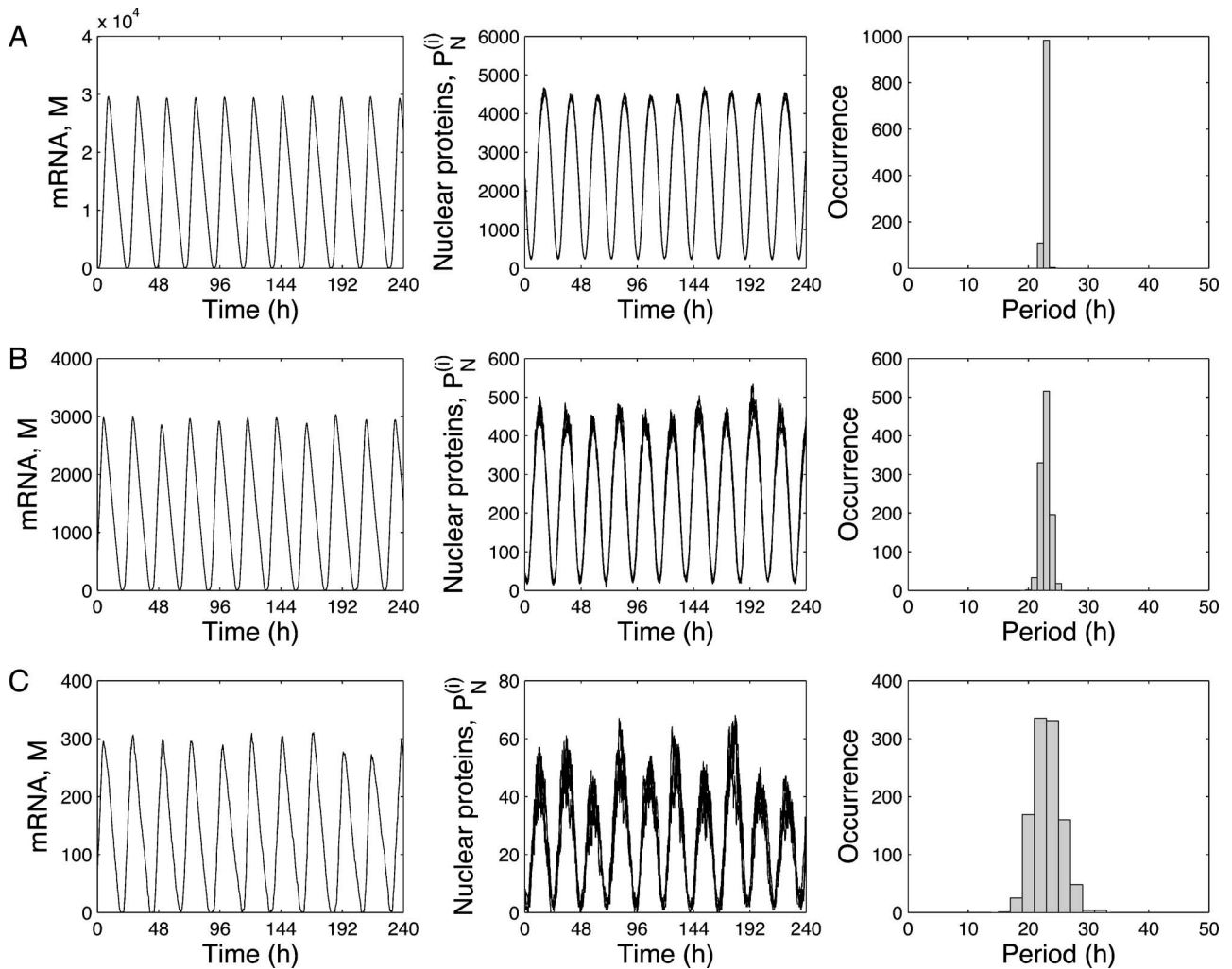


FIG. 10. Stochastic oscillations obtained by simulation of the five coupled oscillators model for circadian rhythms. The panels show oscillations of mRNA concentration, M (left column), and of the five nuclear clock proteins, $P_N^{(i)}$, ($i=1, \dots, 5$) (middle column), and the distribution of periods (right column) for (A) $\Omega=1000$, (B) $\Omega=100$, and (C) $\Omega=10$. Other parameter values are as in Fig. 2 except v_m and v_d which were multiplied by $N=5$ in order to keep sustained oscillatory behavior.

business, we calculated the half-life of the autocorrelation function, as a function of Ω (Fig. 11). As for the single-cell model, a linear relationship between the half-life of the autocorrelation function and Ω is observed [Fig. 11(A)]. The slope of this line gives an estimation of the robustness of the oscillations. The higher the slope, the more robust the oscillations. The comparison of the slopes obtained for the single cell and for the coupled models confirms the visual observation that the coupling leads to more robust oscillations. Similar conclusions are drawn from the linear relation between the standard deviation of the period and $1/\sqrt{\Omega}$ [Fig. 11(B)]. The standard deviation decreases and the oscillations become more coherent as the value of Ω increases.

Finally we assessed the effect of the number N of coupled oscillators on the robustness of the oscillations. In Fig. 12, we plotted the half-life of the autocorrelation function, and standard deviation of the period as a function of N . This result indicates that the robustness of the oscillations increases proportionally with the number of oscillators. In support of this conclusion the standard deviation of the period displays a linear dependence on $1/\sqrt{N}$. Such an increase

in robustness with the number of coupled circadian oscillators was already reported in a more abstract model of locally coupled oscillators.²⁵

X. DISCUSSION

During the last two decades, experimental studies have unraveled the molecular mechanism of circadian rhythms in numerous organisms.¹⁻⁴ Initially pertaining to the fly *Drosophila*²⁶ and the fungus *Neurospora*,²⁷ molecular studies of circadian rhythms have since been extended to cyanobacteria,²⁸ plants,²⁹ and mammals.³⁰ Remarkably, in all cases investigated, the molecular mechanism of circadian oscillations relies on the negative autoregulation exerted by a protein on the expression of its gene.^{3,4} Based on experimentally determined mechanisms, mathematical models of increasing complexity have been proposed for these rhythms.^{5-7,14} Computational approaches throw light on the precise conditions in which circadian oscillations occur as a result of genetic regulation. The models also account for a variety of properties of circadian rhythms, such as phase

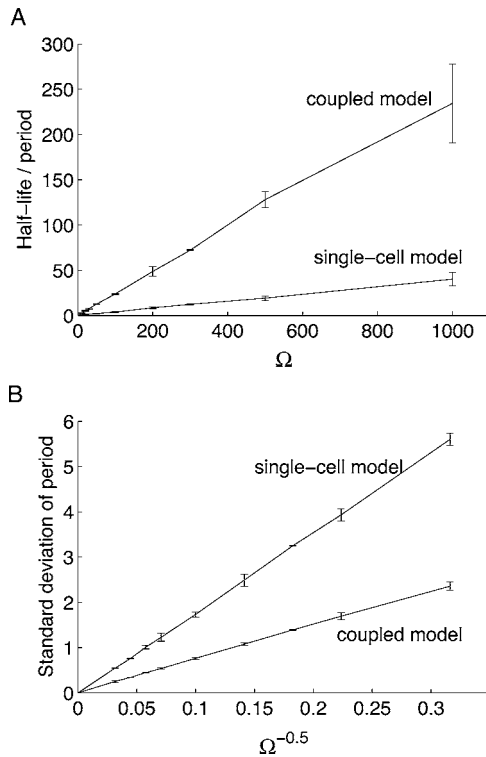


FIG. 11. (A) Half-life of the autocorrelation function as a function of the number of molecules, Ω , and (B) standard deviation of the period distribution as a function of $1/\sqrt{\Omega}$, in the case of five coupled oscillators. Each point is the averaged measure over five runs. Each run corresponds to time series of 25 000 h. The vertical bars indicate the standard deviation of the five measurements. These results have to be compared with those obtained for the single-cell oscillator (Fig. 3).

shifting or long-term suppression by light pulses, entrainment by light-dark cycles, and temperature compensation.

These models generally use ordinary differential equations to describe the time evolution of the concentration of mRNA and proteins. In such a deterministic description, we assume that the molecules are present in sufficiently large amounts so that the fluctuations are averaged out. However, in a cell the number of molecules involved in the oscillatory mechanism might be small. From the data of Bae *et al.*³¹ collected from *Drosophila* heads, when assuming a cell volume of 10^{-13} l, we may estimate that the number of dClock protein molecules in *Drosophila* is about 100 per cell. In *Neurospora* it was shown that a few tens of nuclear *FRQ* protein are sufficient to produce circadian oscillations.³² In view of such small numbers of molecules, the question arises as to whether deterministic models are appropriate for the description of circadian clocks.⁸ Indeed, when the numbers of molecules of protein or mRNA involved in the oscillatory mechanism are very low, the effect of molecular noise ceases to be negligible. To assess the effect of the molecular noise on circadian oscillations, it is necessary to resort to stochastic approaches. In this paper we discussed the role of several factors that affect the robustness of the oscillations with respect to molecular noise. For this purpose we used a simple, three variable model which is based on the negative feedback exerted by a clock protein on the expression of its gene. Similar results have been obtained with the five variable

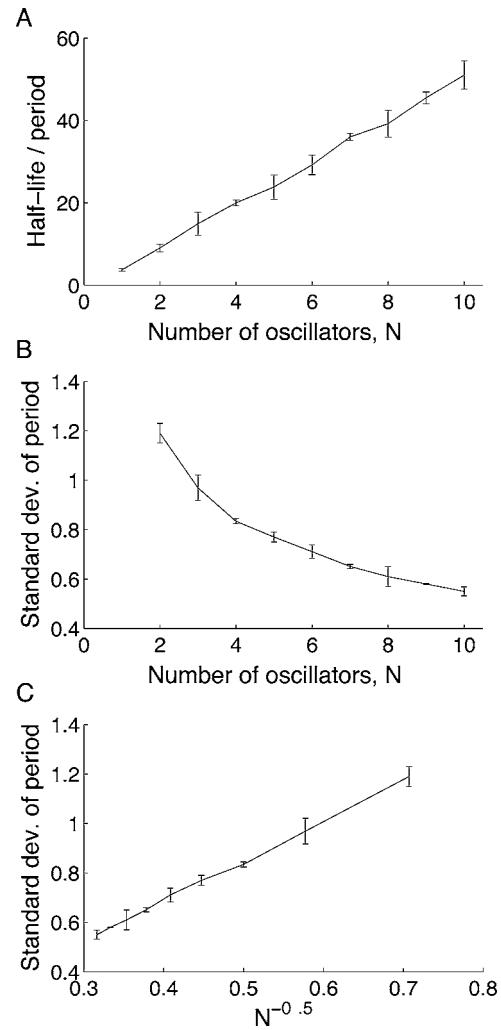


FIG. 12. (A) Half-life of the autocorrelation function as a function of the number of coupled oscillators, N , and (B) standard deviation of the period distribution as a function of N . Each point is the averaged measure over five runs. Each run corresponds to time series of 25 000 h. The vertical bars indicate the standard deviation of the five measurements.

model and the ten variable models proposed for the circadian clock of *Drosophila*.^{9,16,17,33} The stochastic version of the three variable model was simulated using the stochastic algorithm introduced by Gillespie.¹⁹

Our simulations showed that robust circadian oscillations can already occur with a limited number of mRNA and protein molecules, in the range of some hundreds. When the ratio mRNA/protein diminishes upon holding the protein constant, the effect of noise is enhanced because the absolute number of mRNA molecules decreases. This effect can be overcome by increasing the total number of molecules. We showed, as in more complex models,^{9,16,33} that entrainment by light-dark cycles and cooperativity in repression enhance the robustness of circadian oscillations with respect to molecular noise, whereas the proximity of a bifurcation point leads to less robust oscillations. In a more detailed model,¹⁷ we studied the effect of another parameter, the binding/unbinding rate of the inhibitory protein to the promoter of the clock gene. This analysis revealed that decreasing these reaction rates below a threshold value leads to highly erratic

oscillations, because of the excitable nature of the steady state obtained in these conditions.

Finally, we studied the effect of the coupling of circadian oscillators on the robustness of the oscillations. We focused here on the case of the *Neurospora* circadian system. The syncytial morphology of this fungus is characterized by the presence of several nuclei in a single cell. The coupling is thus achieved by considering that, in a given cell, the cytosolic protein can enter any of the nuclei. Stochastic simulations of this model showed that such a coupling further contributes to increasing the robustness of the oscillations. In other organisms, the nature of the coupling is different. In mammals for example, the rhythm-producing neurons in the SCN are coupled through neurotransmitters.³⁴ Corroborating conclusions reached in nonmolecular models for circadian rhythms,²⁵ the present results suggest that such coupling plays an important role in increasing the robustness of circadian oscillations.

ACKNOWLEDGMENTS

This work was supported by Grant No. 3.4636.04 from the *Fonds de la Recherche Scientifique Médicale* (F.R.S.M., Belgium), and by the European Union through the Network of Excellence BioSim, Contract No. LSHB-CT-2004-005137. D.G. is *Chargé de Recherches du Fonds National de la Recherche Scientifique* (F.N.R.S., Belgium).

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