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Journal of Theoretical Biology 242 (2006) 478–488
Journal of Theoretical Biology
www.elsevier.com/locate/yjtbi

Amplitude of circadian oscillations entrained by 24-h light–dark cycles

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Received 16 August 2005; received in revised form 19 March 2006; accepted 21 March 2006
Available online 28 March 2006

Abstract

An intriguing property of circadian clocks is that their free-running period is not exactly 24 h. Using models for circadian rhythms in *Neurospora* and *Drosophila*, we determine how the entrainment of these rhythms is affected by the free-running period and by the amplitude of the external light–dark cycle. We first consider the model for *Neurospora*, in which light acts by inducing the expression of a clock gene. We show that the amplitude of the oscillations of the clock protein entrained by light–dark cycles is maximized when the free-running period is smaller than 24 h. Moreover, if the amplitude of the light–dark cycle is very strong, complex oscillations occur when the free-running period is close to 24 h. In the model for circadian rhythms in *Drosophila*, light acts by enhancing the degradation of a clock protein. We show that while the amplitude of circadian oscillations entrained by light–dark cycles is also maximized if the free-running period is smaller than 24 h, the range of entrainment is centered around 24 h in this model. We discuss the physiological relevance of these results in regard to the setting of the free-running period of the circadian clock.

Keywords: Circadian rhythm; Entrainment; Free-running period; Amplitude; light–dark cycle

1. Introduction

Circadian rhythms are observed in many species, from some prokaryotes to eukaryotes. These endogenous rhythms are caused by feedback regulation of a number of so-called clock genes (Dunlap, 1999). In *Neurospora*, the *frq* gene is transcribed into *frq*-mRNA which is translated into FRQ protein in the cytoplasm. FRQ proteins enter the nucleus to suppress the transcription of the *frq* gene (Crosthwaite et al., 1997). A similar negative feedback regulation of clock genes also underlies circadian rhythm generation in *Drosophila* and mammals, where PER proteins indirectly repress the expression of the *period* (*per*) gene (Glossop et al., 1999; Shearman et al., 2000).

The autonomous oscillation of clock proteins at the cellular level underlies the occurrence of circadian rhythms at the behavioral level. Allada et al. (2003) described a mutant allele of *Drosophila Clk*, Clk^{ar}, that is behaviorally arrhythmic although molecular oscillations persist with reduced amplitude. This experimental finding suggests that large-amplitude oscillations of mRNAs and proteins at the cellular level are required for the generation of circadian rhythms at the behavioral level. Gorl et al. (2001) depicted a *Neurospora* strain lacking the PEST-1 element in the *frq* gene. Overt rhythmicity is lost in this mutant, although molecular oscillations of FRQ persist with a prolonged free-running period of 28 h. How the generation of circadian rhythms at the behavioral level is affected by the change in FRQ remains unknown.

Mathematical models for circadian rhythms based on negative autoregulation of gene expression have first been proposed for circadian rhythms in *Drosophila* (Goldbeter, 1995; Leloup and Goldbeter, 1998). In the first of these models, PER proteins negatively regulate the transcription of the *per* gene. This regulatory mechanism is capable of producing sustained circadian oscillations under constant conditions, e.g. continuous darkness. A second model incorporates the formation of a complex between the PER and TIM proteins, as well as the effect of light, which is to elicit TIM degradation. A variety of theoretical models have subsequently been proposed for circadian rhythms in

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doi:10.1016/j.jtbi.2006.03.016

Drosophila (Kurosawa et al., 2002; Smolen et al., 2002; Tyson et al., 1999; Ueda et al., 2001), *Neurospora* (François, 2005; Gonze and Goldbeter, 2000; Leloup et al., 1999; Ruoff and Rensing, 1996), and mammals (Forger and Peskin, 2003; Leloup and Goldbeter, 2003, 2004). Recent models for *Drosophila* and mammals include positive regulation in addition to negative autoregulatory feedback on gene expression. All of these molecular models for circadian rhythms can account for the generation of sustained oscillations in constant darkness and most can display entrainment by light–dark cycles consisting of a 12 h light phase followed by a 12 h dark phase (abbreviated as 12:12 LD cycles) for appropriate parameter values. A previous theoretical study (Gonze and Goldbeter, 2000) raised the possibility that complex oscillations and even aperiodic oscillations in the form of chaos may occur if the amplitude of the forcing LD cycles becomes sufficiently large.

A conspicuous property of circadian clocks is that their free-running period is not exactly 24 h. Thus the free-running period is about 21.5 h in *Neurospora* (Garceau et al., 1997), about 28 h in *Phaseolus* (Bunning and Moser, 1972), close to 24.4 h in *Drosophila* (Konopka and Benzer, 1971), close to 25.6 h in zebrafish (Cahill et al., 1998), and of the order of 24.18 h in humans (Czeisler et al., 1999). The free-running period thus differs from 24 h for most species. This is quite puzzling because if their free-running period were exactly 24 h, organisms would not need to shift the phase of their endogenous rhythm every day in order to synchronize with the environmental rhythm.

Why is the free-running period often different from 24 h? Pittendrigh and Daan (1976) showed that the stable entrainment to the natural 24 h LD cycle becomes more difficult the closer an animal's natural period is from 24 h (see also Moore-Ede et al., 1982). By using measured phase response curves of rodents and an abstract model for oscillations, they demonstrated that the variation of the phase angle between the circadian pacemaker and the LD cycle becomes very large when the free-running period is close to 24 h (Pittendrigh and Daan, 1976). On the basis of population genetics models, Daido (2001) suggested that it can be beneficial for species to have free-running periods different from 24 h. However, it remains unclear why the free-running period of the circadian clock is of the order of 21.5 h in *Neurospora*, and 24.2 h in humans, instead of precisely 24 h.

In the present paper, we use models for circadian rhythms in *Neurospora* and *Drosophila* to study how the entrainment of these rhythms is affected by the free-running period and by the amplitude of the external LD cycle. We first consider the model for *Neurospora*, in which lights act by inducing the expression of the *frq* gene. By numerical analysis, we show that the amplitude of the oscillations of the clock protein FRQ entrained by LD cycles is maximized when the free-running period is smaller than 24 h. We investigate how complex oscillations—including chaos—occur as a function of the strength and period of the forcing by LD cycles. We then turn to the model for circadian rhythms in *Drosophila*, in which light acts by enhancing the degradation of a clock protein. We show that while the amplitude of PER oscillations entrained by LD cycles is also maximized if the free-running period is smaller than 24 h, the range of entrainment is centered around 24 h in this model. Finally, we discuss the physiological relevance of these results in regard to the setting of the free-running period of circadian oscillations.

2. Minimal model for the *Neurospora* circadian clock

We first consider a minimal, three-variable model for the gene–protein network producing circadian rhythms in *Neurospora* (Gonze et al., 2000; Leloup et al., 1999). The clock gene, *frq*, is transcribed into *frq* mRNA, which is translated into FRQ protein. FRQ proteins enter the nucleus where they inhibit the transcription of the *frq* gene. The products of other genes, such as *wc-1* and *wc-2*, which are known to be involved in the oscillatory mechanism, are disregarded in this minimal model. Moreover, the role of FRQ phosphorylation is not considered.

The dynamics of the gene–protein network are governed by the following kinetic equations (Gonze et al., 2000; Leloup et al., 1999):

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + F_N^n} - v_m \frac{M}{K_M + M}, \tag{1a}$$

$$\frac{dF_c}{dt} = k_s M - v_d \frac{F_c}{K_d + F_c} - k_1 F_c + k_2 F_N, \tag{1b}$$

$$\frac{dF_N}{dt} = k_1 F_c - k_2 F_N, \tag{1c}$$

where the concentrations of *frq* mRNA (M), cytoplasmic FRQ proteins (F_C), and nuclear FRQ proteins (F_N) are defined with respect to the total cell volume. The first term in Eq. (1a) is a Hill function describing negative feedback by F_N on gene transcription, while the other terms are of a linear type for translation of *frq* mRNA and for transport of FRQ into and out of the nucleus, and of Michaelis–Menten type for mRNA and protein degradation. Autonomous oscillations of FRQ proteins and *frq* mRNA can be obtained by numerical integration of Eqs. (1a)–(1c) in conditions corresponding to continuous darkness (DD). These oscillations correspond to the evolution toward a limit cycle, which is shown in Fig. 1B as a projection onto the phase plane (M, F_T) where F_T denotes the total protein concentration $F_C + F_N$. For the parameter set considered by Gonze et al. (2000), the free-running period is 21.5 h, which corresponds to the actual free-running period of circadian rhythms in *Neurospora*.

In order to discuss the effect of the free-running period on the amplitude of FRQ entrained by LD cycles, it is useful to transform Eqs. (1) by multiplying the right-hand side of all Eqs. (1) by τ_0/τ

$$\frac{dM}{dt} = \frac{\tau_0}{\tau}\left[v_s \frac{K_I^n}{K_I^n + F_N^n} - v_m \frac{M}{K_M + M}\right], \tag{2a}$$

$$\frac{dF_c}{dt} = \frac{\tau_0}{\tau}\left[k_s M - v_d \frac{F_c}{K_d + F_c} - k_1 F_c + k_2 F_N\right], \quad (2b)$$

$$\frac{dF_N}{dt} = \frac{\tau_0}{\tau}[k_1 F_c - k_2 F_N]. \quad (2c)$$

In Eqs. (2) τ_0 is 21.5 h, which is the autonomous period given by Eqs. (1) for the parameter set taken by Gonze et al. (2000) (see legend for Fig. 1). Moreover, τ denotes the free-running period (also referred to below as autonomous period) of Eqs. (2). Upon varying τ while all other parameters are fixed in Eqs. (2), autonomous oscillations produced by Eqs. (2a)–(2c) will always have the same amplitudes for M, F_C, and F_N but will be characterized by a different free-running period in DD (see gray curve in Fig. 1B).

2.1. Entrainment of circadian oscillations by 12 h:12 h LD cycles

In *Neurospora*, the precise biochemical processes underlying the light response are still not completely known. It was first shown that light enhances expression of the *frq* gene (Crosthwaite et al., 1995). A light pulse induces a 4–25-fold increase of *frq* mRNA. Here we shall focus on the entrainment by 12 h:12 h LD cycles, which correspond to the natural period of the environment and represent the LD cycle most commonly used in the experiments. We shall not address the effect of variations of the photoperiod.

To take into account the enhancement of the transcription rate during the light phase we consider that in Eq. (2a), the transcription rate v_s is fixed at the constant value $v_{s\max}$ during the light phase, where $v_{s\max}$ is defined by Eq. (3) (Gonze et al., 2000):

$$v_{s\,\max} = v_s(1+\delta) \quad \text{with} \quad \delta > 0. \quad (3)$$

We wish to determine the effect of changes in the free-running period τ on the amplitude of oscillations in FRQ proteins upon entrainment by LD cycles. The amplitude of FRQ in the course of sustained oscillations will be defined as $\mathrm{Max}[F_c + F_N] - \mathrm{Min}[F_c + F_N]$.

Heintzen et al. (2001) demonstrated that abundant *frq* mRNA is quickly degraded after *frq* expression is enhanced by light. Thus degradation of *frq* mRNA is also enhanced during the light phase, which is not incorporated in Eqs. (2) and (3) for simplicity. Taking into account implicitly the fast degradation of *frq* mRNA, we will consider light-triggered increases in gene expression smaller than those observed in the experiments. This is because larger increases in *frq* expression do not allow for entrainment by LD cycles. Later in this article, we will show that the conclusions derived from Eqs. (2) and (3) also hold for the model in which degradation of *frq* mRNA is increased during the light phase.

In Fig. 1A, we show oscillations entrained by a 12:12 LD cycle when the free-running period τ is equal to 21.6 and 25.6 h, respectively. The corresponding limit cycles are shown in Fig. 1B, together with the limit cycle under 12:12 LD when τ is equal to 24 h and the limit cycle in continuous darkness (which is the same regardless of the value of τ).

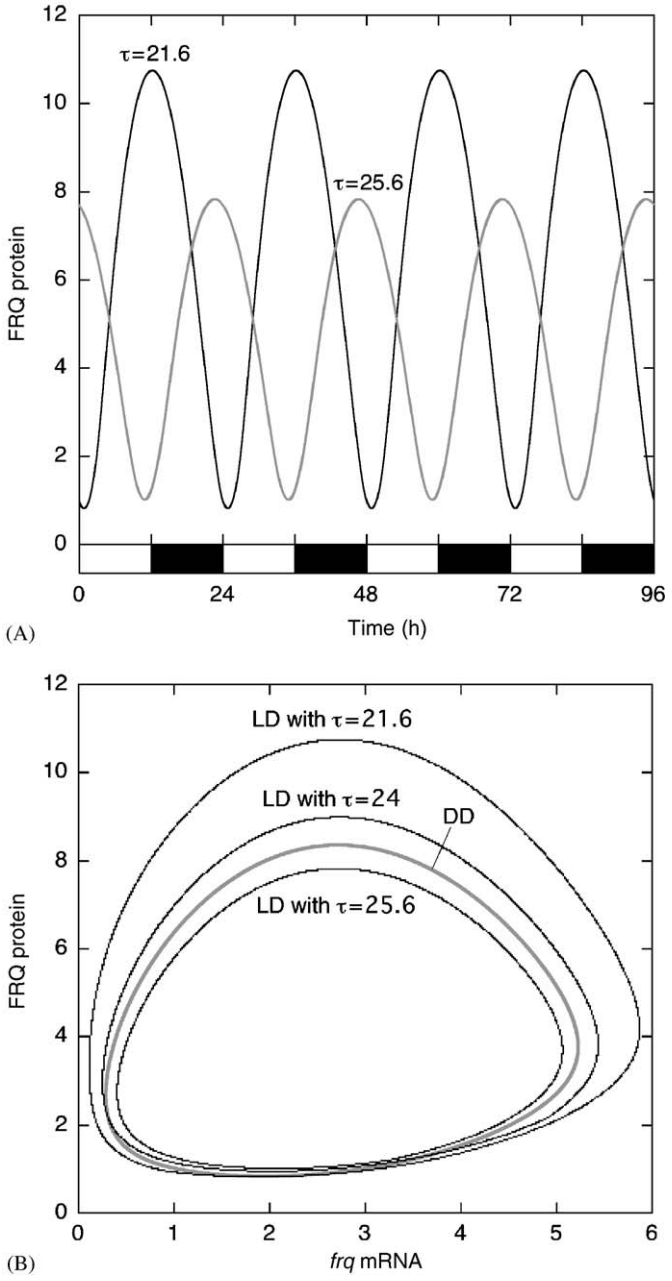


Fig. 1. Dynamics of the circadian oscillator model in *Neurospora* in continuous darkness or upon entrainment by 12 h:12 h light–dark (LD) cycles. (A) FRQ oscillations ($F_C + F_N$) in LD cycles when the free-running period τ is equal to 21.6 and 25.6 h, respectively. (B) The corresponding limit cycles together with the limit cycles under continuous darkness (DD) and under 12:12 LD cycles when τ is equal to 24 h. The limit cycle in DD is the same regardless of the value of τ. The curves are obtained by numerical integration of Eqs. (2). Parameter values are: $n = 4$, $v_m = 0.505\,\mathrm{nM\,h^{-1}}$, $v_d = 1.4\,\mathrm{nM\,h^{-1}}$, $k_s = 0.5\,\mathrm{h^{-1}}$, $k_1 = 0.5\,\mathrm{h^{-1}}$, $k_2 = 0.6\,\mathrm{h^{-1}}$, $K_I = 1.0\,\mathrm{nM}$, $K_m = 0.5\,\mathrm{nM}$, $K_d = 0.13\,\mathrm{nM}$. Parameter v_s (in $\mathrm{nM\,h^{-1}}$) remains constant and equal to 1.6 in panel (B) for DD, and varies in a square wave manner with a minimum value of 1.6 in dark phase and maximum value of 2.0 in light phase (these values correspond to $\delta = 0.25$ in Eq. (3)). The concentration scale in each graph is expressed in nM.

Thus, by varying the free-running period τ in Eqs. (2), different limit cycles entrained by 12:12 LD cycles occur whilst we obtain the same limit cycle under constant dark

conditions (Fig. 1B). The amplitude of FRQ oscillations entrained by LD cycles becomes larger or smaller than the amplitude of FRQ in DD depending on the free-running period, τ (Figs. 1A and 1B). Fig. 2A illustrates the dependency of the amplitude of FRQ oscillations entrained by LD cycles on the free-running period. In this figure, the relative amplitude denotes the amplitude of FRQ in LD divided by the amplitude in DD. Fixing the entrainment strength at the value $\delta = 0.25$—which means that the rate v_s increases by 25% in the light phase—we observe that when the free-running period increases from 20 to 28 h, FRQ oscillations are entrained by 12:12 LD cycles only when the free-running period is in the range 21.0–25.6 h. As the free-running period increases in this range, the amplitude of FRQ oscillations under LD monotonically decreases. When the free-running period is longer than 25.6 h, quasi-periodic oscillations occur under LD conditions, and the period of these oscillations is far from 24 h (see below).

2.2. Effect of entrainment strength on the robustness of oscillations

Studying the same model for *Neurospora* circadian rhythms, Gonze and Goldbeter (2000) previously demonstrated that complex oscillations such as period-2 oscillations and chaos can occur under LD cycles when the enhanced transcription rate of *frq* expression during the light phase is much larger than the transcription rate during the dark phase (the maximum rate of *frq* transcription is denoted by $v_{s\max}$ in Eq. (3)). They showed that the range of period of LD cycles in which FRQ oscillations are successfully entrained decreases as the enhanced transcription rate during the light phase increases. Here (see Fig. 3) we examine the reverse situation in which we vary the free-running period of the oscillations produced by Eqs. (2) while the period of the LD cycle remains fixed at 24 h. We wish to determine the sensitivity of the amplitude of FRQ oscillations entrained by LD cycles with respect to the increase in transcription rate during light phase (measured by parameter δ in Eq. (3)).

Fig. 3 illustrates entrainment of FRQ oscillations by 12:12 LD cycles at increasing values of the entrainment strength ($\delta = 0.1$–0.7). For $\delta = 0.1$, oscillations are entrained by LD cycles when the free-running period τ ranges from 22.8 to 24.6 h. Outside this range, for example for $\tau = 22$ h, quasi-periodic oscillations are observed (Fig. 4B). When the entrainment strength δ increases, the domain of entrainment enlarges. However, if we further increase the entrainment strength (e.g. up to $\delta = 0.5$ or 0.7), complex oscillations occur when the free-running period is close to 24 h. For $\delta = 0.6$ for example, FRQ oscillations are successfully entrained by 12:12 LD cycles when the free-running period ranges from 18.0 to 21.6 h and from 25.8 to 26.0 h. On the other hand, period-2 oscillations under LD cycles occur when the free-running period is in the range 21.8–24.6 h (Fig. 4A and C), and chaos occurs when the free-running period is between 24.8 and 25.6 h (Fig. 4D). Period-2 oscillations (Fig. 4C) consist in the periodic alternation of a small amplitude followed by a large-amplitude peak in FRQ, such that the overall period is very

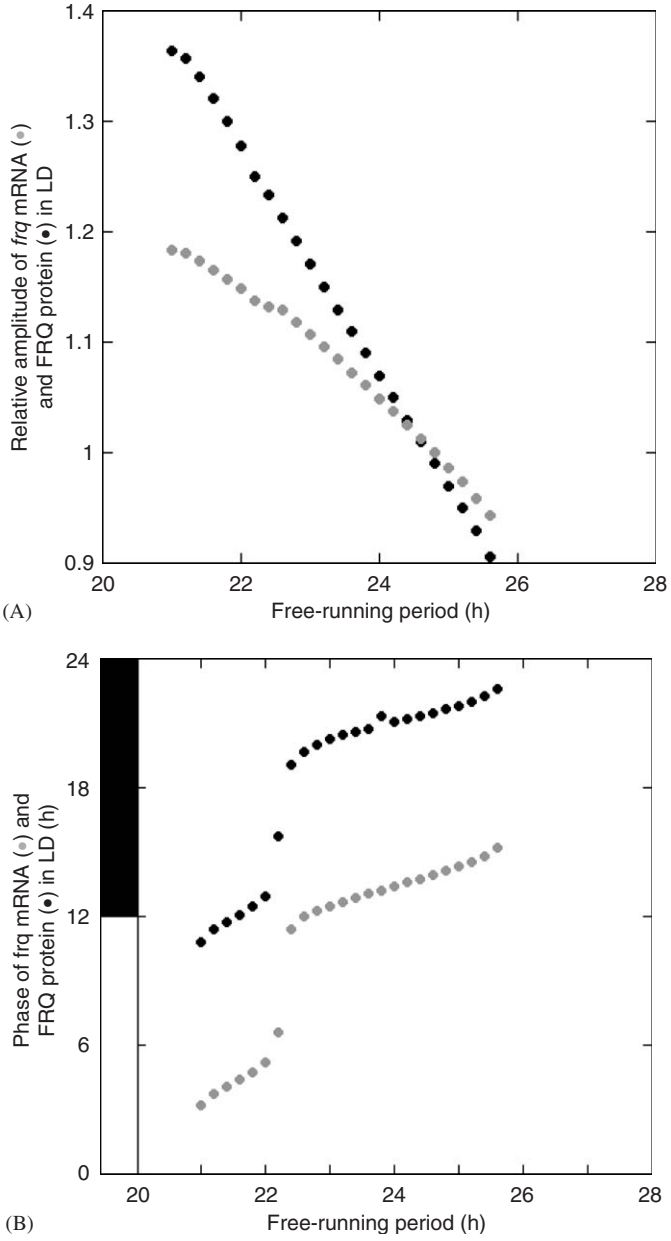


Fig. 2. Effect of free-running period on entrainment by LD cycles. (A) Dependency of the amplitude of FRQ (black dot) and *frq*-mRNA (gray dot) oscillations entrained by LD cycles on the free-running period τ. FRQ oscillations are entrained when the value of τ ranges from 21.0 to 25.6 h. The amplitude of FRQ and *frq*-mRNA entrained by LD cycles is defined as $\mathrm{Max}[F_c + F_N] - \mathrm{Min}[F_c + F_N]$ and $\mathrm{Max}[M] - \mathrm{Min}[M]$, respectively. The relative amplitude of FRQ and *frq*-mRNA under LD cycles is obtained through division by the amplitude in continuous darkness. (B) Phase of the oscillations upon entrainment, as a function of the free-running period τ. The phase of the maximum in FRQ protein ($F_C + F_N$) and *frq*-mRNA (M) is shown with respect to the phase of the 12:12 LD cycle. The white/black bar symbolizes one LD cycle. Parameter values are the same as in Fig. 1. The curves are obtained by numerical integration of Eqs. (2).

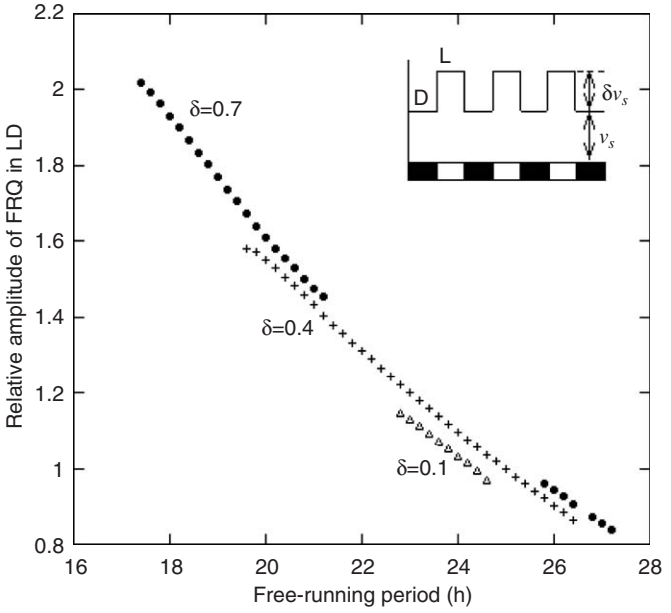


Fig. 3. Effect of entrainment strength on the robustness of circadian FRQ oscillations. The value of the entrainment strength δ in Eq. (3) is increased from 0.1 to 0.7. For $\delta = 0.1$ (open triangles), FRQ oscillations are entrained by LD cycles when the value of free-running period τ extends from 22.8 to 24.6 h. For $\delta = 0.4$ (crosses), FRQ oscillations are entrained when τ ranges from 19.6 to 26.4 h. For $\delta = 0.7$ (filled circles), FRQ oscillations are entrained when τ ranges from 17.4 to 21.2 h, 26.0 to 26.4 h, and 26.8 to 27.2 h. Complex oscillations in the form of period-2 oscillations occur when τ ranges from 21.4 to 22.0 h and from 25.0 to 25.8 h; period-3 oscillations occur when τ is equal to 26.6h, while chaos occurs when τ ranges from 22.2 to 24.8 h. The curves are obtained by numerical integration of Eqs. (2). Parameter values, except δ, are the same as in Fig. 1.

close to 48 h. Quasi-periodic and chaotic oscillations can be distinguished by the fact that the latter display sensitivity to initial conditions: starting from two closely related points in phase space, trajectories will rapidly become decorrelated. Moreover, a Poincaré section established when the trajectory crosses a particular value of *frq* mRNA, M, produces a closed curve in the case of quasi-periodic oscillations (Fig. 4E) and a continuous, open curve in the case of chaos (Fig. 4F).

If the entrainment strength is sufficiently high (e.g. $\delta = 1$), FRQ oscillations are not entrained by 12:12 LD cycles when the free-running period ranges from 20 to 28 h, and complex oscillations occur. However, entrainment can occur for $\delta = 1$ when the free-running period ranges from 15.4 to 15.8 h, and from 18.0 to 19.8 h. Thus the domain of entrainment by 12:12 LD cycles enlarges and then reduces again as δ increases. Moreover, within the domain of entrainment, there is a window in which period-2 oscillations or chaos occur.

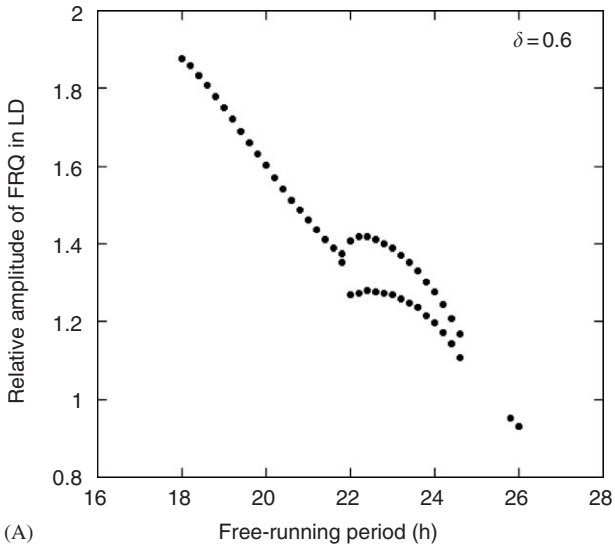
2.3. Why do smaller free-running periods give larger-amplitude circadian oscillations?

To understand why the amplitude of entrained circadian oscillations rises when the free-running period diminishes, it is useful to determine the phase of FRQ oscillations entrained by LD cycles as a function of the free-running period. Plotted in Fig. 2B are the phases corresponding to the maximum in FRQ protein and in *frq* mRNA. In Fig. 2B, the phase of the oscillations varies in a sigmoidal manner as a function of τ. The threshold value characterizing this sigmoidal curve is close to 22.2 h when $\delta = 0.25$. The phase is rapidly delayed when the free-running period increases above this threshold. Parameter values that yield free-running oscillations of 22.2 h in DD give 24 h free-running oscillations in LL conditions, when v_s is held at $v_{s\max}$. For small free-running periods, the peak in *frq* mRNA occurs during the light phase of the LD cycle, in which more mRNA is made than when the peak occurs in the dark phase at larger values of the free-running period. As a result, the amplitude of FRQ oscillations is more pronounced when the free-running period is relatively small (see Figs. 2A and 1A,B).

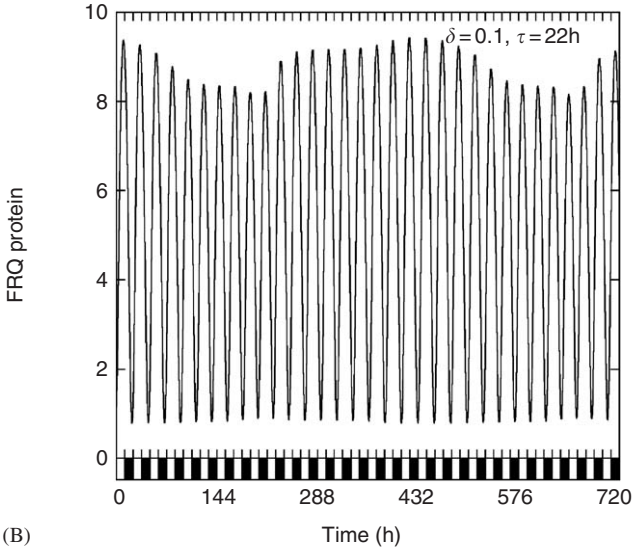
2.4. Influence of light-enhanced mRNA degradation

Heintzen et al. (2001) showed that in addition to the induction of *frq* gene, light increases the expression of *vivid* (*vvd*) gene. This is followed by the translation of *vvd* gene and the peak of VVD abundance appears about 1 h after light-on which indirectly suppresses the expression of *frq* gene. We can incorporate the light-induced degradation of *frq* mRNA if the degradation rate v_m is fixed at the constant value $v_{m\max}$ 30 min after light-on, where $v_{m\max}$ is defined by $v_{m\max} = v_m(1+\delta\varepsilon)$ with $\varepsilon > 0$. Then we can show that FRQ oscillations are entrained by LD cycles for very large entrainment strength (data not shown). For $\varepsilon = 0.62$ and $\delta = 8$ for example, FRQ oscillations are successfully entrained by LD cycles when the free-running period ranges from 21.4 to 22.2 h. The *frq* mRNA oscillations entrained by LD cycles reach an abundance of about 3 times larger than that in DD which may correspond to the report by Crosthwaite et al. (1995) indicating that light pulse

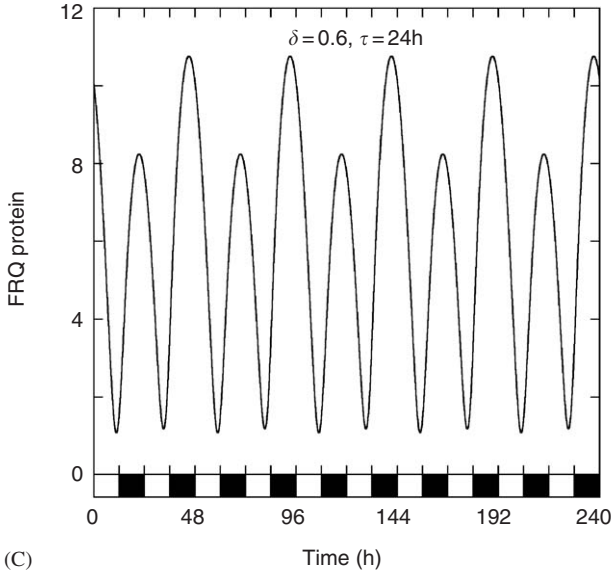
Fig. 4. Complex FRQ oscillations in LD cycles. (A) Amplitude of oscillations entrained by 12:12 LD cycles as a function of free-running period for $\delta = 0.6$. FRQ oscillations are entrained when τ ranges from 18 to 21.6 h and from 25.8 to 26.0 h. In contrast, period-2 oscillations which consist of the alternation of a small peak followed by a large peak appear when τ ranges from 21.8 to 24.6 h, while chaos occurs when τ ranges from 24.8 to 25.6 h. (B) For $\delta = 0.1$, FRQ oscillations are entrained when τ ranges from 22.8 to 24.6 h. For $\tau = 22$ h, complex oscillation in the form of quasi-periodic oscillations are observed. (C) Period-2 oscillations obtained for $\delta = 0.6$, corresponding to $\tau = 24$ h in panel A. (D) Chaos obtained for $\delta = 1.0$, with $\tau = 24$ h. For this value of δ, FRQ oscillations are not entrained by 12:12 LD cycles when τ ranges from 20.0 to 28.0 h. The curves are obtained by numerical integration of Eqs. (2). Parameter values except for entrainment strength δ are the same as in Fig. 1. (E) and (F) Poincaré sections corresponding to quasi-periodic (panel B) and chaotic (panel D) oscillations, respectively. The sections are obtained by plotting the successive values of F_C and F_N when M is on the increase and reaches the value of 3.


δ = 0.6
Relative amplitude of FRQ in LD
Free-running period (h)

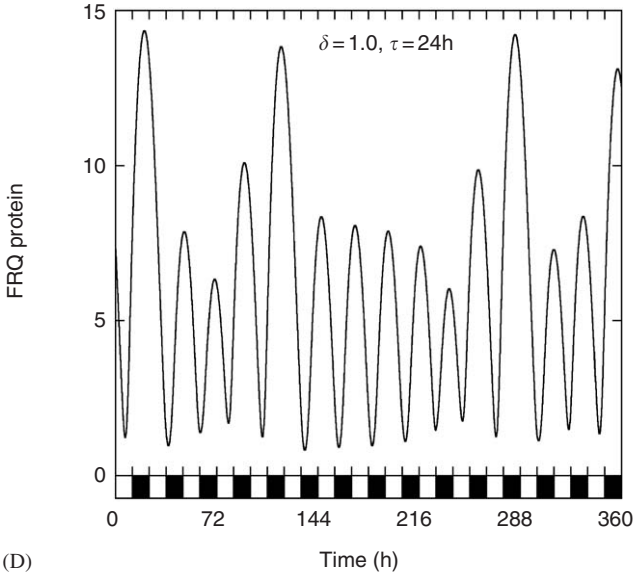
(A)


δ = 0.1, τ = 22h
FRQ protein
Time (h)

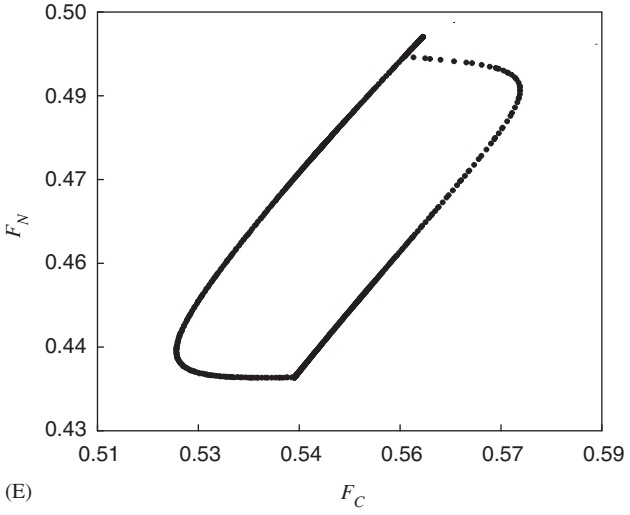
(B)


δ = 0.6, τ = 24h
FRQ protein
Time (h)

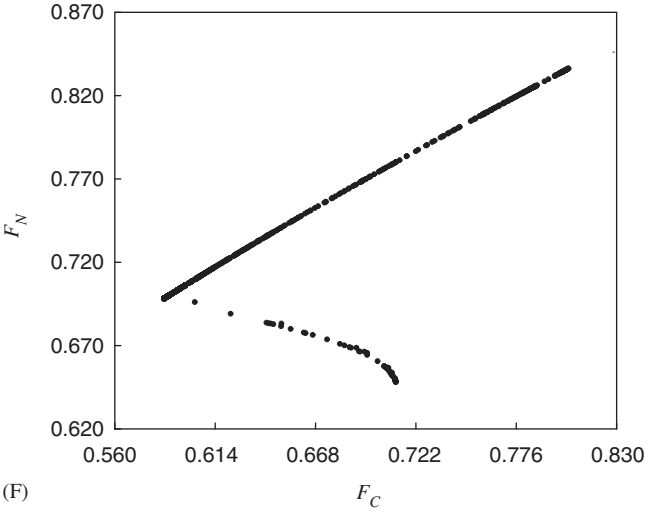
(C)


δ = 1.0, τ = 24h
FRQ protein
Time (h)

(D)


F_N
F_C

(E)


F_N
F_C

(F)

induces the 4–25-fold increase of *frq* mRNA. On the other hand, bimodal oscillations occur under LD cycles when the free-running period is in the range 20.0–21.2 h and quasi-periodic oscillations occur when the free-running period is close to 24 h. If there is no delay in the onset of increased *frq* mRNA degradation during the light phase, we did not observe the entrainment of FRQ oscillations at $\tau = 21.5$ h.

2.5. Influence of the form of period forcing

In the real environment, light intensity undergoes gradual variations in contrast to the all-or-none changes commonly considered in laboratory experiments. A previous study showed (Gonze and Goldbeter, 2000) that chaos is less likely to occur when the form of periodic forcing is sinusoidal instead of being a square wave. This suggests that complex oscillations in the form of period-2 or chaos appear here at very large entrainment strength (see previous sections) because of the use of a square wave for the change of the transcription rate in 12:12 LD conditions. To address this possibility, we consider the case in which the transcription rate is fixed during the dark phase but changes into a sine curve during the light phase in which it is given by

$$v_{s\,\max} = v_s[1 + \delta \sin(2\pi t_l/\tau)], \tag{4}$$

where t_l is the time after start of the light phase ($0 \leqslant t_l \leqslant 12$ h) in 12:12 LD cycles.

In Figs. 5A, we show the effect of the free-running period on the amplitude of circadian oscillations entrained by 12:12 LD cycles when the transcription rate, given by Eq. (4), changes from a low constant value during dark phase into a sine curve during light phase. For $\delta = 0.1$, autonomous oscillations are entrained when the free-running period is around 24 h. For $\delta = 0.5$, the domain of entrainment enlarges. Complex oscillations do not occur for this value of δ, in contrast to what occurs in the case of a square-wave variation of the light-sensitive parameter (Fig. 3). However, period-2 oscillations occur for very large entrainment strengths such as $\delta = 1.2$ when the free-running period is between 21.8 and 24.0 h (Fig. 5). For $\delta \geqslant 1.7$, FRQ oscillations are not entrained by LD cycles when the free-running period ranges from 20 to 28 h, and complex oscillations occur. Entrainment can nevertheless occur for $\delta = 1.7$ when the free-running period ranges from 16.0 to 19.2 h.

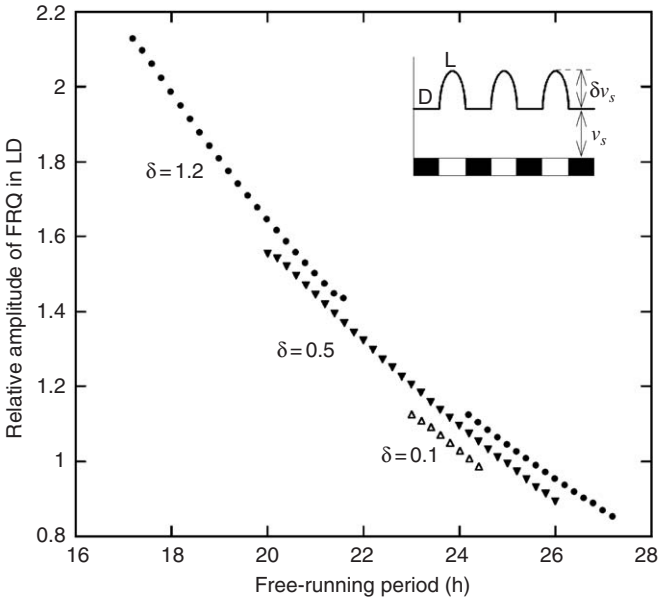


Fig. 5. Influence of the form of periodic forcing on the entrainment of FRQ oscillations by LD cycles. Parameter v_s (in nM h^{-1}) remains constant and equal to 1.6 in dark phase and varies in a sine wave manner from the minimum value of 1.6 up to the maximum value of $1.6(1+\delta)$ in light phase (see Eq. (4)). The amplitude of circadian FRQ oscillations entrained by LD cycles is shown as a function of the free-running period. The value of the entrainment strength δ is increased from 0.1 to 1.2. For $\delta = 0.1$ (open triangles), FRQ oscillations are entrained by LD cycles when the free-running period τ ranges from 23.0 to 24.4 h. For $\delta = 0.5$ (closed triangles), FRQ oscillations are entrained when τ ranges from 20.0 to 26.0 h. For $\delta = 1.2$ (circles), FRQ oscillations are entrained when τ ranges from 17.2 to 21.6 and 24.2 to 27.2 h. Complex oscillations in the form of period-2 oscillations then occur when τ ranges from 21.8 to 24.0 h. The curves are obtained by numerical integration of Eqs. (2). Parameter values except for entrainment strength δ are the same as in Fig. 1.

3. Core model for the *Drosophila* circadian clock

The five-variable model proposed by Goldbeter (1995) for oscillations of the PER protein and *per* mRNA represents a relatively simple model for the circadian clock in *Drosophila*. The model takes into account the transcription of the *per* gene into *per* mRNA, translation of the latter into PER protein, two successive steps of reversible phosphorylation of PER, degradation of the doubly phosphorylated PER and entry of this fully modified form of PER into the nucleus where it represses the expression of the *per* gene. The variables considered are thus the concentration of *per* mRNA (M), unphosphorylated PER protein (P_0), monophosphorylated PER protein (P_1), diphosphorylated PER protein in the cytoplasm (P_2) and in the nucleus (P_N). The time evolution of this model is governed by the following set of kinetic equations (Goldbeter, 1995):

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M}{K_m + M}, \tag{5a}$$

$$\frac{dP_0}{dt} = k_s M - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}, \tag{5b}$$

$$\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}, \tag{5c}$$

$$\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - v_d \frac{P_2}{K_d + P_2}, \tag{5d}$$

$$\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N. \tag{5e}$$

Sustained circadian oscillations occur in this model in an appropriate range of parameter values. For the set of parameter values originally considered (Goldbeter, 1995), the autonomous period τ is 23.6 h, which is close to the free-running period of *Drosophila*. As discussed further below, a major reason for considering the *Drosophila* circadian clock model here is that it illustrates a situation where light acts by inducing protein degradation instead of gene expression as in the *Neurospora* clock model.

As done in the previous section for the *Neurospora* model, we first transform Eqs. (5) to discuss the effect of the free-running period on the amplitude of PER oscillations entrained by LD cycles. When multiplying the right-hand side of all Eqs. (5) by τ_0/τ we obtain system (6):

$$\frac{dM}{dt} = \frac{\tau_0}{\tau}\left[v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M}{K_m + M}\right], \tag{6a}$$

$$\frac{dP_0}{dt} = \frac{\tau_0}{\tau}\left[k_s M - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}\right], \tag{6b}$$

$$\frac{dP_1}{dt} = \frac{\tau_0}{\tau}\left[v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}\right], \tag{6c}$$

$$\frac{dP_2}{dt} = \frac{\tau_0}{\tau}\left[v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - v_d \frac{P_2}{K_d + P_2}\right], \tag{6d}$$

$$\frac{dP_N}{dt} = \frac{\tau_0}{\tau}[k_1 P_2 - k_2 P_N]. \tag{6e}$$

When varying the free-running period τ with all other parameters fixed in Eqs. (6), we obtain the same limit cycle characterized by the period τ. This limit cycle corresponds to autonomous oscillations characterized by the same amplitudes for M, P_0, P_1, P_2 and P_N but a different period (τ) in DD.

3.1. Amplitude of PER oscillations entrained by 12h:12h LD cycles

The mechanism of circadian oscillations in *Drosophila* involves the formation of a heterodimer between the PER and TIM proteins. The PER-TIM complex migrates to the nucleus where it represses in an indirect manner the transcription of *per* and *tim* genes. Light acts by enhancing the rate of degradation of TIM. This light-sensitive mechanism allows for the entrainment of circadian rhythms by LD cycles in *Drosophila* (Myers et al., 1996). A model based on the formation of PER–TIM complexes accounts for the entrainment of circadian rhythms by LD cycles when incorporating the effect of light on TIM degradation (Leloup and Goldbeter, 1998). In this model, the degradation rate of TIM during the light phase, $v_{d\ \max}$ is defined by

$$v_{d\ \max} = v_d(1 + \delta). \tag{7}$$

For simplicity, we will consider the simpler model based on PER alone and will assume that light acts by enhancing the degradation rate of diphosphorylated PER according to Eq. (7). Thus, parameter v_d in Eq. (6d) will be replaced by the higher value $v_{d\ \max}$ during the light phase of the LD cycle.

The dependency of the amplitude of PER oscillations on the free-running period upon entrainment by 12:12 LD cycles is illustrated in Fig. 6A. When the entrainment strength is small (e.g. $\delta = 0.05$ in Eq. (7)), circadian oscillations are entrained when the free-running period τ ranges from 23.0 to 24.4 h. Outside this range PER oscillations in LD do not converge to sustained oscillations of 24 h period. As the entrainment strength δ increases, the domain of free-running period yielding entrainment enlarges. Entrainment occurs in this model over a wide range of δ values, e.g. up to 10, or even larger.

It is useful to determine the phase of phosphorylated PER protein when oscillations are entrained by 12 h:12 h LD cycles. In Fig. 6B, the phase of the peak of doubly phosphorylated PER occurs 3.2 h before the onset of light when the free-running period is 23 h. In this case, most of the degradation of the protein should occur in the dark phase at a low degradation rate. As the free-running period increases, the phase of the protein moves toward the light phase and the protein is degraded at a higher rate. This explains why the amplitude of PER oscillations entrained by LD cycles becomes larger when the free-running period is smaller than 24 h.

4. Discussion

An intriguing characteristic of circadian rhythms is that the free-running period τ under constant dark conditions is often quite different from 24 h in many organisms such as *Neurospora*, for which $\tau = 21.5$ h. One possible reason, pointed out by Pittendrigh and Daan (1976), is that stable entrainment to the natural 24 h light–dark cycle may become more difficult to achieve when the free-running period approaches 24 h.

In the present paper, we determined the free-running period that gives the largest amplitude of circadian oscillations entrained by 12:12 light–dark cycles. We performed this analysis in two models differing by the effect of light, which acts either by inducing the expression of a clock gene, as in *Neurospora*, or by inducing the degradation of a clock protein, as in *Drosophila*. Our main results bear on the effect of the free-running period of circadian rhythms on the amplitude of oscillations entrained by LD cycles, on the range of entrainment, and on the possible occurrence of complex oscillatory behavior.

The three-variable model for *Neurospora* displays entrainment by 12:12 LD cycles in a certain range of free-running period. When the entrainment strength increases, the domain of entrainment enlarges and then reduces again. Indeed, at large entrainment strength, the domain of entrainment is interrupted by a range of τ in

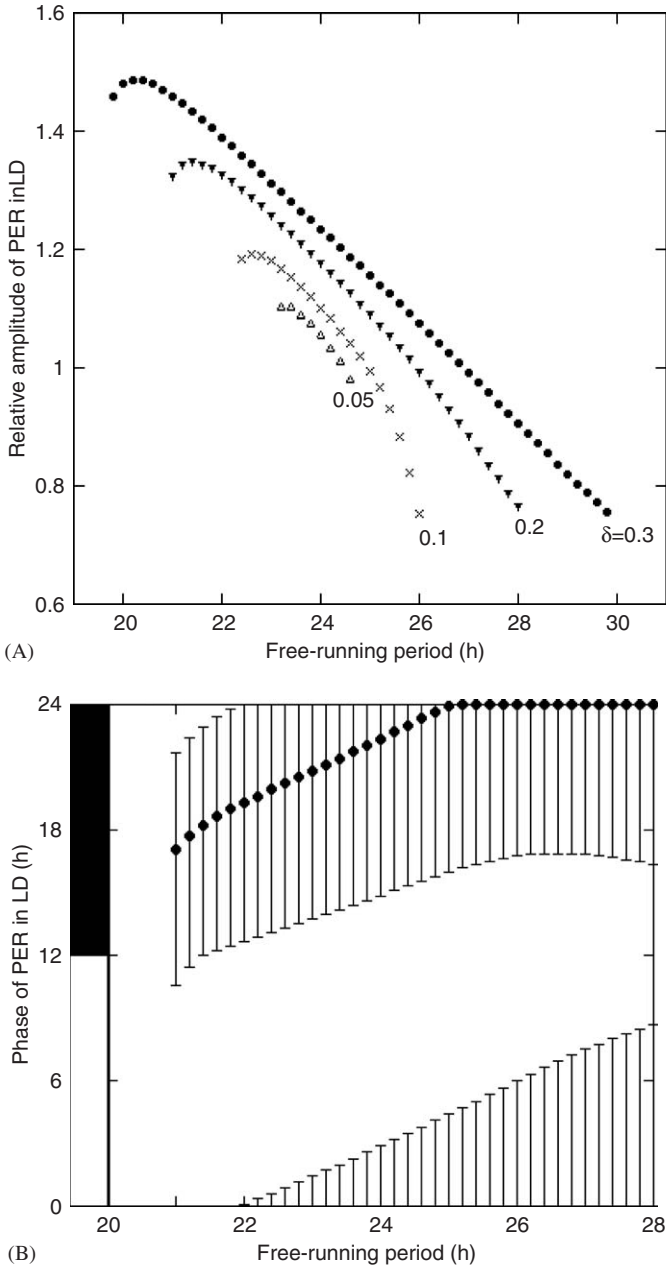


Fig. 6. Effect of the free-running period τ on PER oscillations entrained by LD cycles. (A) Dependency of the amplitude of PER oscillations entrained by LD cycles on τ. The amplitude of total PER oscillations is defined as $\mathrm{Max}[P_0+P_1+P_2+P_N]-\mathrm{Min}[P_0+P_1+P_2+P_N]$ and the relative amplitude under LD cycles is obtained through division by the amplitude in continuous darkness. For $\delta=0.05$, PER oscillations are entrained by LD cycles when τ ranges from 23.0 to 24.4 h. For $\delta=0.1$, PER oscillations are entrained when τ ranges from 22.4 to 25.8 h. For $\delta=0.2$, PER oscillations are entrained by LD cycles when τ ranges from 21.0 to 28.0 h. For $\delta=0.3$, PER oscillations are entrained by LD cycles when τ ranges from 19.8 to 30.0 h. (B) Phase of the oscillations upon entrainment, as a function of the free-running period τ. The value of the entrainment strength δ is equal to 0.2. The phase in which total PER exceeds half of the maximum level reached in the course of sustained oscillations in LD cycles is shown with respect to the phase of 12:12 LD cycles. The black dots represent the phase of the maximum in total PER. The white/black bar symbolizes one LD cycle. Parameter values are: $v_s=0.76\,\mu\mathrm{Mh}^{-1}$, $v_m=0.65\,\mu\mathrm{Mh}^{-1}$, $K_m=0.5\,\mu M$, $k_s=0.38\,\mathrm{h}^{-1}$, $v_d=0.95\,\mu\mathrm{Mh}^{-1}$, $k_1=1.9\,\mathrm{h}^{-1}$, $k_2=1.3\,\mathrm{h}^{-1}$, $K_I=1\,\mu\mathrm{M}$, $K_d=0.2\,\mu\mathrm{M}$, $n=4$, $K_1=K_2=K_3=K_4=2\,\mu\mathrm{M}$, $V_1=3.2\,\mu\mathrm{Mh}^{-1}$, $V_2=1.58\,\mu\mathrm{Mh}^{-1}$, $V_3=5\,\mu\mathrm{Mh}^{-1}$, $V_4=2.5\,\mu\mathrm{Mh}^{-1}$. The curves are obtained by numerical integration of Eqs. (6).

which complex oscillations—i.e. period-2 or chaos—occur (Figs. 3 and 4). The free-running period that gives largest amplitude of entrained circadian oscillations is always smaller than 24 h. This is due to the fact that at small autonomous periods, the peak in *frq* mRNA appears during the light phase, in which more mRNA is synthesized than when the mRNA peak appears during the dark phase at higher values of τ. If $\tau=21.5$ h, close to the free-running period of *Neurospora*, the amplitude of circadian oscillations entrained by LD cycles is much larger than when the free-running period is close to 24 h (see Fig. 2A).

Mutations in *frq* can result in substantial period defects, yielding periods from 16 to 35 h (Dunlap et al., 2004). For example the free-running period in *frq-1* is 16 h and that in *frq-7* is 29 h. The present results predict that the amplitude of FRQ oscillation in wild-type strain under LD cycles would be larger than that in *frq-7*. If the entrainment strength is very large, FRQ oscillations are entrained by LD cycles only for $\tau<20$ h in the model. These results also predict that entrainment of FRQ oscillations might be observed in *frq-1* but not in wild-type and *frq-7* when entrainment strength is very large, corresponding to a very high intensity of light in experiments.

In mammals, light also acts by inducing expression of a clock gene (i.e. mPer1). Numerical results of the three-variable model for *Neurospora* indicate that complex oscillations may appear at large values of δ if τ is close to the value 24.18 h observed for mammals (Fig. 3). However, the responsiveness of mPer1 mRNA to light is gated so that little or no induction of mPer1 occurs during the subjective day under DD whereas robust induction occurs during subjective night (Shigeyoshi et al., 1997). Thus we may have to incorporate such gating of light when addressing the effect of LD cycles on the mammalian circadian clock.

In the five-variable model for *Drosophila*, the domain of entrainment by 12:12 LD cycles also enlarges when the entrainment strength increases, but for this model neither period-2 oscillations nor chaos occur within the domain. The amplitude of clock protein (i.e. PER) entrained by 12:12 LD cycles is again larger when the free-running period is smaller than 24 h. Numerical simulations for this model indicate that PER oscillations are more likely to be entrained by LD cycles at various entrainment strengths if the free-running period is close to 24 h (Fig. 6A). The comparison with the *Neurospora* model suggests that entrainment by LD cycles is more robust and the occurrence of chaos less likely when light acts by inducing protein degradation instead of gene expression.

Experiments in *Drosophila* demonstrated the importance of large-amplitude circadian oscillations (Allada et al., 2003). Indeed, a mutant allele of *Drosophila Clk*, Clk^{ar} displays sustained oscillations of CLOCK protein with reduced amplitude but is arrhythmic at the behavioral level (Allada et al., 2003). This observation implies that behavioral rhythms may require large-amplitude circadian oscillations of clock proteins or mRNAs. If natural selection works in the direction of favoring large-amplitude

oscillations of clock proteins (i.e. FRQ or PER), the present results might explain why the free-running period of circadian rhythms in *Neurospora* is 21.5 h, but not why the free-running period in mammals and *Drosophila* is close to 24 h. The present results show that the amplitude may not be the only or primary issue.

In agreement with the conclusions presented by Pittendrigh and Daan (1976), the stable entrainment to the LD cycle becomes more difficult the closer the free-running period of the *Neurospora* model is from 24 h. For large entrainment strength, the domain of complex oscillations in the form of period-2 or chaos appears when the free-running period is close to 24 h. In the case of chaotic or quasiperiodic oscillations, the phase of the peak of *frq* mRNA of complex oscillations does not settle to a constant value. It is likely that natural selection would not favor such an instability of the phase of circadian oscillations forced by the 12:12 LD cycles. It may be possible for a mutant having a free-running period close to 24 h that FRQ oscillations are not entrained by 12:12 LD cycles. The present results indicate that an advantage of having a free-running period far from 24 h for *Neurospora* may be to secure the occurrence of stable entrainment.

However, the argument of Pittendrigh and Daan (1976) does not hold for the results of the *Drosophila* model examined here. In the *Drosophila* model, in which entrainment is mediated by light-enhanced protein degradation rather than light-enhanced gene expression, the stable entrainment by the LD cycle is always easier when the free-running period is close to 24 h. An advantage of having a free-running period close to 24 h in *Drosophila* may be to secure the occurrence of entrainment over a large range of light intensity.

Acknowledgements

This work was supported by Aihara Complexity Modelling Project, ERATO, JST to G.K., by Grant #3.4636.04 from the *Fonds de la Recherche Scientifique Médicale* (F.R.S.M., Belgium) to A.G. and by the European Union through the Network of Excellence BioSim, Contract No. LSHB-CT-2004-005137.

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