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Theoretical models for circadian rhythms in *Neurospora* and *Drosophila*

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Abstract – We examine theoretical models proposed for the molecular mechanism of circadian rhythms in *Drosophila*. The models are based on the negative feedback exerted by a complex between the PER and TIM proteins on the expression of the *per* and *tim* genes. We show that a similar model can account for circadian oscillations in *Neurospora*, where the protein FRQ negatively regulates the expression of the *frq* gene. The effect of light on the circadian rhythms is included by considering that it elicits a rise in the rate of TIM degradation in *Drosophila*, whereas in *Neurospora* it enhances the rate of *frq* transcription. The models account for the occurrence of sustained circadian oscillations in continuous darkness in *Drosophila* and *Neurospora*. Numerical simulations further indicate that the periodic forcing of circadian oscillations by light–dark cycles can result either in the entrainment to the external periodicity or in aperiodic oscillations (i.e. chaos), depending on the magnitude of the periodic changes in the light-controlled parameter. © 2000 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

circadian rhythms / *Drosophila* / *Neurospora* / PER / TIM / FRQ / model

Résumé – Modèles théoriques pour les rythmes circadiens chez *Neurospora* et chez la drosophile. Nous examinons des modèles théoriques proposés pour le mécanisme moléculaire des rythmes circadiens chez la drosophile. Ces modèles sont fondés sur la rétroaction négative exercée par un complexe entre les protéines PER et TIM sur l'expression des gènes *per* et *tim*. Nous montrons qu'un modèle similaire peut rendre compte des oscillations circadiennes chez *Neurospora*, organisme chez lequel la protéine FRQ exerce un contrôle négatif sur la transcription du gène *frq*. L'effet d'une perturbation lumineuse sur les rythmes circadiens est inclus dans les modèles en considérant que la lumière accroît la vitesse de dégradation de la protéine TIM chez la drosophile et la vitesse de transcription du gène *frq* chez *Neurospora*. Les modèles rendent compte de l'existence d'oscillations circadiennes entretenues en obscurité constante chez la drosophile et *Neurospora*. Les simulations numériques montrent que le forçage périodique des rythmes circadiens par des cycles lumière-obscurité peuvent résulter, selon l'amplitude des changements du paramètre de contrôle induits par la lumière, soit en l'entraînement des oscillations à la période externe, soit en des oscillations apériodiques, c'est-à-dire du chaos. © 2000 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

rythmes circadiens / Drosophile / *Neurospora* / PER / TIM / FRQ / modèle

Version abrégée

Nous proposons des modèles théoriques pour le mécanisme moléculaire des rythmes circadiens chez la drosophile et chez *Neurospora*. C'est pour ces deux organismes que le mécanisme moléculaire de ces rythmes endogènes d'une période proche de 24 h est connu dans le plus de détail. Le modèle pour les

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rythmes circadiens chez la drosophile est fondé sur la rétroaction négative exercée sur l'expression des gènes *per* et *tim* par le complexe entre les protéines PER et TIM, produits de ces deux gènes. Le modèle rend compte des oscillations circadiennes observées en obscurité constante dans le niveau des protéines PER et TIM et de leurs ARN messagers; ces oscillations entretenues sont du type cycle limite. Le modèle permet d'expliquer le comportement périodique du type sauvage et celui des mutants per^{l} et per^{s} de l'horloge circadienne, de même que la faible variation de la période du rythme circadien en fonction de la température (phénomène de « compensation de température »). L'effet de la lumière sur le rythme est incorporé dans le modèle en considérant que la vitesse de dégradation de la protéine TIM augmente pendant la phase lumineuse. La variation périodique de la vitesse de dégradation de TIM sous-tend l'entraînement des oscillations circadiennes par un cycle lumière-obscurité d'une période de l'ordre de 24 h.

Nous montrons qu'un modèle similaire rend compte du rythme circadien observé chez le champignon *Neurospora*. Dans cet organisme, la protéine FRQ exerce un contrôle négatif sur l'expression de son gène *frq*; la vitesse d'expression de ce gène augmente au cours de la phase lumineuse. L'effet différentiel de la lumière chez les deux organismes permet d'expliquer les phases différentes auxquelles se produisent les pics d'ARN messagers correspondant à l'expression des gènes *per* et *tim* d'une part, et *frq* d'autre part. Une extension du modèle pour le rythme chez *Neurospora* est étudiée, afin de rechercher les conditions dans lesquelles le pic de FRQ nucléaire survient avant le pic de la quantité totale de protéine FRQ, comme semblent l'indiquer les résultats expérimentaux disponibles.

Revenant à l'effet des cycles lumière-obscurité sur les rythmes circadiens, nous montrons, à l'aide du modèle proposé pour *Neurospora*, que l'entraînement à la période externe n'est pas le seul type de réponse observé. Lorsque l'amplitude des variations du paramètre contrôlé par la lumière est suffisamment élevée, l'entraînement par la périodicité externe fait place à des oscillations apériodiques. Ce comportement chaotique correspond dans l'espace des concentrations à l'évolution vers un attracteur étrange, alors que le comportement périodique dans des conditions d'obscurité constante ou d'entraînement par le cycle lumière–obscurité correspond à l'évolution vers un cycle limite. Tandis que le chaos résulte ici du forçage de l'oscillateur circadien par un cycle lumière–obscurité, du chaos autonome a été observé en conditions d'obscurité constante dans le modèle pour le rythme circadien chez la drosophile, fondé sur la régulation par le complexe PER-TIM. La prédiction de comportement chaotique résultant du forçage par un cycle lumière-obscurité pourrait être testée de manière expérimentale chez *Neurospora* et chez la drosophile.

1. Introduction

All eukaryotic organisms [1] and some bacterial species [2] possess the capability of adapting to their periodically changing environment by displaying rhythms of periodicity of approximately 24 h. A key property of such circadian rhythms is that they are endogenous as they generally persist in constant conditions, e.g. continuous darkness and/or continuous light [3]. During the last decade, thanks to genetic and biochemical studies, remarkable advances have been made on the molecular mechanism of circadian clocks, particularly in *Drosophila* [4–6] and *Neurospora* [7, 8]. Of particular significance is the recent finding that the *per* and *tim* genes involved in circadian rhythm generation in *Drosophila* are also found in mammals [9, 10], including man. This raises the possibility that the clock mechanism may be largely conserved from the fly to the human circadian system [11].

Theoretical models have long proved useful in clarifying the conditions in which periodic phenomena arise in regulated biological systems [12, 13]. Most models proposed so far pertain to ultradian biochemical oscillations, characterized by periods ranging from seconds to minutes. In regard to circadian rhythms, mathematical models were first of an abstract nature, and were borrowed from the physical literature, as exemplified by the use of the Van der Pol oscillator as an analogue for circadian oscillations [14]. This approach is still used to study the effect of light on the human circadian system [15]. The first model for oscillations resulting from negative feedback on gene expression was due to Goodwin [16]. This model was later used to examine properties of circadian rhythms, including phase response curves yielding the phase shifts triggered by light pulses [17], or temperature compensation [18].

Given the increasing availability of experimental data, more detailed theoretical models can now be proposed for circadian rhythms. Such models have been studied so far for circadian oscillations of the products of the *per* and *tim* genes in *Drosophila* [19, 20]. The purpose of the present article is to examine theoretical models for circadian oscillations based on the experimental observations gathered on the molecular mechanisms of circadian rhythms in *Neurospora* and *Drosophila*. We show that the model previously proposed for circadian oscillations in *Drosophila* [13, 19] can be used, with a few minor modifications, to account for circadian oscillations in *Neurospora*.

Incorporating the effect of light on the circadian mechanism allows the comparison of theoretical predictions with experimental data with regards to the phase relations between the biochemical variables of the circadian clock and the light–dark cycle. Besides pointing to the common features of circadian rhythms which, in both cases, occur in the form of limit cycle oscillations, the models provide a unifying explanation for a variety of experimental observations and lead to the prediction of new modes of oscillations in response to environmental perturbations. Thus, the models show that forcing the circadian oscillatory systems by light–dark cycles may lead to entrainment or to aperiodic behaviour in the form of chaos, depending on the magnitude of the periodic biochemical changes that are modulated by light intensity.

2. Models for circadian oscillations in *Drosophila* and *Neurospora*

Experimental observations indicate that a similar genetic control mechanism underlies circadian rhythm generation in both *Drosophila* and *Neurospora*. In each case it appears that circadian oscillations originate from the negative autoregulation of gene expression [1, 6, 8]. Thus, in *Drosophila*, as schematized in *figure 1.A*, a complex formed by the proteins PER and TIM, products of the *per* and *tim* genes, migrates to the nucleus where it represses the transcription of these genes; light controls the circadian system by inducing the degradation of TIM [21–24]. Similarly, in *Neurospora*, as schematized in *figure 1.B*, a protein known as FRQ enters the nucleus where it represses the transcription of its gene *frq* [8, 25]. Here, in contrast, light controls the circadian system by inducing the transcription of *frq* [26]. The theoretical models presented below for circadian oscillations in *Drosophila* and *Neurospora* incorporate the negative autoregulatory feedback loops involving, respectively, the PER–TIM complex and FRQ, as well as the specific effects of light in these two systems.

2.1. Model for circadian rhythms in *Drosophila*

The first model studied for circadian oscillations in *Drosophila* [13, 19] was based on the sole negative regulation exerted by PER on the expression of the *per* gene. The model also incorporated the multiple phosphorylation of PER. This model was later extended to incorporate the role of the TIM protein which forms a complex with PER [20]. Whereas the dynamic behaviour of the former model was described by a set of five kinetic equations, the behaviour of the extended model schematized in *figure 2* is governed by a set of ten kinetic equations describing the time evolution of the mRNAs of *per* and *tim*, as well as the various phosphorylated or non-phosphorylated forms of PER and TIM and the cytosolic and nuclear forms of the PER–TIM complex. These equations are listed as equations (1a)–(1j) in Leloup and Goldbeter [20]. The

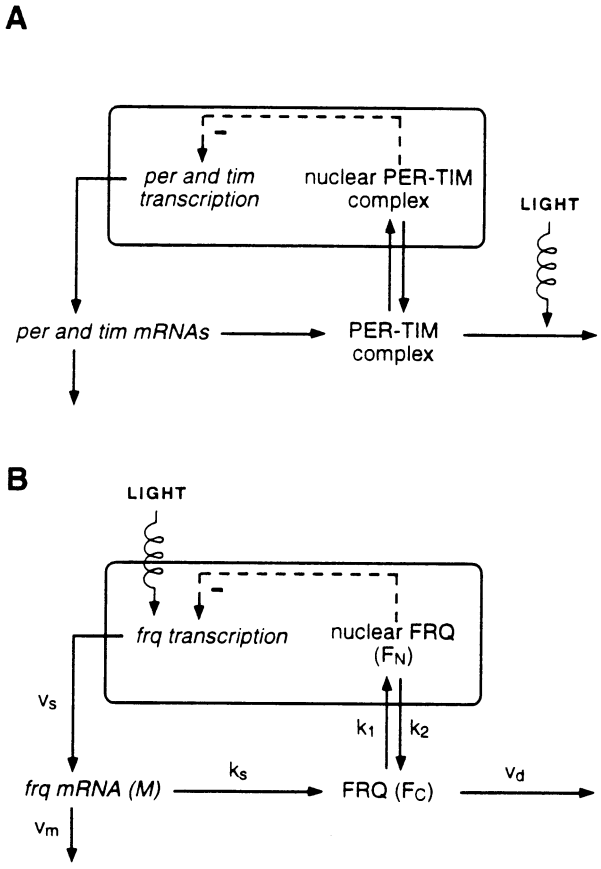

Figure 1. Scheme of the models for circadian oscillations in (A) *Drosophila* and (B) *Neurospora*.
For the fly circadian rhythm, the model is based on the negative regulation exerted by the PER–TIM protein complex on the expression of the *per* and *tim* genes; light controls the rhythm by enhancing the rate of TIM degradation. In *Neurospora*, the model is based on the negative feedback exerted by the protein FRQ on the transcription of the *frq* gene; the rate of gene expression is enhanced by light. In each case the models include gene transcription in the nucleus, accumulation of the corresponding mRNA in the cytosol with the associated protein synthesis, protein transport into and out of the nucleus, and regulation of gene expression by the nuclear form of the PER–TIM complex in *Drosophila* and FRQ in *Neurospora*. Not indicated in these simplified schemes are the phosphorylation reactions involving PER and TIM, or FRQ. A more detailed model for *Drosophila* incorporating the formation of the PER–TIM complex as well as phosphorylations of PER and TIM is represented in *figure 2*.

effect of light is incorporated in this extended model through modulation of parameter v_{dT} which measures the maximum rate of TIM degradation [20].

Shown in *figure 3.A* are the oscillations in total PER protein (P_t), per mRNA (M_P), and nuclear PER–TIM complex (C_N) obtained in conditions corresponding to constant darkness in the *Drosophila* clock model; such conditions are achieved in the extended model by holding parameter v_{dT} at a constant, low value. Although the environmental conditions remain constant, the PER–TIM control system generates autonomous oscillations with a period close to 24 h for the set of parameter values

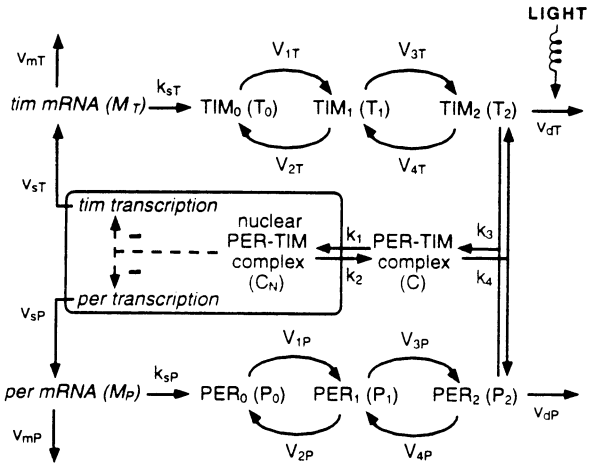

Figure 2. Scheme of the extended model for circadian oscillations in *Drosophila* involving negative regulation of gene expression by a complex between PER and TIM [20].
The *per* (M_P) and *tim* (M_T) mRNAs are synthesized in the nucleus and transferred into the cytosol, where they accumulate at the maximum rates v_{sP} and v_{sT}, respectively; there they are degraded enzymatically at the maximum rates v_{mP} and v_{mT}, with the Michaelis constants K_{mP} and K_{mT}. The rates of synthesis of the PER and TIM proteins, proportional to M_P and M_T, respectively, are characterized by the apparent first-order rate constants k_{sP} and k_{sT}. Parameters V_{iP}, V_{iT} and K_{iP}, K_{iT} (i = 1,...4) denote the maximum rate and Michaelis constant of the kinase(s) and phosphatase(s) involved in the reversible phosphorylation of P_0 (T_0) into P_1 (T_1) and P_1 (T_1) into P_2 (T_2), respectively. The fully phosphorylated forms (P_2 and T_2) are degraded by enzymes of maximum rate v_{dP}, v_{dT} and Michaelis constants K_{dP}, K_{dT}, and reversibly form a complex C (with the forward and reverse rate constants k_3, k_4) which is transported into the nucleus at a rate characterized by the apparent first-order rate constant k_1. Transport of the nuclear form of the PER–TIM complex (C_N) into the cytosol is characterized by the apparent first-order rate constant k_2. The negative feedback exerted by the nuclear PER–TIM complex on *per* and *tim* transcription is described by an equation of the Hill type, in which *n* denotes the degree of co-operativity, and K_{IP} and K_{IT} the threshold constants for repression.

considered. These oscillations are of the limit cycle type (see refs. [13, 19]; the limit cycle is similar to that shown for the *Neurospora* system in section 3). The corresponding oscillations obtained in conditions of entrainment by light–dark cycles are shown in *figure 3.B*. In such conditions, parameter v_{dT} varies in a square-wave manner as it increases up to a higher value during each light phase. As the duration of both the light and dark phases is equal to 12 h in the case considered (this particular light–dark cycle is denoted by 12:12 LD), the system is entrained precisely to the 24-h external periodicity.

The extended model schematized in *figure 2* has been used [20] to account for the altered rhythmic behaviour of mutants of the *Drosophila* circadian clock, such as the long-period (per^l) and short-period (per^s) mutants [4]. This model also accounts for the phase response curves obtained experimentally for the wild type (per^+) and for the per^s mutant [20]. By varying the strength and the duration of the perturbation by light pulses, we used the model to generate a family of phase response curves yielding the phase shift of circadian oscillations as a function of the phase at which the perturbation is applied. The model also allowed us to show that the phosphorylation of PER and TIM and the formation of a complex between the PER and TIM proteins favour rhythmic behaviour as these processes result in the enlargement of the domain of sustained oscillations in parameter space [20]. Finally, we examined in the simpler model based on PER alone the origin of temperature compensation [27]. This property, by which the period remains largely independent from temperature, is characteristic of circadian rhythms and arises in the model from the antagonistic effects exerted on the period by the different kinetic parameters which vary with temperature.

The model based on PER and TIM can further be extended by incorporating the role of newly discovered gene products. Thus, sustained oscillations are still obtained when including the CLOCK and CYCLE proteins which behave as transcriptional activators for *per* and *tim* [28–30]. In this version of the model, the PER–TIM complex binds to the CLOCK–CYCLE complex and thereby inhibits the transcription of the *per* and *tim* genes. The possibility of post-transcriptional regulation suggested by some experiments [31] has also been investigated; the model shows that such a regulatory mechanism can still give rise to oscillations in PER and TIM even if the negative regulation at the genetic level is impaired. Finally, the model accounts for the occurrence of sustained oscillations of PER and TIM in the presence of a constant level of *per* mRNA [32], but only if *tim* mRNA is allowed to vary. The model shows that the oscillations in *tim* mRNA due to the still active feedback loop involving the PER–TIM complex are sufficient to give rise to periodic behaviour (J.-C. Leloup, A. Goldbeter, in prep.).

2.2. Model for circadian rhythms in *Neurospora*

The schemes of the simplified molecular mechanisms of circadian oscillations in *Drosophila* and *Neurospora* shown in panels A and B, respectively, of *figure 1* indicate that these mechanisms are closely related as regards the nature of the feedback loop that governs circadian rhythmicity, even if they differ in the identity of the molecules involved in the regulatory circuit. Thus, the role of the PER–TIM complex in the negative feedback on gene expression in *Drosophila* is played by FRQ in *Neurospora* [25]. A further difference pertains to the effect of light, which controls TIM degradation in the fly [21–24] and *frq* transcription in the fungus [26].

Considering the formal similarity between the models of *figure 1.A* and *B*, it is natural to resort to similar equations in describing the molecular mechanism of circadian oscillations in the two systems. However, since no complex has been found between FRQ and a second protein in *Neurospora*, the model proposed below for the fungal circadian rhythm is closely related to the model initially proposed

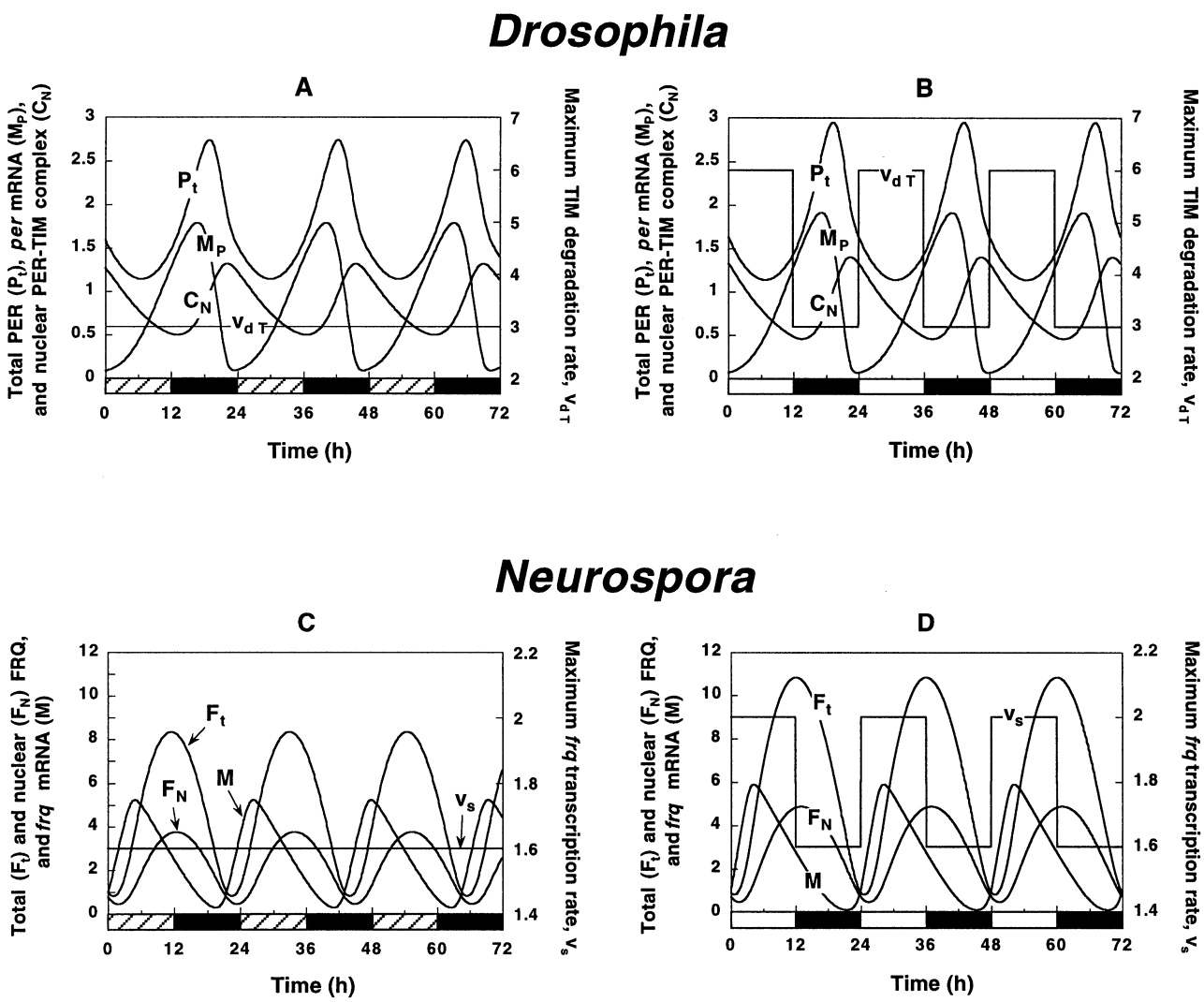

Figure 3. Sustained oscillations generated by the model based on negative control of (A and B) *per* and *tim* expression by a PER–TIM complex in *Drosophila* and (C and D) *frq* expression by the FRQ protein in *Neurospora*.

The curves in panels A and C correspond to continuous darkness, whereas those in panels B and D correspond to entrainment by a light–dark cycle of 24-h periodicity (12:12 LD). The LD cycle is symbolized by the alternation of white and black bars at the bottom of panels B and D; continuous darkness is symbolized in panels A and C by the alternation of hatched and black bars. The curves for *Drosophila* have been obtained by numerical integration of the ten kinetic equations governing the dynamics of the extended model schematized in *figure 2*; these equations are listed as equations (1a)–(1j) in [20]. Shown in panels A and B is the temporal variation in *per* mRNA (M_P) and in the total amount of PER protein (P_t), together with the variation in nuclear PER–TIM complex (C_N). Parameter values are: $n = 4$, $v_{sP} = 1.1$ nMh^{-1}, $v_{sT} = 1$ nMh^{-1}, $v_{mP} = 1.0$ nMh^{-1}, $v_{mT} = 0.7$ nMh^{-1}, $v_{dP} = 2.2$ nMh^{-1}, $k_{sP} = k_{sT} = 0.9$ h^{-1}, $k_1 = 0.8$ h^{-1}, $k_2 = 0.2$ h^{-1}, $k_3 = 1.2$ nM^{-1}h^{-1}, $k_4 = 0.6$ h^{-1}, $K_{mP} = K_{mT} = 0.2$ nM, $K_{IP} = K_{IT} = 1$ nM, $K_{dP} = K_{dT} = 0.2$ nM, $K_{1P} = K_{1T} = K_{2P} = K_{2T} = K_{3P} = K_{3T} = K_{4P} = K_{4T} = 2$ nM, $V_{1P} = V_{1T} = 8$ nMh^{-1}, $V_{2P} = V_{2T} = 1$ nMh^{-1}, $V_{3P} = V_{3T} = 8$ nMh^{-1}, $V_{4P} = V_{4T} = 1$ nMh^{-1}, $k_d = k_{dC} = k_{dN} = 0.01$ nMh^{-1}. Parameter v_{dT} (in nMh^{-1}) remains constant and equal to 3 in (A), and is equal in (B) to 3 and 6 during the dark and light phases, respectively. For the case of *Neurospora* (panels C and D), the curves have been obtained by numerical integration of equations (1a)–(1c). Parameter values are: $v_m = 0.505$ nMh^{-1}, $v_d = 1.4$ nMh^{-1}, $k_s = 0.5$ h^{-1}, $k_1 = 0.5$ h^{-1}, $k_2 = 0.6$ h^{-1}, $K_m = 0.5$ nM, $K_I = 1$ nM, $K_d = 0.13$ nM, $n = 4$. Parameter v_s (in nMh^{-1}) remains constant and equal to 1.6 in (C), and is equal in (D) to 1.6 and 2 during the dark and light phases, respectively. The concentration scale is expressed, tentatively, in nM. Given that quantitative experimental data are still lacking, the above parameter values, which are in a physiological range, have been selected arbitrarily so as to yield a period close to 24 h in *Drosophila* and 21.5 h in *Neurospora*; these are the periods of the oscillations observed in constant darkness in these organisms.

for the *Drosophila* rhythm based on the sole regulation by PER [13, 19].

In *Neurospora*, FRQ can be phosphorylated [33], much as PER and TIM in *Drosophila* [24, 34]. Given that the non-phosphorylated form of FRQ enters the nucleus [33] and that oscillations can occur in the model in the absence of phosphorylation [20], we disregard in a first step the covalent modification of the protein. The minimal model

for circadian oscillations of FRQ and *frq* mRNA in *Neurospora* is then governed by the following set of three kinetic equations:

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + F_N^n} - v_m \frac{M}{K_m + M} \quad (1a)$$

$$\frac{dF_C}{dt} = k_s M - v_d \frac{F_C}{K_d + F_C} - k_1 F_C + k_2 F_N \quad (1b)$$

$$\frac{dF_N}{dt} = k_1 F_C - k_2 F_N \quad (1c)$$

In these equations, the three variables M, F_C and F_N denote, respectively, the concentrations (defined with respect to the total cell volume) of the *frq* mRNA and of the cytosolic and nuclear forms of FRQ. The total, non-conserved concentration of FRQ, equal to $F_C + F_N$, is denoted F_t. Parameter v_s denotes the rate of *frq* transcription; this parameter increases in the light phase. The other parameters appearing in these equations are the constant K_I related to the threshold beyond which nuclear FRQ represses *frq* transcription, the Hill coefficient n characterizing the degree of co-operativity of the repression process, the maximum rate v_m of *frq* mRNA degradation and the Michaelis constant K_m related to the latter process, the apparent first-order rate constant k_s measuring the rate of FRQ synthesis which is assumed to be proportional to the amount of *frq* mRNA present in the cytosol, the maximum rate v_d of FRQ degradation and the Michaelis constant K_d related to this process, and the apparent first-order rate constants k_1 and k_2 characterizing the transport of FRQ into and out of the nucleus. Phosphorylation of FRQ can readily be incorporated, as was done previously for the case of *Drosophila* [13, 19, 20]. In the model this process is not required for sustained oscillations, but it affects their period as well as the magnitude of the domain in which they occur in parameter space [20].

As for the case of oscillations in *Drosophila* illustrated in *figure 3* in conditions of constant darkness (panel A) and 12:12 LD cycle (panel B), we show in panels C and D the corresponding oscillations produced by the model for circadian rhythms in *Neurospora*. Here, the parameter that varies with light is the rate of *frq* transcription, v_s. This parameter remains at a low value during the dark phase and increases up to a higher value during the light phase. In the case considered in *figure 3.D*, the system is entrained precisely to the external period equal to 24 h, whereas it produces autonomous oscillations of a period close to 21.5 h in constant conditions (*figure 3.C*), as observed in the experiments [33].

The curves in *figure 3.C* indicate that the peak in total FRQ precedes by 1.1 h the peak in nuclear FRQ. A qualitatively similar phase relationship between total PER and the nuclear PER–TIM complex is obtained in the model for circadian rhythms in *Drosophila* (*figure 3.A*). While experiments in the fly show that nuclear PER reaches its peak after the total amount of protein [35], the situation is not fully clear in the fungus. There, the only data available so far seem to indicate that nuclear FRQ reaches its peak a few hours before total FRQ [36]. However, the fact that the data have been collected at 4-h intervals does not allow a precise determination of the phase relationships between the two quantities. Since the model predicts a different phase relationship, it would be of particular interest to determine the temporal variation of *frq* mRNA, of the total amount of FRQ, and of both the nuclear and cytoplasmic forms of the protein at shorter time intervals, e.g. every hour, so as to characterize more precisely the phase relationships between these variables.

In the meantime, to investigate conditions in which nuclear FRQ might peak before the total amount of this protein, we have added a step in the mechanism considered in *figure 1.B*. Thus, we assumed that the cytoplasmic FRQ form F_C enters the nucleus but is also transformed into a relatively stable form of the protein, denoted F_S, which does not enter the nucleus; degradation of F_S but not of F_C occurs in the cytosol. We also include the possible degradation of nuclear FRQ, not considered so far, and obtain the following set of kinetic equations for the concentrations of F_C, F_S and F_N; the kinetic equation for the *frq* mRNA (M) remains as given by equation (1a):

$$\frac{dF_C}{dt} = k_s M - k_1 F_C + k_2 F_N - kF_C \quad (2a)$$

$$\frac{dF_s}{dt} = kF_C - v_d \frac{F_s}{K_d + F_s} \quad (2b)$$

$$\frac{dF_N}{dt} = k_1 F_C - k_2 F_N - v_{dN} \frac{F_N}{K_{dN} + F_N} \quad (2c)$$

The total amount of FRQ, F_t, is now equal to the sum $F_C + F_S + F_N$. As shown in *figure 4*, this four-variable model can also produce sustained oscillations. However, the fact that the cytoplasmic form F_S peaks several hours after the nuclear form F_N delays the rise in total FRQ, which peaks 0.4 h after nuclear FRQ in the case considered in *figure 5*. However, this phase difference, which holds only qualitatively with the experimental observation [36], is the largest obtained in the course of numerical simulations of equations (1a) and (2a–c). As the preceding model, the modified version generally produces a peak in nuclear FRQ that follows the peak in total FRQ. This result further underlines the usefulness of obtaining more detailed experimental measurements of the phase relationships between the biochemical variables of the *Neurospora* circadian clock.

3. Effect of light–dark cycles on circadian oscillations: entrainment versus chaos

The effect of light–dark cycles on circadian oscillations has already been discussed in the previous section and illustrated in panels B and D of *figure 3* for the cases of

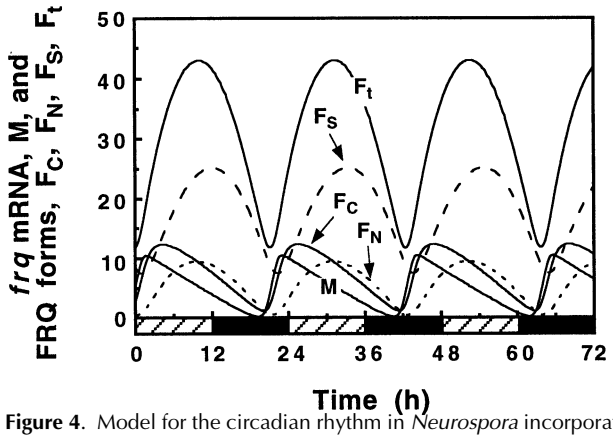

Figure 4. Model for the circadian rhythm in *Neurospora* incorporating the putative formation of an additional form of cytoplasmic FRQ, F_s, which is degradable but unable to enter the nucleus.
The purpose of this modification of the model is to investigate possible mechanisms that might result in advancing the peak in nuclear FRQ, F_N, with respect to the peak in total protein, F_t. The curves are obtained by numerical integration of equations (1a) and (2a)–(2c), for the following parameter values: $v_s = 6$ nMh^{-1}, $v_m = 0.7$ nMh^{-1}, $v_d = 4$ nMh^{-1}, $v_{dN} = 1.5$ nMh^{-1}, $k_s = 1$ h^{-1}, $k = 0.5$ h^{-1}, $k_1 = 0.3$ h^{-1}, $k_2 = 0.15$ h^{-1}, $K_m = 0.4$ nM, $K_I = 1$ nM, $K_d = 1.4$ nM, $K_{dN} = 0.4$ nM, $n = 4$. The period of the oscillations is close to 21.3 h. Continuous darkness is symbolized at the bottom of the panel by the alternation of hatched and black bars.

Drosophila and *Neurospora*, respectively. The circadian models proposed for the two organisms allow us to consider additional aspects of the periodic forcing of circadian clocks by light–dark cycles.

Experiments carried out in *Drosophila* with 8:16, 12:12 and 16:8 LD cycles [37] have shown that the peak of *per* mRNA follows by about 4 h the onset of the dark phase, regardless of the relative duration of the light and dark phases in such light–dark cycles of 24-h periodicity. Simulations of the extended model schematized in *figure 2* account for such observations and indicate that the beginning of the dark phase corresponds to a drop in TIM degradation which allows the rise in TIM; the subsequent increase in the PER–TIM complex up to the level beyond which repression occurs and mRNA begins to decrease takes the same time in all cases, hence the locking of the peak in *per* mRNA to the onset of the dark phase (J.C. Leloup, A. Goldbeter, in prep.).

Whereas the data of *figure 3.B* and *D* correspond to entrainment to the external LD cycle, the models indicate that the periodic forcing of the circadian system does not always result in periodic behaviour. To determine the influence of the intensity of light during the light phase of LD cycles, we have examined the effect of periodic changes of varying amplitude in parameter v_s in the model for circadian oscillations in *Neurospora*. In panel B of *figure 5*, we illustrate again the case of periodic entrainment to the LD 12:12 cycle for the same set of parameter values as in *figure 3.D*, except that v_s (in nMh^{-1}) now increases from the same basal value of 1.6 (which corresponds to an autonomous period close to 21.5 h, as shown in *figure 3.C*) up to the slightly larger value of 2.25 instead of 2. The resulting periodic behaviour corresponds to the limit cycle trajectory shown as a projection in the concentration space (M, F_N, F_t) in panel A of *figure 5*.

When the value of v_s in the light phase is larger, however, entrainment to the external LD cycle fails and aperiodic, chaotic oscillations occur, as exemplified in panel D of *figure 5* where the parameter increases from 1.6 to 4.7 nMh^{-1}. Such sustained oscillations are characterized by irregular changes in both the amplitude and the time intervals between successive peaks, as illustrated in *figure 5.D* for the variation of *frq* mRNA. This chaotic behaviour corresponds to the evolution toward a strange attractor in the phase space (*figure 5.C*), which markedly differs from the limit cycle trajectory shown in *figure 5.A*. The occurrence of chaos was characterized by means of first return maps (data not shown). Upon increasing the maximum value of v_s in the light phase of LD cycles, chaos is reached from periodic behaviour through a sequence of period-doubling bifurcations.

Thus, depending on the intensity of light which affects the amplitude of the biochemical changes brought about by LD cycles in *Neurospora* [26] and incorporated here in the variation of parameter v_s which measures the maximum rate of *frq* transcription, the forcing of circadian oscillations may result in entrainment or chaos. Another possibility, not shown here, is that of quasiperiodic oscillations, which are obtained in the model when the amplitude of the periodic variation in v_s is much reduced.

In the domain of entrainment, the simulations of the model indicate that an additional effect of LD cycles of increasing magnitude – corresponding to increasing light intensity during the light phase – is to shift the phase of the oscillations with respect to the phase of the periodic forcing. Thus, the maximum in *frq* mRNA shifts from 4.2 to 12.2 h after the onset of the light phase (which, as the dark phase, lasts 12 h) as the value of parameter v_s during the light phase increases from 2 to 2.6 nMh^{-1}; for larger v_s values, chaos occurs.

4. Discussion

We have previously proposed models for circadian oscillations of the PER and TIM proteins and of their mRNAs in *Drosophila*. The simpler model is based on the negative feedback exerted in this organism by the PER protein on the expression of the *per* gene. In the extended model schematized in *figure 2*, the negative feedback is exerted on the expression of the *per* and *tim* genes by the PER–TIM complex. In this article we showed that a similar model based on the negative feedback exerted by the protein FRQ on the expression of the *frq* gene may account for the origin of circadian oscillations of FRQ and *frq* mRNA in *Neurospora*. In each case circadian oscillations are of the limit cycle type. The endogenous nature of the oscillations is reflected by the fact that they occur in constant environmental conditions, e.g. continuous darkness (see below).

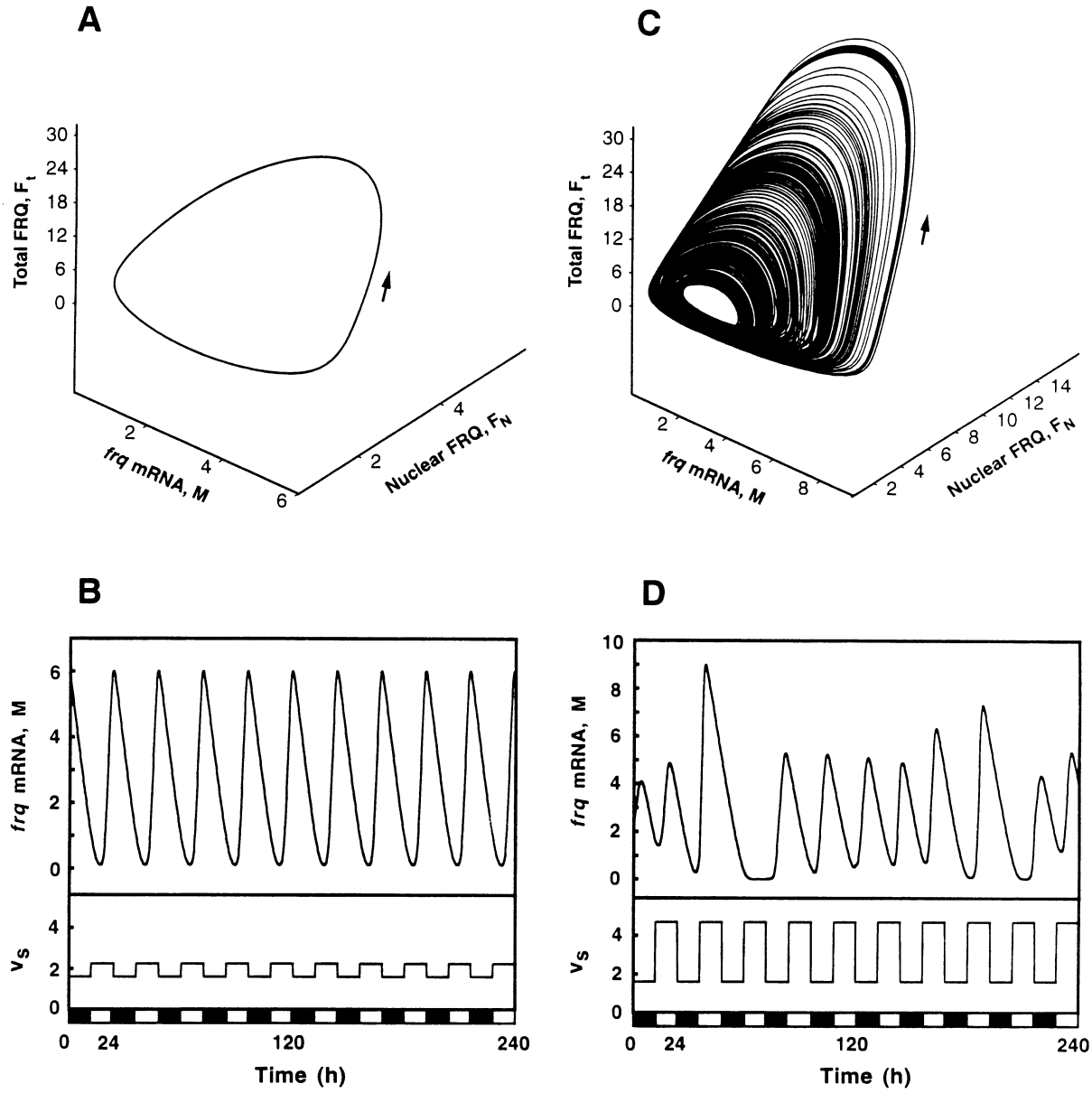

Figure 5. Entrainment (A and B) and chaos (C and D) resulting from the forcing of the model for circadian oscillations in *Neurospora* by periodic variations in the maximum rate of *frq* transcription (v_s) associated with a light–dark cycle of 24-h periodicity.
Shown is the time evolution of *frq* mRNA (*M*) when v_s varies (in nMh^{-1}) from 1.6 to 2.25 in (B), and from 1.6 to 4.7 in (D), during the dark and light phases, respectively. The curves are obtained as in *figure 3.C* and *3D*, for the same set of parameter values, except for the change in v_s just mentioned. The aperiodic oscillations in (D) represent chaotic behaviour. The trajectories in the phase space corresponding to the oscillations shown in (B) and (D) are represented in (A) and (C), respectively, as projections in the space formed by the concentrations of *frq* mRNA, nuclear FRQ and total FRQ. The limit cycle trajectory obtained in (A) at the lower amplitude of v_s forcing transforms into a strange attractor in (C) at the higher amplitude of periodic variations in v_s. The arrows indicate the direction of movement along the phase space trajectories. The light–dark cycle of 24-h periodicity (12:12 LD) is symbolized by the alternation of white and black bars at the bottom of panels B and D.

The models take into account the different effects of light in the two organisms. Thus, in *Drosophila*, light induces the degradation of the TIM protein, whereas in *Neurospora* light induces the expression of the *frq* gene. As a result of this differential effect of light, the peak in *per* (*tim*) and *frq* mRNA occurs during the dark and the light phases, respectively (see *figure 3.B* and *D*), in agreement with experimental observations in *Drosophila* [37] and *Neurospora* [38].

Moreover, the models can help to explain why sustained circadian oscillations occur both in DD (continuous darkness) and, apparently, LL (continuous light) in *Neurospora* [26, 33], while the oscillations are sustained in DD but damped in LL in *Drosophila* [37]. The model for *Drosophila* indeed shows that circadian rhythms occur in a window bounded by two critical, bifurcation values of the light-controlled parameter v_{dT}. Thus, if the value of v_{dT} in the dark lies within the oscillatory domain but goes out

of it upon increasing during the light phase, this would explain why oscillations are sustained in DD but not in LL conditions. In contrast, the study of the model for circadian rhythms in *Neurospora* shows that sustained oscillations occur above a critical value of the light-controlled parameter v_s (although for other parameter values the situation may become analogous to that seen in the *Drosophila* model, with the appearance of a second, larger critical value of v_s providing an upper bound for the oscillatory domain). If the situation is that of a single bifurcation point, and if the values of v_s corresponding to DD and LL are both larger than this single critical value, circadian oscillations will be sustained in continuous darkness or light.

Another conspicuous property of circadian rhythms is that their period remains largely independent of temperature. As shown by the study of the model for circadian oscillations of the PER protein, this property of temperature compensation can in principle arise from the antagonistic effects exerted on the period by the different kinetic parameters of the system. The value of most parameters is expected to increase with temperature. The rise in some parameters results in an increased period while a decreased period results from the rise in other parameters. If these effects roughly counterbalance each other, the period of the rhythm will not change significantly as the temperature changes [27].

The model for circadian rhythms in *Drosophila* incorporating the formation of a PER–TIM complex is so far the most detailed in molecular terms. This model accounts for oscillations in the wild type and in several *per* mutants such as per^{l} and per^{s} [20]. Further extensions of this model are presently being studied, to incorporate the possible role of post-transcriptional regulation as well as the role of recently discovered gene products such as the activators CLOCK and CYCLE which mediate the control exerted by the PER–TIM complex on the expression of the *per* and *tim* genes.

The model for *Neurospora* is less detailed, if only because FRQ does not appear to form a complex with another protein to exert its negative feedback action. In its simple three-variable form obtained when disregarding FRQ phosphorylation, the model is closely related to the model proposed by Goodwin [16] for oscillations due to negative feedback on gene expression. Ruoff et al. [39] have recently applied the Goodwin oscillator model to determine the phase-shifting effect of pulses of cycloheximide and heat shocks on the *Neurospora* circadian clock. Their study focuses on the effect of inhibitors of protein synthesis and degradation, and does not address the control of circadian oscillations by light, which is considered in the present study. In contrast to the model proposed here, which is derived from the simple version of the *Drosophila* oscillator model [13, 19], the version of the Goodwin model used by Ruoff et al. contains only linear terms except the repression function which is characterized by a rather large value of 9 for the Hill coefficient. In the present model, oscillations occur for a Hill coefficient of 4 (*figure 3.C*), but can also occur for smaller values, e.g. 2, and even in the absence of co-operativity of repression when the Hill coefficient is equal to unity [20], although co-operativity definitely favours the occurrence of sustained oscillations.

We have shown in the present paper that light–dark cycles can have different effects on circadian oscillations, depending on the magnitude of the periodic changes in the light-controlled parameter. Thus, quasiperiodic oscillations, entrainment to the external cycle, or aperiodic oscillations in the form of chaos can be observed. Chaos resulting from periodic forcing has been studied in a variety of oscillatory systems, in a chemical, physical or biological context (see, for example, Holden [40]). To our knowledge the present results are the first to pertain to the occurrence of chaos through the periodic forcing of circadian oscillations by light–dark cycles. This prediction, made on the basis of simulations of the model for circadian oscillations in *Neurospora*, has not yet been substantiated by experimental observations. To obtain such data, it would be interesting to perform a detailed study of the effect of LD cycles of varying light intensity during the light phase, to determine whether entrainment can sometimes fail and transform into chaos.

The results of *figure 5* have been obtained by using the model for circadian oscillations in *Neurospora*. Similar results on the effect of LD cycles will likely be obtained with the model for circadian rhythms in *Drosophila*, when incorporating a periodic variation of parameter v_{dT} as in *figure 3.B*. As the autonomous period is close to 24 h in constant darkness in *Drosophila*, the question arises as to whether the period of the external LD cycle might have to differ from 24 h to obtain chaos in this organism. For the case of *Neurospora*, the occurrence of chaos could be favoured by the difference between the autonomous period close to 21.5 h in constant darkness and the 24 h period of the LD cycle considered in *figure 5.D*. However, chaos has also been found when forcing this system by an LD cycle of 21.5 h period. Therefore, we expect that chaos should occur in the *Drosophila* circadian model even for 12:12 LD cycles, provided that the large amplitude of the changes in TIM degradation rate required for chaos can be achieved experimentally by increasing the intensity of light during the light phase.

Forcing the circadian oscillator by LD cycles of sufficient amplitude is not the only scenario leading to chaos in the circadian control system. Thus, autonomous chaos has been found in the extended model for circadian oscillations in *Drosophila* [41]. Such aperiodic oscillations occur in this model in constant environmental conditions corresponding to continuous darkness, as a result of asymmetries in the values of the biochemical parameters characterizing the two branches of the PER–TIM control system schematized in *figure 2*. While it is likely that such autonomous chaos does not underlie the oscillatory behaviour of arrhythmic mutants of the *Drosophila* circadian clock – these mutants generally lack a functional protein of the circadian control system [42, 43] – the results on the

occurrence of chaos through periodic forcing by light–dark cycles are a possibility that warrants experimental investigation both in *Drosophila* and *Neurospora*. If chaos were found in such experiments, the question would arise as to whether the phenomenon possesses any physiological significance.

In the case of *Drosophila*, another question would pertain to the behaviour resulting from coupling chaotic cells. The model for circadian oscillations in PER and TIM accounts for the behaviour of a single pacemaker cell in *Drosophila*. How pacemaker cells are coupled between them and with other rhythm-expressing cells in the organism remains to be established experimentally, and studied theoretically both in the cases of periodic and chaotic behaviour.

We focused in the present study on the modelling of circadian rhythms in *Neurospora* and *Drosophila*. Recent experimental observations indicate that the *per* and *tim* gene products are also found in mammals, including man [9, 10]. This strengthens the possibility that the models proposed for *Drosophila* – which, as shown here, are themselves closely related to that proposed for *Neurospora* – may also hold for circadian rhythms in mammals. It appears that the effect of light might nevertheless be close to that seen in *Neurospora*, since light induces the expression of some of the *per* gene homologues in mammals [44, 45].

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Note added in proof:

We have recently carried out a detailed analysis of the conditions in which chaos occurs in the *Neurospora* circadian clock model driven by light-dark cycles of varying shape, period and amplitude (D. Gonze, A. Goldbeter, manuscript submitted for publication). Modeling the effect of light on circadian rhythms has also been discussed in two recent publications [46, 47].

References

[1] Dunlap J.C., Molecular bases for circadian clocks, Cell 96 (1999) 271–290.

[2] Johnson C.H., Golden S.S., Ishiura M., Kondo T., Circadian clocks in prokaryotes, Mol. Microbiol. 21 (1996) 5–11.

[3] de Mairan J.J. Dortous, Observation botanique, Histoire de l'Académie Royale des Sciences (Paris) (1729) 35.

[4] Konopka R.J., Benzer S., Clock mutants of *Drosophila melanogaster*, Proc. Natl. Acad. Sci. USA 68 (1971) 2112–2116.

[5] Baylies M.K., Weiner L., Vosshall L.B., Saez L., Young M.W., Genetic, molecular, and cellular studies of the per locus and its products in *Drosophila melanogaster*, in: Young M.W. (Ed.), Molecular Genetics of Biological Rhythms, M. Dekker, New York, 1993 , pp. 23–153.

[6] Rosbash M., Molecular control of circadian rhythms, Curr. Opin. Genet. Dev. 5 (1995) 662–668.

[7] Dunlap J.C., Genetic and molecular analysis of circadian rhythms, Annu. Rev. Genet. 30 (1996) 579–601.

[8] Crosthwaite S.K., Dunlap J.C., Loros J.J., *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity, Science 276 (1997) 763–769.

[9] Tei H., Okamura H., Shigeyoshi Y., Fukuhara C., Ozawa R., Hirose M., Sakaki Y., Circadian oscillation of a mammalian homologue of the *Drosophila period* gene, Nature 389 (1997) 512–516.

[10] Zylka M.J., Shearman L.P., Levine J.D., Jin X., Weaver D.R., Reppert S.M., Molecular analysis of mammalian *timeless*, Neuron 21 (1998) 1115–1122.

[11] Dunlap J.C., An end in the beginning, Science 280 (1998) 1548–1549.

[12] Winfree A.T., The Geometry of Biological Time, Springer, New York, 1980.

[13] Goldbeter A., Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of Periodic and Chaotic Behaviour, Cambridge University Press, Cambridge, 1996 .

[14] Wever R.A., Virtual synchronization towards the limits of the range of entrainment, J. Theor. Biol. 36 (1972) 119–132.

[15] Jewett M.E., Kronauer R.E., Refinement of a limit cycle oscillator model of the effects of light on the human circadian pacemaker, J. Theor. Biol. 192 (1998) 455–465.

[16] Goodwin B.C., Oscillatory behavior in enzymatic control processes, Adv. Enzyme Regul. 3 (1965) 425–438.

[17] Drescher K., Cornelius G., Rensing L., Phase response curves obtained by perturbing different variables of a 24 hr model oscillator based on translational control, J. Theor. Biol. 94 (1982) 345–353.

[18] Ruoff P., Mohsenzadeh S., Rensing L., Circadian rhythms and protein turnover: the effect of temperature on the period lengths of clock mutants simulated by the Goodwin oscillator, Naturwissenschaften 83 (1996) 514–517.

[19] Goldbeter A., A model for circadian oscillations in the *Drosophila period* (PER) protein, Proc. R. Soc. Lond. B 261 (1995) 319–324.

[20] Leloup J.C., Goldbeter A., A model for circadian rhythms in *Drosophila* incorporating the formation of a complex between the PER and TIM proteins, J. Biol. Rhythms 13 (1998) 70–87.

[21] Hunter-Ensor M., Ousley A., Sehgal A., Regulation of the *Drosophila* protein Timeless suggests a mechanism for resetting the circadian clock by light, Cell 84 (1996) 677–685.

[22] Lee C., Parikh V., Itsukaichi T., Bae K., Edery I., Resetting the *Drosophila* clock by photic regulation of PER and a PER-TIM complex, Science 271 (1996) 1740–1744.

[23] Myers M.P., Wager-Smith K., Rothenfluh-Hilfiker A., Young M.W., Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock, Science 271 (1996) 1736–1740.

[24] Zeng H., Qian Z., Myers M.P., Rosbash M., A light-entrainment mechanism for the *Drosophila* circadian clock, Nature 380 (1996) 129–135.

[25] Aronson B.D., Johnson K.A., Loros J.J., Dunlap J.C., Negative feedback defining a circadian clock: Autoregulation of the clock gene *frequency*, Science 263 (1994) 1578–1584.

[26] Crosthwaite S.K., Loros J.J., Dunlap J.C., Light-induced resetting of a circadian clock is mediated by a rapid increase in *frequency* transcript, Cell 81 (1995) 1003–1012.

[27] Leloup J.C., Goldbeter A., Temperature compensation of circadian rhythms: control of the period in a model for circadian oscillations of the PER protein in *Drosophila*, Chronobiol. Int. 14 (1997) 511–520.

[28] Allada R., White N.E., So W.V., Hall J.C., Rosbash M., A mutant *Drosophila* homolog of mammalian *clock* disrupts circadian rhythms and transcription of *period* and *timeless*, Cell 93 (1998) 791–804.

[29] Darlington T.K., Wager-Smith K., Ceriani M.F., Staknis D., Gekakis N., Steeves T.D.L., Weitz C.J., Takahashi J.S., Kay S.A., Closing the

circadian loop: CLOCK-induced transcription of its own inhibitors *per* and *tim*, Science 280 (1998) 1599–1603.

[30] Rutila J.E., Suri V., Le M., So W.V., Rosbash M., Hall J.C., CYCLE is a second bHLH-PAS clock protein essential for circadian rhythmicity and transcription of *Drosophila period* and *timeless*, Cell 93 (1998) 805–814.

[31] So W.V., Rosbash M., Post-transcriptional regulation contributes to *Drosophila* clock gene mRNA cycling, EMBO J. 16 (1997) 7146–7155.

[32] Cheng Y., Hardin P.E., *Drosophila* photoreceptors contain an autonomous circadian oscillator that can function without *period* mRNA cycling, J. Neurosci. 18 (1998) 741–750.

[33] Garceau N.Y., Liu Y., Loros J.J., Dunlap J.C., Alternative initiation of translation and time-specific phosphorylation yield multiple forms of the essential clock protein FREQUENCY, Cell 89 (1997) 469–476.

[34] Edery I., Zwiebel L.J., Dembinska M.E., Rosbash M., Temporal phosphorylation of the *Drosophila period* protein, Proc. Natl. Acad. Sci. USA 91 (1994) 2260–2264.

[35] Curtin K.D., Huang Z.J., Rosbash M., Temporally regulated nuclear entry of the *Drosophila period* protein contributes to the circadian clock, Neuron 14 (1995) 365–372.

[36] Luo C., Loros J.J., Dunlap J.C., Nuclear localization is required for function of the essential clock protein FRQ, EMBO J. 17 (1998) 1228–1235.

[37] Qiu J., Hardin P.E., *per* mRNA cycling is locked to lights-off under photoperiodic conditions that support circadian feedback loop function, Mol. Cell. Biol. 16 (1996) 4182–4188.

[38] Iwasaki K., Thomas J.H., Genetics in rhythm, Trends Genet. 13 (1997) 111–115.

[39] Ruoff P., Vinsjevik M., Mohsenzadeh S., Rensing L., The Goodwin model: simulating the effect of cycloheximide and heat shock on the sporulation rhythm of *Neurospora crassa*, J. Theor. Biol. 196 (1999) 483–494.

[40] Holden A.V., Chaos, Manchester Univ. Press, Manchester, 1986.

[41] Leloup J.C., Goldbeter A., Chaos and birhythmicity in a model for circadian oscillations of the PER and TIM proteins in *Drosophila*, J. Theor. Biol. 198 (1999) 445–459.

[42] Yu Q., Jacquier A.C., Citri Y., Hamblen M., Hall J.C., Rosbash M., Molecular mapping of point mutations in the *period* gene that stop or speed up biological clocks in *Drosophila melanogaster*, Proc. Natl. Acad. Sci. USA 84 (1987) 784–788.

[43] Sehgal A., Price J.L., Man B., Young M.W., Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless*, Science 263 (1994) 1603–1606.

[44] Albrecht U., Sun Z.S., Eichele G., Lee C.C., A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light, Cell 91 (1997) 1055–1064.

[45] Shearman L.P., Zylka M.J., Weaver D.R., Kolakowski L.F. Jr, Reppert S.M., Two period homologs: circadian expression and photic regulation in the suprachiasmatic nuclei, Neuron 19 (1997) 1261–1269.

[46] Leloup J.C., Gonze D., Goldbeter A., Limit cycle models for circadian rhythms based on transcriptional regulation in *Drosophila* and *Neurospora*, J. Biol. Rhythms 14 (1999) 433–448.

[47] Leloup J.C., Goldbeter A., Modeling the molecular regulatory mechanism of circadian rhythms in *Drosophila*, BioEssays 22 (2000) 84–93.