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Dependence of the period on the rate of protein degradation in minimal models for circadian oscillations

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Circadian rhythms, which occur spontaneously with a period of about 24 h in a variety of organisms, allow their adaptation to the periodic variations of the environment. These rhythms are generated by a genetic regulatory network involving a negative feedback loop on transcription. Mathematical models based on the negative autoregulation of gene expression by the protein product of a clock gene account for the occurrence of self-sustained circadian oscillations. These models differ by their degree of complexity and, hence, by the number of variables considered. Some of these models can be considered as minimal because they contain a reduced number of biochemical processes and variables capable of producing sustained oscillations. In three of these minimal models, the period of the oscillations significantly changes with the rate of degradation of the clock protein. However, depending on the model considered, the period increases, decreases or passes through a maximum as a function of the protein degradation rate. We clarify the bases for these markedly different results by bringing to light the roles of (i) protein phosphorylation, which is required for protein degradation, and (ii) the velocity and degree of saturation of mRNA and protein degradation. Changes in the parameter values of the more complex of the minimal models can produce the period profiles observed in the other two models. The analysis allows us to reconcile the contradictory predictions for the dependence of the period on the clock protein degradation rate in three minimal models used to describe circadian rhythms.

Keywords: biological rhythms; circadian clocks; computational model; minimal model; PER phosphorylation

1. Introduction

To adapt to the daily variations of the environment, nearly all living organisms have evolved an autonomous clock characterized by a period of about 24 h, which persists even if the organism is maintained in constant conditions (Dunlap *et al.* 2004). This internal clock further allows the organism to anticipate the environmental changes by preparing it for the light-to-dark or

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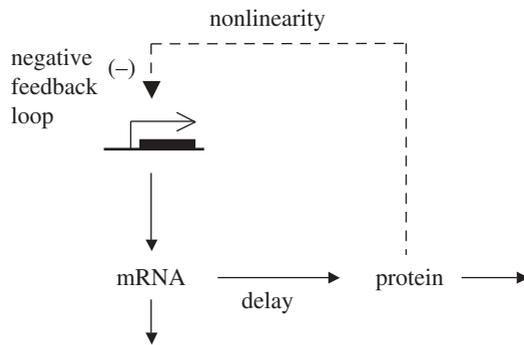


Figure 1. Generic scheme of the core regulatory mechanism of circadian rhythms. A gene is transcribed into mRNA, which is translated into the clock protein. This protein represses the transcription of its own gene, thereby creating a negative feedback loop. In addition to this negative feedback, the presence of at least one nonlinearity in the kinetic equations and a delay—due, for example, to protein phosphorylation or to translocation of the clock protein between cytoplasm and nucleus—are required to generate sustained oscillations.

dark-to-light transition. Genetic studies, initially carried out in the fruit fly *Drosophila* (Hardin *et al.* 1990) and the fungus *Neurospora* (Aronson *et al.* 1994), and later extended to mammals, showed that circadian oscillations are generated at the molecular level. The core molecular mechanism of these oscillations relies on the negative autoregulatory feedback exerted by clock proteins on the expression of their genes (Dunlap 1999; Young & Kay 2001).

A precise control of the period of circadian rhythms is crucial for a good phase relationship between the clock and periodic external signals such as the light–dark cycle. Such a tight control is also important to maintain the period constant at different temperatures, a property referred to as temperature compensation (Ruoff *et al.* 2005). Mathematical models are helpful in predicting how properties such as the period of the oscillations are affected when some control parameters are changed. Several key parameters markedly control the period of the oscillations. Here, we focus on the role of clock protein degradation on the circadian period by means of theoretical modelling and numerical simulations.

A variety of more or less detailed models have been proposed to account for the behaviour of circadian clocks. These models usually take the form of a set of ordinary differential equations that describe the time evolution of the concentrations in clock proteins and their mRNAs. Models that can be considered as minimal possess the minimum number—or a relatively small number—of components required to produce self-sustained oscillations. To produce such oscillations, these models must incorporate three components (figure 1): (i) a negative feedback loop that is achieved through inhibition of the clock gene expression by the clock protein, (ii) nonlinear kinetics, which often characterizes the inhibition term and sometimes other reaction steps, and (iii) a delay, which can take the form of either an implicit delay due to the incorporation of steps describing the post-translational modification of the protein or its translocation into the nucleus (yielding additional, intermediary variables), or an explicit delay leading to a system of delay-differential equations.

Minimal models have been proposed for circadian rhythms in *Drosophila* and *Neurospora* (Goldbeter 1995; Leloup *et al.* 1999; Ruoff *et al.* 1999, 2005; Scheper *et al.* 1999*a,b*; Gonze *et al.* 2000). Despite the fact that they all rely on the same general architecture, these models differ by the number of variables—generally 3 to 5—and by the functions used to describe the kinetics of the various biochemical processes. More recently, following the discovery of additional genes and regulatory interactions, more detailed models have been proposed for the *Drosophila* and the mammalian circadian clock (Leloup & Goldbeter 1998, 2003, 2004; Smolen *et al.* 2001; Forger & Peskin 2003; Becker-Weimann *et al.* 2004). All these models, which contain more than 10, and sometimes tens of variables, still rely on a core-regulatory mechanism involving the inhibition of a clock gene by the corresponding clock protein. Although the genetic components of the circuitry responsible for circadian oscillations are well known, the kinetics of the processes in which they take part have not been characterized experimentally in a precise manner. These processes include transcription, translation, mRNA and protein degradation, nuclear transport, and phosphorylation–dephosphorylation steps.

The dependence of the period on a variety of control parameters has been determined numerically in detailed models for circadian rhythms. Thus, in a model for the mammalian circadian clock, different, often non-monotonous profiles of the period were established as a function of the rates of protein or mRNA degradation (Leloup & Goldbeter 2004). Intriguingly, even in minimal models, the profile of the period as a function of a key parameter such as the clock protein degradation rate can be markedly different, depending on the model considered. If all minimal models for circadian clocks are based on a core regulatory negative feedback and involve at least one nonlinear step, they do not always incorporate protein phosphorylation. Moreover, these models use different, linear or nonlinear kinetic functions to describe the interactions between the variables. As a consequence, their predictions are sometimes contradictory. The goal of this paper is to show how these contradictory predictions can be resolved.

In this study, we compare several minimal models with regard to the profile of period as a function of the protein degradation rate. Thus, the period rises as this degradation rate increases in a five-variable model proposed for circadian oscillations in *Drosophila* (Goldbeter 1995), in which mRNA and protein degradation, as well as phosphorylation steps, are described by Michaelian functions. In contrast, in a three-variable model with linear mRNA and protein degradation rates and no clock protein phosphorylation, known as the Goodwin model, the period decreases when the protein degradation rate increases (Ruoff *et al.* 1999, 2005). In a third model (Leloup *et al.* 1999), similar to the five-variable model for circadian oscillations of the PER (*period*) protein in *Drosophila* but without phosphorylation of the PER protein, the period passes through a maximum as a function of the protein degradation rate. We investigate the kinetic properties responsible for these marked variations and reconcile the contradictory predictions of the three minimal models by showing how they derive from the more general, five-variable model for PER oscillations in *Drosophila*. The different profiles of period can be achieved using the latter model by progressive modification of its parameter values. We show that the degree of saturation of various processes such as mRNA and protein degradation, as well as the kinetic order and velocity of the phosphorylation steps, have crucial effects on the period profile.

The predictions reached by the analysis of the minimal models can be used to modify the period profile both in a simple delay model for circadian oscillations based on negative feedback (Scheper *et al.* 1999*a,b*) and in a more detailed model for circadian oscillations in *Drosophila* based on the formation of a complex between the PER and TIM (*timeless*) proteins (Leloup & Goldbeter 1998). The results highlight the dependence of clock properties on the nature of kinetic functions, and clarify the conditions in which a single mathematical model may account for the distinct profiles obtained for the period in different minimal models for circadian oscillations.

2. Minimal models for circadian clocks

Here, we present three minimal models previously proposed for the molecular mechanism of circadian rhythms. All these models represent a particular implementation of the general molecular mechanism illustrated in figure 1. They all rely on a negative feedback loop exerted by a protein on the expression of its own gene, and include an implicit delay and at least one nonlinear step.

The first model, schematized in figure 2*a*, was initially proposed for circadian oscillations of the PER protein and *per* mRNA in *Drosophila* (Goldbeter 1995). This model, which will be referred to hereafter as model A, is based on the negative feedback exerted by PER on the expression of its gene, and includes two reversible phosphorylation steps. Model A is described by a set of five kinetic equations

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M}{K_m + M}, \quad (2.1)$$

$$\frac{dP_0}{dt} = k_s M - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}, \quad (2.2)$$

$$\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}, \quad (2.3)$$

$$\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N \quad (2.4)$$

and
$$\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N - v_n \frac{P_N}{K_n + P_N}. \quad (2.5)$$

The variables here are the concentrations of *per* mRNA (M), non-phosphorylated cytosolic PER protein (P_0), monophosphorylated (P_1) and biphosphorylated (P_2) cytosolic PER protein, and nuclear PER protein (P_N). For a detailed description of the equations and for a definition of the parameters, see Goldbeter (1995). Note that several steps of the model are of nonlinear nature: the inhibition term takes the form of a Hill function in equation (2.1) with the degree of cooperativity n , whereas mRNA and protein degradation, as well as phosphorylation–dephosphorylation terms, are described by Michaelis–Menten functions.

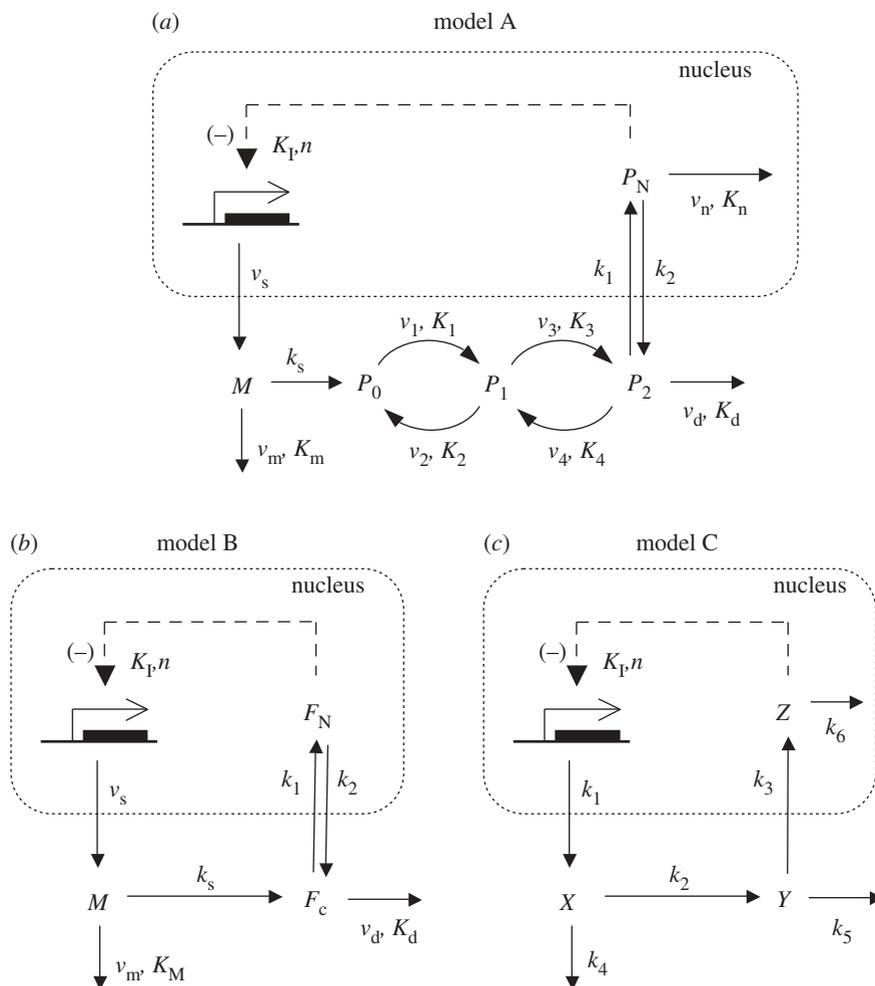


Figure 2. Three minimal models considered for circadian oscillations: (a) In the five-variable model A initially proposed for circadian oscillations of the PER protein in *Drosophila* (Goldbeter 1995), two phosphorylation–dephosphorylation steps producing a delay are considered for PER. The bisphosphorylated form of the protein is either degraded or enters into the nucleus where it represses in a cooperative manner the expression of its gene. Other steps refer to mRNA synthesis, transport into the cytosol and degradation, as well as PER protein synthesis at a rate proportional to the level of mRNA. Nonlinearities occur at the level of transcription, phosphorylation–dephosphorylation, mRNA and PER degradation. (b) The three-variable model B (Leloup *et al.* 1999; Gonze *et al.* 2000) can be seen as a simplification of model A in which the phosphorylation–dephosphorylation steps are omitted. (c) The three-variable model C (Ruoff *et al.* 1999) uses only linear terms except at the level of the transcription where a high degree of cooperativity ($n=9$) for the Hill repression function is considered.

The second model (figure 2b), hereafter referred to as model B, was initially proposed to account for circadian oscillations in the protein FRQ (*frequency*) in *Neurospora* (Leloup *et al.* 1999; Gonze *et al.* 2000). The model is similar to model A, except that it does not incorporate the phosphorylation of the protein and, consequently, contains three instead of five variables. The dynamics of *frq*

mRNA (M), cytosolic FRQ protein (F_c) and nuclear FRQ protein (F_N) are governed by the following system of three kinetic equations:

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + F_N^n} - v_m \frac{M}{K_m + M}, \quad (2.6)$$

$$\frac{dF_c}{dt} = k_s M - v_d \frac{F_c}{K_d + F_c} - k_1 F_c + k_2 F_N \quad (2.7)$$

and
$$\frac{dF_N}{dt} = k_1 F_c - k_2 F_N. \quad (2.8)$$

The third model (figure 2c), hereafter referred to as model C, was also used to account for circadian oscillations in FRQ in *Neurospora* (Ruoff *et al.* 1999, 2005). The model is a variant of the model proposed by Goodwin (1965), which was originally proposed as a generic model for biochemical oscillations based on negative feedback, rather than being designed specifically for circadian rhythms. This three-variable model is described by the following system of kinetic equations:

$$\frac{dX}{dt} = k_1 \frac{K_I^n}{K_I^n + Z^n} - k_4 X, \quad (2.9)$$

$$\frac{dY}{dt} = k_2 X - k_5 Y \quad (2.10)$$

and
$$\frac{dZ}{dt} = k_3 Y - k_6 Z. \quad (2.11)$$

In the context of circadian rhythms, the variables were initially interpreted (Ruoff *et al.* 1999, 2005) as a clock protein (Y), its mRNA (X) and an inhibitor (Z), the synthesis of which is brought about by Y . Here we consider Z as the nuclear form of the clock protein.¹ This will allow us to compare the different models: X , Y and Z would thus correspond to M , P_2 and P_N in model A, and to M , F_c and F_N in model B.

In the following, the dynamics generated by the three models are investigated by means of numerical simulations and bifurcation diagrams. Most results were obtained by means of the program XPP-AUTO (Doedel 1981; Ermentrout 2002).

3. Distinct period profiles as a function of the clock protein degradation rate

Depending on the parameter values, all three models can produce self-sustained oscillations characterized by a period of about 24 h. However, the way the period changes as a function of the parameters markedly depends on the model. Thus, in figure 3, we show the period profile obtained as a function of the maximum protein degradation rate measured by v_d in models A and B, or k_5 in model C. Sustained oscillations occur in a domain bounded by two critical values in the

¹Note that, in this interpretation, k_5 and k_3 would describe the same transport rate and consequently, for consistency, they should be equal. This is not the case in the original parameter values (Ruoff *et al.* 1999; table 1), but this can be solved by a simple rescaling of Z (through rescaling of k_3 and k_6), and K_I .

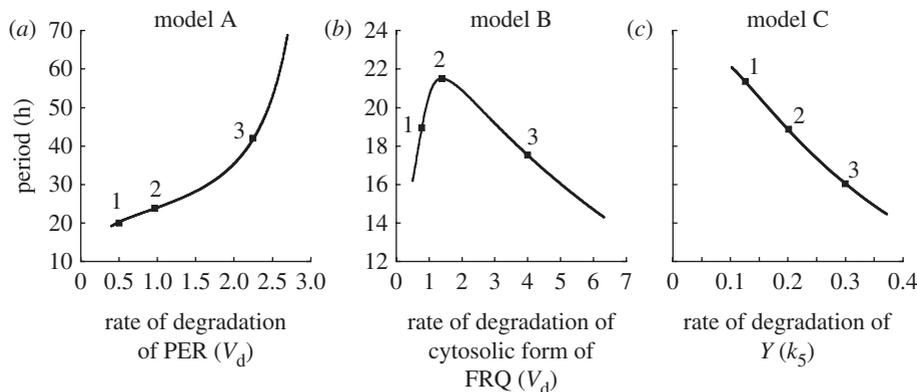


Figure 3. Distinct profiles of the period as a function of the clock protein degradation rate, v_d , in model A and model B, and as a function of the rate constant for protein degradation, k_5 , in model C. As v_d (or k_5) increases, the period rises in model A, passes through a maximum in model B, and decreases in model C. Parameter values are given in tables 1 and 2. Points 1, 2 and 3 refer to situations illustrated for each model by the corresponding time series in figure 4. As in figures 5, 7 and 8, the period profiles have been established by means of the program AUTO (Doedel 1981).

Table 1. List of parameter values used for numerical simulations of model B and model C in figures 3 and 4.

model B (Leloup <i>et al.</i> 1999)		model C (Ruoff <i>et al.</i> 1999)	
v_s (nM h ⁻¹)	1.6	k_1 (nM h ⁻¹)	1
K_I (nM)	1	n	9
n	4	k_2 (h ⁻¹)	1
v_m (nM h ⁻¹)	0.505	k_3 (h ⁻¹)	1
K_M (nM)	0.5	k_4 (h ⁻¹)	0.2
k_s (h ⁻¹)	0.5	k_5 (h ⁻¹)	0.2
v_d (nM h ⁻¹)	1.4	k_6 (h ⁻¹)	0.2
K_d (nM)	0.13	K_I (nM)	1
k_1 (h ⁻¹)	0.5		
k_2 (h ⁻¹)	0.6		

three cases, but the period profile is very different. In model A, the period increases when protein degradation increases. In model C, the period decreases almost linearly with increasing degradation rate. This relation is in agreement with the observation that mutant *Neurospora* strains having a FRQ protein characterized by a shorter life time—i.e. by a higher degradation rate—oscillate with a smaller period (Ruoff *et al.* 2005). Finally, in model B, the profile is biphasic: the period reaches a maximum at some intermediate value of v_d and decreases at higher or lower values of this parameter.

To further illustrate how the oscillations are affected by changes in the clock protein degradation rate, it is useful to plot the time course of mRNA (M) and nuclear clock protein (P_N or F_N) in models A and B, and of the corresponding

Table 2. List of parameter values (column 2) for model A yielding the original period profile as a function of the PER protein degradation rate (see figures 3*a* and 5*a*, and Goldbeter 1995). Listed in columns 3 and 4 are parameter values for which the period profile in model A tends to the period profiles of models B (figure 5*b*) and C (figure 5*c*), respectively.

parameter	model A	model A tends to model B	model A tends to model C
v_s (nM h ⁻¹)	0.76	0.76	0.76
K_1 (nM)	1	1	1
n	4	4	9
v_m (nM h ⁻¹)	0.65	0.65	20
K_m (nM)	0.5	0.5	100
k_s (h ⁻¹)	0.38	0.38	0.38
v_1 (nM h ⁻¹)	3.2	110	110
K_1 (nM)	2	100	100
v_2 (nM h ⁻¹)	1.58	0	0
K_2 (nM)	2	2	2
v_3 (nM h ⁻¹)	5	110	110
K_3 (nM)	2	100	100
v_4 (nM h ⁻¹)	2.5	0	0
K_4 (nM)	2	2	2
v_d (nM h ⁻¹)	0.95	0.95	0.95
K_d (nM)	0.2	0.2	0.2
v_n (nM h ⁻¹)	0	0	0
K_n (nM)	0	0	0
k_1 (h ⁻¹)	1.9	1	1
k_2 (h ⁻¹)	1.3	1.3	0.125

variables X and Z in model C. Time series for three increasing values of v_d (or k_5) for each model (rows 1–3) are shown in figure 4. We notice that not only the period but also the shape of the oscillations are affected when the control parameter is changed.

4. How to reconcile the contradictory predictions of the three minimal models for circadian oscillations

The results described so far have been obtained for each model in a given set of conditions corresponding to a particular set of parameter values. The question arises as to whether other profiles of period could be obtained for other parameter values. Numerical simulations indicate that model A, which is more complex and contains the two other models, can indeed produce period profiles similar to those obtained with models B and C as a function of the clock protein degradation rate. Numerical simulations indicate, however, that the latter models cannot produce the period profile of model A.

Let us illustrate examples of parameter changes through which model A tends to yield the period profiles of models B and C, respectively. At higher values of the maximum rate of phosphorylation, and in the unsaturated range

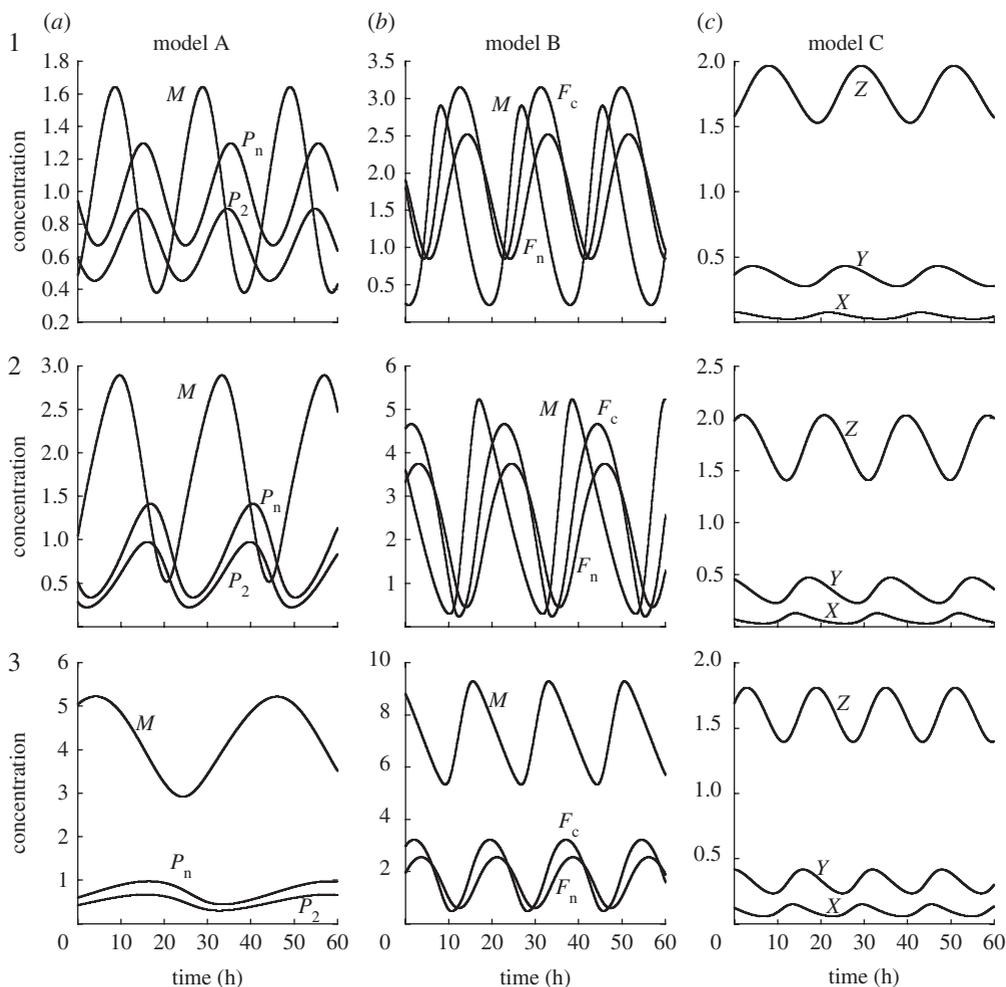


Figure 4. Oscillations obtained for models A, B and C for three values of v_d (or k_5) increasing from rows 1 to 3. Model A (a): $v_d = 0.5, 0.95, 2.25$ (in nM h^{-1}). Model B (b): $v_d = 0.75, 1.4, 4$ (in nM h^{-1}). Model C (c): $k_5 = 0.125, 0.2, 0.3$ (in h^{-1}). The curves, obtained by numerical integration of equations (2.1)–(2.5) for model A, (2.6)–(2.8) for model B, and (2.9)–(2.11) for model C, correspond to the three points marked 1, 2 and 3 on the period profile in figure 3*a–c*. Other parameter values are given in tables 1 and 2.

of the phosphorylation reactions—i.e. at high values of the Michaelis constants characterizing these steps (table 2, second column)—model A tends to model B (figure 5*b*). We then obtain, indeed, a period profile similar to the one obtained for the original model B (figure 3*b*). If, in addition to rapid phosphorylation, we consider linear mRNA degradation, and treat nuclear entry as irreversible by neglecting clock protein export from the nucleus (table 2, third column), the period profile of model A tends to that of model C (compare figures 3*c* and 5*c*). Thus, depending on the values of the kinetic parameters, the three different profiles of period can be obtained for model A, which is sufficiently complex

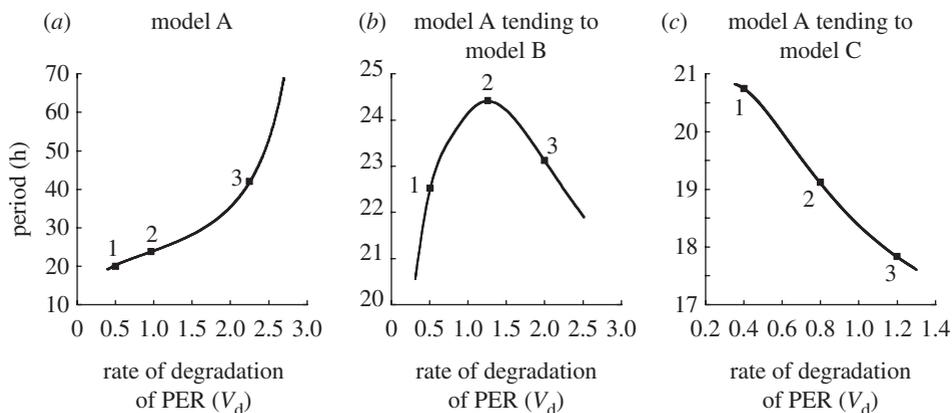


Figure 5. Markedly distinct period profiles obtained in model A for different sets of parameter values. (a) Period profile (same as in figure 3a) obtained for the basal values of model A listed in column 2 of table 2. (b) Period profile, similar to that generated by model B, obtained with model A for the parameter values listed in column 3 of table 2. (c) Period profile, similar to that generated by model C, obtained with model A for the parameter values listed in column 4 of table 2.

to embed the simpler models B and C. The corresponding oscillations obtained in model A for the three cases are shown in figure 6. Having now at hand a single model displaying the different period profiles, we can investigate in more detail the conditions that lead to an increase or decrease of the period as a function of the protein degradation v_d .

One noticeable difference between the various models pertains to the use of Michaelis–Menten functions in models A and B, whereas the corresponding processes are described by linear kinetics in model C. In the former models, the kinetics depends linearly on the substrate concentration when the latter remains small with respect to Michaelian constants, but, when the concentration becomes larger, the enzymes become saturated and their rate reaches a constant, maximum value.

Does the profile of period as a function of the protein degradation rate v_d depend on the degree of saturation of mRNA and protein degradation and of the enzymes that catalyse phosphorylation–dephosphorylation? To address this question, we compute the period profile as a function of v_d by progressively replacing saturating kinetics by linear kinetics, i.e. by moving from the zero-order to the first-order kinetic domain. This situation is achieved by applying conditions (a) and (d) in table 3, and favoured by condition (b) (the increase in parameter K_d , which does not correspond to first-order kinetics, is limited because oscillations disappear at larger values). The corresponding changes in period profile are shown in figure 7. To make the profile in model A tend towards that of model B we need to apply, in addition, conditions (c), (e) or (f) depending on the path chosen, as illustrated in figure 7. As indicated in table 3, conditions (c) and (e) correspond to an increase in the maximum rates of phosphorylation–dephosphorylation and mRNA degradation, respectively. Condition (f) simply corresponds to an increase in the degree of cooperativity n of repression. Different paths may transform the period profile of model A into profiles characteristic of

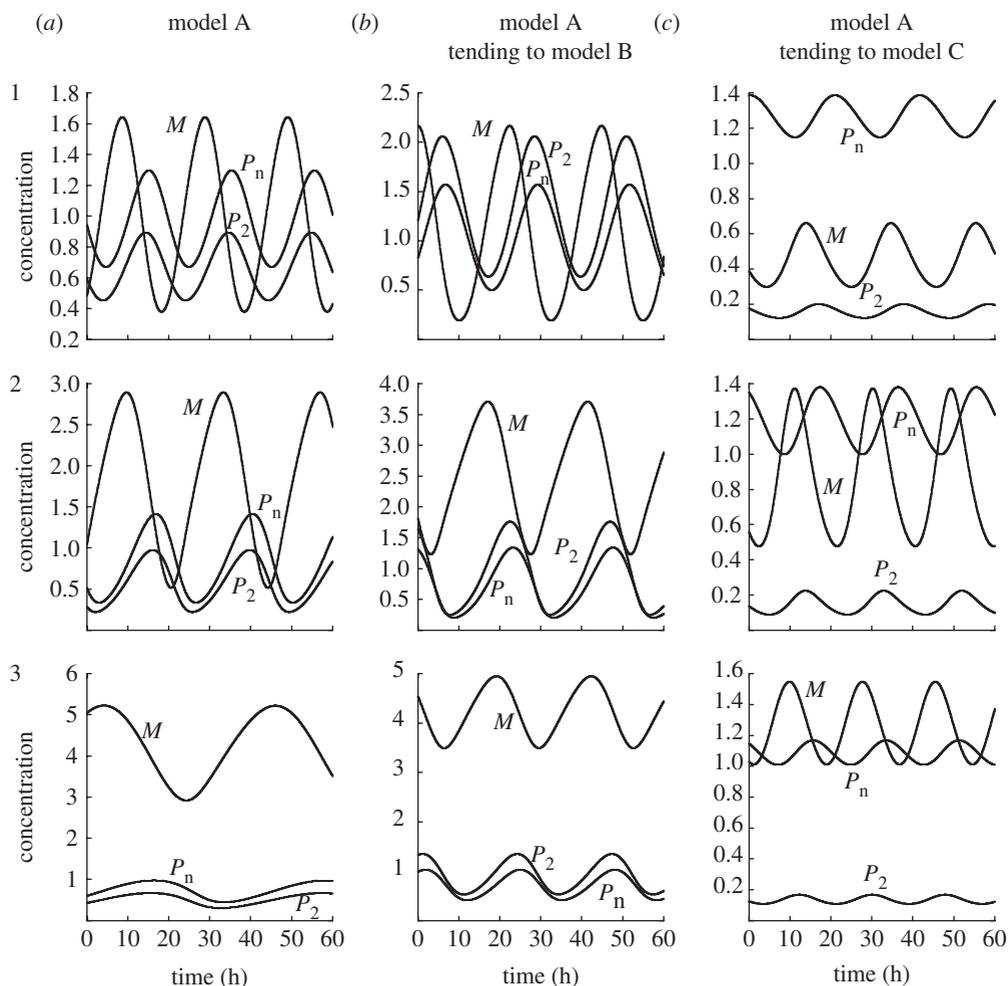


Figure 6. Oscillations obtained in model A for parameter values yielding period profiles similar to those generated by models B and C. The curves are obtained for three values of v_d increasing from rows 1 to 3. Model A ((a), similar to figure 4a): $v_d = 0.5, 0.95, 2.25$ (in nM h^{-1}). Model A in conditions where it tends to yield a period profile as in model B (b): $v_d = 0.5, 1.25, 2$ (in nM h^{-1}). Model A in conditions where it tends to yield a period profile as in model C (c): $v_d = 0.4, 0.8, 1.2$ (in nM h^{-1}). The curves, obtained as described in figure 4, correspond to the three points marked 1, 2 and 3 on the period profile in figure 5a–c. Other parameter values are as in figure 5a–c.

model B. Thus, in figure 7, this result is obtained by the following changes in conditions: $(a + c)$ or $(a + c + d + e + f)$ or $(a + b + c)$. On each of these paths conditions (a) and (c) need to apply, which correspond to high rates and first-order linear kinetics for phosphorylation–dephosphorylation reactions. If these conditions do not apply, the period profile remains the original one obtained for model A in figure 3.

The next step pertains to the changes in conditions needed to transform the period profile of model A into profiles characteristic of model C. As shown in figure 8, one way is to start from conditions that lead to model B

Table 3. Parameter values for which the period profile as a function of the clock protein degradation rate in model A tends to the period profiles characteristic of models B or C. Listed in the second column are the basal values considered for model A (see table 2; Goldbeter 1995). Columns *a–h* refer to changes in parameter values which progressively transform the period profile in model A into profiles characteristic of models B and C, as indicated in figures 7 and 8. A blank indicates that the parameter keeps its basal value indicated in the second column.

parameter	model A	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>h</i>
v_s (nM h ⁻¹)	0.76								
K_1 (nM)	1								
n	4						9		
v_m (nM h ⁻¹)	0.65					4			
K_m (nM)	0.5				25				
k_s (h ⁻¹)	0.38								
v_1 (nM h ⁻¹)	3.2			50					
K_1 (nM)	2	50							
v_2 (nM h ⁻¹)	1.58			50					
K_2 (nM)	2	50							
v_3 (nM h ⁻¹)	5			50					
K_3 (nM)	2	50							
v_4 (nM h ⁻¹)	2.5			50					
K_4 (nM)	2	50							
v_d (nM h ⁻¹)	0.95								
K_d (nM)	0.2		0.6						
v_n (nM h ⁻¹)	0								0.2
K_n (nM)	0								1
k_1 (h ⁻¹)	1.9								
k_2 (h ⁻¹)	1.3							0.4	0

in figure 7, to combine them in the form $(a + b + c + d + e + f)$, and to add conditions (*g*) and (*h*). The latter conditions (table 3) correspond to a quasi-irreversible entry of the repressor protein in the nucleus (condition *g*), and to the incorporation of nuclear protein degradation, to avoid nuclear accumulation of the repressor protein when its entry becomes fully irreversible (condition *h*).

In summary, we observe that fast and non-saturated linear processes are necessary for observing a decrease in the period as v_d increases, as in model C, whereas slow, saturable phosphorylation kinetics of the Michaelian type induce a rise in period as v_d increases.

The rate of protein degradation is but one of the parameters capable of controlling the period of the oscillations. The period profile as a function of the rates of mRNA and protein synthesis also depends on the model considered. Thus, in models A and B, we observe that the period increases with the rate of mRNA synthesis (v_s) and decreases with the rate of protein synthesis (k_s), whereas the opposite occurs for model C, even though the period variation is then restricted to a narrow range (data not shown). It is likely that a whole range of period profiles could also be obtained under different kinetic conditions for models A and B, as shown here for the dependence on the rate of clock protein degradation.

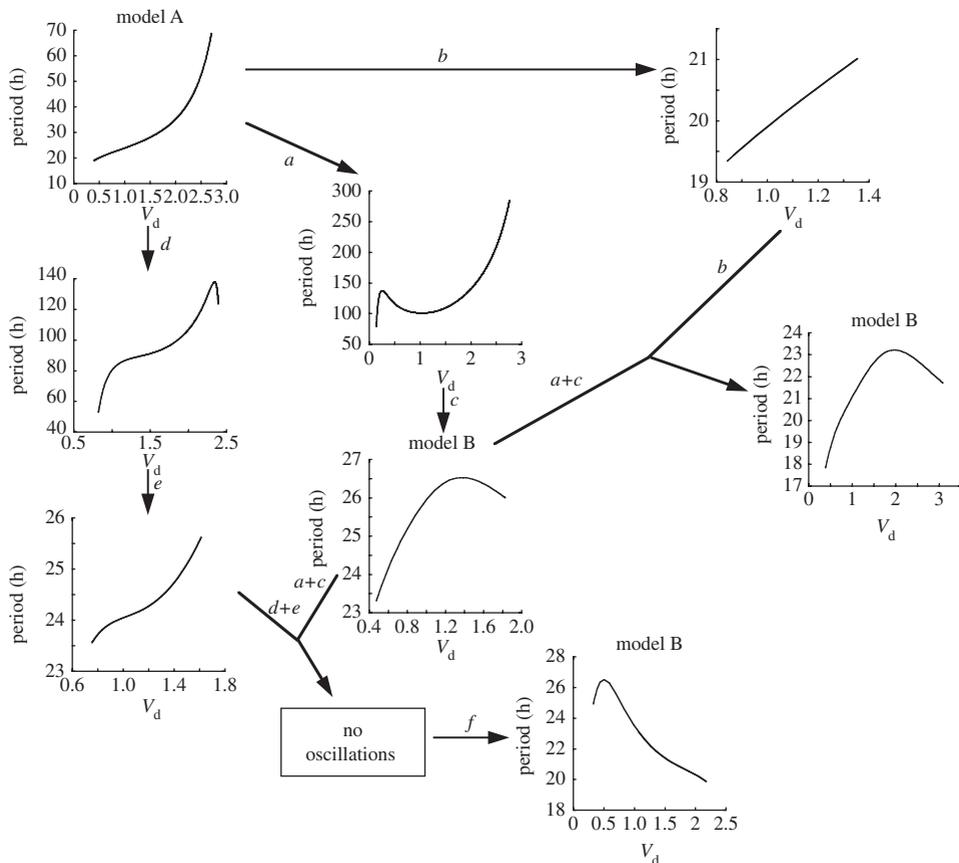


Figure 7. Changes in parameter values that progressively convert the period profile of model A into that of model B as a function of the clock protein degradation rate. Basal parameter values for model A yielding the period profile of figure 3a are listed in table 3 (second column). From model A, which is the most detailed model, we show different ways to pass from the original period profile of model A to the period profile of model B (figure 3b). The different steps (which need not be followed in any prescribed order) are listed in table 3 and explained below (see also text). (a) Non-saturated phosphorylation–dephosphorylation ($K_1 = K_2 = K_3 = K_4 = 50$ nM); (b) decrease in saturation of protein degradation ($K_d = 0.6$ nM); (c) rapid phosphorylation–dephosphorylation kinetics ($v_1 = v_2 = v_3 = v_4 = 50$ nM h⁻¹); (d) non-saturated mRNA degradation ($K_m = 25$ nM); (e) fast mRNA degradation ($v_m = 4$ nM h⁻¹); (f) high degree of cooperativity in repression ($n = 9$).

5. Extending the results to other models for circadian oscillations

Having determined conditions in which changes in the values of kinetic parameters in the more general model A lead to altered period profiles, can we use these results to reverse the period profile for circadian oscillations obtained in a more complex, 10-variable model based on the formation of a complex between the PER and TIM proteins in *Drosophila* (Leloup & Goldbeter 1998). In this model, for the basic set of parameter values considered in the original publication, the period again rises as the PER protein degradation rate (v_{dP})

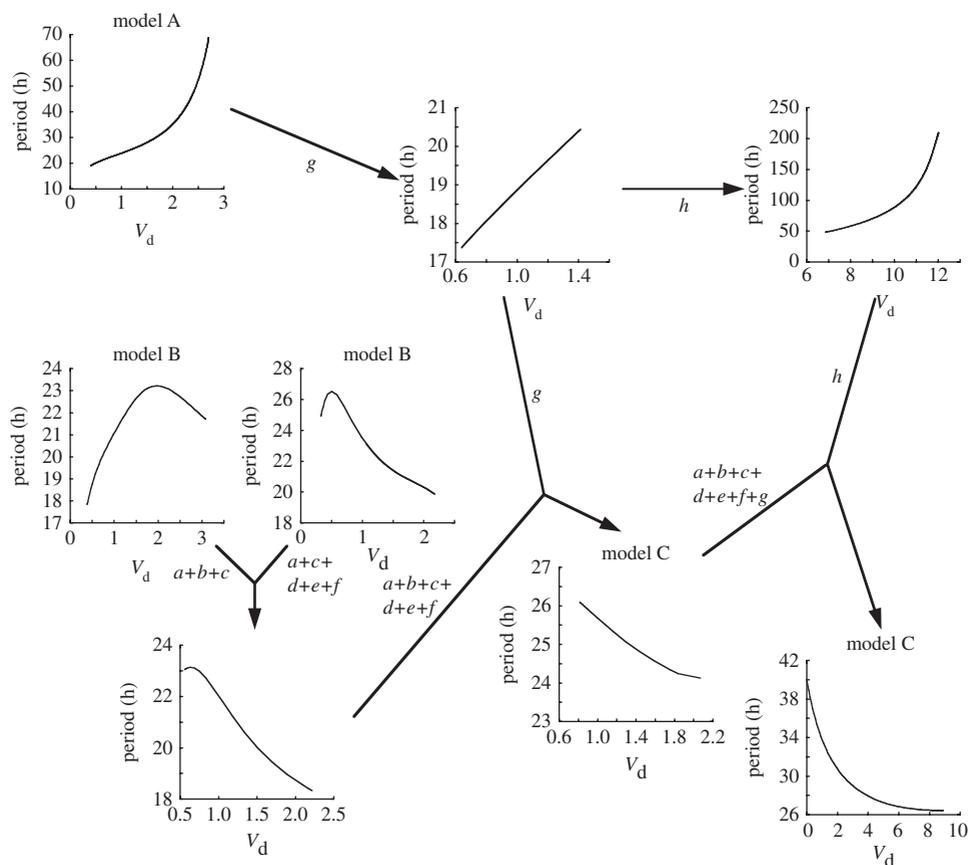


Figure 8. Changes in parameter values that progressively convert the period profile of model A into that of model C as a function of the clock protein degradation rate. As done in figure 7 for the conversion to the period profile of model B, we show ways by which changes in parameter values convert the original period profile of model A to the period profile of model C (figure 3c). The different steps (which need not be followed in any prescribed order) are listed in table 3 and explained below (see also text). Conditions (a)–(f) are as described in figure 7. In addition, (g) corresponds to making the transport of the clock protein to the nucleus less reversible ($k_2 = 0.4 \text{ h}^{-1}$), whereas for condition (h) protein transport to the nucleus becomes irreversible ($k_2 = 0 \text{ h}^{-1}$) and nuclear protein degradation is incorporated to avoid resulting accumulation of the clock protein in the nucleus ($v_n = 0.2 \text{ nM h}^{-1}$, $K_n = 1 \text{ nM}$). The path leading from the period profile of model A to the period profile of model C passes through the profile of model B.

increases, except at very high values of v_{dP} , for which a slight decrease of the period is observed. A biphasic profile in which the period briefly rises and then decreases as a function of the protein degradation rate is observed in this model when considering non-saturated, fast phosphorylation rates, quasi-irreversible transport of the clock protein into the nucleus, and non-saturated mRNA degradation rates. The results obtained for the five-variable model A can thus be used to reverse the profile of the period in the more complex PER–TIM model.

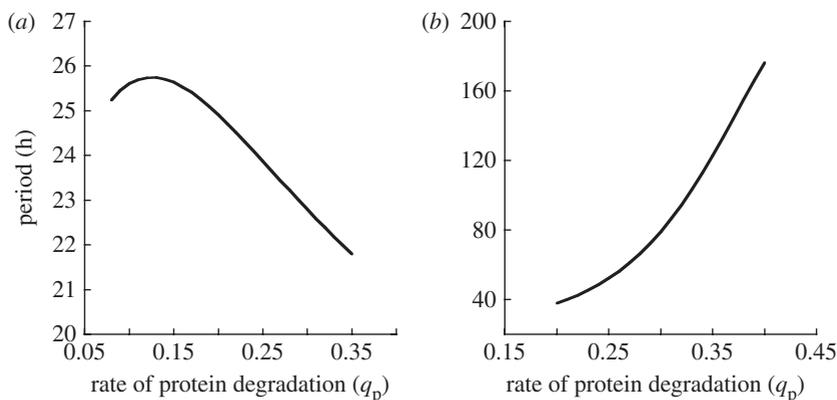


Figure 9. Profile of period obtained as a function of the clock protein degradation rate (q_p) in the delay model for circadian oscillations (Scheper *et al.* 1999*a,b*). (a) Basic parameter values of the model; $r_m = 1 \text{ h}^{-1}$, $q_m = 0.21 \text{ h}^{-1}$, $K = 1$, $n = 2$, $r_p = 1 \text{ h}^{-1}$, $m = 3$, $q_p = 0.21 \text{ h}^{-1}$. (b) The linear protein degradation term in equation (5.2) has been replaced by a Michaelis–Menten function with $K_d = 0.1$ (see text). The curves have been obtained by numerical integration of equations (5.1) and (5.2).

A minimal delay model for circadian oscillations was proposed by Scheper *et al.* (1999*a,b*) (see also Sriram & Gopinathan 2004). It consists of two variables, the mRNA (M) and protein (P) concentrations, and contains a single explicit delay included in the function describing the rate of protein synthesis

$$\frac{dM}{dt} = r_m \frac{1}{K + P^n} - q_m M, \quad (5.1)$$

$$\frac{dP}{dt} = r_p M^m(t - \tau) - q_p P. \quad (5.2)$$

The profile of period as a function of the protein degradation rate q_p obtained with the basic parameter values is shown in figure 9*a*. When the linear protein degradation term is replaced by a Michaelis–Menten function, i.e. when $q_p P$ is replaced by $q_p P / (K_d + P)$ in equation (5.2), the period profile is reversed: the period now rises as q_p increases (figure 9*b*). Thus, in this delay model, saturable protein degradation is sufficient to reverse the period profile.

6. Discussion

The autonomous period of circadian clocks determines its ability to be entrained by light–dark cycles at the appropriate phase. Therefore, it is essential that the period of the circadian clock be appropriately controlled. The effect of parameter changes on the period of the oscillations can be investigated by means of mathematical models. These models, which generally consist of a set of ordinary differential equations, describe the time evolution of the key variables of the system and can be analysed by numerical simulations. Several studies on the sensitivity of models for circadian oscillations give a general picture on *how* the period or other properties of the oscillations change as a result of variations

in parameter values, but do not focus on *why* these changes occur (Leloup & Goldbeter 2004; Stelling *et al.* 2004; Rand *et al.* 2006; Bagheri *et al.* 2007; Jin *et al.* 2008; Rand 2008) and do not compare the consistency between several models. In a detailed comparative study, Kurosawa *et al.* showed that the saturation of several key steps in the regulatory mechanism could strongly favour the occurrence of self-sustained oscillations (Kurosawa & Iwasa 2002; Kurosawa *et al.* 2002). However, these authors did not systematically study the effect of control parameters on the period of the oscillations.

Among the multiple molecular mechanisms that influence the period of circadian rhythms, a major role is played by those that control clock protein degradation (Liu *et al.* 2000; Eide *et al.* 2005; Gallego *et al.* 2006; Busino *et al.* 2007; Reischl *et al.* 2007). Here we compared three minimal models proposed for circadian clocks, showed that they predict opposite profiles of period as a function of the clock protein degradation rate, and indicated how to reconcile these contradictory predictions. All the models considered rely on three common features: negative feedback, delay and presence of at least one nonlinearity in the kinetic equations. The models can be considered as minimal because those features were shown to be sufficient to produce self-sustained oscillations. Our results show that fast and non-saturated processes are necessary for a decrease in period as the clock protein degradation rate increases, whereas slow Michaelian phosphorylations induce a rise in period as this parameter increases.

The fact that the period profile is dictated by the kinetics of the degradation and phosphorylation terms can be used to predict the kinetic conditions in which the circadian system operates. In the case of *Neurospora*, Ruoff *et al.* (2005) observed that strains in which the FRQ protein is mutated have a higher period if the half-life of the protein is longer (smaller degradation rate) and a smaller period if the half-life is shorter (higher degradation rate), as predicted by model C, which is used accordingly by the authors to account for their results. From this observation, according to our conclusions, we can surmise that mRNA degradations and phosphorylations are fast and non-saturated, because in these conditions model A yields the period profile of model C as a function of the clock protein degradation rate.

Although the minimal models discussed here are all capable of self-sustained circadian oscillations, the regulatory mechanism underlying circadian oscillations is much more complex. It involves interlocked feedback loops (Glossop *et al.* 1999; Lee *et al.* 2000; Ripperger & Schibler 2001). Moreover, experiments showed that multiple phosphorylations of the PER protein have differential effects on the protein degradation and translocation in the nucleus (Vanselow *et al.* 2006; Xu *et al.* 2007). Finally, the Michaelis–Menten term used in this study assumes an enzymatic process but other sources of nonlinearity in the degradation kinetics may also be present, such as the cooperative dimerization of proteins, which was shown to affect the dynamics of genetic circuits (Buchler *et al.* 2005). It is likely that these additional controls affect the profile of period as a function of the protein degradation rate.

The minimal models considered here represent a more general class of oscillators based on a negative feedback loop exerted by a protein on the expression of its own gene. Far from being restricted to circadian rhythms such negative autoregulation is indeed encountered in other oscillatory systems such as Hes1 or NF κ B (Monk 2003; Krishna *et al.* 2006), which are characterized by

an ultradian period in the range of a few hours. The Hes1 oscillatory system is closely related to the segmentation clock that controls somitogenesis. Minimal models for the Hes1 system are very similar to the models proposed for circadian rhythms, and are based either on a Goodwin-type model (Hirata *et al.* 2002, 2004; Zeiser *et al.* 2007) or on a simple delay model with negative feedback (Monk 2003; Lewis 2003). The results reported here might thus bear on these other oscillatory systems.

In summary, the present results show that the choice of kinetic functions and the range of parameter values may be crucial for the properties of genetic oscillators based on negative autoregulatory feedback. The use of Michaelis–Menten kinetics encompasses a variety of different situations and is thus appropriate to describe processes such as phosphorylation–dephosphorylation as well as mRNA and protein degradation. The results obtained here cannot be reached by resorting to sheer intuition and verbal description, and can only be predicted on the basis of the time series generated by numerical simulation of mathematical models. This illustrates one role of modelling, which is to make non-intuitive predictions. By pointing to the importance of the precise kinetic form of the biochemical processes involved in the oscillatory mechanism, the present results also allow us to reconcile contradictory predictions generated by different minimal models for circadian rhythms.

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