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The Temperature-Compensated Goodwin Model Simulates Many Circadian Clock Properties

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Goodwin's model oscillator, which consists of a negative feedback loop within a single gene expression pathway (1963, 1965), was modified in order to demonstrate temperature compensation, a characteristic property of circadian oscillators. Temperature effects were introduced into the oscillatory mechanism by means of the Arrhenius equation. This term may provide a simplified estimate of the complicated temperature-dependence of actual cellular processes. Temperature compensation within the oscillatory mechanism was achieved by balancing the effects of different temperatures on amplifying processes and on stabilizing processes (antagonistic balance; Ruoff, 1994). Apart from temperature compensation, the modified Goodwin oscillator showed other characteristic properties of circadian clocks including:

(i) temperature entrainment even by temperature cycles of rather low amplitude (1°C),
(ii) phase shifts by temperature pulses or steps of different signs (up or down) and different amplitudes, giving rise to phase response curves similar to those reported, for example, in *Neurospora crassa* (Francis & Sargent, 1979; Rensing *et al*., 1987; Nakashima, 1987).

A single change in one of the reaction rates of the model oscillator not only caused a change in the period length but also abolished temperature compensation. This closely corresponds to the effects observed after a single point mutation of the clock gene (frq^7) in *Neurospora crassa* (Aronson *et al*., 1994b). Limit cycle oscillations were damped at permanently low temperatures, however the period of the damped oscillations was still temperature compensated.

Introduction

One of the characteristic properties of circadian clocks is the temperature-compensation of their frequency (Pittendrigh & Caldarola, 1973; Balzer & Hardeland, 1988; Edmunds, 1988; Lakin-Thomas *et al*., 1990). This observation implies the presence of a control mechanism that keeps the circadian period unchanged at different but constant temperatures. This mechanism is assumed to compensate for temperature-induced changes in the processes involved in the clock mechanism. Temperature-compensation is necessary for a correct timing of the "hands" of the clock, i.e., the numerous clock-controlled processes and the correct determinations of light or dark periods in photoperiodic responses.

How is temperature-compensation possible in view of the fact that most enzyme-catalysed processes are known to be strongly dependent upon temperature? A variety of theories and specific models have been proposed to solve this problem (Hastings & Sweeney, 1957; Pavlidis *et al*., 1968; Njus *et al*., 1974; Rössler *et al*., 1975). We have previously argued that even chemical oscillators themselves contain all the necessary components for generating temperature-compensation. If the effect of temperature on the

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amplifying and on the inhibiting (stabilizing) parts of the oscillatory reactions are balanced, the oscillator's period length will be temperature compensated. This principle, termed *antagonistic balance* (Ruoff, 1992, 1994), has been applied to oscillators which are usually not temperature-compensated, e.g. the Brusselator (Prigogine & Lefever, 1968), the Kauffman–Wille model (Kauffman & Wille, 1975), and the Oregonator (Field & Noyes, 1974). In this paper we extended this principle to a model of the circadian clock.

A recent detection of a feedback loop between clock gene activity and the amount of its product as a basic mechanism of the circadian clock (Aronson *et al*., 1994a; Zeng *et al*., 1994) has made it attractive to use Goodwin's model oscillator (1965), which is based on a similar feedback mechanism. Aronson *et al*. (1994b) concluded that both changes originate from the same point mutation (amino acid exchange) from the observation that some period length mutants (for example *frq*^{7} in *Neurospora crassa*) also show defects in temperature compensation. Therefore, we used the Goodwin oscillator and the concept of antagonistic balance to test whether a single change of a rate constant, for example a slower degradation of the clock protein, may cause changes in both period length and temperature-compensation. This is actually observed in our model.

We further tested, whether the temperature-compensated Goodwin oscillator can be entrained by periodic temperature changes and whether the oscillator can be phase shifted similar to circadian clocks when perturbed by single temperature steps or pulses. These characteristic properties of circadian clocks were also simulated by the model.

As in chemical oscillators (Ruoff, 1992) we used the Arrhenius equation to describe the influence of temperature on reaction rates in spite of the fact that reaction rates in biological systems are controlled by a number of factors, such as the activity of enzymes, the concentration of the substrates (and products), their transport rates, etc., which are often actively adapting to different temperatures. It is reasonable to assume, however, that the activation energy in the Arrhenius equation can be used as a gross estimate of the temperature dependence of reaction rates in biological systems.

The Model

The model studied is defined by the following three differential equations (Goodwin 1963, 1965; Drescher *et al*., 1982; Rensing & Schill, 1985, 1987):

$$\frac{dX}{dt} = \frac{k_1}{Z^9 + 1} - k_4 X \tag{1}$$

$$\frac{dY}{dt} = k_2 X - k_5 Y \tag{2}$$

$$\frac{dZ}{dt} = k_3 Y - k_6 Z \tag{3}$$

The k_i's are rate constants of processes *Ri* [Fig. 1(a)] and X, Y, and Z represent chemical components and their respective concentration values. In molecular terms, X may represent mRNA that codes for a

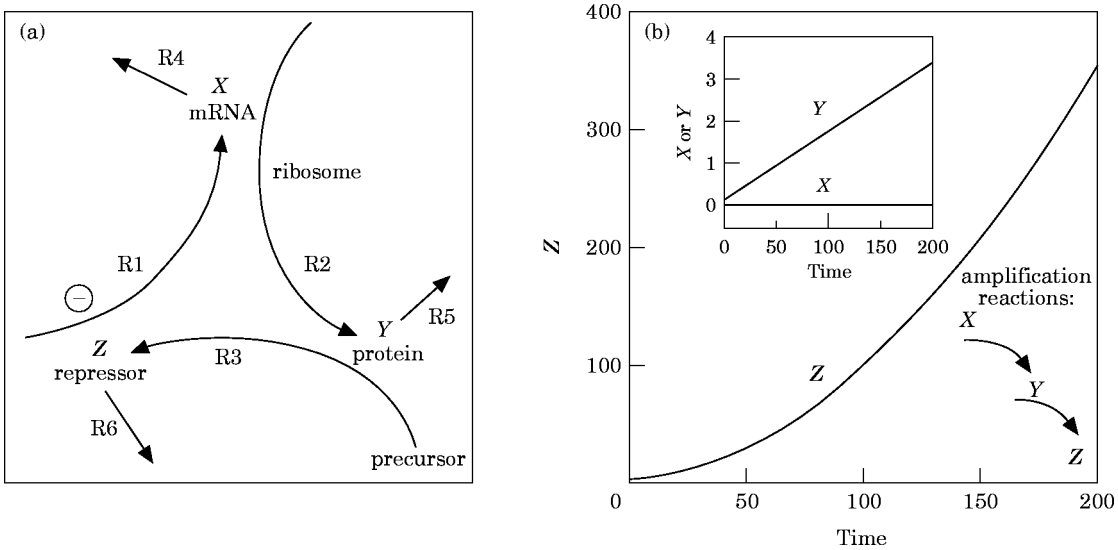

FIG. 1. (a) Inhibition reaction R1, amplifying reactions R2, R3, and stabilizing reactions R4, R5, and R6 in the Gooodwin model. (b) The amplifying reactions lead to a destabilization of the system, which is obtained by setting the rate constants of the stabilizing reactions to zero, i.e., $k_4 = k_5 = k_6 = 0$. The figure shows the (quadratic) increase of Z with time. The inset shows the constant X level and the linear increase in Y.

protein Y. This protein catalyses a reaction leading to the end product Z that inhibits further synthesis of this mRNA species [Fig. 1(a)].

EXPLANATION OF OSCILLATIONS

The "driving force" of the oscillations is due to the amplification reactions R2 and R3. The amplification can be recognized by setting $k_4 = k_5 = k_6 = 0$. When the synthesis of X is inhibited $dX/dt \rightarrow 0$, i.e., X becomes constant and attains a steady-state value X_{SS}. Subsequently, the rate $dY/dt = k_2 X_{SS}$ will also be constant and will lead to a linear increase of Y. Subsequent integration of eqn (3) ($k_6 = 0$) shows that Z increases quadratically with time [Fig. 1(b)].

If reactions R4, R5, and R6 are operative at higher values of Z, the steady-state value X_{SS} will eventually decrease because of reaction R4. When X decreases, the synthesis rate of Y will also decrease (because $k_2 X$ decreases). In addition, the concentration of Y will decrease because of reaction R5. This will finally lead to a decrease in dZ/dt and in the level of Z such that the inhibition of the formation of X will disappear. X will be produced and the cycle restarted.

According to Franck (1978, 1989) the driving forces in oscillatory reactions are based on self-amplifying processes. Because amplification drives the system away from its steady state (destabilizes the system), Franck noticed that in order to obtain oscillations additional *stabilizing* reactions must oppose the amplification. This simultaneous action of stabilizing and destabilizing reactions has been termed *antagonistic feedback* (Franck, 1978, 1989), and is a necessary (but not sufficient) condition for oscillations. In the Goodwin oscillator, R4, R5, and R6 [Fig. 1(a)] are such stabilizing processes.

The existence of antagonistic feedback is not a sufficient condition for oscillations. Depending on the values chosen for the rate constants, we may observe limit cycle oscillations, damped oscillations or a monotonic non-oscillatory approach to a steady state. Besides numerical computations, linear stability analysis (Murray, 1989) has been used to assure whether a certain set of rate constant values result in a stable or in an unstable steady state (damped or limit cycle oscillations).

The exponent in the Z^9 term in eqn (1) is known to be unrealistically high. In order to decrease this exponent, the number of amplifying reactions can be increased (Rensing & Schill, 1985). However, in the present study we wish to keep the number of parameters and rate constants at a minimum and therefore study only the dynamics of eqns (1–3).

With the rate constant values chosen in our computations, "soft" almost sinusoidal oscillations are observed. "Soft" means that the system needs a considerable time and many transient cycles to reach the final state (limit cycle or stable steady state) after a perturbation.

The Temperature-Compensated Goodwin Model

Temperature is introduced into the model by the Arrhenius equation, which links rate constant k_i to temperature T:

$$k_i = A_i \exp\left\{-\frac{E_i}{RT}\right\} \tag{4}$$

in which the pre-exponential factor A_i is treated as a constant and E_i is the activation energy of reaction Ri. Both A_i and E_i are temperature-independent. 283 K or 298 K are used as reference temperatures, which means that the selected values of k_1–k_6 refer to one of these temperatures. The choice of activation energies E_i allows determination of the A_i's. Equation (4) can then be used to calculate the k_i's at different temperatures T. In biological terms, reactions R1–R6 will be enzymatic and involve more temperature-dependent [eqn (4)] rate constants (Laidler & Peterman, 1979). The use of a single rate constant k_i for process Ri as chosen in this work represents an approximation.

For most chemical oscillator models it is impossible to describe the influence of temperature on period length analytically. Therefore, a numerical solution must be sought. An *approximate* analytical expression, however, can be obtained by writing the period length P as a power law function of the rate constants (Ruoff, 1992, 1994, 1995). In case of the Goodwin model the expression takes the form:

$$P_{appr} = \tau_0 \prod_{n=1}^{6} (k_i)^{\alpha_i} \tag{5}$$

where α_i are empirical (but otherwise constant) parameters which can be found by calculating $\partial \log P_{num}/\partial \log k_i$ from numerically determined period lengths, $P_{num} \cdot \tau_0$ is a constant that is dependent on the reference rate constants. We have compared P_{appr} with P_{num} for a variety of rate constant values and found that eqn (5) describes quite well the period of the oscillations as a function of k_i.

The condition for temperature-compensation is given by

$$\frac{\partial P_{appr}}{\partial T} = 0 \tag{6}$$

TABLE 1

Period P *as a function of temperature for antagonistically balanced and non-balanced* E_i *combinations**

E_1 (J/mol)	E_2 (J/mol)	E_3 (J/mol)	E_4 (J/mol)	E_5 (J/mol)	E_6 (J/mol)	P (15°C)	P (20°C)	P (25°C)	P (30°C)	P (35°C)
3.96×10^4	1.34×10^5	9.11×10^4	2.21×10^2	5.93×10^2	9.28×10^2	24.1	23.1	23.1	23.0	22.9
7.66×10^4	4.66×10^4	1.18×10^5	3.44×10^2	3.39×10^2	9.08×10^2	23.7	23.1	23.0	22.7	22.8
2.11×10^5	6.49×10^3	6.11×10^4	7.54×10^2	6.60×10^2	3.97×10^2	24.4	23.0	22.5	22.9	22.1
2.36×10^4	9.26×10^4	6.25×10^4	7.63×10^2	1.92×10^2	2.02×10^2	23.1	23.1	22.9	22.5	22.8
2.36×10^4	9.26×10^4	6.25×10^4	7.63×10^2	1.00×10^5	2.02×10^2	66.7	48.3	36.7	29.0	23.5
2.09×10^4	1.68×10^4	2.42×10^4	5.96×10^4	9.67×10^3	2.79×10^4	37.6	28.4	22.5	18.1	14.9
2.38×10^4	5.69×10^4	1.63×10^4	1.88×10^4	4.77×10^4	3.41×10^4	38.3	28.7	22.6	18.1	14.5
3.68×10^4	4.64×10^4	4.16×10^4	1.16×10^4	2.01×10^4	2.75×10^3	27.5	25.1	22.8	20.7	19.3
3.11×10^4	4.55×10^3	2.58×10^4	3.72×10^3	5.01×10^4	1.09×10^4	32.0	26.3	22.6	19.5	17.2
5.48×10^4	6.83×10^3	1.99×10^4	5.12×10^4	3.76×10^4	2.14×10^3	38.1	28.9	22.7	18.4	15.3
3.49×10^4	3.20×10^2	3.47×10^4	0	0	0	22.9	22.3	22.3	22.6	22.6
1.76×10^4	2.00×10^4	4.05×10^4	0	0	0	22.8	22.6	22.5	22.6	22.6
2.53×10^4	5.15×10^4	7.39×10^3	0	0	0	22.9	22.4	22.4	22.6	22.5
5.17×10^4	2.36×10^4	3.16×10^4	0	0	0	22.9	22.5	22.5	22.6	22.6
4.12×10^4	3.39×10^4	1.73×10^4	0	0	0	23.0	22.4	22.4	22.6	22.6

* $k_1 = k_2 = k_3 = 1.0$; $k_4 = k_5 = 0.2$; $k_6 = 0.1$; $T_{ref} = 298$ K. Rows 1–4: activation energies E_4–E_6 were randomly selected in the range 0–1000 J/mol; activation energies E_2 and E_3 were randomly selected in the range 0–1.5×10^5 J/mol; E_1 was calculated according to eqn (10). Row 5: first period of damped oscillations when rate constant k_5 is decreased to 0.05 ($T_{ref} = 298$ K) and E_5 increased to 1×10^5 J/mol. Rows 6–10: all activation energies were randomly selected in the range 0–6.0×10^4 J/mol. Rows 11–15: activation energies E_1–E_3 were randomly selected in the range 0–6.0×10^4 J/mol.

When inserting the Arrhenius eqn (4) into eqn (5) and making the derivation with respect to temperature, one obtains:

$$\frac{\partial P_{\text{appr}}}{\partial T} = \frac{1}{RT^2}\left[\sum_{i=1}^{6} \alpha_i E_i\right] P_{\text{appr}} \tag{7}$$

The condition for temperature-compensation [eqn (6)] implies that:

$$\sum_{n=1}^{6} \alpha_i E_i = 0 \tag{8}$$

In other words: *whenever the* α_i*-weighted sum of the activation energies is zero* (*or approximately zero*) *the oscillator is temperature-compensated*. The reason why eqn (8) can be zero is due to the fact that all component processes in the oscillator can be divided into period-increasing (P^+) *and* period-decreasing (P^-) reactions. P^+ reactions will have positive α_i values, while P^- reactions will have negative α_i values. Because the P^+ and P^- reactions are associated with the destabilizing (amplifying) and stabilizing reactions of the oscillator, we have termed eqn (8) as *antagonistic balance in temperature* (Ruoff, 1994).

In case eqn (8) is not fulfilled the period will be temperature-dependent [eqn (5)]:

$$P_{\text{appr}} = C \exp\left\{ -\left[\sum_{i=1}^{6} \alpha_i E_i\right] \Big/ RT \right\} \tag{9}$$

where C is a (temperature-independent) constant. Experimental results show that chemical oscillatory reactions follow the exponential temperature dependence of the period [eqn (9)] often with high precision (Ruoff, 1995).

For the Goodwin oscillator the α_i values of the stabilizing reactions R4, R5, R6 are negative, while the α_i values of the amplifying R1, R2, R3 are slightly positive, and eqn (8) can be written as:

$$0.0023(E_1 + E_2 + E_3) = 0.3583(E_4 + E_5) + 0.3416E_6 \tag{10}$$

The first four rows in Table 1 show that for E_i-combinations that follow eqn (10), small changes in the period length are observed as the temperature is varied. On the other hand, when all six E_i values are allowed to change randomly (rows 6–10), and eqn (10) is not obeyed, a strong temperature dependence appears.

Equation (10) implies that the average activation energies E_1, E_2, E_3 should be about 150 times larger than the average value of E_4, E_5, or E_6. If the average, E_1, E_2, E_3 value is about 50 kJ/mol (that would result in rate constants with Q_{10} values of 2), then E_4, E_5 and E_6 should be near 0 kJ/mol to obtain temperature-compensation. This precise situation is shown in Table 1, rows 11–15.

Row 5 in Table 1 illustrates the effect when the rate constant of the degradation of protein Y (process R5) is decreased. In this case temperature-compensation is

TABLE 2
Period P *as a function of temperature for damped and limit cycle oscillations*[a]

Temperature (K)	Period	Stability of steady state
273	23.7	stable[b]
275	23.3	stable[b]
277	22.8	stable[b]
279	22.7	stable[b]
281	22.7	stable[b]
283	22.7	stable[b]
285	22.7	stable[b]
287	22.7	stable[b]
289	22.7	stable[b]
291	22.7	stable[b]
293	22.7	unstable[c]
295	22.7	unstable[c]
297	22.7	unstable[c]
—	—	—
309	22.7	unstable[c]
311	22.7	unstable[c]
313	22.7	unstable[c]

[a] $k_1 = k_2 = k_3 = 1.0$; $k_4 = k_5 = 0.2$; $k_6 = 0.1$; $T_{ref} = 283$ K; $X_{start} = 1.137$; $Y_{start} = 0.1668$; $Z_{start} = 1.128$; Period is measured at 400 time units. $E_1 = 1.83167 \times 10^4$ J/mol; $E_2 = 2.24481 \times 10^5$ J/mol; $E_3 = 1.74848 \times 10^4$ J/mol; $E_4 = 296.434$ J/mol; $E_5 = 201.092$ J/mol; $E_6 = 328.277$ J/mol.

[b] By linear stability analysis. A stable steady state results in damped oscillations.

[c] By linear stability analysis. An unstable steady state results in limit cycle oscillations.

lost and the period length of the oscillator is altered (lengthened).

Temperature-Entrainment of the Temperature-Compensated Model

A temperature-compensated oscillator does not mean that the oscillations are independent of temperature. Here we show that the temperature-compensated Goodwin model can be entrained by sinusoidal temperature cycles with rather small amplitudes.

LIMIT-CYCLE OSCILLATOR CASE

We consider a temperature-compensated limit-cycle Goodwin oscillator having the following rate constant values at 283 K: $k_1 = 1.0$; $k_2 = 1.0$; $k_3 = 1.0$; $k_4 = 0.1$; $k_5 = 0.1$; $k_6 = 0.1$. The activation energies for k_1–k_6 are: $E_1 = 1.83167 \times 10^4$ J/mol; $E_2 = 2.24481 \times 10^5$ J/mol; $E_3 = 1.74848 \times 10^4$ J/mol; $E_4 = 296.434$ J/mol; $E_5 = 201.092$ J/mol; $E_6 = 328.277$ J/mol. In the studied temperature range from 273 K to 313 K the period of the unperturbed oscillator, $P_{c(ircadian)}$, changes from 36.8 to 37.6 with a maximum of 38.0 at 283 K.

When the period of the temperature cycle, $P_{t(emperature)}$, is shorter than P_c, entrainment is observed until P_t reaches about 30 [Fig. 2(A)]. For P_t lower than 30, entrainment is not observed and the oscillator resets to its original period of 38 [Fig. 2(b)]. An increase of the temperature amplitude at low P_t does not enforce entrainment, but leads to a irregular/chaotic response.

When P_t is longer than P_c (38.0) temperature-entrainment can be observed even at rather long P_t values (data not shown). When even longer temperature cycles are applied, one observes oscillations with period P_c superimposed on the entrained (enforced) rhythm. It is also observed that when the amplitude of the entraining temperature rhythm increases, the amplitude of the superimposed oscillations decrease.

TEMPERATURE-COMPENSATION IN DAMPED SYSTEMS AND TEMPERATURE EFFECTS ON NONOSCILLATORY STEADY STATES

Temperature-compensation may also be observed in damped oscillations, i.e., when the Goodwin model approaches a stable steady state (Table 2). From these results we conclude that antagonistic balance applies not only to pure limit cycle oscillations, but apparently also to stable and unstable oscillations!

However non-oscillatory (damped stable steady) states can also be influenced by temperature. Short P_t values in damped systems lead to irregular oscillations, which is similar to the situation when limit cycle oscillations are perturbed.

LOSS OF OSCILLATION STABILITY AT LOW TEMPERATURE

Table 2 illustrates another behavior often observed experimentally: when the temperature is lowered the oscillations of the Goodwin model become damped. In the particular case studied here, the steady-state changes its stability between 291 K and 293 K. Interestingly, the oscillator's temperature-compensation is independent of the stability of the steady state.

Phase Resetting by Temperature Steps

When the temperature is changed from 273 K to 278 K for the temperature-compensated limit cycle oscillator ($k_1 = 1.0$, $k_2 = 1.0$, $k_3 = 1.0$, $k_4 = 0.1$, $k_5 = 0.1$, $k_6 = 0.1$, $T_{ref} = 283$ K, $E_1 = 1.83167 \times 10^4$ J/mol; $E_2 = 2.24481 \times 10^5$ J/mol; $E_3 = 1.74848 \times 10^4$ J/mol; $E_4 = 296.434$ J/mol; $E_5 = 201.092$ J/mol; $E_6 = 328.277$ J/mol) the oscillator approaches a new oscillatory state (limit cycle). Let us assume that the temperature step has been applied at time t_{per} ("Phase of Perturbation"). We now compare the successive maxima in X, Y, or Z for the unperturbed and perturbed oscillation when $t > t_{per}$. Let T_1^u, T_2^u,

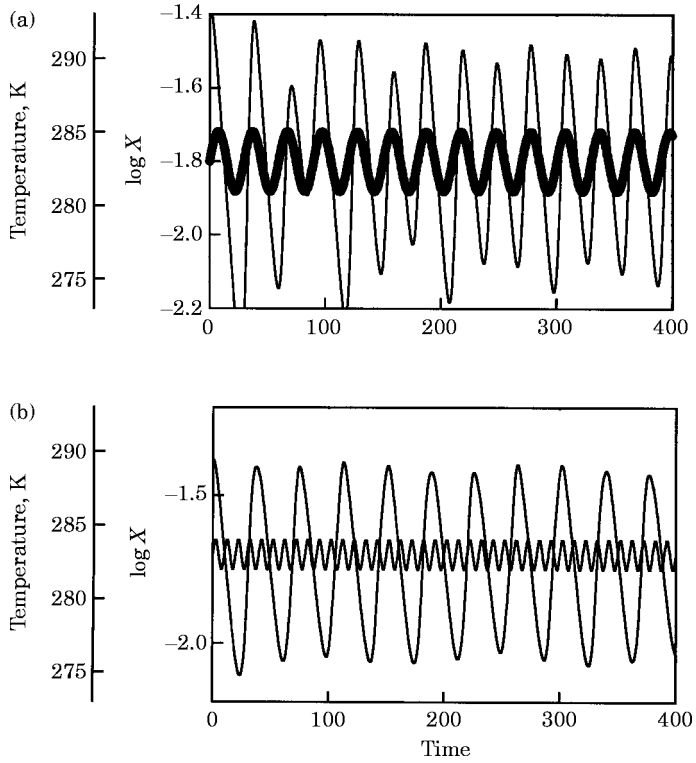

FIG. 2. (a) Temperature-entrainment using a sinusoidal temperature perturbation with $P_t = 30$ and a temperature amplitude of 1°C. The inner oscillation is the perturbing cycling temperature, while the outer oscillation is the responding log X value. (b) When P_t is decreased to 10, the oscillator is no longer able to follow the perturbing rhythm and resets to its original unperturbed period $P_c = 38$.

$T_3^u, \ldots, T_n^u, \ldots$ denote the series of successive (X, Y, or Z) maxima for the unperturbed oscillator when $t > t_{per}$. T_1^p, T_2^p, $T_3^p, \ldots, T_n^p, \ldots$ is the corresponding series for the perturbed oscillator. The phase shift $\Delta\Phi_n$ related to the nth (X, Y, or Z) maximum is defined as

$$\Delta\Phi_n = T_n^u - T_n^p \tag{9}$$

A phase response curve is a relationship that shows any phase shift $\Delta\Phi$ as a function of t_{per}. Negative phase shifts correspond to *phase delays*, while positive phase shifts correspond to *phase advances*.

THE PROBLEM OF ASSIGNING A UNIQUE PHASE SHIFT FOR TEMPERATURE STEPS

Figure 3 shows the calculated $\Delta\Phi_n$ values as a function of n for a 273 K → 278 K step. After an initial fall, $\Delta\Phi_n$ values decrease linearly with n. The reason for this linear decrease in n lies in the fact that despite the oscillator's temperature-compensation the period at 278 K is slightly longer than the period at 273 K. From Fig. 3 it is apparent that there is a problem in assigning a unique $\Delta\Phi$ among the $\Delta\Phi_n$ values that would represent 'the' phase shift. To solve this problem we have made two linear extrapolations from the initial and final $\Delta\Delta\Phi_n/\Delta n$ values and estimate $\Delta\Phi$ as that $\Delta\Phi_n$ value which corresponds to the intersection of the two lines (Fig. 3).

The variation of $\Delta\Phi_n$ with n in Fig. 3 is one of six possibilities that can occur when studying phase shifts

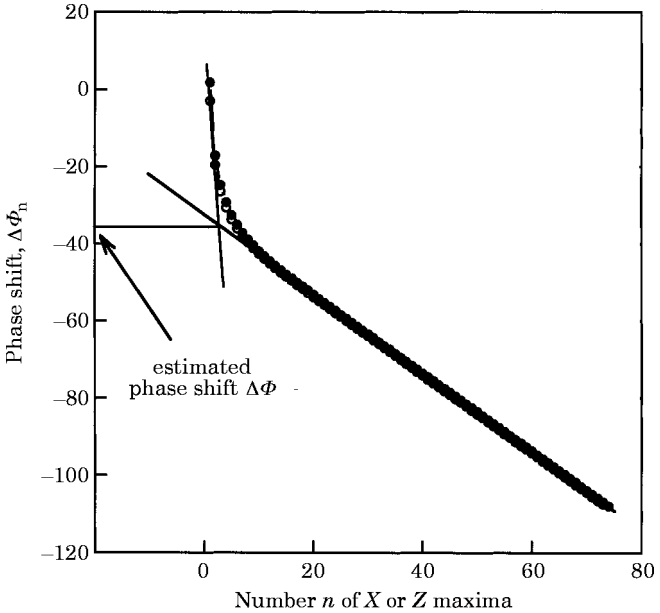

FIG. 3. Phase shifts $\Delta\Phi_n$ as a function of n for X (●) maxima. The estimated phase shift $\Delta\Phi$ is indicated.

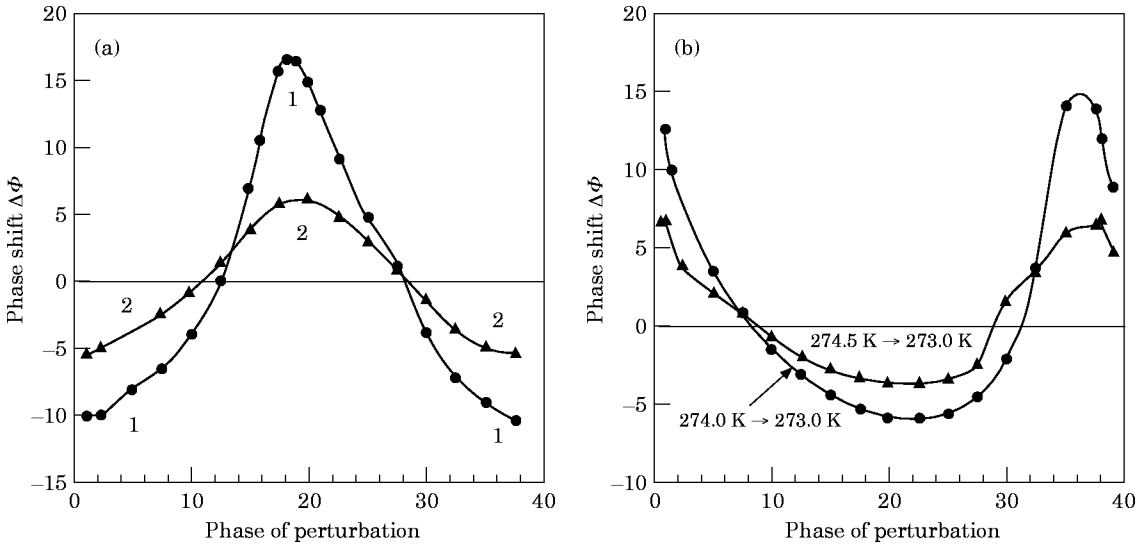

FIG. 4. (a) Estimated phase shifts $\Delta\Phi$ for temperature step-ups. 1: 273 K → 274 K; 2: 273.0 K → 273.5 K. (b) Estimated phase shifts $\Delta\Phi$ for temperature step-downs. Zero corresponds to the X maximum.

due to temperature steps. Three possibilities occur because the final frequency of the perturbed oscillator may be (i) the same, (ii) lower, or (iii) higher than the initial frequency. For each of these three possibilities there are two additional possibilities, because the initial change of $\Delta\Phi_n$ may either increase or decrease.

Figure 4 shows phase shifts determined for temperature step-ups and temperature step-downs. It may be noted, that for an increasing and decreasing temperature step positive and negative phase shifts are observed.

Phase Resetting by Temperature Pulses

PRCs derived from pulsed perturbations of the circadian oscillator are most common in the experimental literature but have also been studied thoroughly from the theoretical point of view (Winfree, 1980). The effect of a temperature pulse on phase resetting is more straightforward to analyse than the effect of a temperature step: after the applied pulse the oscillator returns to its initial oscillatory state (with exactly the same period) and reaches a constant phase difference between unperturbed and perturbed oscillator. The definition of phase shifts $\Delta\Phi_n$ is that of eqn (9). The final phase shift $\Delta\Phi$ is the limiting phase shift for large n, i.e.,

$$\Delta\Phi = \lim_{n\to\infty} \Delta\Phi_n.$$

In practice, it was sufficient to calculate $\Delta\Phi$ as the $\Delta\Phi_n$ value when n = 20, i.e., after 800 time units after the pulse has been applied.

The main variables of the perturbing pulse are its length and height, which are both affecting the resulting degree of $\Delta\Phi$ (for review see Winfree, 1980). However, not only was a dose-dependence of $\Delta\Phi$ observed but also a shift of the position of the PRC with respect to the phase of the applied pulse (Taylor *et al*., 1982). It is, therefore, useful to determine maximal or minimal $\Delta\Phi$ independent of its phase position. When varying length and height of temperature pulses the resulting experimental dose response curves cannot always be identified to a linear or nonlinear relation (Cornelius & Rensing, 1982) which would allow conclusions with respect to the temperature dependence of the involved processes.

In the Goodwin model we observed the following dependencies. Figure 5(a) shows the phase response curves obtained for 273 K → 275 K temperature pulses with variable pulse lengths. Figure 5(b) shows that both the maximal advance and delay phase shifts are linearly dependent on pulse length. Figure 6 shows the behavior of the phase response curve at constant pulse length with varying pulse height ΔT. In contrast to the pulse length, the maximal phase shifts are now exponential functions of the temperature.

Discussion

The Goodwin model [Fig. 1(a)] is a simple oscillatory system containing an inhibitory feedback. Such a feedback system has now been recognized as part of the circadian oscillator of *Drosophila melanogaster* and in *Neurospora crassa* (Hardin *et al*., 1992; Aronson *et al*., 1994; Zeng *et al*., 1994).

Our results show that the modified Goodwin model is in qualitative agreement with the main features

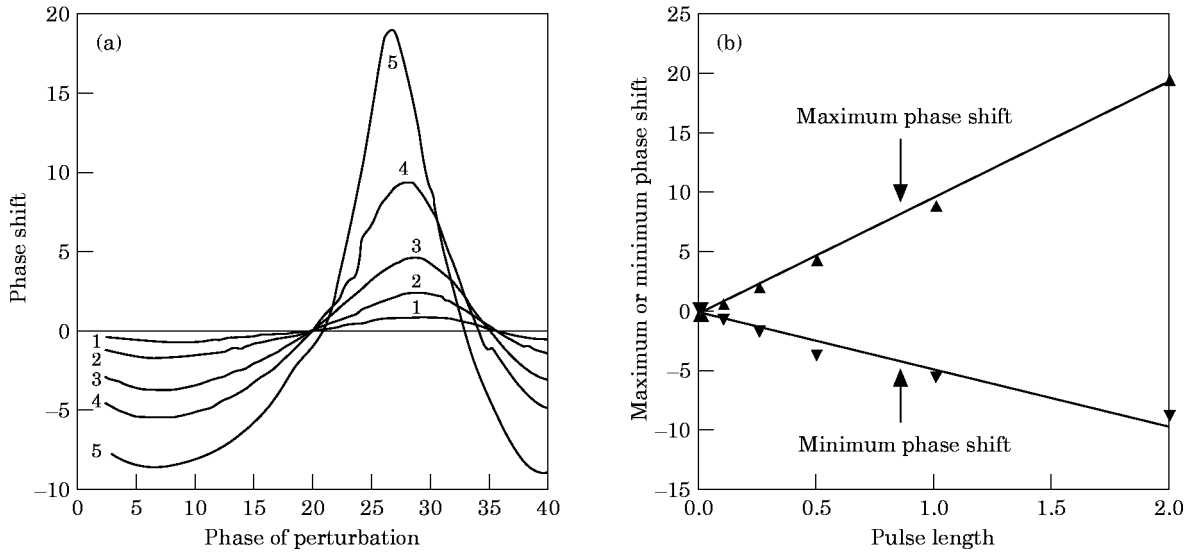

FIG. 5. (a) Phase response curve for 273 K → 275 K temperature pulses with variable pulse length. Zero phase corresponds to the X maximum. Pulse lengths: 1, 0.1; 2, 0.25; 3, 0.5; 4, 1.0; 5, 2.0. (b) Maximal advance and delay phase shift show a linear dependence on pulse lengths.

of the circadian oscillator observed experimentally when studying temperature-entrainment and phase resetting.

ACTIVATION ENERGY REQUIREMENTS FOR TEMPERATURE-COMPENSATION

Antagonistic balance [eqn (8)] implies temperature-compensation for different but constant temperature levels. The loss of temperature compensation by (point) mutations in organisms like *Neurospora* (Lakin-Thomas *et al*., 1990; Aronson *et al*., 1994) could be interpreted in terms of antagonistic balance in the following way: the point mutation alters the degradation rate of a protein that catalyses one of the reactions that participate in the oscillatory mechanism. This causes a change in activation energy and the reaction rate catalysed by the enzyme. A large change in activation energy and reaction rate then destroys the antagonistic balance and leads to a different period length as well as abolishment of temperature-compensation or even of oscillations (Table 1, row 5; Ruoff, 1994; Aronson *et al*., 1994). Equation (8) also implies that whenever the activation energies E_4, E_5, and E_6 of the degradation reactions are low, the

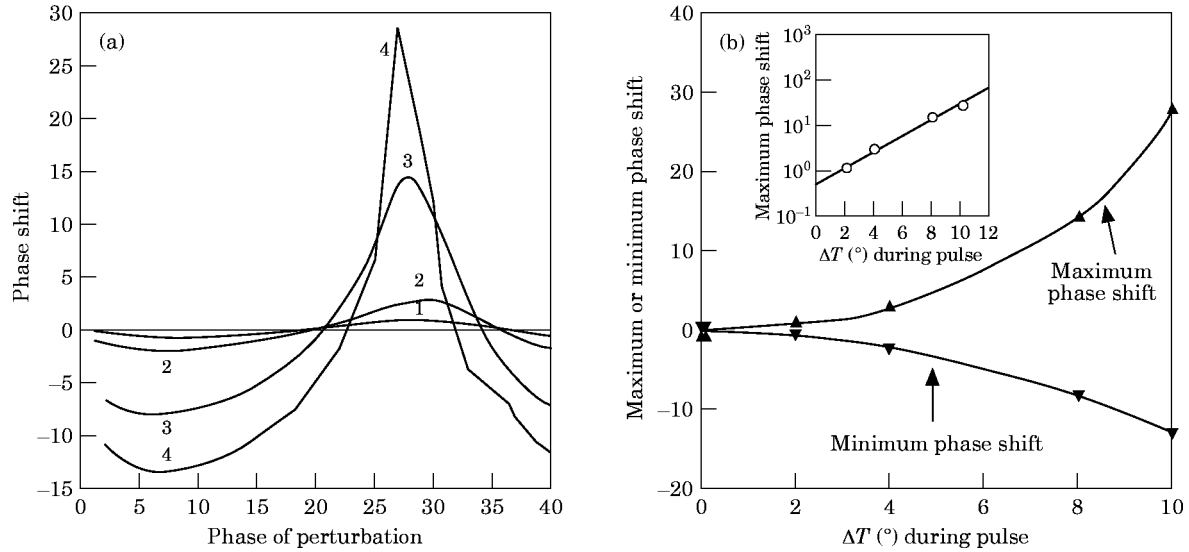

FIG. 6. (a) Effect of temperature pulse height ΔT on phase response curves for temperature pulses with 0.1 time units pulse length. Initial temperature is 273 K. Pulse heights: 1: 2 K; 2: 4 K; 3: 8 K; 4: 10 K. (b) Maximal advance and delay phase shifts show an exponential dependence on pulse heights ΔT. Zero phase corresponds to the X maximum.

activation energies E_1, E_2, and E_3 can be varied in a relatively large range without affecting temperature-compensation (Table 1). It is an interesting question whether this particular way to achieve temperature-compensation is of physiological significance. Mechanisms compensating for the effects of different temperatures, commonly called temperature adaptation, are by no means restricted to circadian rhythms. Protein synthesis and degradation, in particular, are balanced, i.e., moderate temperature changes influence both processes in the same direction, thus leaving the protein level relatively constant after a short transient change (DeMaio *et al*., 1993; Mohsenzadeh *et al*., 1994; Rensing *et al*., 1995). In addition, each of the two processes is apparently adapting, involving protein phosphorylation changes of the initiation or elongation factors (Duncan & Hershey, 1989) and *de novo* synthesis of proteinases (Mohsenzadeh *et al*., 1994). The fluidity of membranes is another property of cells which is often adapting to different temperatures, e.g., kept rather constant, a phenomenon called *homeoviscous adaptation* (Thompson, 1980).

DAMPED TEMPERATURE-COMPENSATED OSCILLATIONS

One of the interesting features of the modified Goodwin Oscillator is that even damped oscillations show temperature-compensation. It may be noted that the transition between damped and limit cycle oscillations occurs smoothly with no apparent changes in period (Table 2). In view of the condition of antagonistic balance, temperature-compensation in damped oscillatory responses should also be observed in other oscillators. This may lead to a slightly alternative view of ''circadian responses'' of organisms: while models for circadian organization mostly included an unspecified limit cycle ''master clock'', there may exist instead a set of entrainable structurally unstable physiological functions that may result in a (more or less damped but temperature-compensated) oscillatory response (Sinha, 1981, 1983). The consideration of damped systems easily accounts for the fact that many circadian rhythms become damped once the organisms are under constant conditions [for example light (Lillo, 1984; Lillo & Ruoff, 1984)]. The consideration of an entrainable and damped-oscillatory physiology is an alternative to the concept of one internal limit cycle master clock.

ENTRAINMENT BY TEMPERATURE CYCLES

Both temperature-compensated limit cycles and a (temperature-compensated) non-oscillatory steady state can readily be entrained or (the latter) forced even by a change in temperature of a few degrees centigrade. This is in agreement with experiments which have shown that circadian rhythms can be entrained by temperature cycles of only a few degrees centigrade amplitude (Oltmanns, 1960; Hoffmann, 1969a, b; Lindberg & Hayden, 1974; Tokura & Oishi, 1985).

However, when the perturbing temperature period becomes too short, the Goodwin oscillator is not able to keep up with the perturbing rhythm and oscillates with its ''natural'' frequency, a well-known phenomenon for circadian rhythms. Under these circumstances an increase in temperature is not able to enforce regular oscillations: the response becomes irregular.

PHASE RESETTING BY TEMPERATURE STEPS AND PULSES

The phase response curves of the Goodwin oscillator are qualitatively similar to temperature step perturbations of the circadian rhythm of eclosion in *Drosophila pseudoobscura* (Zimmerman *et al*., 1968) for small increasing temperature steps. However, studies on models based on autocatalytical amplification (Ruoff, unpublished) show the opposite behavior: temperature step-ups show delayed phase shifts with a minimum, while temperature step-downs show positive phase shifts with a maximum. These results indicate a major difference between the phase response behavior of oscillator models containing an autocatalytical reaction (like the Brusselator or the Kauffman–Wille model) and oscillators containing an inhibitory reaction as the Goodwin model.

A difference, however, between the data observed for *Drosophila pseudoobscura* (Zimmerman *et al*., 1968) and the calculations presented here is that although positive temperature steps show a bell-shaped phase response curve in the model [similar to that observed by Zimmerman *et al*., (1968)] the model calculation shows both negative and positive phase shifts. The same is true for negative temperature steps: the form of the calculated phase response curve [Fig. 4(b)] is quite similar to the experimental PRC (Zimmerman *et al*., 1968), with the exception that in the Goodwin model both positive and negative phase shifts are observed. On the other hand, the data for *Neurospora crassa* by Francis & Sargent (1979) correspond better with our computations.

The PRC of the Goodwin model also shows qualitative agreement with the corresponding experimental analyses of temperature pulses (Zimmerman *et al*., 1968; Engelmann *et al*., 1974; Francis & Sargent, 1979; Rensing *et al*., 1987; Nakashima, 1987; Gooch *et al*., 1994; Goto *et al*., 1994) in contrast to computations performed on autocatalytical models.

Although the maximal advance and delay phase shifts depend linearly on pulse length [Fig. 5(b)], the variation with respect to the height of the temperature pulse is exponential [Fig. 6(b)]. This exponential dependence appears to be due to the Arrhenius-type of dependence between the rate constants and temperature [eqn (4)], and which becomes apparent during the transitory process induced by the temperature perturbation. On the other hand, when the phase shift is studied as a function of the pulse height, it is seen that the relationship phase shift–pulse height is dependent on the phase of perturbation. This can be seen in Fig. 6(a) in which the maximum phase shift is changing its position as a function of the pulse height. The behavior here appears similar to experimental observations by Francis & Sargent (1979), Nakashima (1987) or Taylor *et al*. (1982) in the case of PRCs to protein synthesis inhibitors. Our calculations suggest that in order to see a possible exponential dependence between phase shift and the height of the temperature pulse, ΔT, the maximum (or minimum) phase shift $\Delta\Phi_{max}$ (or $\Delta\Phi_{min}$) should be plotted against ΔT. Existing experimental data of $\Delta\Phi_{max}$ and $\Delta\Phi_{min}$ vs. ΔT (Rensing *et al*., 1987) show an apparent linear relationship, indicating that the actual temperature dependence of the oscillatory processes is not exponential. This question, therefore, needs further experimental and theoretical analysis.

We find it encouraging that the Goodwin oscillator shows a much better qualitative agreement with experimental phase response curves than oscillator models in which the amplification/instability is based on autocatalysis.

Conclusion

The calculations show that a temperature-compensated Goodwin model can qualitatively describe many dynamic features of temperature-perturbed circadian rhythms, including temperature entrainment by small temperature variations and the shape of phase response curves of temperature steps and temperature pulses. Limit cycle oscillations may become damped by decreasing the temperature, however both limit cycle and damped oscillations still show temperature-compensation.

Abbreviations

$\Delta\Phi$: a phase shift.

$\Delta\Phi_{max}$, $\Delta\Phi_{min}$: maximal or minimal phase shift.

$\Delta\Phi_n$: the phase shift between the n^{th} maxima of unperturbed and perturbed oscillator [eqn (9)].

E_i: the activation energy of rate constant k_i [eqn (4)].

k_i: the rate constant of reaction *Ri* (Fig. 1).

P_{appr}: an approximate period length calculated by [eqn (5)].

P_c: the (circadian) period length of an unperturbed oscillator.

P_{num}: the numerically calculated period length by solving differential eqns (1–3).

PRC: phase response curve.

P_t: the period length of a perturbing sinusoidal temperature rhythm.

T: the temperature in Kelvin.

t: time.

t_{per}: the time at which the perturbation of an oscillator is started.

T_{ref}: the (reference) temperature in Kelvin at which a certain set of rate constant values k_i are defined.

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