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Temperature effects on circadian clocks

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Abstract

Periodic temperature changes represent one of the most effective entraining (Zeitgeber) signals for circadian clocks in many organisms. Different constant temperatures affect the circadian amplitude and ultimately the expression of circadian clocks, while the circadian period length (tau) remains approximately constant (temperature compensation). Experimental results and theoretical models are presented that may serve to explain these effects. After introducing the physico-chemical basis of temperature on enzyme-catalyzed and physiological reactions, and after describing mechanisms for temperature adaptation of physiological reactions to different thermal environments, general effects of temperature on chemical and biological oscillators are described. Kinetic models for circadian clocks and temperature compensation are presented and compared with experimental results. Special attention is given to the question how constant but different temperature levels affect clock amplitude, period length and phase. Influences of single and periodic temperature variations (steps or pulses) on circadian clocks are presented together with models which may explain the resulting phase response curves and entrainment patterns. Because temperature compensation is only one aspect of a general homeostatic mechanism that keeps the circadian period rather constant, the influence of other environmental variables and their relationship to temperature are discussed.

Keywords: Circadian clock; Circadian amplitude; Rhythm entrainment; Temperature effects; Temperature compensation; Molecular mechanism; Metabolic control theory; Mathematical modeling

1. Introduction

The circadian clock is essential for the timing of physiological processes, such as circadian rhythms of activity and sleep, hormones, blood components, and cellular processes (Bünning, 1963; Dunlap et al., 2003; Edmunds, 1988). It furthermore participates in adaptation mechanisms which help organisms to cope with extreme seasonal temperature changes by means of seasonal reproduction, hibernation or migration of aquatic or terrestrial animals.

By means of clock mutants and the focus on certain model organisms (such as *Synechococcus, Neurospora*, *Drosophila*, mouse) considerable progress has been made to identify central molecular mechanisms that drive the circadian rhythms in these systems. A common theme in generating circadian rhythms is the presence of transcriptional–translational negative feedback loops, where one or several central clock proteins inhibit their own expression (Dunlap, 1999).

Daily environmental and artificial temperature changes are characteristic Zeitgeber signals for the entrainment of the circadian clock in many organisms

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(Rensing and Ruoff, 2002). Because temperature is one of the most important environmental parameters, which affects practically all parts of an organism's physiology, there is the need for regulatory mechanisms which assure a proper clock function within a certain, for the organism important physiological temperature range. Fig. 1 shows a generalized structure of interlocked positive and negative transcriptional–translational feedback loops of clock genes which were identified in the model organisms mentioned above. Although the actual clock genes and clock proteins differ in various organisms, the dynamic structure appears to be surprisingly conserved (Dunlap, 1999). Temperature effects on the circadian clock can be direct, i.e., affecting the component processes within the clock or indirect by affecting reactions that are peripheral to the clock (Fig. 1).

Before dealing with temperature effects on the circadian clock in more detail, a brief summary of how temperature affects chemical and biochemical processes may be useful.

2. Influence of temperature on chemical and biochemical processes

When investigating the influence of temperature on chemical reactions it is generally observed that an increase of 10 °C leads to an increase in the reaction velocity by about a factor of 2–3. This rule (also known

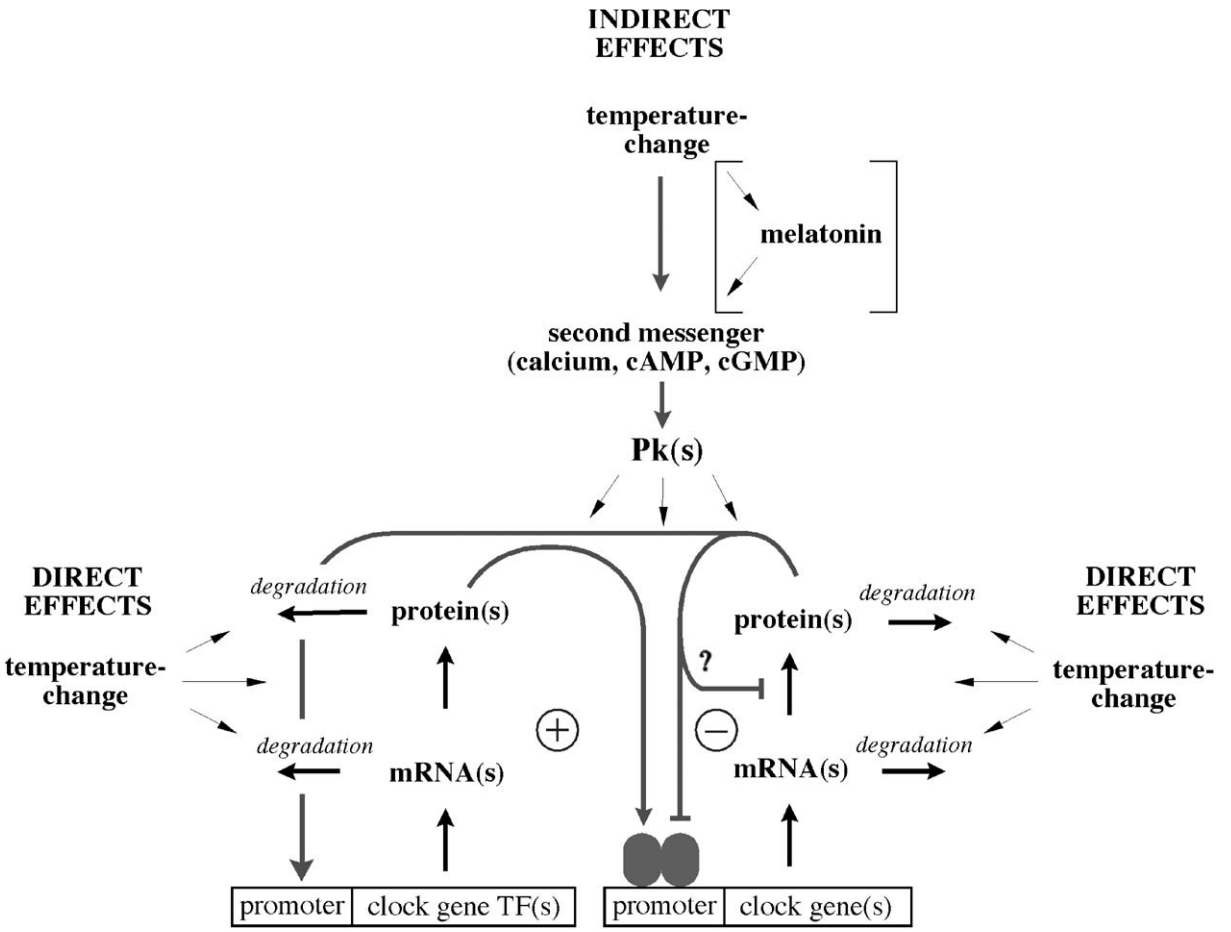

Fig. 1. Indirect and direct effects of temperature changes on the circadian clock (Rensing and Ruoff, 2002): The generalized clock mechanisms consist of clock gene transcription factors (TF), their expression, degradation, and positive effects on clock gene expression (left) as well as the clock protein(s), their expression, degradation and negative effects on the transcription factors (right). In several clock mechanisms, there is also a positive feedback of the clock proteins on the expression of the TFs and possibly on translational control. All these processes may be affected, to different extents, by temperature. A major part of all clock mechanisms consists of protein phosphorylation/dephosphorylation by protein kinases/phosphatases. The latter may be influenced by temperature-induced changes in intracellular messengers and hormones such as melatonin (in ectotherms).

as *Van't Hoff's rule*) is often stated in terms of the Q_{10}, which describes the quotient (ratio) between reaction velocities v at temperatures T and $T+10\,^{\circ}\mathrm{C}$, i.e.,

$$Q_{10} = \frac{v(T+10\,^{\circ}\mathrm{C})}{v(T)}. \tag{1}$$

Although first formulated by Van't Hoff (Laidler, 1993), the *Arrhenius* equation (2) describes the influence of temperature T on the rate constant k_i for a particular process i:

$$k_i = A_i e^{-E_i^a/RT}, \tag{2}$$

where A_i is the so-called pre-exponential factor, E_i^a is the activation energy (both are considered as temperature independent), while R is the gas constant and T is the temperature (in Kelvin). Taking diffusion-controlled reactions aside, it is the influence of temperature on the rate constant (Eq. (2)), which affects the velocity of chemical and physiological processes. For some diffusion-controlled processes the change of viscosity of the reaction medium by temperature plays an important role for the velocity of these processes (Laidler and Meiser, 1995), but these effects will not be considered here.

For elementary (single step) processes, the activation energy is interpreted as a single energy barrier which molecules have to overcome in order to form products, while in more complex processes several intermediates may be formed and several energy barriers must be overcome to form a product (Laidler and Meiser, 1995).

To determine Q_{10} experimentally, it is not necessary to measure velocities or periods at temperatures exactly $10\,^{\circ}\mathrm{C}$ apart. If velocities (or frequencies for circadian or other chemical oscillations) are measured at temperatues T_1 and T_2, then the Q_{10} can be calculated according to

$$Q_{10} = \left(\frac{v(T_2)}{v(T_1)}\right)^{10/(T_2-T_1)}. \tag{3}$$

Most enzyme-catalyzed reactions have Q_{10} values of about 2 (Dixon et al., 1979). When the Q_{10} for a certain process is known at two given temperatures T_1 and T_2, the activation energy can be calculated as

$$E_i^a = R\frac{T_1 T_2}{10}\ln\ Q_{10}. \tag{4}$$

A Q_{10} of 2 around $25\,^{\circ}\mathrm{C}$ (298 K) corresponds to an activation energy of about 50 kJ/mol.

2.1. Evolutionary thermal adaptation of enzymatic processes

The Michaelis–Menten mechanism of an enzyme-catalyzed irreversible process R1

$$\mathrm{S} \xrightarrow{E} \mathrm{P} \tag{R1}$$

is written as

$$E + S \underset{}{\overset{K_M}{\rightleftarrows}} E\cdot S \overset{k_{cat}}{\rightarrow} E + P, \tag{R2}$$

where K_M (Michaelis constant) is the dissociation constant of the enzyme substrate complex $E\cdot S$, which can be either in rapid equilibrium with free E and S (in this case k_{cat} is small compared to the equilibrium process) or ES may be in a steady state, i.e., $\mathrm{d}[E\cdot S]/\mathrm{d}t = 0$ (the turnover defined by k_{cat} is in this case much more rapid than the equilibrium that would have been established between $E\cdot S$ and E, S). In both cases, however, the equation $K_M = [E][S]/[E\cdot S]$ holds true, and the velocity for the formation of P is given by Eq. (5)

$$v = \frac{\mathrm{d}[P]}{\mathrm{d}t} = \frac{k_{cat}[E]_0[S]}{K_M + [S]}. \tag{5}$$

During evolution organisms adapted to different thermal environments by adjusting kinetic parameters of enzymatic reactions to their different needs (Hochachka and Somero, 2002). One of the key events in evolutionary thermal adaptation is apparently conformational flexibility (Somero, 1995). Holland et al. (1997) studied orthologous homologs of lactate dehydrogenase (LDH) of six barracuda species from different thermal environments and showed that different kinetic and thermic stabilities are due to amino acid substitutions at one or a few places outside the active side. Examination of these hypervariable loop regions showed that the most cold-adapted LDH orthologs contain high levels of glycyl residues relative to orthologs of warm adapted species which is consistent with a more flexible "hinge" for the cold-adapted species. Quite recent work (Johns and Somero, 2004) on the Pacific damselfishes (genus Chromis) reports striking evolutionary convergence in temperature adaptation of LDH and presents further support for the hypothesis that enzyme adaptation to temperature involves subtle amino acid changes at a few sites that affect the mobility of those enzyme domains that are involved in rate-determining catalytic conformational changes.

There is evidence that certain-enzyme catalyzed reactions in poikilothermic organisms even show an "instantaneous" temperature compensation (Hazel and Prosser, 1974; Hochachka and Somero, 2002). In terms of Eq. (5) this means that at nonsaturating substrate concentrations, K_M values were increasing with temperature and compensating for the corresponding increase in $V_{max} = k_{cat}\ [E]_0$. This behavior seems also to be based on certain conformational changes of the enzyme. Recently, Andjus et al. (2002) have studied the kinetic requirements for instantaneous temperature compensation in more detail.

3. Temperature compensation

In order to work as correct clocks at different temperatures, circadian clocks are temperature compensated. This means that the period of the rhythm is approximately constant at different (but constant) temperatures with a Q_{10} value close to 1. However, it should not be overlooked that temperature may still have a substantial impact on circadian organization in pacemakers with Q_{10} values "close to 1" (Ruby and Heller, 1996). Temperature compensation occurs normally within a defined temperature range that is relevant for the organism. Outside this temperature range the period is more strongly influenced by temperature change. Temperature compensation is not only observed in circadian oscillators, but also in some ultradian rhythms and certain neuronal oscillations such as wingbeat frequencies in insects or the defecation rhythms of nematodes (Ruoff et al., 2000b). In the fruit fly *Drosophila melanogaster* for example, a temperature compensated ultradian rhythm in the courtship song (period ≈ 50 s) was observed that is determined by the *per* gene, which also plays an important part in the mechanism (negative feedback loop) that defines the circadian rhythm in this organism (Kyriacou, 2002; Kyriacou and Hall, 1980).

It has been suggested that temperature compensation is only one aspect of a general homeostatic mechanism that keeps the circadian period constant upon different environmental parameters such as for example nutritional variations or changes in pH (Pittendrigh and Caldarola, 1973). For example, in *Neurospora crassa* it was recently observed (Fig. 2) that altering the *frequency* (*frq*) gene plays a role both for temperature and pH-compensation of the oscillator (Ruoff et al., 2000a).

Various hypotheses have been put forward to explain temperature compensation (Hong and Tyson, 1997; Leloup and Goldbeter, 1997; Ruoff, 1992, 1997; Ruoff et al., 1997, 2000b). One of the first kinetic approaches was proposed by Hastings and Sweeney (1957), who assumed that temperature change affects two reactions that act antagonistically on the period of the oscillation, a principle that is also observed in mechanical temperature-compensated pendulum clocks and in electronic crystal oscillators (Ruoff et al., 2000b).

When considering a generalized scheme of N component processes which define a chemical or biological oscillator, the requirement for getting temperature compensation in such an oscillator can be formulated as (Ruoff, 1992):

$$\sum_i \left(\frac{\partial \ln P}{\partial \ln k_i}\right) E_i^a = 0, \tag{6}$$

where k_i is the rate constant of process "i" and E_i^{a} is the activation energy of this process. The partial derivatives

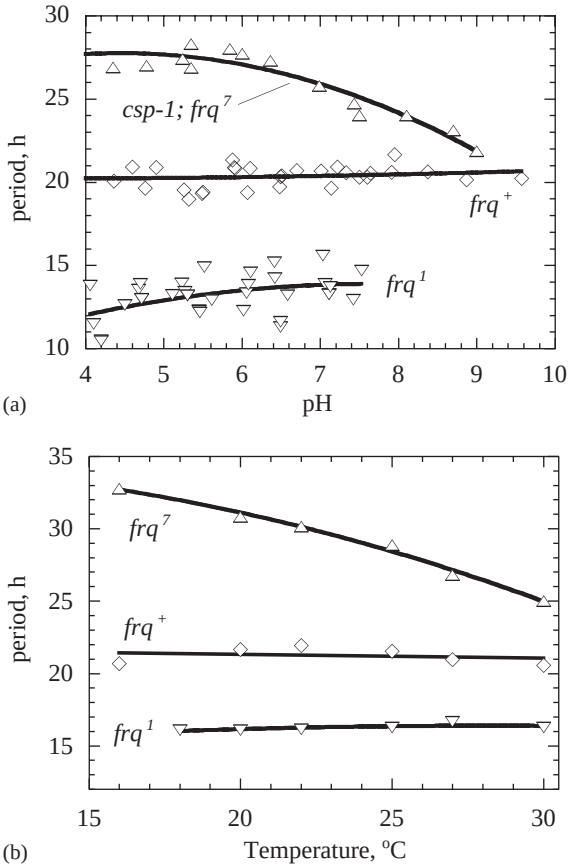

Fig. 2. Correspondence between pH and temperature compensation in *Neurospora crassa.* (a) shows pH compensation in wild-type (frq^+) and in the short period (frq^1) and long period (frq^7) mutants (Ruoff et al., 2000a). (b) shows temperature compensation in the same mutants (Gardner and Feldman, 1981). Interestingly, in the frq^7 mutant both temperature and pH compensation is lost indicating that the *frq* gene (FRQ protein) may play an important role in the general homeostasis of the circadian period (Pittendrigh and Caldarola, 1973) in *Neurospora*.

$(\partial \ln P/\partial \ln k_i)$ are called control coefficients C_i (Fell, 1997; Heinrich and Schuster, 1996; Kacser and Burns, 1973) which, in addition, satisfy the summation rule $\sum_i C_i = -1$ (Heinrich and Schuster, 1996; Ruoff et al., 2003).

In order to satisfy Eq. (6), the control coefficients need to have opposite signs in order to balance positive and negative contributions (note that activation energies E_i^{a} are positive and independent of temperature). It appears that the opposite signs of the control coefficients in reaction kinetic oscillators are closely related to the positive and negative feedbacks which are necessary to generate physico-chemical oscillations (Franck, 1980; Ruoff, 1992).

For a given reaction kinetic oscillator with a corresponding set of control coefficients C_i an infinite number of activation energy combinations are possible to satisfy Eq. (6) and to generate temperature compensation (Ruoff, 1992, 1994, 1995; Ruoff et al., 2003). Recently, an inorganic chemical oscillator was found, which showed temperature compensation within a temperature range of about 10 °C (Kóvacs and Rábai, 2002; Rábai and Hanazaki, 1999). These findings demonstrate that even in "*in vitro*" chemical oscillators activation energy combinations may be found that result in temperature compensation.

How did the property of temperature compensation arise in biological clocks? It appears reasonable to assume that certain combinations of adapted E_i^a values may have emerged during evolution, similar to those found in the thermal or instantaneous adaptation of certain enzymes. The above theory of temperature compensation has been applied in a simple model of the *Neurospora* circadian clock (the Goodwin oscillator, Fig. 3a). This model is able to predict how period changes in certain clock mutants correspond to partial losses in temperature compensation. Fig. 3b shows the results of three tested strains among which two have partially lost temperature compensation. In accordance with model predictions (Ruoff et al., 1996, 1999b) it was found that in the long period *frq* mutants the longer period appears to be the result of a more stable FRQ protein due to an increased activation energy for FRQ protein degradation. Because the activation energies of FRQ degradation are associated with relatively high negative control coefficients, temperature compensation should be less effective, i.e., dP/dT should become more negative with increased activation energies of FRQ degradation and increased period lengths. Recent experimental results support this view (Ruoff et al., 2004). For more detailed reviews of temperature compensation also in ultradian clocks as well as of other aspects of temperature compensation, we refer to the work by Rensing and coworkers (Rensing et al., 1987b, 1995, 1997; Rensing and Monnerjahn, 1996; Ruoff et al., 2000c) and to an earlier review by Hazel and Prosser (Hazel and Prosser, 1974).

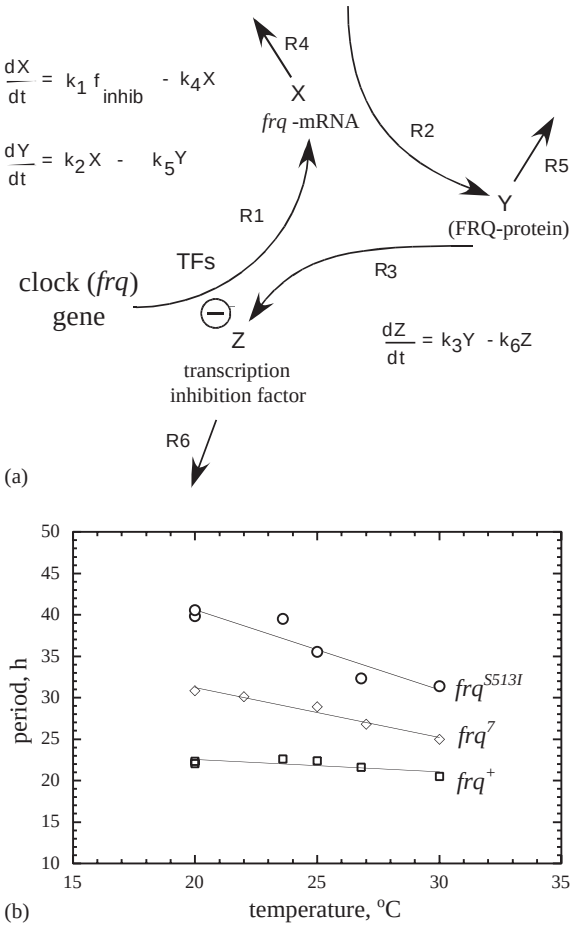

Fig. 3. (a) The Goodwin oscillator can model the *Neurospora* circadian clock by three coupled differential equations. Variables are X (*frq*-mRNA), Y (FRQ-protein) and Z (transcription inhibition factor causing a negative feedback). Temperature compensation is obtained in this model when $RT^2dP/dT = 0.0023(E_1+E_2+E_3)-0.3583(E_4+E_5)-0.3414E_6 = 0$. (b) Temperature compensation in three *Neurospora* strains: wild type (frq^+) and the two long period mutants frq^7 and frq^{S513I}. Consistent with predictions by this model is the increased loss of temperature compensation, i.e., the slope dP/dT is getting more negative, when FRQ protein stability increases due to an increase in the activation energy E_5 in the FRQ degradation process R5 (Fig. 3a). An increase in E_5 results also in a lower FRQ-degradation rate constant k_5 and an increased period.

4. Initiation and "oscillation restart"

Temperature steps usually lead to phase shifts or a new start (initiation) of the circadian clock. If the clock was kept in a stationary (but also in an oscillatory) state during the initial temperature, a temperature step to another temperature may cause a "restart" of the oscillator with a defined new initial phase. This phenomenon has been used in so-called "release assays" to test for the initial phase. In *Neurospora*, for example, a temperature shift causes a change in the level of FRQ-protein oscillations and is interpreted by the cell as a start from a defined phase. A temperature-induced change from low to high level in *Neurospora* means that the oscillations start at the minimum of FRQ, while a change from high to low level means that the oscillations start at the maximum of FRQ (Fig. 4) (Loros and Dunlap, 2001).

As already indicated above for temperature compensation, circadian oscillations are expressed only within a

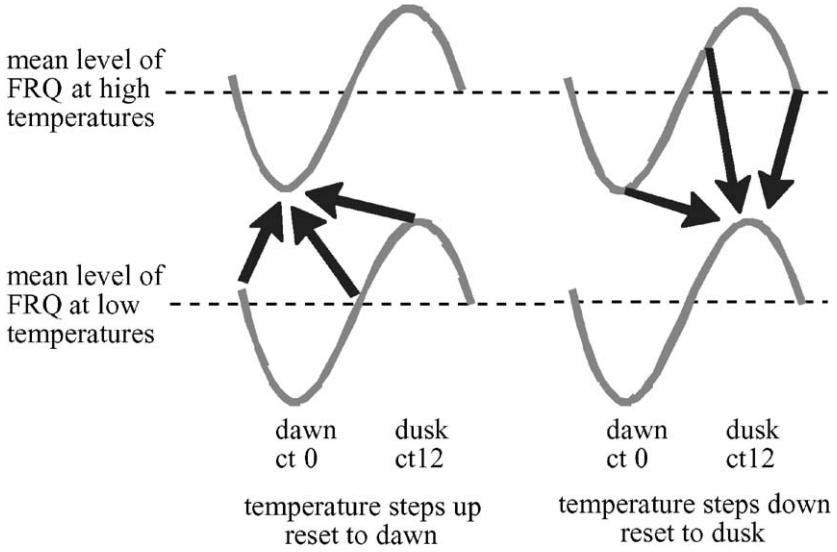

Fig. 4. Model for the phase determination after step-up and step-down experiments based on the different levels of FRQ-oscillation. Left: after a step-up all phases are interpreted as minimum of FRQ (at dawn), and the rhythm starts at this position. Right: after a step-down all phases are interpreted as maximum of FRQ (at dusk) and the rhythm starts from this position (Loros and Dunlap, 2001).

certain temperature range/limit. The limits of expression vary according to species and may depend on the latitude of the species' habitat.

Temperature steps may lead to transient changes of the circadian period length, which may be caused by larger or longer adaptation times to approach an oscillatory steady state at the new temperature. Long-term effects of a temperature step on the period length are similar to those described for light pulses (Sharma and Daan, 2002) and depicted as "tau response curves" (TRCs). Sometimes the period length undergoes transient shortening and lengthening (or vice versa) after a temperature step before arriving at a steady state (Rensing and Ruoff, 2002).

5. Phase shifts

Temperature changes (steps/pulses up or down) lead to phase-dependent phase shifts, which are described as so-called "phase response curves" (PRCs) or "phase transition curves" (PTCs). In PRCs (Fig. 5), the phase shift (ideally after transients have disappeared) is plotted against the phase where the perturbation was applied, while in PTCs the new phase is plotted against the old (unperturbed) phase (Winfree, 2000). To make phase response curves easier comparable between organisms with different period lengths, the term "circadian time" (ct) is introduced. One ct unit is the 1/24 part of the actual period length, such that the phase for any circadian oscillator varies between ct 0 and ct 24. ct 0 is defined as the beginning of the subjective light time and ct 12 is defined as the change from subjective light to subjective night conditions. The extend of the phase shift depends on the strength ("amplitude") and duration of the temperature pulse. The PRCs to small (or short) temperature changes generally result in weak (after Winfree so-called "type 1") PRCs (Winfree, 2000), while larger temperature changes cause strong (type 0) PRCs.

One class of PRCs is derived from step-up or step-down treatments of the oscillator at different phases and registration of the subsequent phase shifts of the oscillations. Step-up or down of 5 °C treatments of *Neurospora crassa* showed PRCs of the strong resetting types (type 0, see above). The temporal position of the step-up and the step-down PRCs differ by about 9 h when comparing the position of zero phase shifts: they occurred at about ct 8 (step-ups), and at about ct 0 (step-downs) (Francis and Sargent, 1979). Whether the step-up PRC primarily represents advances and the step-down PRC primarily delays is a matter of how one interprets advance or delay shifts that are greater than 12 h, as delay or advances, respectively.

Another class of PRCs consists of pulse-up or pulse-down treatments of the oscillator and the subsequent registration of the phase shift. This class is based on a complex response because a pulse consists of a step-up and step-down signal (or vice versa), which both affect the phase. The sum of both signals, in turn, is dependent on their strength and the adaptation process that occurs between the first and second signal. As concluded from a mathematical model (the "Goodwin Oscillator", Fig. 3a), however, a significant temporal relationship exists between the phase of the PRC and the perturbed

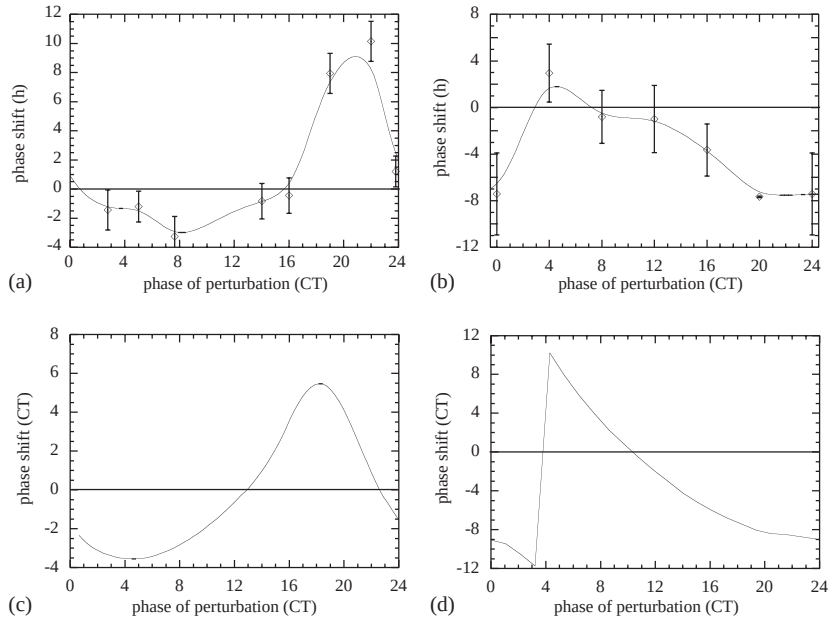

Fig. 5. Experimental and calculated PRCs for moderate temperature pulses and heat shock pulses for the *Neurospora* circadian clock. (a) experimental PRC using a 3 h 30 °C pulse (Rensing et al., 1987a); (b) experimental PRC using a 1 h 45 °C pulse; (c) calculated PRC using a low temperature pulse; (d) calculated PRC using a heat shock pulse. In case of the heat shock inhibition of protein synthesis and protein degradation reactions are assumed. The same type of phase response curve is obtained when using the protein synthesis inhibitor cycloheximide (Ruoff et al., 1999a).

clock variable (Drescher et al., 1982; Rensing and Schill, 1987).

PRCs to positive temperature pulses are surprisingly similar to those of light pulses: both PRCs show a gradual change of the phase shift from positive values (advances) to negative values (delays) with increasing phase, where the crossover from advances to delays occur at about ct 0–8. At larger phases often an abrupt change from delays to advances (''break point'') occurs at about ct 12–20. This type of behavior has qualitatively been described by the temperature compensated Goodwin oscillator when comparing with PRCs from *Drosophila* and *Neurospora* (Ruoff and Rensing, 1996). Particularly high positive temperatures lead to heat shock responses which suppress protein synthesis and results in PRCs similar to those of protein synthesis inhibitors (Fig. 5).

Sometimes ''after effects'' of a temperature treatment are observed. For example, pulses of low (2 °C) temperature caused delay phase shifts in the singing activity of crickets, which sometimes is associated with a permanent change of the free-running period length (Wiedenmann and Loher, 1984). In *Tenebrio molitor* a down-shift of temperature (5 °C) caused a transient shortening followed by a lengthening of the period of the locomotor activity rhythm before the steady state period length was reached (Lohmann, 1964). These ''after effects'' of a temperature treatment are not yet understood in molecular terms, even though the temperature effects on the phase seem to be instantaneous in the case of 40 °C pulses as determined by a subsequent light PRC (Maier, 1973).

A low temperature pulse (6 °C for 2 h) given 30 min before a light pulse in *Drosophila* delayed the light PRC for 1.5 h. This was attributed to the slowing of processes between the photoreceptor and the clock (Hamm et al., 1975).

6. Amplitude levels and phase

Although circadian rhythms show an approximate constancy of the period length within a physiological temperature range, the corresponding amplitudes are generally not constant and vary with temperature. In fact, the amplitude in circadian clocks has been used as a criterion how temperature compensation may be understood. The argument (''amplitude model'') is as follows (Lakin-Thomas et al., 1991). The assumed limit cycle oscillations of circadian rhythms can be described as a closed trajectory in the concentration space of the oscillator, where an increase of temperature should lead to an increase in the velocity by which the system moves along this trajectory. In order to maintain a constant period the area/length which is swept by the trajectory during one cycle has therefore to increase at higher

temperatures, which therefore should result in an increased amplitude. Although model calculations on temperature compensated oscillatory systems have mainly supported this view (Ruoff, 1992; Ruoff et al., 2003), there are counter examples both theoretically as well as experimentally. In a model for the *Drosophila* circadian clock Leloup and Goldbeter (1999) found two coexisting limit cycles where one limit cycle had a smaller amplitude but surprisingly a considerably longer period. An experimental counter example to the "amplitude model" was found in *Gonyaulax*, where different temperatures influenced the amplitudes of the flashing and glow bioluminescent rhythms in different directions: the amplitude of flashing decreased, while that of the glow rhythm increased when the temperature was shifted from 15 °C to 25 °C (von der Heyde et al., 1992). These output amplitudes, however, may be not representative for the amplitude of the underlying clock variable.

In *Neurospora* the amplitude of the conidiation rhythm (an output rhythm of the circadian clock) is maximal at about 25 °C and decreases toward higher and lower temperatures. Interestingly, two forms of the FRQ protein are made, where the longer form dominates at higher temperatures while the shorter form is more abundant at lower temperatures. In strains where only the long form of FRQ is present the conidiation amplitude is diminished or absent at lower temperatures, while in strains which express only the short form the amplitude is lower or absent at higher temperatures (Liu et al., 1997). Different temperatures (21 °C and 28 °C) also influence the level of the FRQ oscillations (more than two-fold at the higher temperature), but not of the *frq*-mRNA (Liu et al., 1997).

In birds and mammals the temperature dependence of the circadian amplitude (or level) was often determined by the locomotor activity. An early review (Pohl, 1968b) on available data concluded that there is probably an optimum temperature as well an optimum light intensity for activity, but that the temperature and light dependencies are not necessarily correlated. The optimum in temperature seems to depend on energy expenditure and body temperature regulation. For example, in the light-active chaffinch *Fringilla coelebs* an increase of the temperature from 10 °C to 25 °C is associated with an increase in the amount of activity (15–20%) and a slight shortening (0.3 h) of the period length. In the dark-active Fat Dormouse (*Glis glis* L.) higher temperatures lead to an activity decrease as much as 25% and to an increase of the period length by 0.5 h per 10 °C (Pohl, 1968a).

In cultured chick pineal cells the highest amplitude of the melatonin secretion rhythm in LD and DD was observed at 40 °C and decreased with lower temperatures (Barrett and Takahashi, 1995; Zatz et al., 1994) while at about 47 °C the melatonin production stopped within a few hours (Zatz et al., 1994). The period length of this rhythm was temperature compensated over the range of 34–40 °C with a Q_{10} of 0.83 (Barrett and Takahashi, 1995). Cultured rat SCN slices also showed a higher amplitude of the circadian firing rate at 37 °C compared to 31 °C, while the period length was temperature compensated with a Q_{10} of 0.99 (Ruby et al., 1999; Ruby and Heller, 1996). From the few systematic data, one may conclude that temperature has a definite effect on the amplitude, but that maximum amplitude and level is species dependent and may vary also in a seasonal and development-specific way (Rensing and Ruoff, 2002).

Different constant temperatures may also change the phasing of the circadian rhythm with respect to the light-dark cycle. This effect may cause organisms to become day-active at one temperature and night-active at another. In addition, different temperatures may affect the light signal perception and thus the phasing of the oscillations.

7. Entrainment

Most organisms, to some extent also homeothermic animals, can be entrained by periodic temperature changes. In several groups of organisms, including plants, fungi, ectothermic and heterothermic vertebrates, very small temperature differences of 0.7–2.0 °C are sufficient. Temperature cycles with period lengths differing from 24 h are able to entrain the circadian clock up to species-specific limits of entrainment. These limits are dependent on the amplitude of the temperature cycle (Zeitgeber strength). In ectothermic vertebrates, 24 h temperature cycles can entrain the circadian clock(s) as demonstrated for the locomotor activity of lizards, but it is not easy to distinguish in these cases between entrainment and masking (Rensing and Ruoff, 2002). Masking means that an organism is able to act immediately and in an appropriate way to changes of the environment, which dominates the internally produced rhythmicity (Rietveld et al., 1993).

When combined with a light-dark cycle, a temperature cycle usually enhances the amplitude when applied in phase with the light dark cycle, i.e., warm phase during light and cold phase during night. Entrainment by temperature changes occurs also in organisms in which the light pathway has been blocked. The dual effects of Zeitgeber signals on the organism, one that acts directly (masking) and the other that acts as entraining signal eventually result in the optimal adaptation of the organism to the day/night changes of the environment (Redlin, 2001; Rietveld et al., 1993).

In mammals (Table 1) periodic temperature differences between 4 °C and 14 °C entrained the rhythmicity in some species, but often not all individuals of the

Table 1
Entrainment by 24 h temperature cycles in birds and mammals

Species	T °C difference[a]	Degree of entrainment[b]	c/w active[c]	References
Birds				
House sparrow (*Passer domesticus*)	37–41	+		(Hoffmann, 1970)
	32–35	±		
	17–23	−		
House finch (*Carpodacus mexicanus*)	18			(Enright, 1966)
Mammals normotherms				
Rock pocket mouse (Perognathus intermedius)	9	−		(Steward and Reeder, 1968)
Flying squirrel (*Glaucomys volans*)	10	−		(DeCoursey, 1960)
Rat (*Rattus norvegicus*)	13	− (+)		(Francis and Coleman, 1988)
Squirrel monkey (*Saimiri sciurius*)	8	−		(Aschoff and Tokura, 1986;
	15–16	+ (−)	c (w)	Sulzman et al., 1977)
Bat (*Phyllostomus discolor*)	10	−		(Erkert and Rothmund, 1980)
Common marmoset (*Callithrix j. jacchus*)	10	+ (+)	w	(Pàlkovà et al., 1999)
Marsupial mouse–stripe-faced dunnart (*Sminthopsis macroura*)	14	− (+)	c	(Francis and Coleman, 1990)
Antelope ground squirrel (*Ammospermophilus leucurus*)	6–12	+ (−)	c	(Pohl, 1998)
	4	− (+)		
Common mole-rat (*Cryptomys Hottentotus*)	6	+	w	(Fritsche et al., 1997)
Pig-tailed macaque (*Macaca nemistrina*)	15	+ (−)	w	(Tokura and Aschoff, 1983)
Eutamias sibiricus	2	−		(Hoffmann, 1969)
Mouse (*Apodemus sylvaticus*) (*A. flavicollis*)	15	− (+)		(Hoffmann, 1970)
	26			
Microtus oeconomus	6.5	− (+)		(Swade and Pittendrigh, 1967)
Eutamias sibiricus	8			
Citellus undulates	12.5			
Palm squirrel (*Funambulus pennanti*)	10–16	+ (−)	c (w)	(Rajaratnam and Redman, 1998)
Mammals heterotherms				
Little pocket mouse (*Perognathus longimembris*)	1.5–10	+	c	(Lindberg and Hayden, 1974)
	1.1	−		
Bat (*Molossus ater*)	10	+ (−)	c (w)	(Erkert and Rothmund, 1980)
Syrian hamster (*Mesocricetus auratus*)	6–12	+ (−)	w	(Pohl, 1998)
	4	− (+)		

[a]Temperature difference applied.
[b]+:most individuals are entrained; (+): a few individuals are entrained; (−): a few individuals are not entrained; −: most individuals are not entrained.
[c]c: cold active; w: warm active.

tested group. Other species did not respond. The activity of the responding individuals is confined to the warm or cold phase, which probably depends on the respective temperatures during the warm and cold phase and the body temperature regulation. Among mammals, a group of species, so-called heterotherms show daily (example: little pocket mouse (Lindberg and Hayden, 1974)) or seasonal (example: Syrian hamster (Pohl, 1998)) variations in body temperature regulation. The little pocket mouse can be entrained to temperature differences of only 1.5 °C, and the PRC of 10 °C temperature pulses were of type 0 (strong resetting). It is presently not clear whether it is possible to make a clear distinction between hetero- and normothermic mammals based on such temperature responses (Erkert and Rothmund, 1980; Lindberg and Hayden, 1974; Pohl, 1998).

One may speculate that the self-selected rhythm of body temperature in reptiles or the endogenously controlled body temperature in homeotherms (some of which show temperature differences of more than 2 °C) may, in itself, serve as an internal entraining system. In warm-blooded animals, including humans, the body and skin temperature cycles under the control of a central oscillator, the suprachiasmatic nucleus (SCN). This

rhythm of the body and skin temperature can entrain peripheral oscillators in other organs of the body (Brown et al., 2002), influence sleep propensity by modulating thermosensitive neurons in sleep-related brain areas (Van Someren, 2000), and even entrain the central oscillator itself (Van Someren, 2003).

Model computations with the Goodwin oscillator showed that this oscillator, apart from showing qualitatively correct PRCs on temperature pulses (see above) can also describe essential features of temperature entrainment of circadian clocks: within a certain interval around the oscillator's natural period the oscillator can easily adjust to the entrainment period; however if the entrainment period is outside this interval the oscillator resets to a period which is close to the period of the unperturbed oscillator (Ruoff and Rensing, 1996). Although mathematical modeling of circadian rhythms has progressed considerably during recent years (Goldbeter, 2002), the Goodwin oscillator (Ruoff and Rensing, 1996) appears to be the only example in which temperature entrainment has been studied in a temperature compensated circadian clock model.

8. Outlook

As more details of the molecular processes of biological oscillators are being identified, there will be an increased need for more quantitative kinetic and mechanistic description by reaction kinetic models. Such an approach should reflect the knowledge of the kinetics of the involved processes, but should also contain a minimal amount of assumptions that may be needed in order to explain the characteristics of the system, a principle, which is known as Ockham's razor. Although biological oscillators are of great complexitiy, their understanding implies not only the consideration of key events but also their correct kinetic description by appropriate models and the predictions of new aspects by such models that can be verified experimentally.

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