Temperature Compensation in Biological Oscillators: A Challenge for Joint Experimental and Theoretical Analysis

Temperature has a profound effect on practically all chemical and biochemical reactions. However, despite this strong influence many biological oscillations, circadian clocks, ultradian rhythms, as well as a variety of neuronal oscillators are temperature-compensated, i.e., show a homeostatic mechanism that keeps the period unaffected by environmental temperature changes. This review describes our current understanding of mechanisms that may lead to temperature-compensation, theoretical approaches and possible future directions to study temperature-compensation.

Key Words: *temperature-compensation, circadian clock, ultradian rhythms, neuronal oscillations, Goodwin oscillator, Neurospora crassa, Drosophila*

Many physiological oscillators have developed homeostatic mechanisms to compensate for environmental changes in temperature, because some of these oscillators acts as clocks, such as circadian rhythms,[1–7] while others have important physical or physiological functions as for example the wing beat frequency in insects or the defecation rhythm in nematodes. This article deals with the question how temperature-compensation is achieved in biological oscillators, with theoretical approaches to understand the control mechanisms, and with possible future directions for expanding the analytical techniques.

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INFLUENCE OF TEMPERATURE ON CHEMICAL PROCESSES

It is now generally believed that all chemical change, no matter how complex, is the consequence of a number of *elementary processes*.[8] An elementary process takes place in a single step and involves at most three reactant or product molecules. Kinetic orders in each reaction direction are equal with the stoichiometric coefficients of the elementary process, and the net direction of the process is determined by the free energy of formation and the concentrations of reacting species. All elementary reactions can again be grouped into a set of component stoichiometric processes, which together describe the overall change from reactants to products in a complex reacting system.[8] The influence of temperature on an elementary process or a component stoichiometric process is generally described by the Arrhenius equation,[9] which connects the rate constant k_i of process "i" with the absolute temperature T measured in Kelvin:

$$k_i = A_i \exp\left(-\frac{E_i^a}{RT}\right) \tag{1}$$

A_i is the so-called pre-exponential factor. In most cases A_i can be considered to be independent of temperature. E_i^a is the activation energy of reaction "i" which can be visualized as an energy-barrier the molecules have to overcome in order to form a product.[10]

In many cases, Eq. 1 describes the influence of temperature with sufficiently high precision, although sometimes (for example radical reactions with low E_i^a) A_i becomes slightly temperature dependent.[11] For the sake of simplicity, however, we will assume here that A_i is independent of temperature.

To describe the influence of temperature on a particular biochemical or physiological process Q_{10} values are often used instead of activation energies. Q_{10} is defined as the ratio between the velocities (rates) v of a reaction at temperature $T + 10°C$ and T, i.e.,

$$Q_{10} = \frac{v(T + 10°C)}{v(T)}. \tag{2}$$

To determine Q_{10} experimentally, it is not necessary to measure the velocities at temperatures exactly 10°C apart. If velocities are measured

at temperatures T_1 and T_2, then 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 biochemical reactions (including enzyme-catalyzed reactions) have Q_{10} values of about 2.[12] The relationship between Q_{10} and the activation energy E_i^a is given by[12,13]

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

OCCURENCE OF TEMPERATURE-COMPENSATED PROCESSES

Despite the fact that biochemical reactions are highly dependent on temperature, there are physiological processes that have been found to be practically independent of environmental temperatures. Many of these physiological processes involve oscillations at different time scales like circadian rhythms with period lengths of about one day (*circa*, about, *dies* day) down to the millisecond domain of neuronal oscillators. In the following we give a brief overview of the major classes of temperature-compensated oscillators.

Circadian Rhythms

Pittendrigh's[2,4] discovery that circadian oscillations are homeostatic and compensate for temperature variations and other environmental influences (for example pH, nutrient supply)[14] in order to allow the rhythm to function as a reliable chronometer (clock), led to a focus on the biochemical/physiological requirements to maintain period homeostasis. In fact, temperature-compensation is now considered as one of the characteristic properties of the circadian clock.[1–7] The Q_{10}'s of circadian oscillations fall within the range of 0.8–1.2. In some organisms, especially from areas with large environmental temperature changes, even Q_{10}'s very near 1.0 exist.[3] On the other hand, organisms from areas with small environmental temperature variations (tropics) tend to have a less effective temperature-compensation.[3]

The best understood molecular mechanisms of circadian oscillators so far come from studies of the filamentous fungus *Neurosopora crassa* and the fruit fly *Drosophila*. In both *Neurospora* and *Drosophila* so-called clock mutants were found, i.e., mutants in which the oscillator's period is altered together with the ability to maintain temperature-compensation. Konopka and Benzer[15] described the first clock mutants in *Drosophila* with mutations in the *period* (*per*) gene. In the long period mutant (per^L) the activity rhythm shows a longer period length (≈29 h, 25°C), while in the short period mutant (per^S) the period length is considerably shorter (≈18 h, 25°C) compared to wild-type (per^+).[16] Both per^L and per^S show attenuated temperature-compensation (Figure 1a).

In *Neurospora*, the first clock-mutants with mutations in the *frequency* (*frq*) gene were reported by Feldman and Hoyle.[17] The different alleles are designated by superscripts: the frq^1 mutant shows a short period length (≈16 h) and is temperature-compensated, while the frq^7 mutant has a long period length (≈ 29 h, 25°C) and is less temperature-compensated compared to the wild-type (21.5 h period length at 25°C, Figure 1b).[18,19]

Both in *Drosophila*[20] and *Neurospora*[21] there is good experimental evidence that the core circadian clock (or a major part of it) consists of a negative feedback loop in the expression of one or two clock genes where the clock-protein(s) inhibit(s) their own gene(s) (Figure 2). In *Drosophila* recent work has shown that the PER-protein associates with a protein TIM (from the gene *timeless* (*tim*)) and that the PER–TIM complex – probably its phosphorylated form – inhibits the expression of both *tim* and *per*.[22,23] PER/TIM apparently interfere with the activating transcription factors (dCLK··CYC protein complex) which bind to the so-called E-box of the *per* and *tim* promoters. Interesting is the fact that *per* in *Drosophila* as well as *frq* and *wc* (*white-collar*) in *Neurospora* encode threonine-glycin (Thr-Gly) repeats.[24] The functions of these repeats is not understood but may be somehow related to temperature-compensation. In *Drosophila* it was shown that the Thr-Gly repeats vary with the geographical location of the flies and are correlated with the flies' ability to maintain a circadian period at different temperatures.[25]

In *Neurospora*, the clock mechanism consists probably of a simpler feedback loop, i.e., the FRQ-protein (or its phosphorylated forms) inhibits its own expression by interfering with activating factors (WC-1, WC-2 protein?).[24] One of the mutated *frq* alleles (frq^7) is less temperature-compensated, as mentioned above. In addition, other clock mutants have been found besides *frq* which affect temperature-compensation.[19,26]

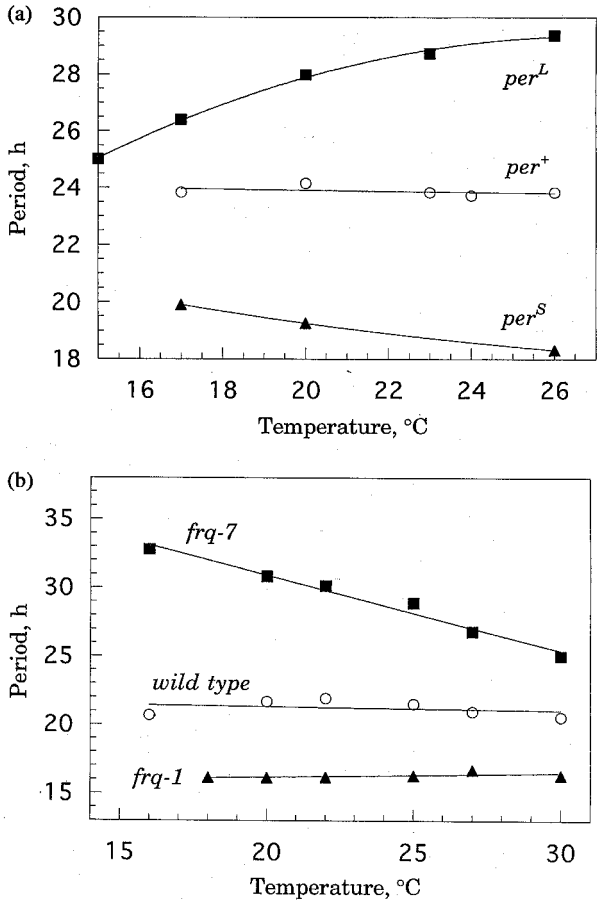

FIGURE 1 (a) Temperature-compensation of the locomotory rhythm in per^+, per^L and per^S mutants in *D. melanogaster*. Redrawn from Ref. 16. (b) Temperature-compensation of the sporulation rhythm in frq^+, frq^7 and frq^1-mutants of *N. crassa*. Redrawn from Ref. 18.

The *cel* mutant[27] shows defects in temperature-compensation and lipid metabolism and may thus indicate that membrane properties contribute to temperature-compensation. When saturated fatty acids were supplied in the growth medium normal growth and temperature-compensation of the conidiation rhythm resumed. Another mutant defective in membrane lipid synthesis and temperature-compensation (*chol-1*) requires choline

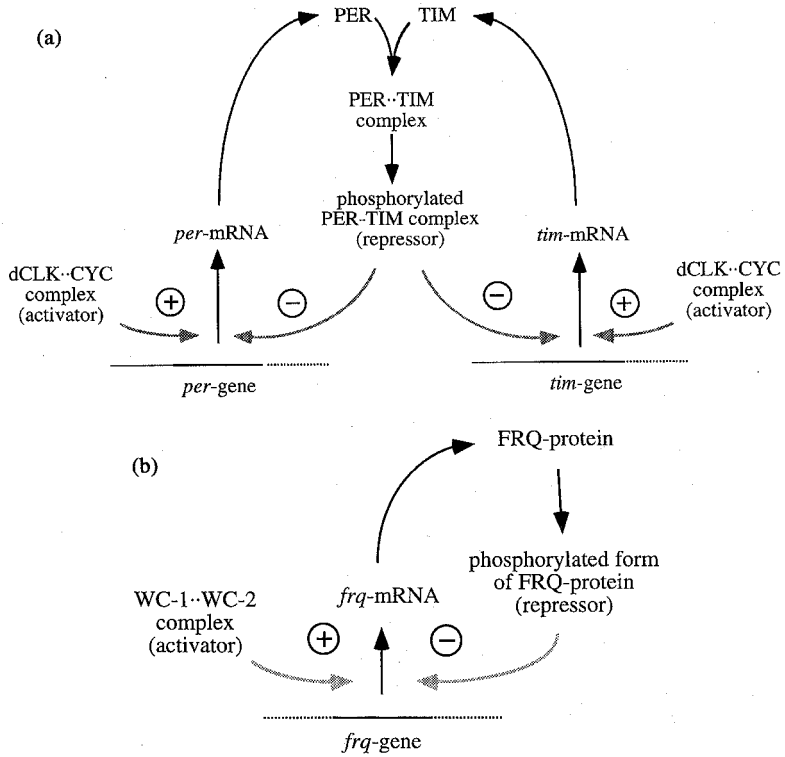

FIGURE 2 (a) Negative feedback loop in *Drosophila*. The *per* and *tim* genes are activated by protein-complexes (dCLK/CYC) belonging to the family of PAS-proteins.[24] Activated gene expression leads to the PER and TIM-proteins which form a PER··TIM heterodimer. Probably one or several phosphorylated forms of the PER··TIM complex inhibit further transcription of both the *per* and *tim* genes. (b) Negative feedback loop in *Neurospora crassa*. The *frq*-gene is assumed to be activated by the WC-1·· WC-2 proteins both containing a PAS-domain. Again, the phosphorylated forms of the FRQ-protein may actually inhibit the transcription of the *frq*-gene.

for normal growth.[27–29] The mutant is defective in the synthesis of the phospholipid PtdCho.[27] On the other hand, *prd-1*, a mutant with an abnormal phospholipid fatty acid composition, still shows a circadian temperature-compensated period.[26,27] Lipid composition of membranes, therefore, may thus represent an indirect way to affect temperature-compensation, possibly via membrane permeability, intracellular ion concentrations and degradation pathways of the clock proteins.

Ultradian Rhythms

Ultradian rhythms[30] represent a heterogeneous class of oscillations varying in period lengths from approximately one minute to several hours. Some ultradian rhythms show temperature-compensation.[31] An important class of these oscillations are referred to as "epigenetic",[30,32] showing a period in the time domain of hours. They are assumed to involve feedback loops at the transcriptional and translational levels. Temperature-compensated oscillations with period lengths of approximately one or several minutes have also been observed.[31] Even for very small period lengths there may be a link between the genetic information for circadian rhythms on one side and ultradian rhythms on the other. For example, in the fruit fly *Drosophila melanogaster*, a temperature-compensated ultradian rhythm in the courtship song (period $\approx$50 seconds) was observed that is determined by the *per* gene which also defines the (temperature-compensated) circadian rhythm in this organism.[33]

Besides temperature-compensation, ultradian rhythms in yeasts have been claimed to show general homeostasis of the period[34] and time-keeping functions,[30] for example, in "gating" the cell division cycle.[31]

Neuronal Oscillators

Ambient temperature can have a considerable impact on the performance of neuronal circuits and their oscillatory behavior, especially in poikilothermic organisms. There is evidence that many neuronal oscillators are temperature-compensated, as for example the vestibulo-ocular reflex in fish,[35] flight neurons[36] and wing-beat frequencies[37] in insects, defecation rhythms of nematodes,[38] the output of interneurons that receive input from other sensor neurons,[39] the suprachiasmatic nucleus (SCN) neurons in the central circadian pacemaker in the brain of mammals,[40,41] or the melatonin production in the retina of the golden hamster. The time scale of periods in these temperature-compensated neuronal oscillations range from a few milliseconds up to the circadian time scale in SCN neurons, in the isolated eye of *Aplysia*,[42] or in isolated *Bulla* basal retinal neurons.[43]

Tosini and Menaker have recently found that the circadian rhythm of melatonin synthesis in retinal neurons of the golden hamster (*Mesocricetus auratus*) is temperature-compensated and that the *tau* mutation affects temperature-compensation and period length.[44]

Analogous to *frq*[7,18] the period length in tau mutants decreases with increasing temperature.

THEORETICAL APPROACHES TO UNDERSTAND TEMPERATURE-COMPENSATION

Concept of Opposing Reactions

One of the first theoretical approaches to explain temperature-compensation in circadian rhythms was proposed by Hastings and Sweeney.[45] They assumed two reactions R1 and R2 to act oppositely on the period of the oscillations. Reaction R1 was proposed to control the period of the rhythm, while reaction R2 produced an inhibitor of reaction R1. Variations in temperature that simultaneously influence R1 and R2 will lead to opposing actions and thus result in temperature-compensation.

An analogous principle was applied in the construction of a temperature-compensated mechanical pendulum and of electronic crystal oscillators. In the mechanical compensation pendulum a variety of approaches exist which differ in the type of arrangement to keep the bob of the pendulum at approximately the same length.[46] In the *Invar-pendulum* (Figure 3) the increase of the pendulum-rod is compensated by a sleeve that expands in opposite direction to the expansion of the rod.

In electronic crystal oscillators it has long been known that the resonance frequency can be shifted by applying a voltage in series with the oscillating quartz crystal. A thermistor-induced voltage applied at the condensator counteracts the frequency shift of the crystal-oscillations induced by temperature, thus leading to temperature-compensation. By this method frequency stabilization in the range of ± 10 to ± 0.5 ppm in the temperature range between $-40°C$ and $+90°C$ was achieved.[47,48]

Opposing reactions are apparently also the basis of temperature-compensation in certain neuronal oscillations. While the Hastings and Sweeney approach puts the control mechanism outside of the oscillator, there is evidence that for circadian rhythms and for certain ultradian rhythms the molecular mechanism of temperature-compensation ("the opposing mechanism") may be an integral part of the oscillator.

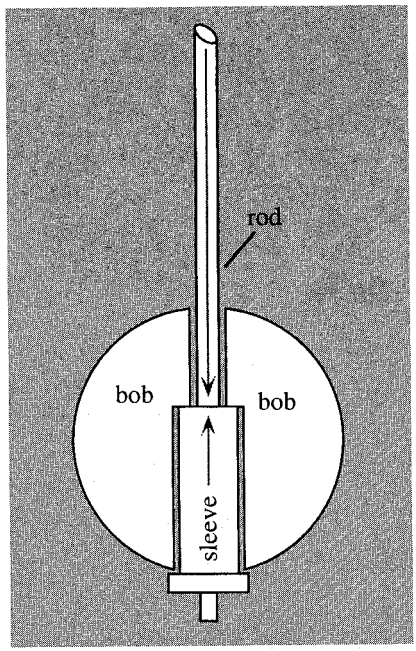

FIGURE 3 The Invar compensation pendulum. The rod increases with increasing temperature, but the center of gravity of the bob, which defines the period–length of the pendulum, is compensated by the expansion of the sleeve in the opposite direction of the expanding rod.[46]

Rate Constant Ratios and Diffusion Controlled Reactions

In another approach, Pavlidis and Kauzman[49] proposed a biochemical oscillator model for circadian rhythms. This model contained the activation and inhibition of an enzyme species. In order to provide temperature-compensation, certain assumptions had to be met: (i) rate constant ratios have to be assumed to be temperature independent; (ii) the product between a rate constant k_i and the (steady state) concentration of an enzyme species e_l, $k_i e_l$, has to be temperature independent; (iii) "single" rate constants have to be assumed to be associated with diffusion-controlled reactions and thus practically independent of temperature.

In fact, there is now evidence that certain enzyme-catalyzed reactions in poikilothermic organisms show an "instantaneous"

temperature-compensation.[50–52] In terms of the simple system

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

it was found that at nonsaturating substrate concentrations S, $K_M = (k_{-1}+k_2)/k_1$ values were increasing with temperature and compensating for the corresponding increase in $V_{max} = k_2[E]_0$, where $[E]_0$ is the total concentration of the enzyme. The origin of this behavior lies apparently in conformational changes of the enzyme.

Thermal Adaptation

A related phenomenon is the adaptation of organisms to different thermal environments. One of the key events in thermal adaptation is apparently conformational flexibility.[51–53] Holland *et al.*[54] have studied orthologous homologs of lactate dehydrogenase-A of six barracuda species from different thermal environments and have shown that different kinetic and thermic stability differences are due to amino acid substitutions at one or a few places outside the active site. The apparent K_M values (at 25°C) have been found to be highest for the cold-adapted species, intermediate values for the subtropical species and lowest K_m values for the tropical species. As a consequence of these differences, K_M is highly conserved among these species at their midrange body temperatures. Thus, evolutionary adaptation of proteins to temperature may be achieved by minor changes in sequence at locations even far from the active site, and these changes may independently affect kinetic properties and thermal stabilities.[54]

Acquired Thermotolerance

The term "acquired thermotolerance" is applied to an ubiquitous adaptive mechanism which protects organisms and cells against higher (heat shock) temperatures.[55] Exposure to heat shock, i.e., usually 8–10°C above the normal range of temperatures, leads to a transient inhibition of many cellular processes, such as gene expression or cell cycle progression, but leads also to increased synthesis of heat shock proteins (HSPs).[56] HSPs together with their constitutive isoforms, the "molecular chaperones", act to stabilize protein conformation, transport, synthesis and degradation and thus counteract the effects of elevated temperature.[57] A few hours after

the temperature shift cells and organisms adapt to continuous moderately elevated temperatures, at least partially due to the increased amounts of HSPs.[58] This temperature adaptation is also observed after a first "priming" heat shock and subsequent recovery as assayed by means of a second heat shock applied some hours after the first one. The response to the second heat treatment is considerably smaller: lethal effects are decreased,[55] protein synthesis inhibition reduced. In *Neurospora crassa*, for example, an inhibitory effect on protein synthesis after a 42°C exposure is observed only after the first but not after the second heat shock.[59] Furthermore, the cell cycle arrest in mammalian cells induced by a priming shock is longer than after a second shock.[60] These diminished responses to a repeated temperature elevation led to the term "acquired thermotolerance". It generally characterizes the ability of cells to protect themselves against heat, but also against other types of stress (alcohols, heavy metals, virus infection, and numerous other factors)[56] which perturb protein conformation and translocation.

Membranes and Temperature-Compensation

Some biological membranes were shown to adapt to different temperatures.[61] They maintain a relatively constant fluidity at different temperatues by varying the ratio between saturated and unsaturated lipids (homeoviscous adaptation). The possibility that temperature-compensation of circadian clocks may be related to this membrane property[62] was originally proposed by Sweeney[63] and Njus *et al.*[64]

Very little is known about the actual mechanisms how membrane composition may affect temperature-compensation of circadian oscillators. The levels of polyunsaturated fatty acids in membrane lipids of *Neurospora crassa* wild-type and in the slime mutant (a mutant defective in cell wall formation) increased dramatically as growth temperature decreased.[65] In the same organism mutants (for example *cel* and *chol-1*) which were defective in the membrane lipid synthesis were also defective in temperature-compensation. The *cel* strain needs fatty acid supplement and the *chol-1* strain choline for normal growth and for maintaining temperature-compensation, indicating that membrane-composition is strongly, but possibly indirectly related to temperature-compensation.[26–29] Experiments with *Gonyaulax polyhedra*, on the other hand, revealed considerable changes of membrane fluidity at different

temperatures, but stable temperature compensation of the period length.[66] It may be of interest to investigate whether a relation exists between defects in lipid metabolism and the dynamics of *frq*-mRNA or FRQ-protein turnover.

Amplitude Model

Lakin-Thomas *et al.*[67] proposed a topological model of temperature-compensation related to two-dimensional limit cycles. According to this theory the amplitude of the limit cycle is altered in accordance with temperature changes. As temperature increases or decreases, the speed by which the trajectory of the system travels through phase space will also vary. A constant period implies that the limit cycle's size (i.e., its amplitude) has to change accordingly, i.e., an increase in temperature is expected to lead to an increased amplitude, while a decrease in temperature has the opposite effect. This behavior has been observed for the temperature-compensated Brusselator[68] and the Kauffman-Wille model.[69] However, it is not clear whether higher-dimensional limit cycles will obey such a simple rule.

Introducing Temperature-Compensation in any Reaction-Kinetic Oscillator

Any physico-chemical oscillator model contains already the necessary elements for temperature-compensation. In general, the oscillator's period depends on the temperature through the rate constants k_i of each of the assumed N component processes Ri in the oscillator (Eq. 1), i.e.,

$$P = f(k_1, k_2, \ldots, k_i, \ldots, k_N) \tag{4}$$

Some of the rate constants will increase the period length, while others will decrease it. Franck[70] has used the concept of *antagonistic feedback* to explain the origin of chemical/biochemical oscillators. According to this theory both positive and negative elements act simultaneously on certain dynamical variables. Actually, there is an unlimited number of activation energies E_i^a for which the period will show little variation. In other words, temperature-compensation is expected to occur as long as period-increasing reactions balance the period-decreasing reactions within a certain temperature interval. We termed this condition "antagonistic

balance". Sets of activation energies that lead to antagonistic balance and to temperature-compensation are derived as follows.

Calculating $\partial P/\partial T$ from Eq. 4 and sorting for period-increasing and period-decreasing contributions leads to the antagonistic balance condition for temperature:[71,72]

$$\sum_i b_i E_i^a = \quad -\sum_j b_j E_j^a \tag{5}$$

(P-increasing) (P-decreasing)

In order to calculate the individual b-coefficients, the function f (Eq. 4) must be known. In most cases no analytical description of f exists, and numerical approaches must be sought. A convenient "Ansatz" is to describe f as

$$f \approx P_0 \prod_i k_i^{b_i} \tag{6}$$

where exponents b_i are calculated numerically. This approach has been tested for a variety of models, including the Brusselator,[68] Oregonator,[73] the Kauffman-Wille model,[69] and the Goodwin model.[74]

The Temperature-Compensated Goodwin Oscillator

More than 30 years ago Goodwin described an oscillator model for a transcription–translation sequence with negative feedback.[75] The 3-dimensional representation of this negative feedback loop needs an inhibition term with a large exponent (greater than 8) in the inhibitor concentration (Figure 4).[76] The X-variable represents the clock mRNA, Y is the clock protein, while Z is the inhibition factor causing the negative feedback due to the inhibition term $1/(1 + Z^9)$. This model is able to describe many dynamic responses of circadian oscillators including temperature-compensation, the effect of protein-synthesis inhibitors and phase response curves[77] of moderate temperature pulses and heat shock.[74,78] The 3-dimensional Goodwin-model has often been criticized because of the unrealistic high exponent in the inhibition term. However, it should be realized that this model describes only the very basic dynamics of a negative feedback oscillator and not all properties, as amplitude or limit-cycle behavior. However, despite the simplicity of this model, it is indeed astonishing how well some of the phase response data can be

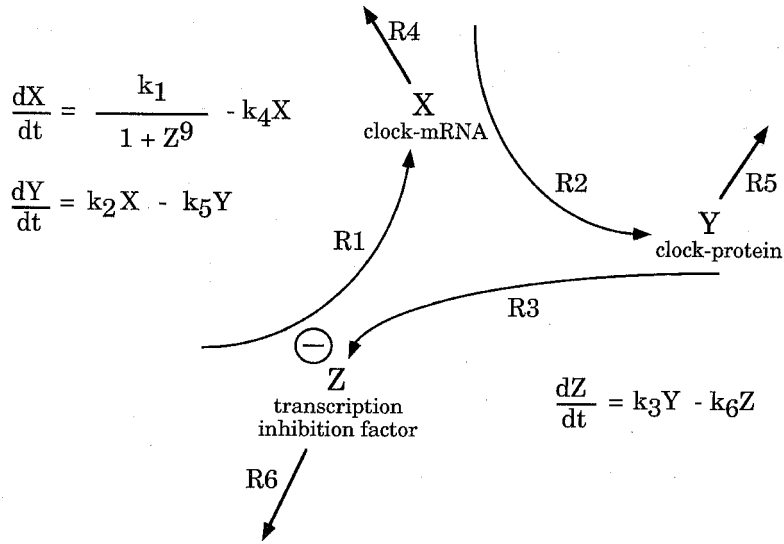

FIGURE 4 The Goodwin model. Reaction R1 is the formation of clock mRNA (X); reaction R2 is the synthesis of clock protein (Y) and R3 is the production of a transcription inhibitor (Z). R4, R5, and R6 represent degradation reactions.

simulated. Furthermore, predictions from this model appear to be worth further investigation in the laboratory as will be discussed below.

When calculating the b_i's (Eqs. 5, 6) it became clear that the period is only marginally dependent on the synthesis rate constants k_1, k_2, and k_3. On the other hand, the degradation rate constants appeared to have a major influence on the period.[72,74] This suggests to investigate whether the *stability* of the clock-mRNA or the clock-protein plays a major role in controlling, for example, the *Neurospora frq*-circadian oscillator. While the activation energies of the degradation rate constants are considerably lower than the synthesis rate constants in the temperature-compensated Goodwin oscillator[74] (which indicates that the turnover of *frq*-mRNA or FRQ-protein is relatively rapid) a point mutation (like in the frq^7-mutant) may lead to a *slower* degradation of either *frq*-mRNA or FRQ-protein (or both) accompanied with an *increase* in activation energy in one of these processes.[79] However, this increase in activation energy does not only explain the increased period length in the frq^7 mutant, it also shows that the period will *decrease* with increasing temperature, demonstrating

the intimate relationship between the factor (degradation) that determines period length *and* temperature-compensation. In terms of the Goodwin model, long period mutants are less temperature-compensated than short period mutants, because for short period mutants (like frq^1) an even lower activation energy will not substantially alter the contribution to the antagonistic balance/temperature-compensation because the activation energies of wild-type degradation rates were assumed to be already quite low.

The conditions for the existence and loss of temperature-compensation in *Drosophila* wild-type and mutants were analyzed in models based also on the negative feedback loops in *per* expression.[79–82] Leloup and Goldbeter[80,81] found that different parameters have opposing effects on the period. Temperature induced similar changes in the values of these parameters, leading to a cancellation in their effects. In the approach by Hong and Tyson,[82] temperature-compensation is brought about by PER dimerization. In order to achieve temperature-compensation the authors proposed that the nuclear import of PER should be a decreasing function of T. Ruoff *et al.*[79] used a similar idea and explained the temperature behavior of per^L and per^S mutants in terms of the 3-dimensional Goodwin model by introducing a temperature-dependent equilibrium between two forms (monomer-dimer?) of the inhibitory PER-protein. Concerning clock-mRNA stability, So and Rosbash have recently found evidence that *per*-mRNA stability differs during its synthesis and degradation and that *per*-mRNA stability is subject to post-transcriptional regulation.[83] Edery *et al.*[84] studied the influence of temperature pulses on the protein and mRNA products of *per* and *tim*. The results indicate that heat signals always induce a rapid decrease of PER and TIM proteins. Surprisingly, when heat pulses are applied at late subjective night an additional transient and rapid increase in the speed of the PER–TIM cycles in abundance and phosphorylation have been observed. This rapid increase compensates for the (rapid) decrease of PER and TIM levels leading to phase shifts that are practically unaltered compared to the unperturbed controls. It appears of interest to investigate whether kinetic models[79–82] are able to account for this temporary speed increase of the rhythm.

Although ultradian rhythms appear as widespread as circadian rhythms very little is known about their molecular mechanism and what may lead to temperature-compensation. Recently, Iwasaki et al.[38] described mutations in 12 genes that cause abnormal defecation cycle periodicities (*dec* phenotype) in *Caenorhabditis elegans*. In the presence of enough

food, the defecation motor program in the wild-type is activated every 45 sec with little variation. As in *Neurospora* and *Drosophila* the mutations caused short and long period mutants: short *dec* (*dec-s*) and long *dec* (*dec-l*). Again corresponding to *Neurospora* and *Drosophila* clock mutants, changes in period length in the *dec* mutants also affected temperature-compensation: the greatest loss in temperature compensation is observed in the long period mutants, especially *dec-1*.[38] The authors suggested that temperature-compensation in *Caenorhabditis elegans* defecation rhythm is an intrinsic feature of the clock mechanism rather than a separate circuit that regulates cycle periodicity. With increasing experimental knowledge models may become a useful tool to test various assumptions concerning regulatory steps that control period and temperature-compensation.

Neuronal Oscillators

In neuronal oscillators temperature-compensation is often achieved "externally" by the simultaneous action of temperature-dependent excitory and inhibitiory inputs to a neuron. These two antagonistic inputs cancel each such that temperature has practically no net effect on the neuron output. For example, in the grasshopper *Schistocerca americana*[39] the interneuron tritocerebral commissure giant (TCG) receives input from wind hair neurons. Both excitatory and inhibitory wind hairs are temperature-sensitive, but simultaneous stimulation by both excitatory and inhibitory wind hairs lead to a TCG output which is temperature-compensated.[39] Thus, temperature-induced changes in excitatory inputs to the TCG appear to be effectively compensated by simultaneous changes in the inhibitory inputs. A similar situation was observed in the flight system of the locust *Locusta migratoria*[37] which is capable of operating in the range between 24°C and 42°C. The wingbeat frequency changes minimally with the ambient temperature. For the same species evidence was presented that excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) exert opposite effects on the central flight rhythm. Equal temperature effects on both EPSPs and IPSPs thus automatically compensate each other, resulting in the low thermosensitivity of the wingbeat frequency.

Little understood is the situation in the circadian neuronal firing rate observed in brain tissues of higher organisms. For homeothermic

animals, body temperature is regulated within a narrow temperature range. Therefore, it is unclear whether circadian rhythms in these animals are temperature-compensated or just "protected" by homeostatic thermoregulation. *In vitro* preparations of ground squirrel neurons of the suprachiasmatic nucleus[41] (SCN) and the chick pineal[85] showed a temperature-compensated circadian rhythm of neuron firing rate. In squirrels, the observed temperature-compensation may be explained by interactions between warm- and cold sensitive neurons. The fact that cold-sensitive SCN neurons are found in greater numbers in hibernating squirrels than in normothermic animals may support this hypothesis.[41] Another possibility to achieve temperature-compensation in the rat SCN may arise by temperature-dependent synaptic inhibition of a temperature-sensitive postsynaptic neuron.[86]

Compound action potentials (CAPs) in eye preparations of *Aplysia* are consistent with the notion that pacemakers represent a multi-oscillatory system.[42] However, so far no indications have been reported that temperature-compensation is due to an antagonistic action of cold- and warm-sensitive neurons. Thompson *et al.*[87] studied temperature-compensated bursting pacemaker neurons in *Tritonia* and *Aplysia* and found that the slow outward tail current (which contributes to the pacemaker activity) is predominantly a potassium(K)-current at cold temperatures (10–15°C), but becomes partly K-insensitive at higher temperatures (20–23°C).

In *Bulla*,[43] the circadian rhythm in spontaneous optic nerve impulses show temperature-compensation with a Q_{10} of about 0.98 (12–25°C). The large circadian pulses recorded from the optic nerve are compound action potentials produced by the basal retinal neurons. In fact, basal retinal neurons continue to express circadian rhythms in membrane conductance either in cell-culture or isolated in a microwell dish.[43] As assumed for *Aplysia*,[42] the *Bulla* retina contains about 100 circadian pacemaker neurons that generate impulses in synchrony as compound action potentials. At present, it is unclear whether temperature-compensation is already an intrinsic property of the basal retinal neuron or whether temperature-compensation is due to the opposing effect of cold- and warm-sensitive neurons.

Recently Dowse *et al.*[88] came to the remarkable result that the heart rate in *Drosophila melanogaster*, which is dependent on temperature, becomes temperature-compensated in nap^{ts} (*no action potential temperature sensitive*) flies with a Q_{10} about 1.1 (14–25°C). The

heart rate of nap^{ts} is the same as that of the wild-type at 14°C, the coldest temperature at which any heartbeat is observed (1.24 Hz). In wild-type flies the increase of the heart rate at temperatures between 14°C and 25°C is nearly double that of nap^{ts}. Dowse *et al.*[78] speculated that the pacemaker mechanism in both mutant and wild-type flies may be temperature-compensated much like the circadian clock, but that the heart requires additional information about ambient temperature through some sensory module to regulate the frequency appropriately. It may be that this pathway is damaged in nap^{ts}.

Outlook

During evolution organisms and cells developed a number of adaptive mechanisms to cope with different environmental temperatures dependent on seasonal, daily or weather-dependent changes, or changes due to drifts within different latitudes or altitudes. On the one hand, these mechanisms include the development of homeothermic organisms which sustain their own autonomous temperature and avoidance behavior, such as bird migration. On the other hand, several mechanisms evolved which allow transient adaptation to fluctuating temperatures, some of which were discussed above.

In general, the latter mechanisms allow to keep cellular variables, such as certain enzyme activities, fluidity of membranes as well as gene expression and cell cycle processes rather constant over a limited range of temperatures. This is obviously of particular importance to frequency-coded neuronal signals and biological clocks signalling the time of day. Technical systems confronted with the same need for temperature-compensation contain similar adaptive devices.

One of the main mechanisms applied in the compensation of different temperatures – or any other perturbation – is a negative feedback arrangement which provides homeostasis of a controlled variable by sensing and counteracting changes of this variable. This is apparently realized in the adaptive process of heat shock protein synthesis during continuous heat treatment.[58] Another common mechanism consists of two opposing processes affecting a third – such as synthesis and degradation rates which determine the amount of a protein species or two metal rods which set the position of the pendulum weight. When both processes are affected by temperature changes in the same direction – positive or

negative – their effect on the third variable cancel each other (antagonistic balance). Alternatively, if the two processes are affected by temperature changes differently, i.e., one positive, one negative, the sum of both activities on the third process remains constant – as is the case in various neuronal temperature compensating systems.

As more details of the molecular events in biological oscillators are being identified, there will be an increased need for a more quantitative kinetic and mechanistic description by reaction kinetic models involving elementary and component processes. Such an approach should reflect the knowledge of the kinetics of the involved processes, but 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.[89] Such an approach was found very powerful in the elucidation of reaction mechanisms of chemical oscillatory reactions, probably the most complicated chemical processes that have yet been elucidated in detail.[8,90] Although biological oscillators are of still greater complexity, 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 experimentally be verified.

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