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REVIEW

TEMPERATURE EFFECT ON ENTRAINMENT, PHASE SHIFTING, AND AMPLITUDE OF CIRCADIAN CLOCKS AND ITS MOLECULAR BASES

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ABSTRACT

Effects of temperature and temperature changes on circadian clocks in cyanobacteria, unicellular algae, and plants, as well as fungi, arthropods, and vertebrates are reviewed. Periodic temperature with periods around 24 h even in the low range of 1–2°C (strong Zeitgeber effect) can entrain all ectothermic (poikilothermic) organisms. This is also reflected by the phase shifts—recorded by phase response curves (PRCs)—that are elicited by step- or pulsewise changes in the temperature. The amount of phase shift (weak or strong type of PRC) depends on the amplitude of the temperature change and on its duration when applied as a pulse. Form and position of the PRC to temperature pulses are similar to those of the PRC to light pulses. A combined high/low temperature and light/dark cycle leads to a stabile phase and maximal amplitude of the circadian rhythm—when applied in phase (i.e., warm/light and cold/dark). When the two Zeitgeber cycles are phase-shifted against each other the phase of the circadian rhythm is determined by either Zeitgeber or by both, depending on the relative strength (amplitude) of both Zeitgeber signals and the sensitivity of the species/individual toward them. A

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phase jump of the circadian rhythm has been observed in several organisms at a certain phase relationship of the two Zeitgeber cycles.

Ectothermic organisms show inter- and intraspecies plus seasonal variations in the temperature limits for the expression of the clock, either of the basic molecular mechanism, and/or the dependent variables. A step-down from higher temperatures or a step-up from lower temperatures to moderate temperatures often results in initiation of oscillations from phase positions that are about 180° different. This may be explained by holding the clock at different phase positions (maximum or minimum of a clock component) or by significantly different levels of clock components at the higher or lower temperatures. Different permissive temperatures result in different circadian amplitudes, that usually show a species-specific optimum.

In endothermic (homeothermic) organisms periodic temperature changes of about 24 h often cause entrainment, although with considerable individual differences, only if they are of rather high amplitudes (weak Zeitgeber effects). The same applies to the phase-shifting effects of temperature pulses. Isolated bird pineals and rat suprachiasmatic nuclei tissues on the other hand, respond to medium high temperature pulses and reveal PRCs similar to that of light signals. Therefore, one may speculate that the self-selected circadian 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. The so-called heterothermic mammals (undergoing low body temperature states in a daily or seasonal pattern) may be more sensitive to temperature changes.

Effects of temperature elevation on the molecular clock mechanisms have been shown in *Neurospora* (induction of the frequency (FRQ) protein) and in *Drosophila* (degradation of the period (PER) and timeless (TIM) protein) and can explain observed phase shifts of rhythms in conidiation and locomotor activity, respectively.

Temperature changes probably act directly on all processes of the clock mechanism some being more sensitive than the others. Temperature changes affect membrane properties, ion homeostasis, calcium influx, and other signal cascades (cAMP, cGMP, and the protein kinases A and C) (indirect effects) and may thus influence, in particular, protein phosphorylation processes of the clock mechanism. The temperature effects resemble to some degree those induced by light or by light-transducing neurons and their transmitters. In ectothermic vertebrates temperature changes significantly affect the melatonin rhythm, which in turn exerts entraining (phase shifting) functions. (*Chronobiology International, 19(5), 807–864, 2002*)

Key Words: Circadian amplitude; Circadian clock; Circadian level; Input pathways; Molecular mechanism; Rhythm entrainment; Temperature effects

INTRODUCTION

Daily environmental and artificial temperature changes are characteristic Zeitgeber signals of the circadian clock of many organisms as observed and reviewed earlier.[1–11] While the other prominent Zeitgeber, light, is perceived by eyes or cellular photoreceptors and is transmitted by neural, hormonal, and intracellular signal pathways to the molecular clock, temperature changes can directly affect the clock mechanism by accelerating or slowing component processes,[12–14] as almost any process in a cell. However, there may be additional effects of temperature changes on the clock variables, for example, through second messengers or influences on the intracellular clock environment such as ion or metabolite concentrations,[15] which we designated, somewhat arbitrarily, as indirect effects (Fig. 1).

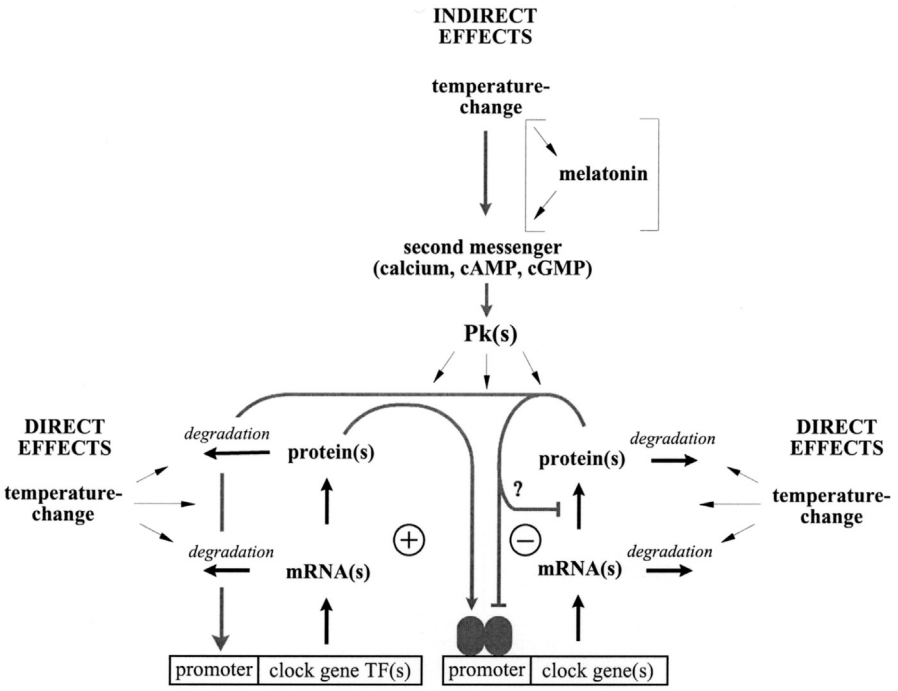

Figure 1. Indirect and direct effects of temperature changes on the circadian clock: the generalized clock mechanisms consist of clock gene transcription factors (TF), their expression, degradation, and positive effects on clock gene expression (left) as well as the clock protein(s), their expression, 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 directly, 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).

Earlier experiments focused on entrainment of the clock by periodic temperature changes, on the effects of temperature steps or pulses on the phase of the circadian oscillator [phase response curve (PRC) or phase transition curves (PTC)[16–18]], or the phase determination after release of the circadian oscillator from one temperature into another (release assay[19,20]). Time units for the determination circadian phases are "circadian time" (ct) units, i.e., the period length of the oscillation divided by 24 (ct 0 = beginning of subjective light time) or "Zeitgeber time" (zt), which designates the external time. Within the PRCs two points with zero phase shifts can be distinguished: crossover from advance to delay (zero phase shift) and crossover from delay to advance (also called "break point"). Some of the phase-shifting effects results can be explained, at least partly, by studying the temperature effects on the clock mechanism, for example of *Neurospora*[13] and *Drosophila*.[21]

In addition to the effects of temperature changes (phasic effects) and the duration of temperature treatments (tonic effects) on phase and frequency, the temperature limits for the expression of the clock and the temperature dependence of the amplitude and level of the circadian oscillation have been analyzed. The amplitude is defined as one half of the range between maximum and minimum values while the level designates the average value over the period(s). These effects of temperature changes were mainly studied in dependent variables (hands of the clock), but some were determined in the clock processes themselves. The phenomenon of "temperature compensation" of the period length[22] and the other reviews cited above as well as the review by Ruoff[23] and the phenomenon of "thermoperiodism"[24,25] will not be discussed here. This article reviews the data collected from different systematic groups of organisms with the aim of deriving common features of the circadian clock responses among them.

The molecular mechanisms of the well-known clocks (e.g., *Synechococcus*, *Neurospora*, *Drosophila*, mouse) consist of feedback systems[12,26,27] (Fig. 1): a strong negative feedback of clock proteins on their own gene expression by means of inhibitory actions on the relevant transcription factors (some of which also oscillate) as well as a positive feedback of the clock proteins on the synthesis of transcription factors. There is also evidence for additional interlocked feedback systems from output variables, thus generating a complex clock system.

In this review, we discuss the known and putative direct effects of temperature on the molecular clock processes as well as possible indirect pathways. The indirect pathways also include temperature effects on the hormonal level—particularly well analyzed in the case of the melatonin production in ectothermic vertebrates (Fig. 1).

CYANOBACTERIA, UNICELLULAR ALGAE, AND PLANTS

In this section, we review the effects of temperature changes and temperature on the circadian clocks of cyanobacteria, unicellular algae, and plants. Within

these heterogeneous groups a few species, such as *Synechococcus*, *Euglena*, *Gonyaulax*, *Kalanchoë*, *Phaseolus*, and *Arabidopsis* are particularly well analyzed for either their response to temperature changes and/or clock mechanism. The early literature on temperature effects on unicellular algae and plants has been discussed in general overviews (see Introduction), a particularly comprehensive one was published by Wilkins.[3]

Entrainment by Periodic Temperature Changes, Phase Shifts by Temperature Steps or Pulses, and Effects of Temperature on Expression, Amplitude, and Level

Entrainment

In the cyanobacterium *Synechococcus* the circadian rhythm of nitrogenase activity and protein synthesis is entrainable by a temperature cycle of 5°C difference.[28] In a situation in which the temperature and the light/dark cycles were 180° out of phase with respect to the normal phase relation, the light/dark cycle dominated as the entraining signal. This dominance may, however, depend on the relative strength of the light and temperature signals applied.

In algae and plants, entrainment of the circadian rhythm by periodic temperature changes was observed rather early, for example in *Phaseolus*,[29] *Oedogonium*,[30] and *Kalanchoë*.[31] The rhythms in *Oedogonium* were entrained by a 2.5°C difference, that of *Kalanchoë* by an even smaller cycle difference of 1°C. Temperature cycles different from 24 h, such as an 18h cycle, but not as short as 12 h or as long as 30h cycles, are able to entrain the rhythm of *Oedogonium*.[30] The circadian phototaxis rhythm of *Euglena* was entrained by a temperature cycle of 13°C difference. When this cycle was shifted with respect to a light/dark cycle, the phase of the circadian rhythm changed to an intermediate position between the two Zeitgeber cycles and "jumped" at a defined phase relationship of the Zeitgeber.[1] Temperature cycles of 7°C difference at high, medium, or low mean temperatures-induced synchrony of cell division and settling in autotrophic *Euglena* cultures, a phenomenon that persisted in constant light and temperature.[32,33]

In the higher plant *Bryophyllum fedtschenkoi*, the circadian rhythm of carbon dioxide metabolism was entrained by short 0.5 or 1h temperature pulses of 20°C difference (from 15 to 35°C).[34]

Entrainment of circadian gene expression by a temperature cycle was shown in meristematic tissues of *Sinapis alba* L. for Sagrp1 and Sagrp1 mRNA-encoding as yet unknown nuclear proteins,[35] in *Arabidopsis* for *Lhcb*[36] and *cat3* transcription (Michael and McClung unpublished citation from Ref. [37]) and in tomato seedlings of *Lysopersicon esculentum* for the amount of mRNA of the light harvesting complex proteins (*Lhc* mRNA).[38] It is likely that the circadian transcription of about 453 genes (6% of all genes) in *Arabidopsis*[39] are entrainable by temperature changes.

Phase Shifts

Step-type changes of temperature led to transient changes of the period and to permanent shifts of the phase in *Bryophyllum*[3] and *Phaseolus*.[4] Extensive temperature step-up experiments of 5–15°C difference with the circadian dark mobility rhythm of autotrophic cultures of *Euglena gracilis* showed strong phase shifts:[40] maximal (12 h) phase shifts resulted when the step occurred at minimal mobility, while no phase shifts occurred when the temperature step was applied at maximal mobility. This step-type temperature treatment thus led to synchronization of cell populations that initially showed differently phased circadian oscillations. The observed phase shifts were caused by transient changes in period length. Step-down experiments, on the other hand, caused strong phase shifts (of 12 h) only when applied at a small phase segment. In both types of experiments, increase or decrease in amplitude was observed after step-up and step-down temperature changes, respectively. Interestingly, the phase shifts after temperature steps were not observed in mixotrophic cultures.

Step-up from 14 to 20°C and step-down from 27 to 20°C experiments on *Gonyaulax* also led to transient changes of the period length (as an after effect) of the circadian rhythm of bioluminescence, a phenomenon that is well known also for light pulses and plotted as so-called tau response curves (TRCs). In addition a desensitization of the clock against the action of protein synthesis inhibitors was observed.[41] The latter effect of a stepwise temperature change was also observed at different higher constant temperatures, which attenuated the phase shifting of *Gonyaulax* rhythms by cycloheximide and anisomycin.[42] Correspondingly, a higher sensitivity against pulses of cycloheximide (100 and 10 μM) was observed at lower temperature in *Phaseolus*, resulting in arrhythmicity at 23°C, but not at 30°C. However, Mayer and Knoll[43] reported only minor differences between the PRCs to cycloheximide at these two temperatures.

Pulse-type changes of the temperature (up or down) and their phase-shifting effects have been analyzed in several algae and plant species (reviews: Refs. [8,17,44]). In *Gonyaulax polyedra* PRCs against positive (+7°C) and negative (−5 and −9°C) pulses of 4 h were shown to be of the weak type and were interestingly in phase with each other as opposed to 180° out of phase as is usually the case for positive and negative pulses.[45] This may be due to equally inhibitory or activating effects of elevated and reduced temperatures on the basic oscillator when the cells were kept before at an optimum temperature. Earlier experiments also showed that low temperature pulses of 8.5°C difference (from 20 to 11.5°C) for 3 h shifted the phase of the bioluminescence rhythm of *Gonyaulax* only by about 2 h, i.e., elicited only weak responses.[46] Zero phase shifts in both studies occurred around ct 0–4.

In *Bryophyllum* leaves, the circadian rhythm of carbon dioxide metabolism was phase-shifted by a 1 or 3h exposure to temperature pulses of +20°C, which resulted in PRCs of the weak and strong type, respectively. Zero phase shifts

occurred at ct 0 and a break point was observed at ct 12.[34] These PCRs were similar to the light PRCs of the same species.[47]

Phase shifting by temperature pulses were explored in *Kalanchoë* already by Schwemmle[48] in 1957 and more recently by Engelmann et al.[49] The experiments of the latter authors revealed temperature pulses of 40°C (+17.5°C difference) caused PRCs of the strong type when the pulses lasted 3 h, and of the weak type when they lasted 2 or 1 h. The PRCs of *Kalanchoë* to temperature pulses show zero phase shifts around ct 3 and a break point around ct 15–18, which is almost identical to the phase-shifting effects of light pulses. This approximate identity of the PRCs was also observed for the circadian rhythm of leave movements in *Phaseolus multiflorus*.[50] On the basis of their experiments and deductions from a model, Engelmann et al.[49] concluded that the temperature signals should act on the same signal input pathway to the clock and on the same clock component as light signals.

Two circadian rhythms (chilling resistance and cotyledon movement) of cotton seedlings (*Gossypium hirsutum* L.) were subjected to cold pulses of 5–19°C difference (from 33°C). Cold pulses during the subjective day time caused phase delays, while pulses given during the subjective night did not.[51] This was related to different chilling resistance (low at day time, higher at night) because prior exposure to low temperature during the night made the cotyledons more resistant to a subsequent cold pulse during the day.

Expression, Amplitude, and Level

The rhythm-initiating temperature pulses or steps as discussed by Wilkins[3] correspond to the so-called release assays (see *Neurospora*): the rhythms of different cells or organisms may be held stationary at a certain phase by a certain temperature and synchronously released from this phase after a shift to normal temperature. Different temperatures, alternatively, may not hold the clock but shift the oscillation to different levels. A subsequent step-up or step-down of temperature also leads to synchronous oscillations (see *Neurospora* below).

The expression of the glow rhythmicity of *Gonyaulax* disappeared at a critical temperature of 12°C. Subjecting synchronous cultures for different times to 11.5°C and subsequent release of these cultures to 21°C resulted in oscillations of bioluminescence that were phase-determined by the time of release, i.e., the first maximum occurred always after a constant time interval.[46] Resumption and phasing of rhythmicity after step-up of the temperature was the same as upon a transfer from bright light to darkness. It was shown that light and low temperature act additively in their effect to cause arrhythmicity.[46] This shows that *Gonyaulax* reacts to temperature changes in a different way as many other organisms in which darkness and low temperature usually act synergistically.

Different temperatures, furthermore, influenced the amplitude of the two bioluminescence rhythms of flashing and glow in different directions: the amplitude of flashing decreased while that of the glow rhythm increased when the temperature was shifted from 15 to 25°C.[52] It is not yet known whether or not this phenomenon is related to the existence of more than one clock (see below).

Initiation of rhythmicity by a temperature change is well analyzed also for the circadian rhythm of carbon dioxide fixation in the leaves of *B. fedtschenkoi*.[53] Beyond the range 10–30°C the rhythm is inhibited, however, it re-starts when the temperature is changed to 15°C. Longer exposure to this inhibitory range of temperatures drives the oscillator to defined phases, which differ by 180° as concluded from the phase at re-start.

Under continuous light the crassulacean acid metabolism (CAM) rhythm of *Kalanchoë daigremontiana* disappears at high (>29°C) and low (<8°C) temperatures. At both temperatures, phosphoenolpyruvate carboxylase (PEPC) activity is low, whereas at low temperatures high leaf malate concentrations were observed and vice versa. After small temperature increases or decreases from low or high temperatures, respectively, the phases of the reinitiated rhythms were 180° out of phase with each other.[54] Temperature change must occur at a fast rate in order to reinitiate the rhythm or synchronize a desynchronized population of oscillators.[55,56]

In tomato seedlings, the amplitudes of the *Lhc* mRNA and of the small subunit of RuBPC/Oase mRNA oscillations were strong at 24°C. At 10 and 30°C under constant light they were low or absent and were attenuated under a light/dark cycle.[57]

Effects of Temperature on the Molecular Mechanism of the Clock

Molecular Mechanism

The molecular mechanism of the circadian clock of cyanobacteria has been primarily explored in *Synechococcus*. Its core consists of a gene cluster, *kai*, composed of three genes *kaiA*, *kaiB*, and *kaiC*.[58,59] Both the *kaiA* and *kaiBC* operons are expressed rhythmically, and the translated proteins (KAI A, B, and C) interact: the KAI C protein serves as a negative feedback repressor of its own gene transcription, while KAI A acts positively on *kaiBC* transcription. This oscillatory system shows functional analogies to those known in *Neurospora*, *Drosophila*, and the mouse.[12] The involved genes, however, are not homologous, and the KAI proteins do not show DNA binding motifs. Phosphorylation changes of KAI C seem to play an important role—part of which is apparently controlled by a histidine kinase (SasA), which forms a complex and interacts with KAI C. Disruption of SasA drastically attenuated circadian expression pattern of all tested genes such that some became arrhythmic.[60] This kinase may also play a role in light transmission to the clock, but it is probably not essential for the entrainment by temperature pulses.[60] Since direct effects of temperature changes

on the expression of *kaiA*, *B*, *C* are as yet undetermined, it is unclear how temperature entrainment occurs.

Unicellular Algae

The molecular mechanisms of the circadian clocks of *Gonyaulax*, *Chlamydomonas*, *Acetabularia*, and *Euglena* have not yet been unraveled in detail.[61] However, in *Gonyaulax* one may derive some conclusions as to the mechanism from the PRCs to various inhibitors. The phase-shifting effects of translational inhibitors[62,63] and inhibitors of phosphorylation/dephosphorylation[64] suggest a role of protein synthesis and phosphorylation in the clock mechanism, which is analogous to the role of these processes in the mechanisms already known in other organisms.[12] One may thus speculate that temperature changes might affect the clock mechanism of *Gonyaulax* similarly, i.e., by increasing or decreasing the level of clock protein(s) or their phosphorylation. It is not yet known whether the temperature changes act on protein levels by influencing transcriptional, translational, or posttranslational processes of the clock mechanism. It is noteworthy, however, that the circadian expression of proteins involved in bioluminescence (one of the prominent hands of the clock) is strongly controlled at the translational level.[61]

In *Gonyaulax*, there is good evidence that a single cell contains more than one oscillator—as concluded from different period lengths of the circadian oscillations of flashing and glow and mobility.[52,65] It is unknown, however, where these different clocks may be located and how they respond to temperature changes.

Plants

Although the components of the basic oscillator(s) of plants are not precisely known, considerable progress in the genetic and molecular analysis has been made recently, particularly in *Arabidopsis* (reviews: Refs. [37,66]). It appears likely that the basic oscillator(s) also consists of feedback mechanisms on the transcriptional, translational, and posttranslational level. Two Myb transcription factors, circadian clock associated-1 (CCA-1)[67] and late elongated hypocotyl (LHY)[68] plus other members of this family have been identified as likely candidates for negative loops to their own expression. The CCA-1 DNA binding is affected by phosphorylation by casein kinase II[69], which may also phosphorylate LHY. Another gene that seems to be involved in the basic mechanism is the *timing of CAB* (*toc1*) gene, whose product TOC1 feeds back to control its own oscillation[70] and which represents a pseudo-response (APRR) regulator.[71,72] All these possible components of the clock may also function as input and/or output transmitter and may thus generate several interlocked oscillatory feedback systems.[37] There

is also considerable evidence in plants that support the existence of more than a single clock (review: Ref. [37]).

Input Pathways

The input pathways for the Zeitgeber signal light is complex but well analyzed (review: Ref. [37]) and seem to involve second messenger molecules (cGMP, Ca^{2+}) and phosphorylation changes.

Input pathways for temperature signals to the plant clocks are not yet clear. Temperature change probably acts directly on the components of the basic oscillator(s) by affecting structural properties of molecules and speed of processes. From the similarity of the PRCs of several plant species toward light and temperature change, it seems that both signals eventually influence the same components, such as the synthesis and degradation of clock proteins or their phosphorylation.[8,73]

The question of just how temperature changes are perceived by the plant cell was addressed by several studies, which are only briefly reviewed here. The transitions from the arrhythmic to the rhythmic state (and vice versa) after temperature steps were experimentally and theoretically analyzed mainly in CAM plants such as *Kalanchoë*. A particular role in the perception of temperature change was assigned to the rate of change: the reappearance of rhythmicity after a temperature decrease was observed only when the change was rapid.[56] Important roles for the disappearance or appearance of rhythmicity were assigned to the tonoplast, the passive efflux of malate and the malate concentration. Leaf malate concentration and osmolarity attain high and low values at low and high temperatures, respectively,[34,54] while the activity of the PEPC is inhibited by different mechanisms at both high and low temperatures.[54,74] Whether or not the holding and release of the carbon metabolism oscillation after temperature steps is due to an independent oscillatory feedback mechanism within this metabolic branch or to the control by a basic circadian clock or to both is not yet known. The perception of chilling, in particular, is attributed to membrane changes, especially to fluidity changes, putative alterations of protein protein kinase C activity, rearrangement of the cytoskeleton as well as to opening of calcium channels.[75–77] A sensor His-protein kinase may be involved in *Synechocystis*.[77] A His-protein kinase was shown to perceive osmolarity changes in yeast and to transduce this information to the mitogen-activated protein kinase (MAPK) cascade.[78] A temperature-dependent calcium influx is apparently involved in the activation of specific cold response genes,[79] which provide chilling tolerance to cells.

While these mechanisms may be involved in the transduction of signals from rather large temperature drops to the clock and in the establishment of an arrhythmic state, the question whether these signal pathways may also play a role in the transduction of rather small temperature signals that entrain or phase shift the circadian clock cannot be answered at present. Since some evidence implies

that light signal transduction involves calcium signals, and that experimentally induced calcium changes shift the clock (review: Ref. [37]), calcium may also play a role in the transduction of temperature signals.

A drastic rise in the temperature causes equally numerous changes in the plant cell (see Ref. [80] for the earlier literature). These changes include the fluidity of membranes, ion homeostasis (such as influx of protons and calcium), cytoskeleton breakdown, effects on protein kinases, and the induction of stress (heat shock) gene expression. In the latter case, a sensing of the temperature changes may be due to activation of the heat shock transcription factor (HSF1) (see for e.g., Ref. [81]). Again, these reactions may be involved in the determination of the oscillatory vs. the arrested state of the clock, but they are not likely to play a role in sensing 1–2°C temperature differences sufficient to entrain the circadian clock. It is not yet known whether these small changes are directly perceived by specific components of the clock and/or are transduced by particular sensors. The effects of different temperatures on the responsiveness to protein synthesis inhibitors as observed in *Gonyaulax* (see above) may be due to different amplitudes or levels of components of the basic oscillator, in a similar way as the lower sensitivity toward protein synthesis inhibitors in the *frq7* mutant of *Neurospora* as discussed subsequently.

FUNGI

In fungi, noncircadian periodicities of spore formation with shorter or longer period lengths have been observed in various species.[16,82] Their basic mechanisms are in some cases attributed to metabolic oscillator(s).[83] In *Pilobolus sphaerosporus* a circadian rhythm of spore formation was already observed in 1954 by Übelmesser.[84] This rhythm was entrained by a 24h temperature cycle of 5°C difference (although not by a 1°C difference) or by a 10°C positive pulse of 1h duration every 12 h. In constant conditions following the latter treatment, a sporulation rhythm of 12h period length was observed that eventually reverted to a circadian period.[84] However, with respect to the circadian oscillation, its properties and molecular mechanism *Neurospora crassa* became the most well-known fungal species (reviewed in Refs. [12,85–87]).

Entrainment by Periodic Temperature Changes, Phase Shifts by Temperature Steps or Pulses, and Effects of Temperature on Expression, Amplitude, and Level

Entrainment

Entrainment of the circadian rhythm of conidiation (vegetative spore formation) in *N. crassa* by periodic (12:12h) temperature changes was clearly shown by Francis and Sargent,[88] the maximum of conidiation occurring 8 h after

the temperature decrease, i.e., during the cold phase of the temperature cycle. These authors also showed that small temperature changes of 2°C were sufficient to entrain the oscillation, which we corroborated by a 2.5°C difference in DD as well as in LL. The control at 25°C showed a period length of 21.4 h while all temperature cycles with differences ≥ 2.5°C entrained the conidiation rhythm to 24.0 h (see also Fig. 2a). Temperature changes of 10°C proved to be stronger entraining signals than periodic light signals of 1500 lux when the Zeitgeber periodicities were 180° out of phase, i.e., when the cold phase of the temperature cycle occurred during the light phase of the light–dark cycle.[89] This may be explained on the basis of stronger responses of clock components to these temperature changes (see Ref. [13]).

Phase Shifts

Zeitgeber signals as per definition must be able to shift the phase of the oscillator (i.e., transiently shorten or lengthen the period) when given at different phases of the oscillation. In *N. crassa* the circadian rhythm of conidiation has been used to establish the phase-shifting effects of light and temperature change. Apart from determining the midpoint of conidiation, computerized density analysis from video recordings were used and led to principally identical results.[90]

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 oscillation. Step-up or down of 5°C treatments of *N. crassa* showed PRCs of the strong resetting type (type 0, see Ref. [16]). The temporal position of the step-up and the step-down PRCs differed 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).[88] 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 phase shifts. 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 processes that occur between the first and the second signal. As concluded from a mathematical model (the "Goodwin Oscillator"), however, a significant temporal relationship exists between the phase of the PRC and the perturbed clock variable.[73,91]

The pulse-up PRC (25.5–30.5°C for 6 h) of Francis and Sargent[88] shows zero phase shifts at around ct 4 and a break point at about ct 18–20 (Fig. 2c). These results are similar to those described for pulse-up experiments of 9°C difference, which show zero shifts at around ct 6 and a break point at around ct 21 which is also seen in the long period mutant *frq*^{7}.[92] The PRCs from temperature-sensitive mutants of *N. crassa* showed very similar PRCs when treated with the same pulse amplitude of 9°C.[93]

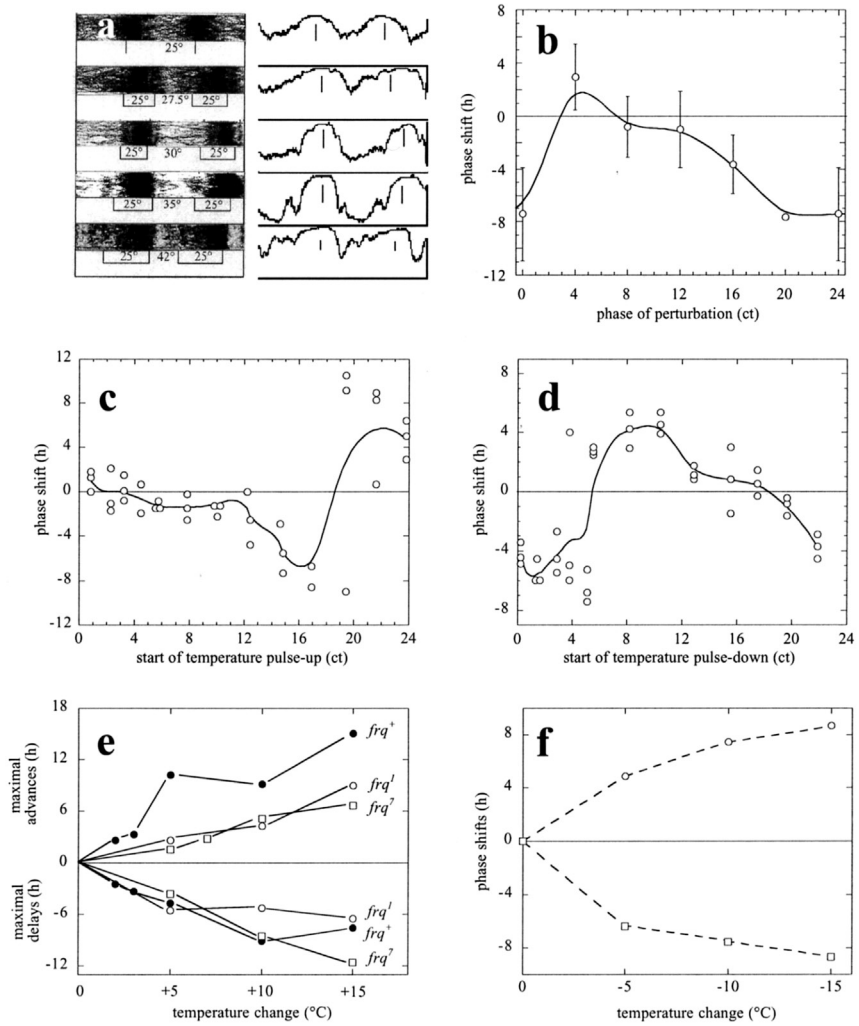

Figure 2. Entrainment and phase shifting by temperature changes in *N. crassa*. (a) Circadian rhythm of conidiation in constant darkness at 25°C (top row tau = 21.4 h) and 12 : 12h temperature cycles of 25/27.5, 25/30, 25/30, 25/42°C (second row to bottom row, tau always 24.0 h). Left: glass tubes with cultures (dark: conidiation), right: densitometric recordings of the tubes. Interval between vertical lines: 24 h. (b) PRC for 1h pulses of 45°C (+20° difference) in the *frq* $^{+}$ strain with indicated standard deviations (after Ref. [96]). (c) PRC for 6h pulses of 30.5°C (+5° difference) in the *frq* $^{+}$ strain (after Ref. [88]) slightly modified: advance shifts beyond 12 h (between ct 16 and 18) were plotted here as delays; the curve shows the result of smoothing over three values. (d) PCR for 6h pulses of 20.5°C (−5° difference) in the *frq* $^{+}$ strain (after Ref. [88]), smoothed curve (see c). (e) Maximal phase advances and delays after 3h pulses of increasingly higher temperatures in *frq* $^{+}$, *frq* ^{1}, and *frq* ^{7} strains. Abscissa: temperature differences (after Ref. [94]). (f) Phase advances after 6h pulses (starting at ct 8) and phase delays after 6h pulses (starting at ct 3) of increasing negative temperature changes. Abscissa: temperature differences (after Ref. [88]).

The pulse-down PRC of Francis and Sargent[88] (25.5–20.5°C, 6 h) is shifted in its position compared to the pulse-up PRC by about 10–12 ct units, i.e., about 180° (Fig. 2d). This PRC differs slightly from that of Nakashima[92] who used pulses of −11°C.

The different phasing for temperature pulse-up and pulse-down PRCs can also be observed with a different plot (new vs. old phase) and the derived "reset zone":[18] they are not exactly 180°C out of phase but show slightly asymmetric positions similar to the step-up vs. step-down PRCs. This observation may indicate a corresponding asymmetry between the positions of the maximum and minimum FRQ levels (see subsequently).

The amount of phase shift is a function of the magnitude and duration of the applied temperature pulse: pulse-up-induced maximal phase advances increase from 3 h (after pulses of 2 or 3°C, 3 h) to about 12 h (after pulses of 15°C, 3 h) (see Ref. [94], Fig. 2e), while maximal delays (or shifts at ct 11) increase from 2 to 6–8 h when the temperature increment is raised from 3 to 15°C.[88,94] The same almost linear relationship between temperature difference and extent of advance and delay phase shift was also observed in the frequency mutants *frq*^{1} and *frq*^{7}[94] as well as in pulse-down experiments with the wild type (see Ref. [88], Fig. 2f). The amount of phase shift also increased linearly with increased duration (2–8 h) of the temperature pulse until the maximum shift (12 h).[88]

Higher temperature (heat shock) pulses of 45°C, which strongly inhibit protein synthesis and induce the heat shock response[95] cause a PRC with different characteristics: zero phase shifts occur at about ct 8–12 and the break point at about ct 2–4 (see Fig. 2b, Ref. [96]). This PRC resembles the PRCs to cold pulses (see Fig. 2d, Ref. [88]) and cycloheximide, an inhibitor of protein synthesis.[97,98]

Initiation, Expression, Amplitude, and Level

In the so-called release assays the Zeitgeber signal (light or darkness, elevated or reduced temperatures) is applied for different intervals of time, for example, for 1–48 h, to organisms or cells. These organisms or cells usually enter the Zeitgeber treatment in synchrony, but are released from the treatment into constant conditions at different time intervals after entry. The phase of the oscillation is then determined and related to the phase of entry and the time of release.[19,20] This assay was frequently applied using light or temperature conditions that either do not allow oscillation, i.e., "hold" the clock in a defined phase, or shift the level significantly. The results of this assay are similar to step-up or step-down experiments (see earlier).

When *N. crassa* was exposed to high temperatures (40 and 45°C) for more than a few hours and then released into 25°C, the first band occurs about 10 h after the transition from high to medium temperature[19,20] (Fig. 3a, b). The warm-induced phase thus seems to be the phase located at ct 10–12, while low

temperatures (3, 4, and 15°C) for longer than 12 h causes an interval of 22.8 h between the transition from low to medium temperature and the first band, i.e., the cold-induced phase seems to be the phase expressed at around ct 20–22.[19,20,88] These different phases approximately correspond to the maximum (ct 10–12) and minimum (ct 20–24) of FRQ (see Ref. [13]).

Temperatures of 31 or 21°C before release to 25°C result in a different phasing of the subsequent conidiation rhythm with advances and delays corresponding to step-up or step-down experiments, while temperatures of 23 and 28°C before the release result in only small (type I) phase shifts[16] suggestive of singularity behavior.[20] A surprising observation is that heat shock temperature of 45°C, which should inhibit protein synthesis, causes the same 10h interval as 40°C.[19,20]

As in the case of a light to dark transition, a temperature shift from 38 to 25°C (or from 15 to 25°C) initiates the circadian rhythm of conidiation (Fig. 3a). As already noted in the release assays, the oscillation starts at a different phase, depending on the exposure to high or low temperatures (Ref. [19], see earlier). The limits of expression for the conidiation rhythmicity are about 34 and 15°C.[88] These limits may be explained by the responses of components of the molecular mechanism to different temperatures. The total amount of FRQ increases about 4-fold from 18 to 29°C. However, FRQ consists of two forms, a longer one translated from an upstream AUG codon of the *frq*-mRNA and a shorter one from a more downstream AUG.[99,100] By means of a translational control mechanism the longer form predominates at elevated temperatures and is required for the expression of rhythmicity at higher (27 and 30°C) temperatures, while the shorter FRQ is relatively dominant at lower (22–18°C) temperatures. For the action of both FRQ forms, Liu et al.[99] postulated different thresholds which increase with temperature and which must be reached in order to sustain a circadian oscillation.

The amplitude of the circadian rhythm of conidiation is maximal at about 25°C and decreases toward higher and lower temperatures. In strains where only the long form of FRQ is present the amplitude is diminished or absent at lower temperatures while in strains with only the short form the amplitude is lower or absent at higher temperatures.[99]

Different temperatures (21 and 28°C) also influence the level of the FRQ oscillation (more than two-fold greater at the higher temperature), but not of the *frq*-mRNA.[13] It is not clear whether the amplitudes of both rhythms also change with the temperature.

Effects of Temperature on the Molecular Mechanism of the Clock

Molecular Mechanism

On the basis of the presently available data the molecular mechanism of the *Neurospora* clock consists of the following components:[87] the frequency (*frq*) gene is expressed in a circadian rhythmic fashion with cycling amounts of *frq*-mRNA and FRQ-protein that reach their peaks at about ct 5 and 12, respectively. The

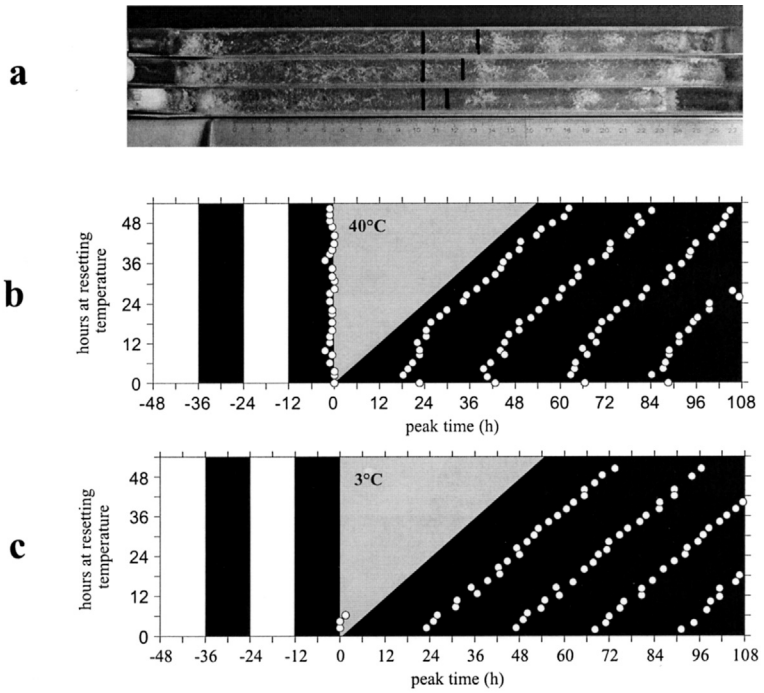

Figure 3. Effect of temperature steps on the initiation and phase of the circadian rhythm in *N. crassa*. (a) Tubes with the *frq* $^{+}$ strain were kept for 2 d at 38°C. First marking corresponds to 08:00h at day 3. Second markings (from bottom to top) correspond to the transition from 38 to 25°C at 15:00, 18:00, and 21:00h, respectively (P. Ruoff unpublished). (b, c) Release assay experiments. Nonshaded areas represent light exposure at 25°C, darkened areas represent dark exposure at 25°C, shaded areas represent dark exposure at the indicated temperatures. After inoculation (at − 48 h) into the race tubes, all *Neurospora* race tubes were synchronized by a 2d exposure to LD 12:12 at 25°C. At time 0, one set of race tubes (five race tubes per set) was maintained in the 25°C dark chamber as a control, and all other sets were put into a dark chamber of the resetting temperature. After 2 h of exposure to the resetting temperature, one set of race tubes was removed and placed into the control 25°C dark chamber; at hour 4 and every 2 h thereafter, another set was transferred. At the completion of the experiment, the tubes were analyzed for the times at which conidiation peaked ("Peak Time") (after Ref. [19]). (d) Model for the phase determination after step-up and step-down experiments based on the different levels of the FRQ-oscillation. Left: after a step-up all phases are interpreted as minimum of FRQ (at dawn), and the rhythm starts from this position (see Fig. 2b). Right: after a step-down all phases are interpreted as maximum of FRQ (at dusk) and the rhythm starts from this position (see Fig. 2a, c) (after Ref. [87]).

FRQ-protein acts as a dimer[101] and is a negative regulator of *frq* transcription, probably by binding to and attenuating the activity of the white collar (WC-1 and WC-2) protein complex, which stimulates *frq* transcription. The WC-1 and WC-2 are also essential for the light input pathway to the clock. The amount of WC-1 shows a circadian rhythm with a peak at around ct 0, although its mRNA level does not oscillate, suggesting posttranscriptional control. The overall levels of WC-1 and WC-2 are depressed in the absence of FRQ,[102] which shows that FRQ is essential for

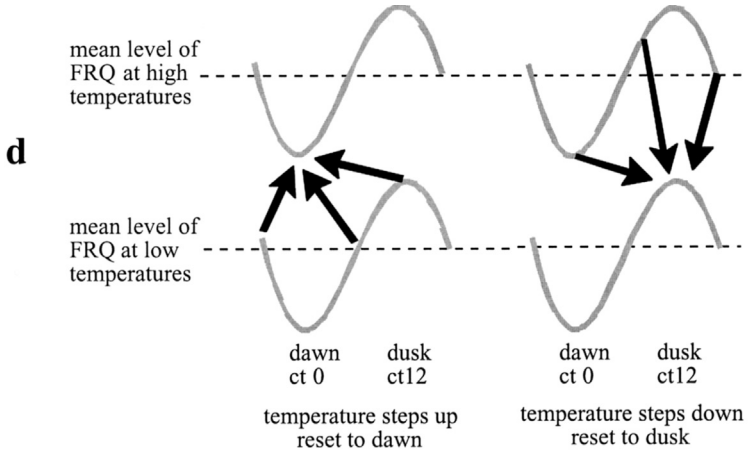

Figure 3. Continued.

two feedback systems: a negative one depressing the activity of its own gene and a positive one that stimulates WC-1 and WC-2 synthesis. The amplitude of the FRQ-oscillation is low at low levels of WCs and high at high levels of WCs.[102] Both the amount of FRQ and of WC-1 are also controlled at the level of translation and/or degradation. The FRQ is translocalized into the nucleus and interacts with the WC-1/WC-2 complex (FRQ–WCC). Eventually FRQ is destroyed by proteasome-catalyzed degradation. The FRQ hyperphosphorylation seems to play an important role in the latter process.[103] Part of this phosphorylation is due to a calcium/calmodulin dependent kinase (CAMK-1).[104]

The WC-1 and WC-2 proteins interact by means of PAS-domains, which is the case of many proteins involved in the transmission of light (and other signals). Upon light exposure WC-1 is phosphorylated, degraded, and replaced by newly synthesized forms.[105] Possibly, these processes may also be initiated by temperature changes, but this is not yet known. Another PAS-containing protein (VIVID) was described in *Neurospora* that represses the light input and dampens the phase-resetting response to light.[106]

Effects of temperature changes on *frq* mRNA and FRQ protein levels provide valid explanations for the phase-shifting responses as described earlier. When *Neurospora* is normally kept at 25°C and then transferred to other temperatures FRQ oscillates at 28°C at a higher and at 21°C at a lower level. All temperature step-ups (at any phase) caused shifts from a low to a high level of FRQ that are interpreted by the cell as a start from the minimum concentration of FRQ (i.e., from ct 0–4) to the maximum which is reached 8–12 h later. All step downs (at any phase) cause shifts from a high to a low level of FRQ that are interpreted as a start from the maximum concentration of FRQ (i.e., ct 8–12[100]). This means that every phase of the basic oscillator at 21°C is interpreted as ct 0–4 when the temperature is elevated (step-up) and every phase at 28°C as ct 8–12 when the temperature is lowered (step-down)[13] (Fig. 3d). It is interesting to note

that both, *frq*-mRNA and FRQ protein, increase after a step-up and decrease after a step-down, but that only the FRQ level remains higher during higher temperatures whereas the *frq*-mRNA level returns to the initial level. The permanently higher level of FRQ at 28°C is thus based on a posttranscriptional control mechanism, also involving the predominance of the longer isoform of FRQ,[99] whereas the mRNA level remains constant in the long run, which represents a case of temperature compensation of the level whose mechanism is not clear.

Exposure of mycelia to an even higher temperature (38°C) for 24 h, which is above the permissive range of rhythmicity, seems to keep FRQ at a high level and thus sets the clock to the state of maximum FRQ (i.e., ct 8–12 as concluded from the phase at which the clock starts when shifted to 21°C).

A corresponding time at 12°C, which is a temperature below the permissive range of rhythmicity, apparently sets the FRQ oscillation to its minimum by the same reasoning as above. These assumptions were verified by measuring the changes of FRQ after these shifts.[13] A conclusion drawn from these experiments is that the phase of the oscillation is not determined by the absolute amounts of the molecular components but by their mutual relationship. It is interesting to note that the levels of *frq* mRNA at high temperature were low and at low temperature high, i.e., that they changed inversely with FRQ levels.

Another result of earlier experiments may be explained by the recent molecular findings relating to (constant) higher temperatures diminishing the phase-shifting effects of light pulses.[107] This may be due to the higher level (or amplitude) of FRQ at a higher temperature,[13] which will reduce the relative induction of *frq*-mRNA and FRQ by light (see also Ref. [108]).

When comparing the effects of light with the effects of temperature some similarities appear. High light intensities enhance the expression of *frq*[109,110]—as does elevated temperature. The two Zeitgeber thus not only cause similar phase shifts but also cause similar effects on the molecular level.[13] When both Zeitgeber were applied to conidiating cultures in synchrony (light–dark, high–low temperature) they reinforce each other, when applied 180° out of phase (light–low, dark–high) the regimen of the temperature Zeitgeber dominated over the light regimen—consistent with the stronger (transcriptional and posttranscriptional) activation of *frq*-expression.[13]

FRQ-less Oscillators

Experimental studies and model calculations have shown that phase resetting, temperature- or light-entrainment, plus other dynamic behaviors of the circadian sporulation rhythm in *Neurospora* can be explained by the above described FRQ–WCC feedback loop. However, the existence of sporulation rhythms in *frq* null strains such as in *frq* ^{9}[111,112] as well as in *frq* null double mutants,[113] shows that endogenous sporulation rhythmicity can occur even in the absence of an operative FRQ protein.

This oscillator, often described as FRQ-less oscillator (FLO), has now come into focus by several studies as researchers try to understand the role of the FLO and its relation to the FRQ–WCC feedback loop.[87,113] Merrow et al.[114] found that the *frq* ^{9}-mutant can be entrained by temperature cycles, but not by light cycles, and that a functional FRQ-protein appears not necessary for temperature-entrainment.

Lakin-Thomas and Brody[113] who constructed the FRQ-less double mutants strains *chol-1 frq* ^{9}, *chol-1 frq* ^{10}, *chol-1 wc-1*, *chol-1 wc-2*, *cel frq* ^{9}, *cel frq* ^{10}, and *cel wc-2* found that these double mutant strains were robustly rhythmic when assayed under lipid-deficient conditions, indicating that free-running rhythmicity does not require the *frq*, *wc-1*, and *wc-2* gene products. However, all FLO-strains so far investigated appear to have poor or no temperature-compensation together with poor or no nutritional compensation.[112,113] This indicates that the FRQ/WCC is necessary to maintain these important circadian properties. Thus, the FRQ–WCC feedback loop appears to be important not only for light and temperature regulation but also for nutritional compensation of *Neurospora's* circadian clock.

Input Pathways

Various cellular second messenger levels (Ca^{2+}, cAMP, cGMP, and IP_3) in *N. crassa* are influenced by elevated temperatures.[115] Higher temperatures, furthermore, caused an increased release of cAMP through the plasma membrane.[116] A putative involvement of second messenger and protein phosphorylation in phase shifting of the clock may be deduced from phase shifts caused by pulses of calcium channel blockers, calmodulin inhibitors, and cAMP analogs.[117] The temperature-induced changes in the amount of second messenger molecules may change the activity of kinases involved in the phosphorylation of FRQ and thereby reset the clock mechanism. This possibility was suggested earlier by phase-shifting effects of the phosphatase inhibitors vanadate and molybdate.[98] Later it was experimentally shown that FRQ phosphorylation affects clock properties.[103] Other indirect temperature effects may exist, for example by changes of membrane fluidity, ion homeostasis, or metabolite levels but are not yet identified. Again, a particular problem is to explain the perception of small (2°C) temperature differences.

Heat shock temperatures such as 45°C inhibit the translational apparatus as well as transcription and processing of mRNA[80] and will thus inhibit these processes of the circadian clock mechanism.

ARTHROPODS: CRUSTACEANS

Crustaceans have been analyzed with respect to the entrainment of circadian as well as tidal rhythms of (mainly) locomotor activity. Temperature changes may act as Zeitgeber in both cases. For the tidal clock, however, temperature changes seem to be enhanced by other signals such as changes in salinity, mechanical

stimulation (turbidity) hydrostatic pressure, or wetting.[118–120] Little is known presently on the molecular bases of these two clocks.

Among the littoral crustaceans the circadian rhythm shown by the fiddler crab *Uca* is rephased by low temperature pulses[121,122] indicating a Zeitgeber function. Constant lower temperature (9°C) increased the amplitude of the ERG rhythmicity in *Procambarus clarkii*.[123]

Experiments with different littoral species showed that their tidal rhythmicity of about 12.4 h can be entrained by corresponding (for e.g., 6.2h:6.2h) temperature changes (*Carcinus*,[124,125] *Corophium volutator*,[119] *Penaeus indicus*, and *P. monodon*[120]). Application of a temperature difference of 10°C, in combination with other Zeitgeber, such as different salinities, enhanced the entraining effect.[120]

Phase-shifting effects of single temperature changes were also observed in *Synchelidium*,[126] *Carcinus*,[125,127] and *Corophium*.[128] In particular, chilling pulses of 5°C or sub zero temperatures were applied and led to phase-dependent phase shifts of the tidal rhythm of *Corophium*,[128] *Bathyporeia*,[129] and *Eurydice*.[130] In *Carcinus* and *Eurydice* the induced phase shift was proportional to the duration of the chilling period. The rhythm reentrained to the end of the cold pulse.

INSECTS

Two "hands" of the clock were mainly analyzed in insects: one is a developmental event such as the emergence of the imago from the pupal case (eclosion), the other is locomotor activity (mostly of the adult form).[4] When development progresses to eclosion, its realization is often circadian phase-dependent, a phenomenon called "gating." One may suspect that the circadian timing optimizes this developmental step with respect to the daily environmental changes. The disadvantage of measuring a developmental event, which occurs only once during the lifetime, is that the rhythmicity is apparent only in a population of insects. Therefore, a perturbing pulse that is applied in order to measure the subsequent phase shifts of the oscillation may act at different developmental stages of individuals and may also interfere with the developmental progress. Locomotor activity, in contrast, can be determined in an individual insect for several to many days.

With respect to the temperature effects on circadian rhythms *Drosophila* species have been the most researched insects (for the earlier literature see Ref. [131]).

Entrainment by Periodic Temperature Changes and Phase Shifts by Temperature Steps or Pulses

Entrainment

The circadian rhythm of the eclosion of adult *Drosophila* flies from the pupal case was first shown to be entrainable by temperature cycles of 10 or 8°C

differences[22,132] while a light–dark cycle is the main Zeitgeber. In contrast, the eclosion rhythm of two Japanese strains of *Chymomyza costata* (Drosophilidae) collected in Sapporo revealed a higher entrainability by temperature cycles of 4°C difference as compared to light/dark cycles. In these species, the majority of eclosions occurred at the beginning of the higher temperature (or light) phase, with a maximal amplitude at rather low mean temperatures (9–13°C). These features were interpreted as adaptation to higher latitudes where temperature changes might be more pronounced compared to light–dark differences.[133] In high altitude Himalayan strains of *D. ananassae* eclosion maxima always occurred during the thermophase of temperature cycles (21/13°C) whether they were imposed in LL, DD, or during the dark or light phase of LD.[134] In these strains, no rhythm was expressed at 13 and 17°C, in contrast to wildtype strains and the case described earlier.[133] There is, furthermore, a latitudinal cline in the variation of the pacemaker amplitude in *D. auraria* and *littoralis*,[135] which in addition may depend on the length of the photoperiod. The circadian amplitude therefore, was suggested to be a sensor of photoperiodic (and thermoperiodic?) changes. It is an interesting question on which molecular mechanisms these effects of different ambient temperatures are based on. There is evidence that the different period lengths and different temperature compensation of *Drosophila melanogaster* strains living at different latitudes are related to different lengths of the (Thr-Gly) sequence within the *per* gene (see Ref. [136], see also thermosensitive splicing events below). As in many other cases a direct effect of temperature (or light), i.e., masking effects, may also play a role.

Temperature cycles of 4°C difference were sufficient to entrain the eclosion rhythm of *Chironomus thummi*.[137] A 14°C difference was applied to entrain eclosion in a butterfly (*Iphiclides podalirius* L.)[138] or a single cold pulse of 18°C difference in the leafcutter bee, *Megachile rotundata* F.[139] Interestingly, the latter species is apparently not responsive to light/dark Zeitgeber signals.

The circadian locomotor activity rhythm of *D. melanogaster* was entrainable by temperature changes of only 2°C difference, at least in several (55%) of the individuals.[140] Entrainment was also observed in blind flies subjected to a temperature cycle of 3°C.[141] Periodic temperatures of 12:12h (5°C difference) entrained the activity rhythm of wild type *D. melanogaster* as well as the arrhythmic mutant per° both under constant light (LL) or constant darkness (DD). On the other hand, the long and short period mutants per^{L} and per^{S} were entrained to the temperature cycle only in LL but not in DD.[142] Small temperature changes of 2°C were sufficient to entrain the locomotor activity of 60% of the individuals of *Musca domestica*[140] while a light/dark Zeitgeber of 3 lux difference was more efficient.

Larger temperature differences were applied to entrain the activity rhythm of cockroaches[143,144] and crickets (*Teleogryllus commodus*).[145] In both species a temperature cycle entrained the activity rhythm after bilobectomy, which abolished the free running rhythmicity.[144,145] The presence of transient periods before entrainment as well as changing phase angles at the beginning of activity

with varying period lengths of the temperature cycle led to the conclusion that the temperature cycle does not induce activity directly (masking effect) but entrained a rhythm independent of the optic lobes. This rhythm, however, was not capable of maintaining a rhythmic activity under constant conditions.[144,145]

When light/dark and temperature cycles were applied simultaneously with the temperature minimum at the end of the dark time (i.e., the natural case) the eclosion peak of *Drosophila pseudoobscura* remained at its position at dawn. However, a successive shift of the temperature minimum to later times in the light–dark cycle successively shifted the phase of the eclosion peak up to about zt 15 (early dark time). Further shifts of the temperature cycle resulted in a phase jump of the oscillator to the dawn position.[22] A similar behavior was observed with the rhythms of cockroaches[22] and *Pectinophora gossypiella*.[146] Such "forbidden" zones[22] in the phase relations of the two Zeitgeber lead apparently to an unstable situation in the entrained oscillator, a situation which may also explain the behavior of the eclosion rhythm of *C. thummi* at different phase relations of the two Zeitgeber cycles.[137]

Different constant temperatures applied with a 12:12h light/dark cycle led to different patterns of activity during the light or dark time in *D. melanogaster*.[142]

A semilunar emergence rhythm of about 15 d in a population of the intertidal midge *Clunio marinus* was shown to be regulated by a combination of two endogenous rhythms: (i) a circatidal rhythm entrained by tidal (12.4 h) temperature cycles (about 4°C difference) and (ii) a circadian rhythm entrained by a 24h light/dark cycle.[147] The end of the warming interval seemed to be of decisive importance as a time cue.

Phase Shifts

Temperature changes have been applied in the form of step-up and step-down experiments, pulses and release assays. In *D. pseudoobscura* step-up (20–28°C) experiments led to different phase-dependent advance phase shifts while step-down experiments led to only small and almost phase-independent delay phase shifts of the circadian rhythm of eclosion.[132] The rather small phase shifts reported in the latter article resulted from initially large phase shifts that were successively reduced during subsequent cycles. This reversal was explained by assuming two oscillators (A and B), of which the B oscillator was assumed to be temperature sensitive, but resynchronized by the light-sensitive A oscillator.[22]

Winfree's[148] experiments included release assays in which the *D. pseudoobscura* cultures (kept at 20°C) were exposed for different time intervals to 29°C and then released. Eclosion occurred rhythmically about 12 h (+24 h) after the return to 20°C regardless of the duration of temperature exposure. The phase at the time of release would correspond approximately to the phase at ct 12, i.e., close to the minimal PER concentration (see later).

When applying temperature pulses of 10°C up or down for 12, 6, or 3 h, Chandrashekaran[149] found PRCs for the 12h pulses that were principally similar

to those of Zimmerman et al.,[132] however, with larger delays but not as large as those of Winfree.[148]

Altogether the PRCs with different exposure times to different temperatures seem to belong to the weak (type 1) with maximally 6h phase shifts. The PRCs differed in their position, when the beginning of the pulse was plotted at the ct times of its application. However, using the midpoint or end of the pulse as reference resulted in more consistent PRCs whose zero shift (crossover) for high temperature pulses occurred at around ct 10 (low level of PER see later). Zero phase shifts after low temperature pulses occurred later at ct 20–22, i.e., about 180° out of phase with the PRC to positive pulses. In addition, these PRCs also confirmed the dependency of the extent of phase shift on the duration of the pulse.

High temperature pulses in the range of 20–40°C for a short duration of 4 min led to a PRC of phase-dependent different delay shifts (no advances) with a maximum delay at about ct 12.[150] This difference in the position of the PRC compared to the other PRCs[132,149] may be due to the high temperature amplitude of the pulses that evoke heat shock response and strongly inhibit protein synthesis.

The phase-shifting effects of temperature pulses were also observed when the circadian oscillations of the amounts of mRNA from the *Drosophila* clock genes *period* (*dper*) and *timeless* (*dtim*) were measured.[21] A 30min exposure to 37°C (from 26°C) at zt 15 delayed both oscillations by about 4 h without any change of the amplitudes, whereas the same temperature pulse at zt 21.5 did not show effects on phase and amplitude of both oscillations. The CLOCK oscillation will be shifted together with the phase shifting of the activity rhythm and of *per* and *tim* mRNA. The oscillation of CLOCK, on the other hand, controls the oscillation of 134 genes as concluded from microarray analysis,[151] which should be shifted (and entrained) by temperature changes as well.

Pulses of low (2°C) temperature caused delay phase shifts in the singing activity of crickets, which is sometimes associated with a permanent change of the free-running period length.[152] 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.[153] 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° pulses as determined by a subsequent light PRC.[150]

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 clock.[154]

Effects of Temperature on the Molecular Mechanism of the Clock

The basic molecular mechanism of the *Drosophila* clock consists of at least one negative and one positive feedback system.[12,26] The negative feedback

comprises the genes *dper* and *dtim* and their cycling products *dper*-mRNA and dPER as well as *dtim*-mRNA and dTIM. The mRNAs peak during the early night (ct 14), the proteins at about ct 18. dPER and dTIM interact via a PAS domain and are transported into the nucleus. They interact negatively with transcription factors encoded by *dclock* (*dclk*) and *cycle* (*cyc*) which both contain basic helix–loop–helix (bHLH) DNA binding domains and PAS domains. *dclk*-mRNA and dCLK exhibit circadian concentration changes with peaks around dawn (ct 0) while *cyc*-mRNA and CYC do not cycle. dCLK/CYC heterodimers bind to E-boxes in the promoters of *dper* and *dtim* and thus activate their transcription. dPER and dTIM antagonize this activation by binding to CLK/CYC and thus form a negative feedback loop.

dCLK and CYC somehow repress *clk* transcription, which is antagonized by dPER and dTIM—two negative actions that amount to a positive loop. Another transcription factor VRILLE (VRI) is also apparently involved in these loops. dPER protein stability and degradation is controlled by a protein kinase (double time (DBT)—a homolog of casein kinase Iε (CKIε). Light treatment causes tyrosine phosphorylation and ubiquitination of dTIM, which is then degraded by proteasomes. The entrainment by light is mediated by cryptochrome (dCRY) that interacts with dTIM in a light-dependent manner[155] and correlates with dTIM degradation. Because dTIM stabilizes dPER, the latter protein is also increasingly degraded by the action of light. A *cryb* mutation renders the mutants blind, i.e., they are no longer entrained by light/dark cycle but only by a temperature cycle.[156]

Temperature pulses of 37°C and also pulses of 34 and 31°C, applied at zt 15 or 21.5, led to dose-dependent reductions in the amounts of PER and TIM proteins,[21] which suggests posttranscriptional effects of higher temperature on the synthesis and/or degradation of these proteins. In spite of these drastic effects, only heat treatment at zt 15 led to a phase delay of several hours of the PER and TIM oscillations. After high temperature treatment at zt 15 the amplitudes of both PER and TIM abundance were reduced about 2-fold. Heat treatment also retarded PER (and perhaps TIM) phosphorylation. Heat treatment at this circadian phase thus caused similar effects as light treatment.[157–160]

At zt 21.5 the induced decrease of PER and TIM is followed by a rapid increase in PER (and to a less extent in TIM) but did not cause lasting phase shifts of their oscillations. This is in contrast to the effects of light pulses at this time, which cause a 2h phase advance of the overt rhythm and premature disappearance of TIM and then PER.[157–160]

The mechanisms responsible for the heat-induced decrease of PER and TIM are not clear: inhibition of synthesis, increased degradation, or both. Also, it is not known, why a rapid recovery particularly in the amount of PER occurs after a heat pulse at zt 21.5, which may be the major reason for the lack of phase shift at this time. Heat shock proteins seem not to play a role in the phase shifting by temperature pulses, as shown by a mutant, which is defective in HSF activity.[161] Such an involvement of HSPs was hypothesized earlier on the basis of similarities

between heat shock-inducing and phase-shifting agents (see Ref. [94], also Ref. [162]).

A comparison between the position of the PRC toward temperature changes of 8[149] or 20°C[150] with the oscillatory levels of PER and TIM which peak around ct 18–21 reveals that maximal phase delays are observed when pulses were applied during the increasing limb of the oscillation. Zero phase shifts were observed at around ct 8–10 corresponding to the minimum of PER/TIM levels—the phase at which the clock is probably held when exposed to 28°C for a longer period of time.[148]

Apart from the temperature effects on the amount of PER/TIM a possible influence of temperature changes on dimerization (PER/PER or PER/TIM) and the translocation of the dimers into the nucleus were discussed,[163] an effect which may eventually be involved in temperature compensation.

Recently, it was shown that a thermosensitive splicing event in the 3′ untranslated region (UTR) of the *per*-mRNA plays an important role in the adaptation of *Drosophila* to low temperatures. Low temperatures cause an enhanced splicing of this intron and advances *per*-mRNA accumulation.[164]

It is not yet clear, how many master clocks (or submaster clocks) determine the behavioral and physiological circadian rhythms of *Drosophila*. Even though it is now clear that the main clock for the activity rhythm is located in the optic lobes,[165] and that the molecular mechanism of this clock is temperature sensitive, clocks also exist in other parts of the brain and in various tissues of *Drosophila*,[166] which may be responsible for the additional clocks postulated by Pittendrigh,[22] Page,[144] Rence and Loher,[145] and Mack and Engelmann.[167] A major circadian pacemaker seems to exist in the prothoracic glands (PG) of *Rhodnius prolixus* that is photosensitive and maintains a circadian rhythmicity of ecdysteroid synthesis.[168] The PGs receive rhythmic signals from the brain via a rhythmic release of prothoracicotropic hormone (PTTH)[169] and generate a circadian rhythm of ecdysteroids in the hemolymph,[170] which, in turn, leads to rhythmic ecdysis[171] and activation of ecdysteroid-dependent developmental genes.

VERTEBRATES

The function of temperature changes as Zeitgeber signals for the vertebrate circadian clocks have been analyzed in the past 40 yr mainly in reptiles and mammals, representing ectothermic (poikilothermic) and endothermic (homeothermic) animals. Hoffmann[6] summarized the 1969 available data and came to the conclusion that "temperature cycles are strong Zeitgebers for poikilothermic organisms, but only very weak ones for homoiotherms." He further stated that entrainment by temperature cycles depends on the Zeitgeber strength (i.e., amplitude and form), on the difference between the endogenous and Zeitgeber period length, and on species-specific and intraspecific (seasonal, age or development-specific) differences in sensitivity toward temperature changes. He

also emphasized that temperature cycles often affect circadian variables, such as locomotor activity, directly ("masking"[172,173]) and thus simulate entrainment. The above conclusions by Hoffmann[6] were corroborated and differentiated in the following decades. Many studies dealt with the hormonal (particularly melatonin) response as well as cellular and molecular reactions to the temperature changes plus the effects of the temperature-induced and experimental melatonin changes on the clock. In addition, the effects of different constant temperatures on the amplitude of circadian oscillations were explored.

FISH, AMPHIBIA, AND REPTILES: ECTOTHERMS (POIKILOTHERMS)

The reptiles have been the main subjects of research among the ectothermic group of vertebrates. Initially, the entraining effects of temperature cycles on the circadian rhythm of locomotor activity were analyzed. Subsequent studies assessed the influence of constant or cyclic temperatures on plasma and pineal melatonin rhythmicity as well as the entraining effects of periodic melatonin changes.

Entrainment by Periodic Temperature Changes, Phase Shifts by Temperature Steps or Pulses, and Effects of Temperature on Expression, Amplitude, and Level

Entrainment

In 1968, Hoffmann demonstrated that 24h temperature cycles of rather low amplitude entrained the locomotor activity rhythm of *Lacerta sicula*: Cyclic 24h temperature differences of 7.2, 3.6, and 1.6°C resulted in synchronization of almost all tested individuals, while a difference of 0.9°C still entrained 2 of 8 lizards. A so-called relative coordination, i.e., partial entrainment at certain phase relationships was observed in several individuals under temperature cycles of low amplitude.[174] Hoffmann also discussed and applied rigid criteria for entrainment by temperature changes in contrast to masking effects and demonstrated true temperature entrainment in these lizards.

Evans[175] exposed lizards (*Uta stansburiana*) to light and temperature cycles of different frequencies and found that they were entrained better by the temperature cycles. Recently, it was confirmed that the circadian locomotor activity rhythm of ruin lizards (*Podarcis sicula campestris*) were entrained by temperature cycles of 3 and 1.7°C difference. In addition, it was shown that a bimodal (splitting) pattern occurred under these conditions that persisted in subsequent constant conditions, although its expression depended on the respective season.[176]

Later research on temperature effects on the circadian clocks of ectotherms predominantly focused on the melatonin rhythmicity in retinae, pineal, parietal

eye, and plasma. With respect to entrainment, it is important to determine whether the melatonin-producing tissue possesses an autonomous clock and whether the temperature effects are due to masking. The different melatonin-producing organs show class and perhaps species-specific as well as seasonal differences in their clock autonomy. Some indirect evidence for the existence of an autonomous clock was reported for the isolated pineal of the lamprey (*Lampetra japonica*),[177] while no significant rhythmicity of melatonin secretion was observed in the pineal complex of the lamprey *Petromyzon marinus* in vitro.[178] It is, however, not easy to decide whether the in vitro conditions allowed or suppressed rhythmicity. A "circadian-like" secretion pattern was described in the cultured pineal organs of a teleost fish (*Catostomus commersoni*) in DD at 20°C, but not at 10°C,[179] and in *Esox lucius*,[180] whereas the cultured pineal of the rainbow trout did not show a rhythmic modulation of melatonin production.[181]

Divergent results on the existence of an autonomous clock were also obtained with cultured pineals of reptiles. An autonomous clock was documented in the lizard *Anolis carolinensis*[182] but not in the gecko (*Christinus marmoratus*).[183]

The observed in vitro effects of thermocycles on pineal organs lacking an autonomous clock thus must be mediated directly, whereas the effects on pineal organs possessing clock(s) may be partly due to entrainment and partly due to direct effects (masking). The same is true for in vivo experiments, where pacemaker located elsewhere may control the melatonin rhythmicity. In all cases it is difficult to apply the rigid criteria for true entrainment postulated by Hoffmann.[5,6]

In spite of these difficulties there is evidence that temperature cycles entrain the autonomous melatonin rhythmicity of isolated pineals of *C. commersoni*[184] and *E. lucius*[185] as well as the in vivo pineal melatonin rhythm of *A. carolinensis*.[186,187] This was evident even with temperature cycles of only 2°C difference in otherwise constant conditions (LL or DD). Entrainment by a temperature cycle was also shown for the blood plasma melatonin rhythm of the lizard *Tiliqua rugosa*, in this case with a high amplitude cycle of 18°C difference (33–15°C).[188]

Under a temperature cycle, the melatonin peak in most cases (except in *E. lucius*) occurs during the cold (cryo)phase, which corresponds to the dark phase under normal conditions. Since there appears to be no autonomous clock in the pineal explants of the gecko *C. marmoratus* the observed daily rhythm of melatonin production under a thermocycle of (30/15°C) must be a direct effect of the temperature change.[189]

Many experiments were conducted to elucidate the combined effects of temperature and light–dark cycles on the melatonin rhythm, either in phase or in various different phase relations of the two Zeitgeber cycles. Again, it is difficult to decide which of the observed effects were due to entrainment or due to a direct (masking) effect on melatonin production. In isolated eyecups of *Rana perezi*, the phase of the melatonin production rhythmicity appears to be determined only by

the light–dark cycle (maximum melatonin in the dark) while the amplitude is highest when the thermocycle (25/15°C) is in phase with the light–dark cycle and lower when the thermocycle is 180° out of phase.[190] A predominant determination of the phase by a thermocycle (32/20°C) (maximum melatonin in cryophase), on the other hand, was found in the pineals of *A. carolinensis*.[186] The influence of both cycles on the phase of the melatonin rhythm, however, depended on their respective amplitudes.[187] Different phase relationships between light/dark and temperature (33/16°C) cycles resulted in different phases and amplitudes of the blood plasma melatonin rhythm in *T. rugosa* with a maximum when the cryophase was aligned with the beginning of the dark phase.[188,191] There was also an effect of the duration of the thermophase on the amplitude of the melatonin rhythmicity.[192]

Also in the case of solely direct effects of light and temperature signals on the explanted pineals of *C. marmoratus*, highest amplitude of the induced melatonin rhythm is observed when dark and cryophase coincide; lowest amplitude (and a shifted phase) occur when the cycles are 180° out of phase.[189]

Expression, Amplitude, and Level

Different constant temperatures limit the expression of circadian rhythmicity and influence its amplitude within the permissive range of temperatures. In different lamprey species, the amplitude of the melatonin rhythm in cultured pineals under LD was low at 10°C[178] or absent,[177] but high at 20°C, which is similar to results with isolated pineals of a teleost (*C. commersoni*).[179] In cultured pineals of the trout kept in DD an increase in the temperature from 9 to 27°C resulted in an increase in melatonin production up to an optimum at 18°C and decrease at higher temperatures. The sensitivity to the suppression by light also changed with the different temperatures.[181]

The amplitude of the melatonin rhythm in the pineal, lateral eyes and plasma of the frog *R. perezi* under DD was greater at a high constant temperature of 25°C and abolished at low temperatures of 8°C.[193] The melatonin rhythm in isolated eyecups of *R. perezi* in LD displayed its highest amplitude at 15°C and lower amplitudes at 5 and 25°C.[190] Similar temperature dependencies of the amplitude of the melatonin rhythm were observed in *Necturus maculosus*[194] and in reptiles, such as *Nerodia rhombifera*.[195] In the lizard *Trachydosaurus rugosus* the melatonin rhythm in the pineal, the parietal eye, and blood plasma was abolished at constant 16°C but was present at 30°C.[196] Abolishment of the pineal melatonin rhythm in *A. carolinensis* was observed at 10°C.[187] Clear effects of the environmental temperature on the daily rhythm of melatonin production were also reported for the box turtle (*Terrapene carolina*).[197]

These observations suggest a species (and probably season and development) specific optimum temperature for the maximal amplitude of melatonin production and release. This may involve temperature dependencies of the amplitude (or level) of clock variables as for example in *Neurospora* (see earlier), but may also

represent direct temperature effects on synthesis or degradation of the melatonin-synthesizing enzymes, second messenger and other signal pathways as well as on the responsiveness to light (see later). The lower and upper limits of the expression of rhythmicity also appear to be species-specific but they also seem to depend on other factors.

Effect of Temperature on the Molecular Mechanism of the Clock

Molecular Mechanism

In reptiles, a multi-oscillatory system of autonomous clocks exists consisting of the tissues of the pineal, parietal eye, and retinae, which produce a rhythmic melatonin output, whose importance varies with species and season.[198–200] As concluded from suprachiasmatic nuclei (SCN) lesion experiments,[201] the SCN of the lizard (*Podarcis sicula*) apparently play a similar role as pacemaker as in mammals. They control the locomotor activity rhythm and mediate the entrainment of this rhythm by light. The molecular mechanisms of these autonomous (or dependent) clocks are not yet known but they are probably similar to those in mammals (see later).

Input Pathways

As in plants, fungi, and arthropods, different temperatures and temperature changes in ectothermic vertebrates may act directly on the various autonomous clocks in the tissues mentioned earlier, and probably also act on the as yet unknown peripheral clocks in other tissues. In addition to these direct effects a signal pathway for temperature changes to the clock apparently exists via melatonin changes, which are known to be affected by temperature and to be involved in the mediation of light signals. Daily melatonin injections were able to entrain the locomotor activity of *P. sicula* (in the summer) that was abolished by SCN ablation.[200] Similar results were obtained with daily melatonin infusions in pinealectomized and pineal-intact lizards (*Sceloporus occidentalis*).[202] Continuous administration of low doses of melatonin to the lizard *A. carolinensis* caused either arrhythmicity, shortening of the free running periodlength, or suppression of activity, indicating that melatonin can affect both the expression of the activity rhythm as well as properties of the circadian clock driving the activity rhythm.[203] Melatonin also affects the body temperature rhythm: Pinealectomized iguana (*Iguana iguana*) displayed an attenuated amplitude of the (self-selected) body temperature rhythm while daily intraperitoneal injections of melatonin a few minutes after the light to dark transition increased the amplitude of the body temperature rhythm.[204] Since the selected body temperature rhythm shows a rather high difference of about 10°C, about 38°C at day time and 28°C at night[204] and varies with season,[205] this rhythm represents an endogenous thermocycle that may itself serve as internal entraining mechanism for autonomous and

peripheral clocks and may thus feed back on the melatonin and activity rhythm (Fig. 4).

The melatonin production in the eye cups of *R. perezi* is directly affected by temperature changes. Temperature increments induced equivalent reductions of serotonin *N*-acetyltransferase (NAT) activity, which appears to be due to a greater increase in the rate of enzyme degradation compared to the rate of synthesis.[190,206] Other possible temperature effects on melatonin production may involve second messenger changes as were shown for *Neurospora* and mammalian cells. The effects of melatonin on the SCN and other targets, on the other hand, are mediated by melatonin receptors, G-proteins, and various kinases that may act on components of the clock (see mammals later). Apart from melatonin, other hormonal glands and their rhythmic output may be influenced by temperature changes and may be involved in the mediation of temperature signals, but are not yet analyzed.

BIRDS, MAMMALS: ENDOTHERMS (HOMEOTHERMS)

Temperature changes are generally less-important Zeitgeber signals for the circadian clocks of endothermic (homeothermic) than ectothermic (poikilothermic)

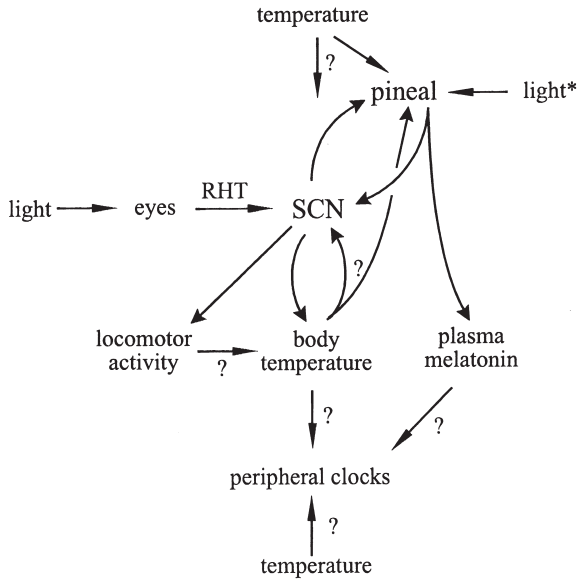

Figure 4. Schematic picture of temperature (and light) effects on the eyes, the pineal, and SCN of vertebrates. The body temperature rhythm may be controlled by temperature selection in lizards or autonomously by birds and mammals. Changes of body temperature by external temperatures, by self-selection, locomotor activity, or endogenous rhythmicity may entrain or shift the pacemaker and peripheral clocks. The same applies to the melatonin rhythmicity which, in turn, may also be entrained by periodic signals from the SCN, light (*except mammals), and body temperature.

animals.[1,6,173] This observation can also be deduced from a priori reasoning: a homeostatic regulation of the body temperature should render the organism less sensitive to environmental temperature changes. This was principally corroborated by a series of studies published during the last three decades. Among mammals, however, a group of so-called heterothermic animals appear to be more sensitive to temperature signals. They are adapted to seasonal low temperatures by lowering their body temperature correspondingly or show a daily topor (low-temperature phase), which in many species occurs during the day. Even in normothermic mammals, the effects of temperature cycles and of different constant temperatures on the phase, amplitude, and level of the circadian rhythm cannot be neglected. As in ectotherms, several studies on the effects of temperature changes in endothermic animals were conducted with cell or organ cultures of pineals or the SCN in order to test their responses. As yet no direct influences on the molecular mechanisms of the clock have been analyzed.

Entrainment by Periodic Temperature Changes, Phase Shifts by Temperature Pulses, and Effects of Temperature on Expression, Amplitude, and Level

Entrainment and Phase Shifts

Entrainment by periodic temperature changes has been studied primarily by measuring the locomotor activity mainly of mammals. As already discussed in the previous section, it is difficult to determine true entrainment because of strong positive or negative masking of locomotor activity. This problem can in principle be overcome by the criteria proposed by Hoffmann[6] and by establishing PRCs.

When reviewing the literature some 30 yr ago, Hoffmann[6,7] registered a lack of clear entrainment by periodic temperature changes in mammals and birds. Since then additional species have been studied with respect to the influence of periodic temperature changes on the circadian rhythm. The results of these studies revealed, apart from some exceptions, equally large individual differences as previously stated by Hoffmann.[7] Only limited data are available on temperature entrainment of birds (Table 1).

In normothermic mammals (Table 1) periodic temperature differences between 4 and 14°C entrained the rhythmicity in some species, but often not all individuals of the tested group. Other species did not respond. The activity of the responding individuals is confined to the warm or cold phase, which, however, does not always correspond to confinement of activity to the light- or dark-phase, respectively. The confinement probably depends on the respective temperatures during the warm and cold phase and the body temperature regulation. The activity is often heavily masked, either positively or negatively (see Refs. [6,173,207,208] and many other cases). However, the release of the activity rhythm into constant conditions as well as PRCs to temperature pulses, for example with rats[209] show a true Zeitgeber effect of the temperature changes. In marmosets, however, no PRCs

Table 1. Entrainment by 24h Temperature Cycles in Birds and Mammals (Normotherms, Heterotherms)

Species	*T*°C Difference	Entrained	c/w Active	References
Birds				
House sparrow (*Passer domesticus*)	37–41	+		[7]
	32–35	±		
	17–23	−		
House finch (*Carpodacus mexicanus*)	18			[214]
Mammals normotherms				
Rock pocket mouse (*Perognathus intermedius*)	9	−		[215]
Flying squirrel (*Glaucomys volans*)	10	−		[173]
Rat (*Rattus norvegicus*)	13	− (+)		[216]
Squirrel monkey (*Saimiri sciurius*)	8	−		[217]
	15–16	+ (−)	c (w)	[218]
Bat (*Phyllostomus discolor*)	10	−		[213]
Common marmoset (*Callithrix j. jacchus*)	10	+ (+)	w	[210]
Marsupial mouse—stripe-faced dunnart (*Sminthopsis macroura*)	14	− (+)	c	[207]
Antelope ground squirrel (*Ammospermophilus leucurus*)	6–12	+ (−)	c	[212]
	4	− (+)		
Common mole-rat (*Cryptomys hottentotus*)	6	+	w	[219]
Pig-tailed macaque (*Macaca nemistrina*)	15	+ (−)	w	[220]
Eutamias sibiricus	2	−		[6]
Mouse	15	− (+)		[7]
(*Apodemus sylvaticus*)	26			
(*A. flavicollis*)				
Microtus oeconomus	6.5	− (+)		[221]
Eutamias sibiricus	8			
Citellus undulates	12.5			
Palm Squirrel (*Funambulus pennanti*)	10–16	+ (−)	c (w)	[222]
Mammals heterotherms				
Little pocket mouse (*Perognathus longimembris*)	1.5–10	+	c	[211]
	1.1	−		
Bat (*Molossus ater*)	10	+ (−)	c (w)	[213]
Syrian hamster (*Mesocricetus auratus*)	6–12	+ (−)	w	[212]
	4	− (+)		

T°C—temperature difference applied, entrained—degree of entrainment: +—most, (+)—a few, (−)—a few not, −—most individuals not entrained, c—cold active, w—warm active.

were observed after temperature pulses even though other temperature effects were observed.[210]

Among mammals, a heterogeneous group of species, the so-called heterotherms show a variable body temperature regulation with daily (little

pocket mouse, see Ref. [211]) or seasonal (Syrian hamster, see Ref. [212]) lower body temperature. At least the little pocket mouse showed high entrainment sensitivity to temperature cycles and responded to differences of only 1.5°C. The PRC to 10°C pulses belonged to the strong (0) type.[211] Whether or not these responses allow a rigid distinction between hetero- and normothermic mammals cannot be answered presently on the basis of a limited number of species tested (see Refs. [212,213]).

Cultured pineal cells of chicks (*Gallus domesticus*) contain an autonomous clock as concluded from a continued rhythm of melatonin production in constant conditions,[223] its temperature compensation,[224] and response to light (see, e.g., Ref. [225]). This melatonin rhythm can be entrained by a temperature cycle of 18 h at 37°C and 6 h at 42°C as judged from the phase of the melatonin rhythm after release into constant conditions.[224] In addition, 10°C warm temperature pulses (6 h 42°C) in constant darkness shift the phase of the rhythm almost exactly as a 6h light pulse (type 0 PRC, Fig. 5a, see Ref. [224]). Similar PRCs were earlier described by Zatz et al.[226] The most important autonomous clock of mammals, the SCN, also showed continued circadian oscillations in neuronal activity when kept as slices in tissue culture.[227] The period of the rhythm is temperature compensated ($Q_{10} = 0.99$) and can be phase-shifted by 2h temperature pulses of 3°C difference, from 34 to 37°C.[228] The resulting PRC (Fig. 5b) is similar to the PRC of light signals for the locomotor activity rhythm.[229] There is also a linear relationship between temperature pulse duration and magnitude of the phase shift (Fig. 5c, seeRef. [228]).

Expression, Amplitude, and Level

The limits of expression of circadian rhythms under different temperatures as well as the initiation of expression by a temperature shift are not usually easy to test in endothermic animals, even though the circadian rhythm of locomotor activity is suppressed by high as well as by low temperatures. Heterothermic mammals with daily or seasonal topor, however, apparently possess a functioning clock in the SCN during this state, since the arousal from topor usually occurs at a certain circadian phase.[211,231] Disappearance of the rhythmicity at lower temperatures was observed in chick pineals cells in culture; the circadian melatonin rhythm was abolished at 31°C.[224]

The temperature dependence of the circadian amplitude or level in vivo was often determined in the locomotor activity. For the locomotor activity rhythm Aschoff and Wever[232] introduced parameters such as the duration of activity and rest time (α, ς) and their ratio as well as the total amount of activity. Both parameters may correlate with amplitude and level of locomotor activity and are thus no clear indicators for either parameter. In the light-active chaffinch *Fringilla coelebs*, e.g., an increase of the temperature from 10 to 25°C was associated with an increase in the amount of activity (15–20%), a

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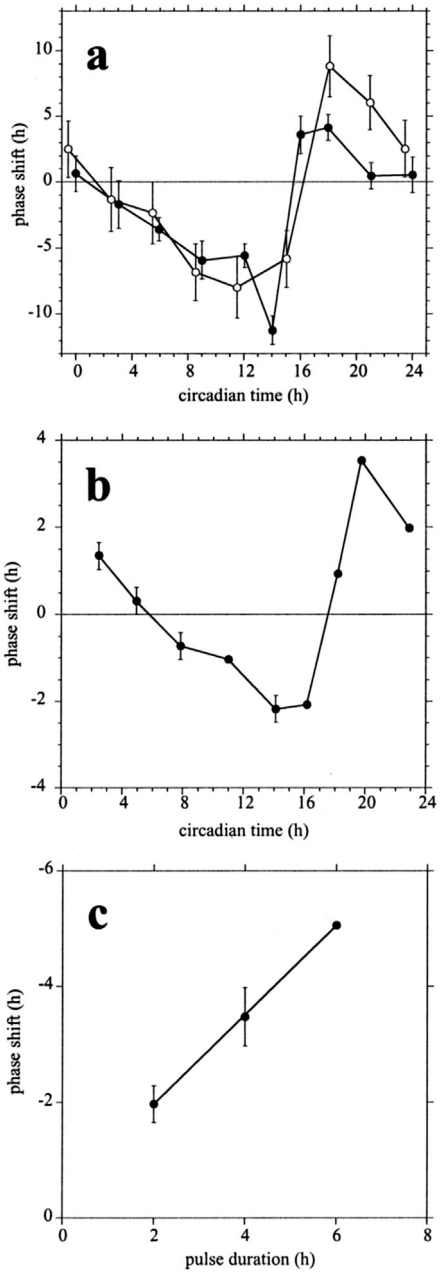

Figure 5. PRCs to positive temperature (and light) pulses in chick pineal cells and rat SCN in culture. (a) PRCs to 6h pulses of 42°C (+10°C difference), solid circles (after Ref. [224]), and to 6h light, open circles (after Ref. [230]) in chick pineal cells in culture. Points and bars represent the mean ± 95% confidence limits; plotted is the time where the pulse is initiated. (b) PRC to 2h pulses of 37°C (+3°C difference) in rat SCN in culture. Points and bars represent the mean ± SE of 3–6 trials, except at ct 16 and 18 ($n = 2$ each). Plotted is the time of the midpoint of the temperature pulse (after Ref. [228]). (c) Phase shift magnitude (max delay) increases linearly with pulse durations (abscissa): Solid line: first order regression ($r = 0.95$; $p < 0.0001$) (after Ref. [228]).

shortening of the period length by 0.3 h and individually different changes of α/s. However, in the dark-active Fat Dormouse (*Glis glis* L.) higher temperatures led to a decrease in the amount of activity by as much as 25%, and to an increase of the period length by 0.5 h per 10°C.[233] The changes of the period length correlate with phase alterations with respect to a light–dark cycle, the chaffinch increases the positive phase angle between beginning of activity and light-on with increasing temperature, while the Fat dormouse increases the negative phase angle.[208] In the common marmoset *Calithrix j. jacchus* the amount of activity was highest at 25° and was lower at 20°C (76%) and 30°C (69%) while the period length changed individually in different directions.[210] The amount of activity in the Colombian Owl Monkey *Aotus lemurinus griseimembra* was highest at 20°C and lower at 25 and 30°C.[234] In the latter case, activity was recorded in LD and turned out to be restricted to the dark phase. Since light suppressed the locomotor activity, the observed activity may well be due to changes in the level rather than the amplitude.

Pohl[208] reviewing the available data on the temperature dependence of the amount of activity in birds and mammals concluded that there is probably an optimum temperature as well as an optimum light intensity for activity, but that the temperature and light dependencies are not necessarily correlated. The temperature-optimum seems to depend on energy expenditure and body temperature regulation.

In chick pineal cells in culture, the highest amplitude of the rhythm of melatonin secretion in LD and DD was observed at 40°C and decreased with lower temperatures,[224,226] while 46.7°C stopped melatonin production within a few hours.[226] The period length of this rhythm was temperature compensated over the range of 34–40°C ($Q_{10} = 0.83$).[224]

Rat SCN slices in culture also showed a higher amplitude of circadian rhythm of the firing rate at 37°C compared to 31°C, while the period length was compensated ($Q_{10} = 0.99$, see Ref. [228]).

From the few systematic data relating to the temperature effects on the amplitude, one may conclude that temperature has a definite influence on this parameter. The maximum amplitude (and level), however, seems to vary in a species and possibly seasonal and development-specific way.

Effects of Temperature on the Molecular Mechanism of the Clock

Molecular Mechanism

In birds, the autonomous circadian system, i.e., the pacemaking clocks are localized in the SCN, in the pineal,[235] and in the eyes.[236] A mutual interaction (coupling) between these pacemaker sites as well as interactions with other hormonal glands may be a prerequisite for the maintenance of a stable circadian organization even under constant conditions (Fig. 4). This probably includes as well the synchronization of peripheral clocks in various tissues that exist in

mammals (see later). The significance of the pineal and eyes for this coupled system may vary in a species-specific way. The molecular mechanism of the autonomous and peripheral clocks are not yet known in detail but are probably similar to that unraveled in mice (see later), which is supported by an analysis of the chick pineal clock.[237]

The autonomous pacemaker sites in mammals are restricted, as presently understood, to the SCN and, at least in some species, to the eyes,[238] while the food-entrainable oscillator (FEO) is not yet localized.[239] The pineal is apparently not autonomously oscillating in its melatonin production and secretion but depends on rhythmic neuronal input from the SCN. The SCN may contain more than one master oscillator each consisting of a number of coupled clock-containing neurons.[240,241] When hamster SCN were sectioned in a vertical plane, the anterior and posterior parts showed two peaks of neuronal firing activity, one tied to the morning (light on) the other to the evening (light off) signals.[242] Also, the left and right portions of the SCN oscillators may oscillate 180° out of phase in desynchronized hamsters.[243] The often observed phenomenon of "splitting," i.e., the appearance of two tracks of locomotor activity for example under a thermocycle,[212] or under low light intensities, sometimes with different period lengths,[244] may thus be due to a desynchronization of clocks in the SCN.

The peripheral clocks in the various tissues of a mammal are entrained by the SCN, probably by means of melatonin and other hormones, by food intake,[245,246] serum components,[247] drugs affecting the actions of neurotransmitters,[248] and possibly by rhythmic changes of the body temperature (see later).

The molecular mechanism of the mammalian SCN clock shows several components that are homologous to the *Drosophila* clock but seem to be functionally and structurally different (reviews: see Refs. [26,249–252]). The clock genes include 3 *period* homologs (*mper* 1, 2, 3) a *clk* homolog (mclk) and a *cyc* homolog (*bmal1* or *mop3*). The *mper* homologs all cycle in their expression at the mRNA and protein level, the latter peaking near the end of the subjective day and beginning of subjective night (~ct 10–13). The positive elements of the clock mechanism are the transcription factors mCLK and BMAL1, interacting with each other by the PAS domain and forming a bHLH DNA binding motif. In rodents, *bmal1* mRNA and protein level cycle with a peak during the subjective night (ct 18) while *mclk* mRNA does not cycle.

All mPER proteins exert negative effects on mCLK/BMAL1-mediated gene activation, however, not as effective as PER/TIM in *Drosophila*. Perhaps, the negative feedback of mPER is supplied by two mammalian cryptochromes, mCRY1- and mCRY2, which strongly inhibit mCLK/BMAL1. Their messages as well as their proteins cycle in synchrony with those of the *per* genes. All three mPERs associate with each other and with the mCRYs. They all seem to bind to mCLK/BMAL1, suggesting the existence of higher order complexes that negatively regulate their own genes but may affect other processes as well. There also seems to exist a positive feedback between mPER2 (plus CRY1 and CRY2)

and the activation of *Bmal1* which oscillates 180° out of phase with *mper* expression, while BMAL1/CLOCK represses *Bmal1*.[253]

Input Pathways

Since light signals reset the (light sensitive) SCN clock in mammals by rapid induction of *mper1* transcription[254] and since light and positive temperature pulses shift the clock in isolated SCN slices almost identically (Fig. 5b), it appears likely that positive temperature pulses may exert the same inductive function on *mper1* as light. In rats, light also triggers the degradation of BMAL1.[255] These effects of light are transmitted by neurons of the retino-hypothalamic tract via glutamate as neurotransmitter and NMDA-receptors.[256]

Several intracellular signal pathways may be involved in the transmission of light signals from the NMDA-receptors to the clock mechanism (reviews: see Refs. [26,252]) which eventually results in the activation of genes such as those for the transcription factor AP-1 (*c-fos*, *c-jun*, and *jun-B*) and for the genes *mper1* and *mper2*. An early step in the transmission of the light signal seems to be the influx of calcium and the activation of nitric oxide synthase.[257] Intermediate steps involve phosphorylation of cAMP-response element binding protein (CREB) by protein kinase A, as well as the activation of Ca^{2+}-calmodulin-dependent kinase, and MAP kinase. It is not yet known whether phospho CREB directly induces transcription from the *per-1* promoter which contains multiple CRE. Protein kinase C, glucocorticoid hormones, and Ca^{2+} can all induce a transient increase of *mper1* suggesting several signal pathways involved in the induction. Another pathway for external signals may act on the stability or degradation of mPER1 which depends on its phosphorylation by a casein kinase (CKIε,[258]), the mammalian homolog of DOUBLE TIME in *Drosophila*. The mPER1 and mPER2, CLOCK and BMAL1 undergo robust circadian changes in phosphorylation.[259] Over-expression of CKIε leads to decreased mPER1 protein half-life and alters the nucleocytoplasmic localization of proteins. The CKIε binds to PER proteins and possibly also to higher order complexes with CRY1, CRY2, and BMAL1 which are also phosphorylated by this enzyme.[260] It is interesting to note that an important output of the SCN clock is apparently modulation of the calcium current and corresponding change of the membrane potential and spike frequency.[261] A circadian modulation of calcium levels in the SCN cells is also described[262] and may be subject to temperature changes.

External temperature changes may act on exposed tissues of birds and mammals such as skin and connective tissues, eyes, and brain,[210,228] more than on core tissues (Fig. 4). In the in vitro experiments with bird pineals or rat SCN slices (see earlier), the temperature changes act directly on the cells and on the molecular mechanism of the clock. In the latter cases a rise of temperature may also elicit effects on ion and second messenger levels, which are known from heat shock experiments with human epidermoid A431-cells[263,264] and rat C6 glioma cells[265], which are at least partly

similar to the effects of light. Similar also is the elevated expression of *c-fos* proto-oncogene after heat shock as observed in HeLa cells.[266] Beyond a certain cell specific temperature threshold, however, high temperatures may inhibit transcription and translation and lead to arrhythmicity as observed in bird pineals.[224]

It is not known, whether neuronal pathways from temperature receptors to pacemaker clocks exist that might transduce temperature signals. In view of the low sensitivity of birds and mammals to rather high external temperature changes, the existence of such pathways appears unlikely.

An interesting possibility of temperature entrainment was suggested by Tosini and Menaker[204] and Ruby et al.[228] that the internal temperature cycle in reptiles (by self-selected external temperatures) and in birds and mammals (by neuronal/hormonal and/or behavioral control) may represent an internal periodic entrainment signal that influences for example the melatonin rhythmicity and vice versa (Fig. 4). The differences between maximum and minimum of the body temperature varies in different species from rather low values (0.6–1°C in humans) to considerably higher values (3–4°C in *Callithrix*,[210] or 3.5°C in desert mice (*Acomys cahirinus*) during all seasons, and 6.5°C (*Acomys russatus*) during the hot season,[267] see also Ref. [268]). As most cells from plants, fungi, ectothermic reptiles, and heterothermic mammal were shown to perceive temperature differences of about 1.5–2°C, a body and brain temperature rhythm with a corresponding amplitude may be sufficient to drive at least the peripheral clocks or even the master clocks. Pineal enzymes involved in the synthesis of melatonin appear to be highly sensitive to temperature changes: The biosynthesis of melatonin in chick pineal cells shows a Q_{10} of $>$ II[224] which may be due to the activities of NAT as well as of the tryptophan hydroxylase (TPH, see Ref. [269]), while temperature pulses of 42°C (or higher) acutely inhibit melatonin release at every phase tested.[224,226]

The autonomous melatonin rhythmicity in eyes and pineals of birds and in the eyes of mammals, as well as the neuronally controlled melatonin rhythmicity in the pineals of mammals, may thus be influenced not only by light signals but in addition by exogenous and endogenous thermal signals. This type of internal synchronization would represent a multiple feedback system where periodic outputs feed back to the oscillator. Locomotor activity was shown to act as a nonphotic Zeitgeber of the clock,[270] which also might act by way of increasing the body temperature (Fig. 4).

The melatonin rhythmicity, on the other hand is able to entrain the locomotor activity rhythm of birds and mammals as shown by daily melatonin administration[271,272] and to enhance adaptation during and after transmeridian flights of humans (2–5 mg taken at the bedtime of destination) as revealed from many studies.[273] Melatonin mediates its effects on the SCN as well as on other tissues through G protein-coupled receptors MT(1), MT(2) and, possibly, MT(3).[274] In GnRH neurons melatonin led to inhibition of forskolin-stimulated cAMP accumulation through an inhibitory G protein. It induced PKC activity as well as the extracellular signal-regulated kinase 1 and 2 (ERK1, 2) pathway, and

thus seemed to suppress GnRH secretion by about 45%.[275] In neonatal rats, melatonin inhibited GnRH-stimulated increase in intracellular calcium in most gonadotropic cells but potentiated it in others.[276] These intracellular effects, if they apply also to the SCN, indicate that they are (mainly) antagonistic to the effects of light. This is corroborated by the fact, that melatonin inhibits GnRH-induced increase of cFOS in neonatal rat pituitary.[277] Since light and high melatonin act about 180° out of phase on the clock and when given as pulse elicit PRCs that are 180° out of phase[278] one may expect also antagonistic effects of elevated temperature and melatonin levels on the clock mechanism.

CONCLUSIONS

Entrainment

Most organisms, to some extent also homeothermic animals can be entrained by periodic temperature changes. In several groups of organisms, including plants, fungi, arthropods, 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 Zeitgeber strength, i.e., the amplitude of the temperature cycle, as was observed with periodic light entrainment. In ectothermic vertebrates, 24h temperature cycles can entrain the circadian clock(s) as demonstrated for the locomotor activity of lizards; they probably also entrain melatonin rhythms in vivo and in vitro, but it is not easy to distinguish in these cases between entrainment and masking. 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 dark. Entrainment by temperature changes occurs also in organisms in which the light pathway had been blocked. Because of the high sensitivity toward temperature changes of many organisms perhaps the circadian body temperature changes in homeothermic organisms (and in reptiles by self-selection) serve as internal Zeitgeber signals for peripheral (and pacemaker) clocks. Nonphotic Zeitgeber such as locomotor activity may, at least in part, act on the clock via an increase in body temperature (Fig. 4).

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 (review: see Ref. [279]).

When the temperature cycle is phase-shifted with respect to the light–dark cycle, either the temperature or the light cycle may dominate in determining the phase of the circadian clock, or an intermediate phase is chosen. The phase determination depends probably on the strength of the signals and the sensitivity of the organism to one or the other Zeitgeber. In several instances a “phase jump” of

the circadian clock has been observed at a certain phase relation of the two Zeitgeber cycles.

Initiation and Phase Shifts

Temperature steps usually (except mixotrophic *Euglena* cultures) lead to phase shifts or a new start (initiation) of the circadian clock. If the clock was held stationary or oscillated at a much higher or lower level during the initial temperature, a temperature step to more moderate temperatures may cause a start from a particular phase of the circadian oscillator. This phenomenon has been used for testing this phase by the so-called "release assay." The temperature limits up or down to which the circadian rhythmicity is expressed are defined quite precisely in some species tested—for example in *Neurospora*, where the limits of about 34 and 15°C are probably determined by the amounts of a longer FRQ-protein at higher temperatures and of a shorter FRQ protein at lower temperatures. A temperature shift causes a change in the level of the FRQ oscillation 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 oscillation starts at the minimum of FRQ, while a change from high to low level means that the oscillations starts at the maximum of FRQ (see Ref. [13]). The limits of expression vary according to species and may depend on the latitude of the species or strain distribution.

Stepwise changes of the temperature also lead to transient short-term or long-term changes of the circadian period length. These transient changes of the period length may be caused by a shorter or longer adaptation (compensation) time at the new temperature. These long-term effects of a temperature change are similar to those described for light pulses in mammals[280] 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.

Pulsed temperature changes lead to phase-dependent phase shifts [depicted as so-called PRCs or phase transition curves (PTCs)]. The PRCs to positive pulses are usually about 180° out of phase with PRCs to negative pulses. The extent of the phase shifts depends on the strength (amplitude) and duration of the temperature pulses. The PRCs to small (or short) temperature changes generally result in weak (type 1) PRCs,[16] while higher (and longer) temperature changes cause strong (type 0) PRCs. The position of the PRC to positive temperature-pulses is similar to the position of PRCs to light pulses: Both PRCs are characterized by zero phase shifts (crossover from advance to delay) at about ct 0–8 and crossover from delay to advance shifts ("break point") at about ct 12–20. This fact suggests that positive temperature changes and light signals act on the same process within the molecular clockwork—an assumption that is supported by the molecular analyses in *Neurospora* and *Drosophila* in which they act either positively or negatively on the amount of FRQ or PER/TIM, respectively. Particularly high positive

temperatures lead to heat shock responses which suppress protein synthesis and result in PRCs similar of those to protein synthesis inhibitors.

Amplitude Level and Phase

Different constant temperatures lead to permanent or transient changes of the amplitude and level of circadian rhythms of which mainly the hands were measured. In several instances maximal amplitudes and levels were observed at a species specific temperature. These temperature-induced changes of the oscillator may explain different responses to the application of light signals or protein synthesis inhibitors after temperature changes.

Different constant temperatures may also change the phasing of the circadian clock with respect to the light–dark cycle. This effect may cause organisms to become day-active at one temperature and night-active at another temperature. This effect of temperature may partly depend on an influence on the period length of the clock, in spite of the fact this influence is rather small because of temperature compensation. In addition, different temperatures may affect the light signal perception and thus the phasing of the oscillation.

Molecular Mechanisms

The direct effects of temperature changes on the clock mechanism (Fig. 1) may involve a general acceleration or slowing of enzyme-regulated processes involved in transcription, processing of gene products, translation as well as posttranslational modifications of proteins (e.g., phosphorylation/dephosphorylation). Particularly, degradation processes may be affected and may play an important role in the determination of clock properties.[281] Degradation processes have been shown to be especially sensitive to elevated temperatures in the *Drosophila* clock (PER/TIM), while an activation of transcription and a differential translation apparently are the primarily temperature sensitive processes in *Neurospora*.

More than one clock exists in a single cell in unicellular algae and plants, and multiple pacemaker tissues exist in multicellular animals. In multicellular animal organisms in addition, peripheral clocks exist in most tissues that can be entrained by various internal (and external) periodic signals.

Input Pathways

Temperature signals have a direct influence on clock processes such as transcription, translation, protein phosphorylation, and degradation. In addition, temperature changes affect membrane properties,[282] ion (particularly calcium) levels, and second messenger concentrations (cAMP and cGMP) (Fig. 1). Calcium

changes may function as transmitter of both light and temperature signals. Putative pathways for both signals may be activity alterations of PKC, PKA, and MAPK as well as the activation of transcription factors. In ectothermic vertebrates temperature-induced changes in melatonin levels apparently mediate temperature changes. Melatonin increments are transmitted into cells presumably by negative effects on calcium and cAMP levels.

Even less is known about the sensing mechanisms of small temperature changes of sometimes only 1–2°C, which are sufficient to entrain the circadian clock of many organisms. Possibly, specific components of the clock mechanism are functioning as sensors (for example specific kinases?). These temperature effects, however, must act only transiently in order to maintain the clock property of "temperature compensation."

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REFERENCES

1. Bruce, V.G. Environmental Entrainment of Circadian Rhythms. Cold Spring Harbor Symp. Quant. Biol. **1960**, *25*, 29–48.
2. Sweeney, B.M.; Hastings, J.W. Effects of Temperature upon Diurnal Rhythms. Cold Spring Harbor Symp. Quant. Biol. **1960**, *25*, 87–104.
3. Wilkins, M.B. The Influence of Temperature and Temperature Changes on Biological Clocks. In *Circadian Clocks*; Aschoff, J., Ed.; North Holland Publ. Co.: Amsterdam, 1965; 146–163.
4. Bünning, E. *The Physiological Clock*; Springer Verlag: Berlin, 1967.
5. Hoffmann, K. Temperaturcyclen als Zeitgeber der Circadianen Periodik. Verh. Dtsch. Zool. Ges. Innsbruck **1968**,.
6. Hoffmann, K. Die Relative Wirksamkeit von Zeitgebern. Oecologia **1969**, *3*, 184–206.
7. Hoffmann, K. Zur Synchronisation Biologischer Rhythmen. Verh. Dtsch. Zool. Ges. **1970**, 266–273.
8. Cornelius, G.; Rensing, L. Can Phase Response Curves of Various Treatments of Circadian Rhythms Be Explained by Effects on Protein Synthesis and Degradation? Biosystems **1982**, *15*, 35–42.
9. Balzer, I.; Hardeland, R. Influence of Temperature on Biological Rhythms. Int. J. Biometeorol. **1998**, *32*, 231–241.
10. Edmunds, L., Jr. *Cellular and Molecular Bases of Biological Clocks*; Springer Verlag: Berlin, 1988.
11. Hastings, J.W.; Rusak, B.; Boulos, Z. Circadian Rhythms: The Physiology of Biological Timing. In *Neural and Integrative Animal Physiology*; Prosser, L., Ed.; Wiley: New York, 1991; 435–546.

12. Dunlap, J.C. Molecular Bases for Circadian Clocks. Cell **1999**, *96*, 271–290.
13. Liu, Y.; Merrow, M.; Loros, J.J.; Dunlap, J.C. How Temperature Changes Reset a Circadian Oscillator. Science **1998**, *281*, 825–829.
14. Edery, J. Role of Posttranscriptional Regulation in Circadian Clocks: Lessons from *Drosophila*. Chronobiol. Int. **1999**, *16*, 377–414.
15. Rensing, L.; Kallies, A.; Gebauer, G.; Mohsenzadeh, S. The Effects of Temperature Change on the Circadian Clock of *Neurospora*. Circadian Clocks and Their Adjustment; Ciba Foundation Symposium, J. Wiley: Chichester, 1995; Vol. 183, 26–50.
16. Winfree, A. *The Geometry of Biological Time*; Springer Verlag: Berlin, 1980.
17. Johnson, C. Forty Years of PRCs—What Have We Learned? Chronobiol. Int. **1999**, *16*, 711–743.
18. Shaw, J.; Brody, S. Circadian Rhythms in *Neurospora*: A New Measurement, the Reset Zone. J. Biol. Rhythms **2000**, *15*, 1–16.
19. Gooch, V.D. Effects of Light and Temperature Steps on Circadian Rhythms of *Neurospora* and *Gonyaulax*. In *Temporal Order*; Rensing, L., Jaeger, N., Eds.; Springer Verlag: Berlin, 1985; 232–237.
20. Gooch, V.D.; Wehseler, R.A.; Gross, C.G. Temperature Effects on the Resetting of the Phase of the *Neurospora* Circadian Rhythm. J. Biol. Rhythms **1994**, *9*, 83–94.
21. Sidote, D.; Majercak, J.; Parikh, V.; Edery, I. Differential Effects of Light and Heat on the *Drosophila* Circadian Clock Proteins PER and TIM. Mol. Cell Biol. **1998**, *18*, 2004–2013.
22. Pittendrigh, C.S. Circadian Rhythms and the Circadian Organization of Living Systems. Cold Spring Harbor Symp. Quant. Biol. **1960**, *25*, 159–184.
23. Temperature Compensation of Circadian and Ultradian Rhythms. Spec. Issue Chronobiol. Int. **1997**, *14*, 445–536.
24. Schwemmle, B. Thermoperiodic Effects and Circadian Rhythms in Flowering of Plants. Cold Spring Harbor Symp. Quant. Biol. **1960**, *25*, 239–243.
25. Beck, S.D. Insect Thermoperiodism. Annu. Rev. Entomol. **1983**, *28*, 91–108.
26. Harmer, S.L.; Panda, S.; Kay, S.A. Molecular Bases of Circadian Rhythms. Annu. Rev. Cell Dev. Biol. **2001**, *17*, 215–253.
27. Rensing, L.; Meyer-Grahle, U.; Ruoff, P. Biological Timing and the Clock Metaphor: Oscillatory and Hourglass Mechanisms. Chronobiol. Int. **2001**, *18*, 329–369.
28. Lin, R.F.; Chou, H.M.; Huang, T.C. Priority of Light/Dark Entrainment over Temperature in Setting the Circadian Rhythms of the Prokaryote *Synechococcus* RF-1. Planta **1999**, *209*, 202–206.
29. Stern, K.; Bünning, E. Über die Tagesperiodischen Bewegungen der Primarblätter von *Phaseolus multiflorus*. I. Der Einfluß der Temperatur auf die Bewegungen. Ber. Dtsch. Biol. Ges. **1929**, *47*, 565–584.
30. Bühnemann, F. Das Endodiurnale System der Oedogoniumzelle. III. Über den Temperatureinfluß. Z. Naturforsch. **1955**, *206*, 305–310.
31. Oltmanns, O. Über den Einfluß der Temperatur auf die Endogene Tagesrhythmik und die Blühinduktion bei der Kurztagspflanze *Kalanchoë blossfeldiana*. Planta **1960**, *54*, 233–264.
32. Terry, O.W.; Edmuns, L.N., Jr. Phasing of Cell Division by Temperature Cycles in *Euglena* Cultured Autotrophically Under Continuous Illumination. Planta **1970**, *93*, 106–127.

33. Terry, O.W.; Edmunds, L.N., Jr. Rhythmic Settlings Induced by Temperature Cycles in Continuously-Stirred Autotrophic Cultures of *Euglena gracilis* (Z strain). Planta **1970**, *93*, 128–142.
34. Wilkins, M.B. The Circadian Rhythm of Carbon-dioxide Metabolism in *Bryophyllum*: The Mechanism of Phase Shift Induction by Thermal Stimuli. Planta **1983**, *157*, 471–480.
35. Heintzen, C.; Melzer, S.; Fischer, R.; Kappeler, S.; Apel, K.; Staiger, D. A Light- and Temperature-Entrained Circadian Clock Controls Expression of Transcripts Encoding Nuclear Proteins with Homology to RNA-Binding Proteins in Meristematic Tissue. Plant J. **1994**, *5*, 799–813.
36. Somers, D.E.; Webb, A.R.; Pearson, M.; Kay, S.A. The Short-Period Mutant, *toc1-1*, Alters Circadian Clock Regulation of Multiple Outputs Throughout Development in *Arabidopsis thaliana*. Development **1998**, *125*, 485–494.
37. McClung, C.R. Circadian Rhythms in Plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **2001**, *52*, 139–162.
38. Piechulla, B.; Riesselmann, S. Effect of Temperature Alterations on the Diurnal Expression Pattern of the Chlorophyll a/b Binding Proteins in Tomato Seedlings. Plant Physiol. **1990**, *94*, 1903–1906.
39. Harmer, S.L.; Hogenesch, J.B.; Straume, M.; Chang, H.S.; Han, B.; Zhu, T.; Wang, X.; Kreps, J.A.; Kay, S.A. Orchestrated Transcription of Key Pathways in *Arabidopsis* by the Circadian Clock. Science **2000**, *290*, 2110–2113.
40. Brinkmann, K. Temperatureinflüsse auf die Circadiane Rhythmik von *Euglena gracilis* bei Mixotrophie und Autotrophie. Planta **1966**, *70*, 344–389.
41. Balzer, I.; Possehl, C.; Rode, I.; Hardeland, R. Novel Temperature Effects on the Circadian Rhythmicity of *Gonyaulax*. In *Proc. 11th ISB-Congress*; Driscoll, P., Box, E.O., Eds.; SPB Academic Publishing: The Hague, 1989; 273–279.
42. Broda, H.; Johnson, C.H.; Taylor, W.R.; Hastings, J.W. Temperature Dependence of Phase Response Curves for Drug-Induced Phase Shifts. J. Biol. Rhythms **1989**, *4*, 327–333.
43. Mayer, W.E.; Knoll, U. Temperature Compensation of Cycloheximide-Sensitive Phases of the Circadian Clock in the *Phaseolus pulvinus*. Z. Pflanzenphysiol. **1981**, *103*, 413–425.
44. Johnson, C.H. PRC Atlas. http//johnsonlab.biology.vanderbilt.edu.prcatlasihtm 2000.
45. Scholübbers, H.G.; Taylor, W.; Rensing, L. Are Membrane Properties Essential for the Circadian Rhythm of *Gonyaulax*? Am. J. Physiol. **1984**, *247*, R250–R256.
46. Njus, D.; McMurray, L.; Hastings, J.W. Conditionality of Circadian Rhythmicity: Synergistic Action of Light and Temperature. J. Comp. Physiol. **1977**, *117*, 335–344.
47. Harris, P.J.; Wilkins, M.B. The Circadian Rhythm in *Bryophyllum* Leaves: Phase Control by Radiant Energy. Planta **1978**, *143*, 323–328.
48. Schwemmle, B. Zur Temperaturabhängigkeit der Blütenbildung und der Endogenen Tagesrhythmik bei *Kalanchoë blossfeldiana*. Naturwiss **1957**, *44*, 356.
49. Engelmann, W.; Eger, I.; Johnsson, A.; Karlsson, H.G. Effect of Temperature Pulses on the Petal Rhythm of *Kalanchoë*: An Experimental and Theoretical Study. Int. J. Chronobiol. **1974**, *2*, 347–358.
50. Moser, I. Phasenverschiebungen der Endogenen Tagesrhythmik bei *Phaseolus* Durch Temperatur- und Lichtintensitätsänderungen. Planta **1962**, *58*, 199–219.

51. Rikin, A. Temperature-Induced Phase Shifting of Circadian Rhythms in Cotton Seedlings as Related to Variations in Chilling Resistance. Planta **1991**, *185*, 407–414.
52. von der Heyde, F.; Wilkens, A.; Rensing, L. The Effects of Temperature on the Circadian Rhythms of Flashing and Glow in *Gonyaulax polyedra*: Are the Two Rhythms Controlled by Two Oscillators? J. Biol. Rhythms **1992**, *7*, 115–123.
53. Anderson, C.M.; Wilkins, M.B. Period and Phase Control by Temperature in the Circadian Rhythm of Carbon Dioxide Fixation in Illuminated Leaves of *Bryophyllum fedtschenkoi*. Planta **1989**, *177*, 456–469.
54. Grams, T.E.E.; Borland, A.M.; Roberts, A.; Griffiths, H.; Beck, F.; Lüttge, U. On the Mechanisms of Reinitiation of Endogenous Crassulacean Acid Metabolism Rhythm by Temperature Changes. Plant Physiol. **1997**, *113*, 1309–1317.
55. Grams, T.E.E.; Beck, F.; Lüttge, U. Generation of Rhythmic and Arrhythmic Behaviour of Crassulacean Acid Metabolism in *Kalanchoë daigremontiana* Under Continuous Light by Varying the Irradiance or Temperature: Measurements In Vivo and Model Simulations. Planta **1996**, *198*, 110–117.
56. Rascher, U.; Blasius, B.; Beck, F.; Lüttge, U. Temperature Profiles for the Expression of Endogenous Rhythmicity and Arrhythmicity of CO_2 Exchange in the CAM Plant *Kalanchoë daigremontiana* Can Be Shifted by Slow Temperature Changes. Planta **1998**, *207*, 76–82.
57. Riesselmann, S.; Piechulla, B. Effect of Dark Phases and Temperature on the Chlorophyll a/b Binding Protein mRNA Level Oscillations in Tomato Seedlings. Plant Mol. Biol. **1990**, *14*, 605–616.
58. Iwasaki, H.; Dunlap, J.C. Microbial Circadian Oscillatory Systems in *Neurospora* and *Synechococcus*: Models for Cellular Clocks. Curr. Opin. Microbiol. **2000**, *3*, 189–196.
59. Iwasaki, H.; Kondo, T. The Current State and Problems of Circadian Clock Studies in Cyanobacteria. Plant Cell Physiol. **2000**, *41*, 1013–1020.
60. Iwasaki, H.; Williams, S.B.; Kitayama, Y.; Ishiura, M.; Golden, S.S.; Kondo, T. A KaiC-Interacting Sensory Histidine Kinase, SasA, Necessary to Sustain Robust Circadian Oscillation in Cyanobacteria. Cell **2000**, *101*, 223–233.
61. Mittag, M. Circadian Rhythms in Microalgae. Int. Rev. Cytol. **2001**, *206*, 213–247.
62. Taylor, W.; Dunlap, J.C.; Hastings, J.W. Inhibitors of Protein Synthesis on 80S Ribosomes Phase Shift the *Gonyaulax* Clock. J. Exp. Biol. **1982**, *97*, 121–136.
63. Rensing, L.; Taylor, W.; Dunlap, J.C.; Hasting, J.W. The Effects of Protein Synthesis Inhibitors on the *Gonyaulax* Clock. J. Comp. Physiol. **1980**, *138*, 9–18.
64. Comolli, J.; Taylor, W.; Rehman, J.; Hastings, J.W. An Inhibitor of Protein Phosphorylation Stops the Circadian Oscillator and Blocks Light-Induced Phase Shifting in *Gonyaulax polyedra*. J. Biol. Rhythms **1994**, *9*, 13–26.
65. Roenneberg, T.; Morse, D. Two Circadian Oscillators in One Cell. Nature **1993**, *362*, 362–364.
66. Barak, S.; Tobin, E.M.; Andronis, C.; Sugano, S.; Green, R.M. All in Good Time: The *Arabidopsis* Circadian Clock. Trends Plant Sci. **2000**, *5*, 517–522.
67. Wang, Z.Y.; Tobin, E.M. Constitutive Expression of the *Circadian Clock Associated 1* (CCA1) Gene Disrupts Circadian Rhythms and Suppresses Its Own Expression. Cell **1998**, *93*, 1207–1217.
68. Schaffer, R.; Ramsay, N.; Samach, A.; Corden, S.; Putterill, J.; Carré, I.A.; Couplaud, G. *Late Elongated Hypocotyl*, an *Arabidopsis* Gene Encoding a MYB

Transcription Factor, Regulates Circadian Rhythmicity and Photoperiodic Responses. Cell **1998**, *93*, 1219–1229.
69. Sugano, S.; Adronis, C.; Ong, M.S.; Green, R.M.; Tobin, E.M. The Protein Kinase CK2 Is Involved in Regulation of Circadian Rhythms in *Arabidopsis*. Proc. Natl Acad. Sci. USA **1999**, *96*, 1262–1266.
70. Strayer, C.; Oyama, T.; Schultz, T.F.; Ramanujam, R.; Somers, D.E.; Más, P.; Panda, J.A.; Kay, S.A. Cloning of the *Arabidopsis* Clock Gene TOC1, an Autoregulatory Response Regulator Homolog. Science **2000**, *289*, 768–771.
71. Matsushika, A.; Makino, S.; Kojima, M.; Mizuna, T. Circadian Waves of Expression of the APRR1/TOC1 Family of Pseudoresponse Regulators in *Arabidopsis thaliana*: Insight into the Plant Circadian Clock. Plant Cell Physiol. **2000**, *41*, 1002–1012.
72. Makino, S.; Matsushika, A.; Kojima, M.; Oda, Y.; Mizuno, T. Light Response of the Circadian Waves the APRR1/TOC1 Quintet: When Does the Quintett Start Singing Rhythmically in *Arabidopsis*. Plant Cell Physiol. **2001**, *42*, 334–339.
73. Drescher, K.; Cornelius, G.; Rensing, L. Phase Response Curves Obtained by Perturbing Different Variables of a 24h Model Oscillator Based on Translational Control. J. Theor. Biol. **1982**, *94*, 345–353.
74. Friemert, V.; Heininger, D.; Kluge, M.; Ziegler, H. Temperature Effects on Malic-Acid Efflux from the Vacuoles and on the Carboxylation Pathways in Crassulacean-Acid-Metabolism Plants. Planta **1988**, *174*, 453–461.
75. Minorsky, P.V. Temperature Sensing by Plants: A Review and Hypothesis. Plant Cell Environ. **1989**, *12*, 119–135.
76. Murata, N.; Los, D.A. Membrane Fluidity and Temperature Perception. Plant Physiol. **1997**, *115*, 875–879.
77. Browse, J.; Xin, Z. Temperature Sensing and Cold Acclimation. Curr. Opin. Plant Biol. **2001**, *4*, 241–246.
78. Maeda, M.; Wurgler-Murphy, S.M.; Saito, H. A Two-Component System That Regulates an Osmosensing MAP Kinase Cascade in Yeast. Nature **1994**, *309*, 242–245.
79. Monroy, A.F.; Dhindsa, R.S. Low-Temperature Signal Transduction: Induction of Cold Acclimation-Specific Genes of Alfalfa by Calcium at 25°C. Plant Cell **1995**, *7*, 321–331.
80. Nover, L. *Heat Shock Response*; CRC: Boca Raton, FL, 1991.
81. Ahn, S.G.; Liu, P.C.; Klyachko, K.; Morimoto, R.I.; Thiele, D.J. The Loop Domain of Heat Shock Transcription Factor1 Dictates DNA-Binding Specificity and Responses to Heat Stress. Genes Dev. **2001**, *15*, 2134–2145.
82. Jerebzoff, S.; Jerebzoff-Quintin, S.; Labert, E. *Aspergillus niger*: Characteristics of Endogenous and Low Frequency Rhythms. Int. J. Chronobiol. **1975**, *2*, 131–144.
83. Jerebzoff, S.; Jerebzoff-Quintin, S. Cyclic Activity of L-Asparaginase Through Reversible Phosphorylation in *Leptosphaeria michotii*. FEBS Lett. **1984**, *171*, 67–71.
84. Übelmesser, E.R. Über den Endonomen Rhythmus der Sporangienträgerbildung von *Pilobolus*. Arch. Microbiol. **1954**, *20*, 1–33.
85. Feldman, J.F.; Dunlap, J.C. *Neurospora crassa*: A Unique System for Studying Circadian Rhythms. Photochem. Photobiol. **1983**, *7*, 319–368.
86. Lakin-Thomas, P.L.; Coté, G.G.; Brody, S. Circadian Rhythms in *Neurospora crassa*: Biochemistry and Genetics. Crit. Rev. Microbiol. **1990**, *17*, 365–416.

87. Loros, J.J.; Dunlap, J.C. Genetic and Molecular Analysis of Circadian Rhythms in *Neurospora*. Annu. Rev. Physiol. **2001**, *63*, 757–794.
88. Francis, C.D.; Sargent, M.L. Effects of Temperature Perturbation on Circadian Conidiation in *Neurospora*. Plant Physiol. **1979**, *64*, 1000–1004.
89. Rensing, L. Is "Masking" an Appropriate Term? Chronobiol. Int. **1989**, *6*, 297–300.
90. Loxtercamp, C.D.; Gooch, V.D. Comparison of Analytical Methods for Temperature Pulse Phase Shifting on the Conidiation Circadian Rhythm of *Neurospora crassa*, National Conference on Undergraduate Research. Salisbury, Maryland, 1998.
91. Rensing, L.; Schill, W. Perturbations of Cellular Circadian Rhythms by Light and Temperature. In *Temporal Disorder in Human Oscillatory Systems*; Rensing, L., An der Heiden, U., Mackey, M., Eds.; Springer Verlag: Berlin, 1987; 233–245.
92. Nakashima, H. Comparison of Phase Shifting by Temperature of Wild Type *Neurospora crassa* and the Clock Mutant, *frq-7*. J. Interdiscip. Cycle Res. **1987**, *18*, 1–8.
93. Goto, R.; Kaue, R.; Morishita, M.; Nakashima, H. Effect of Temperature on the Circadian Conidiation Rhythm of Temperature-Sensitive Mutants of *Neurospora crassa*. Plant Cell Physiol. **1994**, *35*, 613–618.
94. Rensing, L.; Bos, A.; Kroeger, J.; Cornelius, G. Possible Link Between Circadian Rhythm and Heat Shock Response in *Neurospora crassa*. Chronobiol. Int. **1987**, *4*, 543–549.
95. Fracella, F.; Scholle, C.; Kallies, A.; Häfker, T.; Schroeder, T.; Rensing, L. Differential HSC70 Expression During Asexual Development of *Neurospora crassa*. Microbiology **1997**, *143*, 3615–3624.
96. Ruoff, P.; Vinsjevik, M.; Mohsenzadeh, S.; Rensing, L. The Goodwin Model: Simulating the Effect of Cycloheximide and Heat Shock on the Sporulation Rhythm of *Neurospora crassa*. J. Theor. Biol. **1999**, *196*, 483–494.
97. Nakashima, H.; Perlman, J.; Feldman, J.F. Cycloheximide-Induced Phase Shifting of the Circadian Clock of *Neurospora*. Am. J. Physiol. **1981**, *241*, R31–R35.
98. Schulz, R.; Pilatus, U.; Rensing, L. On the Role of Energy Metabolism in *Neurospora* Circadian Clock Function. Chronobiol. Int. **1985**, *2*, 223–233.
99. Liu, Y.; Garceau, N.Y.; Loros, J.J.; Dunlap, J.C. Thermally Regulated Translational Control of FRQ Mediates Aspects of Temperature Responses in the *Neurospora* Circadian Clock. Cell **1997**, *89*, 477–486.
100. Garceau, N.Y.; Liu, Y.; Loros, J.J.; Dunlap, J.C. Alternative Initiation of Translation and Time-Specific Phosphorylation Yield Multiple Forms of the Essential Clock Protein FREQUENCY. Cell **1997**, *89*, 469–476.
101. Cheng, P.; Yang, Y.; Heintzen, C.; Liu, Y. Coiled-Coil Domain-Mediated FRQ–FRQ Interaction Is Essential for Its Circadian Clock Function in *Neurospora*. EMBO J. **2001**, *20*, 101–108.
102. Cheng, P.; Yang, Y.; Liu, Y. Interlocked Feedback Loops Contribute to the Robustness of the *Neurospora* Clock. Proc. Natl Acad. Sci. USA **2001**, *98*, 7408–7413.
103. Liu, Y.; Loros, J.J.; Dunlap, J.C. Phosphorylation of the *Neurospora* Clock Protein FREQUENCY Determines Its Degradation Rate and Strongly Influences the Period Length of the Circadian Clock. Proc. Natl Acad. Sci. USA **2000**, *97*, 234–239.

104. Yang, Y.; Cheng, P.; Zhi, G.; Liu, Y. Identification of a Calcium/Calmodulin-Dependent Kinase That Phosphorylates the *Neurospora* Circadian Clock Protein FREQUENCY. J. Biol. Chem. **2001**, *276*, 41064–41072.
105. Talora, C.; Franchi, L.; Linden, H.; Ballario, P.; Macino, G. Role of White Collar-1–White Collar-2 Complex in Blue Light Signal Transduction. EMBO J. **1999**, *18*, 4961–4968.
106. Heintzen, C.; Loros, J.J.; Dunlap, J.C. The PAS Protein VIVID Defines a Clock-Associated Feedback Loop That Represses Light Input, Modulates Gating and Regulates Clock Resetting. Cell **2001**, *104*, 453–464.
107. Nakashima, H.; Feldman, J.F. Temperature Sensitivity of Light-Induced Phase Shifting of the Circadian Clock of *Neurospora*. Photochem. Photobiol. **1980**, *32*, 247–251.
108. Lakin-Thomas, P.L.; Brody, S.; Coté, G.G. Amplitude Model for the Effects of Mutations and Temperature on Period and Phase Resetting of the *Neurospora* Circadian Oscillator. J. Biol. Rhythms **1991**, *6*, 281–297.
109. Crosthwaite, S.C.; Loros, J.J.; Dunlap, J.C. Light-Induced Resetting of a Circadian Clock Is Mediated by a Rapid Increase in Frequency Transcript. Cell **1995**, *81*, 1003–1012.
110. Crosthwaite, S.C.; Dunlap, J.C.; Loros, J.J. *Neurospora wc-1* and *wc-1*: Transcription, Photoresponses, and the Origin of Circadian Rhythmicity. Science **1997**, *276*, 763–769.
111. Loros, J.J.; Feldman, J.F. Loss of Temperature Compensation of Circadian Period Length in the *frq-9* Mutant of *Neurospora crassa*. J. Biol. Rhythms **1986**, *1*, 187–198.
112. Loros, F.F.; Richman, A.; Feldman, J.F. A Recessive Circadian Clock Mutation at the *frq* Locus of *Neurospora crassa*. Genetics **1986**, *114*, 1095–1110.
113. Lakin-Thomas, P.L.; Brody, S. Circadian Rhythms in *Neurospora crassa*: Lipid Deficiencies Restore Robust Rhythmicity to Null *Frequency* and *White-Collar* Mutants. PNAS **2000**, *97*, 256–261.
114. Merrow, M.; Brunner, M.; Roenneberg, T. Assignment of Circadian Function for the *Neurospora* Clock Gene Frequency. Nature **1999**, *399*, 584–586.
115. Kallies, A.; Gebauer, G.; Rensing, L. Heat Shock Effects on Second Messenger Systems of *Neurospora crassa*. Arch. Microbiol. **1998**, *170*, 191–200.
116. Shaw, N.M.; Harding, R.W. Intracellular and Extracellular Cyclic Nucleotides in Wild-Type and White Collar Mutant Strains of *Neurospora crassa*. Temperature Dependent Efflux of Cyclic AMP from Mycelia. Plant Physiol. **1987**, *83*, 377–383.
117. Techel, D.; Gebauer, G.; Kohler, W.; Braumann, T.; Jastorff, B.; Rensing, L. On the Role of Ca^{2+}-Calmodulin-dependent and cAMP-Dependent Protein Phosphorylation in the Circadian Rhythm of *Neurospora crassa*. J. Comp. Physiol. B **1990**, *159*, 695–706.
118. Palmer, J.P. *The Biological Rhythms and Clocks of Intertidal Animals*; Oxford University Press: Oxford, 1995.
119. Holmström, W.F.; Morgan, E. Laboratory Entrainment of the Rhythmic Swimming Activity of *Corophium volutator* (PALLAS) to Cycles of Temperature and Periodic Inundation. J. Mar. Biol. Assoc. UK **1983**, *63*, 861–870.
120. Natarajan, P. External Synchronizers of Tidal Activity Rhythms in the Prawns *Penaeus indicus* and *P. monodon*. Mar. Biol. **1989**, *101*, 347–354.

121. Brown, F.A., Jr.; Webb, H.M. Temperature Relations of an Endogenous Daily Rhythmicity in the Fiddler Crab, *Uca*. Physiol. Zool. **1948**, *21*, 371–381.
122. Stephens, G.C. Influence of Temperature Fluctuations on the Diurnal Melanophor Rhythm in the Fiddler Crab, *Uca*. Physiol. Zool. **1957**, *30*, 55–69.
123. Fanjul-Moles, M.; Prieto-Sagredo, J. Effect of the Temperature upon Ultradian and Circadian ERG Amplitude Rhythms During Ontogeny of the Crayfish *Procambarus clarkii*. Bol. Estud. Med. y Biol. **1996**, *44*, 11–18.
124. Williams, B.G.; Naylor, E. Synchronization of the Locomotor Tidal Rhythm of *Carcinus*. J. Exp. Biol. **1969**, *51*, 715–725.
125. Naylor, E.; Atkinson, R.J.A.; Williams, B.G. External Factors Influencing the Tidal Rhythm of Shore Crabs. J. Interdiscip. Cycle Res. **1971**, *2*, 173–180.
126. Enright, J.T. The Tidal Rhythm of Activity of a Sand Beach Amphipod. Z. Vgl. Physiol. **1963**, *46*, 276–313.
127. Naylor, E. Temperature Relationships of the Locomotor Rhythms of *Carcinus*. J. Exp. Biol. **1963**, *40*, 669–679.
128. Holmström, W.F.; Morgan, E. The Effects of Low Temperature Pulses in Rephasing the Endogenous Activity Rhythm of *Corophium volutator* (PALLAS). J. Mar. Biol. Assoc. UK **1983**, *63*, 851–860.
129. Fincham, A.A. Rhythmic Behaviour of the Intertidal Amphipod *Bathyporeia pelagica*. J. Mar. Biol. Ass. UK **1970**, *50*, 1057–1068.
130. Jones, D.A.; Naylor, E. The Swimming Rhythm of the Beach Isopod *Eurydice pulchra*. J. Exp. Mar. Biol. Ecol. **1970**, *4*, 188–199.
131. Saunders, D.S., (Ed.) *Insect Clocks*; Pergamon Press: Oxford, 1982.
132. Zimmerman, W.F.; Pittendrigh, C.S.; Pavlidis, Th. Temperature Compensation of the Circadian Oscillation in *Drosophila pseudoobscura* and Its Entrainment by Temperature Cycles. J. Insect Physiol. **1968**, *14*, 669–684.
133. Lankinen, P.; Riihimaa, A. Effects of Temperature on Weak Circadian Eclosion Rhythmicity in *Chymomyza costata* (Diptera: Drosophilidae). J. Insect Physiol. **1997**, *43*, 251–260.
134. Khare, P.V.; Barnabas, M.; Kanojiya, M.; Kulkarni, A.D.; Joshi, D.S. Temperature Dependent Eclosion Rhythmicity in the High Altitude Himalayan Strains of *Drosophila ananassae*. Chronobiol. Int. **2002**, *19*, *in press.*
135. Pittendrigh, C.S.; Kyner, W.T.; Takamura, T. The Amplitude of Circadian Oscillations: Temperature Dependence, Latitudinal Clines, and the Photoperiodic Time Measurement. J. Biol. Rhythms **1991**, *6*, 299–313.
136. Sawyer, L.A.; Hennessy, J.M.; Peixoto, A.A.; Rosato, E.; Parkinson, H.; Costa, R.; Kyriacou, C.P. Natural Variation in a *Drosophila* Clock Gene and Temperature Compensation. Science **1997**, *278*, 2117–2120.
137. Kureck, A. Circadian Eclosion Rhythm in *Chironomus thummi*: Ecological Adjustment to Different Temperature Cycles. In *Chironomidae*; Murray, D.A., Ed.; Pergamon Press: Oxford, 1980; 73–79.
138. Wohlfahrt, Th.A. Wärme als Potentieller Zeitgeber für das Schlüpfen des Segelfalters *Iphiclides podalirius* (L.). Naturwissenschaften **1967**, *54*, 121–122.
139. Tweedy, D.G.; Stephen, W.P. Temperature Pulses Synchronize Emergence Rhythm in Bees. Experientia **1970**, *26*, 377–379.
140. Helfrich, Ch. Untersuchungen über das Circadiane System von Fliegen Dissertation, Universität Tübingen,.

141. Wheeler, D.A.; Hamblen-Coyle, M.J.; Dushay, M.S.; Hall, J.C. Behavior in Light Dark Cycles of *Drosophila* Mutants That Are Arrhythmic, Blind or Both. J. Biol. Rhythms **1993**, *8*, 67–94.
142. Tomioka, K.; Sakamoto, M.; Harni, Y.; Matsumoto, N.; Matsumoto, A. Light and Temperature Cooperate to Regulate the Circadian Locomotor Rhythm of Wild Type and *Period* Mutants of *Drosophila melanogaster*. J. Insect Physiol. **1998**, *44*, 587–596.
143. Roberts, S.K. Phase Shifting and Entrainment by Temperature in Cockroaches. J. Cell. Comp. Physiol. **1962**, *58*, 175–186.
144. Page, T.L. Temperature Effects on Lobectomized Cockroaches. J. Insect Physiol. **1985**, *31*, 235–242.
145. Rence, B.; Loher, W. Arrhythmically Singing Crickets: Thermoperiodic Reentrainment After Bilobectomy. Science **1975**, *190*, 383–387.
146. Pittendrigh, C.S.; Minis, D. The Photoperiodic Time Measurement in *Pectinophora gossypiella* and Its Relation to the Circadian System in That Species. In *Biochronometry*; Menaker, M., Ed.; National Academy of Sciences: Washington, 1971; 212–250.
147. Neumann, D.; Heimbach, F. Time Cues for Semilunar Reproduction Rhythms in the European Populations of *Clunio marinus*. II. The Influence of Tidal Temperature Cycles. Biol. Bull. **1984**, *166*, 509–524.
148. Winfree, A.T. Acute Temperature Sensitivity of the Circadian Rhythm in *Drosophila*. J. Insect Physiol. **1972**, *18*, 181–185.
149. Chandrashekaran, M.K. Phase Shifts in the *Drosophila pseudoobscura* Circadian Rhythm Evoked by Temperature Pulses of Varying Durations. J. Interdiscip. Cycle Res. **1974**, *5*, 371–380.
150. Maier, R.W. Phase Shifting of the Circadian Rhythm of Eclosion in *Drosophila pseudoobscura* with Temperature Pulses. J. Interdiscip. Cycle Res. **1973**, *4*, 125–135.
151. McDonald, M.J.; Rosbash, M. Microarray Analysis and Organisation of Circadian Gene Expression in *Drosophila*. Cell **2001**, *107*, 567–578.
152. Wiedenmann, G.; Loher, W. Circadian Control of Singing in Crickets: Two Different Pacemakers for Early-Evening and Before-Dawn Activity. J. Insect Physiol. **1984**, *30*, 145–151.
153. Lohmann, M. Der Einfluß von Beleuchtungsstärke und Temperatur auf die Tagesperiodische Laufaktivität des Mehlkäfers *Tenebrio molitor* L. Z. Vgl. Physiol. **1964**, *49*, 341–389.
154. Hamm, U.; Chandrashekaran, M.K.; Engelmann, W. Temperature Sensitive Events Between Photoreceptor and Circadian Clock? Z. Naturforsch. **1975**, *30c*, 240–244.
155. Ceriani, M.F.; Darlington, T.K.; Staknis, D.; Mas, P.; Petti, A.A.; Weitz, C.J.; Kay, S.A. Light-Dependent Sequestration of TIMELESS by CRYPTOCHROME. Science **1999**, *285*, 553–556.
156. Stanewsky, R.; Kaneko, M.; Emery, P.; Beretta, B.; Wager-Smith, K.; Kay, S.A.; Rosbash, M.; Hall, J.C. The *cryb* Mutation Identifies Cytochrome as a Circadian Photoreceptor in *Drosophila*. Cell **1998**, *95*, 681–692.
157. Hunter-Ensor, M.; Ousley, A.; Sengal, A. Regulation of the *Drosophila* Protein Timeless Suggests a Mechanism for Resetting the Circadian Clock by Light. Cell **1996**, *84*, 677–685.

158. Lee, C.; Parikh, V.; Itsukaichi, T.; Bae, K.; Edery, I. Resetting the *Drosophila* Clock by Photic Regulation of PER and a PER/TIM Complex. Science **1996**, *271*, 1740–1744.
159. Myers, M.P.; Wager-Smith, K.; Rothefluh-Hilfiker, A.; Young, M.W. Light-Induced Degradation of TIMELESS and Entrainment of the *Drosophila* Circadian Clock. Science **1996**, *271*, 1736–1740.
160. Zeng, H.; Qian, Z.; Myers, M.P.; Rosbash, M. A Light-Entrainment Mechanism for the *Drosophila* Circadian Clock. Nature **1996**, *380*, 129–135.
161. Sidote, D.; Edery, I. Heat-Induced Degradation of PER and TIM in *Drosophila* Bearing a Conditional Allele of the Heat Shock Transcription Factor Gene. Chronobiol. Int. **1999**, *16*, 519–525.
162. Rensing, L.; Monnerjahn, C. Heat Shock Proteins and Circadian Rhythms. Chronobiol. Int. **1996**, *13*, 239–250.
163. Price, J.L. Are Competing Intermolecular and Intramolecular Interactions of PERIOD Protein Important for the Regulation of Circadian Rhythms in *Drosophila*? Bioessays **1995**, *17*, 583–586.
164. Majercak, J.; Sidote, D.; Hardin, P.E.; Edery, I. How a Circadian Clock Adapts to Seasonal Decreases in Temperature and Day-Length. Neuron **1999**, *24*, 219–230.
165. Helfrich-Förster, Ch.; Stengl, M.; Homberg, U. Organization of the Circadian System in Insects. Chronobiol. Int. **1998**, *15*, 567–594.
166. Ewer, J.; Frisch, B.; Hamblen-Coyle, J.; Rosbash, M.; Hall, J.C. Expression of the Period Clock Gene Within Different Cell Types in the Brain of *Drosophila* Adults and Mosaic Analysis of These Cells Influence on Circadian Behavioral Rhythms. J. Neurosci. **1992**, *12*, 3321–3349.
167. Mack, J.; Engelmann, W. Different Oscillators Control the Circadian Rhythm of Eclosion and Activity in *Drosophila*. J. Comp. Physiol. **1983**, *127*, 229–237.
168. Vafopoulou, X.; Steel, C.G.H. A Photosensitive Circadian Oscillator in an Insect Endocrine Gland: Photic Induction of Rhythmic Steroidogenesis In Vitro. J. Comp. Physiol. A **1998**, *182*, 343–349.
169. Pelc, D.; Steel, C.G.H. Rhythmic Steroidogenesis by the Prothoracic Glands of the Insect *Rhodnius prolixus* in the Absence of Rhythmic Neuropeptide Input: Implications for the Role of Prothoracicotropic Hormone. Gen. Comp. Endocrinol. **1997**, *108*, 356–365.
170. Ampleford, E.J.; Steel, C.G.H. Circadian Control of a Daily Rhythm in Hemolymph Ecdysteroid Titer in the Insect *Rhodnius prolixus* (Hemiptera). Gen. Comp. Endocrinol. **1985**, *59*, 453–459.
171. Ampleford, E.J.; Steel, C.G.H. Circadian Control of Ecdysis in *Rhodnius prolixus* (Hemiptera). J. Comp. Physiol. A **1982**, *147*, 281–288.
172. Aschoff, J. Exogenous and Endogenous Components in Circadian Rhythms. Cold Spring Harbor Symp. Quant. Biol. **1960**, *25*, 11–28.
173. DeCoursey, P.J. Phase Control of Activity in a Rodent. Cold Spring Habor Symp. Quant. Biol. **1960**, *25*, 49–55.
174. Hoffmann, K. Synchronisation der Circadianen Aktivitätsperiodik von Eidechsen Durch Temperaturcyclen Verschiedener Amplitude. Z. Vgl. Physiol. **1968**, *58*, 225–228.
175. Evans, K.J. Responses of the Locomotor Activity Rhythms of Lizards to Simultaneous Light and Temperature Cycles. Comp. Biochem. Physiol. **1966**, *19*, 91–103.

176. Foa, A.; Bertolucci, C. Temperature Cycles Induce a Bimodal Activtiy Pattern in Ruin Lizards: Masking or Clock-Controlled Event? A Seasonal Problem. J. Biol. Rhythms **2001**, *16*, 574–584.
177. Samejima, M.; Shavali, S.; Tamotsu, S.; Uchida, K.; Morita, Y.; Fukuda, A. Light- and Temperature-Dependence of the Melatonin Secretion Rhythm in the Pineal Organ of the Lamprey, *Lampetra japonica*. Jpn. J. Physiol. **2000**, *50*, 437–442.
178. Bolliet, V.; Ali, M.A.; Anctil, M.; Zachmann, A. Melatonin Secretion In Vitro from the Pineal Complex of the Lamprey *Petromyzon marinus*. Gen. Comp. Endocrinol. **1993**, *89*, 101–106.
179. Zachmann, A.; Falcón, J.; Knijff, S.C.; Bolliet, V.; Ali, M.A. Effects of Photoperiod and Temperature on Rhythmic Melatonin Secretion from the Pineal Organ of the White Sucker (*Catostomus commersoni*) In Vitro. Gen. Comp. Endocrinol. **1992**, *86*, 26–33.
180. Bolliet, V.; Bégay, V.; Ravault, J.P.; Ali, M.A.; Collin, J.P.; Falcón, J. Multiple Circadian Oscillators in the Photosensitive Pike Pineal Gland: A Study Using Organ and Cell Culture. J. Pineal Res. **1994**, *16*, 77–84.
181. Max, M.; Menaker, M. Regulation of Melatonin Production by Light, Darkness, and Temperature in the Trout Pineal. Comp. Physiol. A **1992**, *170*, 479–489.
182. Menaker, M.; Wisner, S. Temperature Compensated Circadian Clock in the Pineal of *Anolis*. Proc. Natl Acad. Sci. USA **1983**, *80*, 6119–6121.
183. Moyer, R.W.; Firth, B.T.; Kennaway, D.J. Effect of Constant Temperatures, Darkness and Light on the Secretion of Melatonin by Pineal Explants and Retinas in the Gecko, *Christinus marmoratus*. Brain Res. **1995**, *675*, 345–348.
184. Zachmann, A.; Knijff, S.C.M.; Bolliet, V.; Ali, M.A. Effects of Temperature Cycles and Photoperiod on Rhythmic Melatonin Secretion from the Pineal Organ of a Teleost (*Catostomus commersoni*) In Vitro. Neuroendocrinol. Lett. **1991**, *13*, 325–330.
185. Falcón, J.; Bolliet, V.; Ravault, J.P.; Chesneau, D.; Ali, M.A.; Collin, J.P. Rhythmic Secretion of Melatonin by the Superfused Pike Pineal Organ: Thermo- and Photoperiod Interaction. Neuroendocrinology **1994**, *60*, 535–543.
186. Underwood, H. Pineal Melatonin Rhythms in the Lizard *Anolis carolinensis*: Effects of Light and Temperature Cycles. J. Comp. Physiol. A **1985**, *157*, 57–65.
187. Underwood, H.; Calaban, M. Pineal Melatonin Rhythms in the Lizard *Anolis carolinensis*: I. Response to Light and Temperature Cycles. J. Biol. Rhythms **1987**, *2*, 179–193.
188. Firth, B.T.; Belan, I.; Kennaway, D.J.; Moyer, R.W. Thermocyclic Entrainment of Lizard Blood Plasma Melatonin Rhythms in Constant and Cyclic Photic Environments. Am. J. Physiol. **1999**, *277*, R1620–R1626.
189. Moyer, R.W.; Firth, P.T.; Kennaway, D.J. Effect of Variable Temperatures, Darkness and Light on the Secretion of Melatonin by Pineal Explants in the Gecko, *Christinus marmoratus*. Brain Res. **1997**, *747*, 230–235.
190. Valenciano, I.; Alonso-Gómez, A.L.; Alonso-Bedate, M.; Delgado, M.J. Effect of Constant and Fluctuating Temperature on Daily Melatonin Production by Eyecups from *Rana perezi*. Comp. Physiol. B **1997**, *167*, 221–228.
191. Firth, B.T.; Kennaway, D.J. Thermoperiod and Photoperiod Interact to Affect the Phase of the Plasma Melatonin Rhythm in the Lizard, *Tiliqua rugosa*. Neurosci. Lett. **1989**, *106*, 125–130.

192. Firth, B.T.; Kennaway, D.J.; Belan, I. Thermoperiodic Influences on Plasma Melatonin Rhythms in the Lizard *Tiliqua rugosa*: Effect of Thermophase Duration. Neurosci. Lett. **1991**, *121*, 139–142.
193. Delgado, M.J.; Vivien-Roels, B. Effect of Environmental Temperature and Photoperiod on the Melatonin Levels in the Pineal, Lateral Eye, and Plasma of the Frog, *Rana perezi*: Importance of Ocular Melatonin. Gen. Comp. Endocrinol. **1989**, *75*, 46–53.
194. Radwing, R.S.; Hutchison, V.H. Influence of Temperature and Photoperiod on Plasma Melatonin in the Mudpuppy, *Necturus maculosus*. Gen. Comp. Endocrinol. **1992**, *88*, 364–374.
195. Tilden, A.R.; Hutchison, V.H. Influence of Photoperiod and Temperature on Serum Melatonin in the Diamondback Water Snake, *Nerodia rhombifera*. Gen. Comp. Endocrinol. **1993**, *92*, 347–354.
196. Firth, B.T.; Kennaway, D.J. Melatonin Content of the Pineal, Parietal Eye and Blood Plasma of the Lizard, *Trachydosaurus rugosus*. Effect of Constant and Fluctuating Temperature. Brain Res. **1987**, *404*, 313–318.
197. Vivien-Roels, B.; Pevet, P.; Claustrat, B. Pineal and Circulating Melatonin Rhythms in the Box Turtle, *Terrapene carolina triunguis*: Effect of Photoperiod, Light Pulse, and Environmental Temperature. Gen. Comp. Endocrinol. **1988**, *69*, 163–173.
198. Tosini, G.; Bertolucci, C.; Foa, A. The Circadian System of Reptiles: A Multioscillatory and Multiphotoreceptive System. Physiol. Behav. **2001**, *72*, 461–471.
199. Innocenti, A.; Bertolucci, C.; Minutini, L.; Foà, A. Seasonal Variations of Pineal Involvement in the Circadian Organization of the Ruin Lizard *Podarcis sicula*. J. Exp. Biol. **1996**, *199*, 1189–1194.
200. Bertolucci, C.; Foa, A. Seasonality and Role of SCN in Entrainment of Lizard Circadian Rhythms to Daily Melatonin Injections. Am. J. Physiol. **1998**, *274*, 1004–1014.
201. Bertolucci, C.; Sovrano, V.A.; Magnone, M.C.; Foa, A. Role of Suprachiasmatic Nuclei in Circadian and Light-Entrained Behavioral Rhythms of Lizards. Am. J. Physiol. Regul. Integr. Comp. Physiol. **2000**, *279*, 2122–2131.
202. Hyde, L.L.; Underwood, H. Daily Melatonin Infusions Entrain the Locomotor Activity of Pinealectomized Lizards. Physiol. Behav. **1995**, *58*, 943–951.
203. Hyde, L.L.; Underwood, H. Effects of Melatonin Administration on the Circadian Activity Rhythm of the Lizard *Anolis carolinensis*. Physiol. Behav. **2000**, *71*, 183–192.
204. Tosini, G.; Menaker, M. The Role of Pineal Complex and Melatonin in the Daily Rhythm of Behavioral Thermoregulation in the Green Iguana. J. Comp. Physiol. A **1996**, *179*, 135–142.
205. Firth, B.T.; Belan, I. Daily and Seasonal Rhythms in Selected Body Temperatures in the Australian Lizard *Tiliqua rugosa* (Scincidae): Field and Laboratory Observations. Physiol. Zool. **1998**, *71*, 303–311.
206. Valenciano, A.I.; Alonso-Gómez, A.L.; De Pedro, N.; Alonso-Bedate, M.; Delgado, M.J. Serotonin *N*-Acetyltransferase Activity as a Target for Temperature in the Regulation of Melatonin Production by Frog Retina. Pflügers Arch. **1994**, *429*, 153–159.

207. Francis, A.J.P.; Coleman, G.J. Ambient Temperature Cycles Entrain the Free-Running Circadian Rhythms of the Stripe-Faced Dunnart, *Sminthopsis macroura*. J. Comp. Physiol. A **1990**, *167*, 357–362.
208. Pohl, H. Wirkung der Temperatur auf die mit Licht Synchronisierte Aktivitätsperiodik bei Warmblütern. Z. Vgl. Physiol. **1968**, *58*, 381–394.
209. Francis, A.J.P.; Coleman, G.J. Phase Response Curves to Ambient Temperature Pulses in Rats. Physiol. Behav. **1997**, *62*, 1000–1004.
210. Pàlkovà, M.; Sigmund, L.; Erkert, H.G. Effect of Ambient Temperature on the Circadian Activity Rhythm in Common Marmosets, *Callithrix j. jacchus* (Primates). Chronobiol. Int. **1999**, *16*, 149–161.
211. Lindberg, R.G.; Hayden, P. Thermoperiodic Entrainment of Arousal from Torpor in the Little Pocket Mouse, *Perognathus longimembris*. Chronobiologia **1974**, *1*, 356–361.
212. Pohl, H. Temperature Cycles as Zeitgeber for the Circadian Clock of Two Burrowing Rodents, the Normothermic Antelope Ground Squirrel and the Heterothermic Syrian Hamster. Biol. Rhythm Res. **1998**, *29*, 311–325.
213. Erkert, H.G.; Rothmund, E. Differences in Temperature Sensitivity of the Circadian Systems of Homoeothermic and Heterothermic Neotropical Bats. Comp. Biochem. Physiol. **1980**, *68A*, 383–390.
214. Enright, J.T. Temperature and the Free-Running Rhythm of the House Finch. Comp. Biochem. Physiol. **1966**, *18*, 463–475.
215. Steward, M.C.; Reeder, W.G. Temperature and Light Synchronisation Experiments with Circadian Activity Rhythms in Two Color Forms of the Rock Pocket Mouse. Physiol. Zool. **1968**, *41*, 149–156.
216. Francis, A.J.P.; Coleman, G.J. The Effects of Ambient Temperature Cycles upon Circadian Running and Drinking Activity in Male and Female Laboratory Rats. Physiol. Behav. **1988**, *43*, 471–477.
217. Sulzman, F.M.; Fuller, C.A.; Moore-Ede, M.C. Environmental Synchronizers of Squirrel Monkey Circadian Rhythms. J. Appl. Physiol. **1977**, *43*, 795–800.
218. Aschoff, J.; Tokura, H. Circadian Activity Rhythms in Squirrel Monkeys: Entrainment by Temperature Cycles. J. Biol. Rhythm **1986**, *1*, 91–99.
219. Fritsche, P.; Miethe, G.; Gattermann, R. Circadian Activity Rhythm and Entrainment in the Subterranean Common Mole-Rat (*Cryptomys hottentotus*). Biol. Rhythm Res. **1997**, *28*, 116.
220. Tokura, H.; Aschoff, J. Effects of Temperature on the Circadian Rhythm of Pig-Tailed Macaques *Macaca nemestrina*. Am. J. Physiol. **1983**, *245*, R800–R804.
221. Swade, R.H.; Pittendrigh, C.S. Circadian Locomotor Rhythms in the Arctic. Am. Nat. **1967**, *101*, 431–466.
222. Rajaratnam, S.M.W.; Redman, J.R. Entrainment of Activity Rhythms to Temperature Cycles in Diurnal Palm Squirrels. Physiol. Behav. **1998**, *63*, 271–277.
223. Deguchi, T. A Circadian Oscillator in Cultured Cells of Chicken Pineal Gland. Nature **1997**, *282*, 94–96.
224. Barrett, R.K.; Takahashi, J.S. Temperature Compensation and Temperature Entrainment of the Chick Pineal Cell Circadian Clock. J. Neurosci. **1995**, *15*, 5681–5692.

225. Zatz, M.; Mullen, D.A. Two Mechanisms of Photoendocrine Transduction in Cultured Chick Pineal Cells: Pertussis Toxin Blocks the Acute But Not the Phase-Shifting Effects of Light on the Melatonin Rhythm. Brain Res. **1988**, *453*, 63–71.
226. Zatz, M.; Lange, G.D.; Rollag, M.D. What Does Changing the Temperature Do to the Melatonin Rhythm in Cultured Chick Pineal Cells? Am. J. Physiol. **1994**, *35*, R50–R58.
227. Gillette, M.U. SCN Electrophysiology In Vitro: Rhythmic Activity and Endogenous Clock Properties. In *Suprachiasmatic Nucleus: The Mind's Clock*; Klein, D.C., Moore, R.Y., Reppert, S.M., Eds.; Oxford University Press: New York, 1991; 125–143.
228. Ruby, N.F.; Burns, D.E.; Heller, H.C. Circadian Rhythms in the Suprachiasmatic Nucleus Are Temperature-Compensated and Phase-Shifted by Heat Pulses In Vitro. J. Neurosci. **1999**, *19*, 8630–8636.
229. Gander, P.H.; Lewis, R.D. Phase-Resetting Action of Light on the Circadian Activity Rhythm of *Rattus exulans*. Am. J. Physiol. **1983**, *245*, R10–R17.
230. Robertson, L.M.; Takahashi, J.S. Circadian Clock in Cell Culture II. In Vitro Photic Entrainment of Melatonin Oscillation from Dissociated Chick Pineal Cells. J. Neurosci. **1988**, *8*, 22–30.
231. French, A.R. Periodicity of Recurrent Hypothermia During Hibernations in the Pocket Mouse, *Perognathus longimembris*. J. Comp. Physiol. B **1977**, *115*, 87–100.
232. Aschoff, J.; Wever, R. Aktivitätsmenge und α: ϛ-Verhältnis als Meßgröße der Tagesperiodik. Z. Vgl. Physiol. **1962**, *46*, 88–101.
233. Pohl, H. Einfluß der Temperatur auf die Freilaufende Circadiane Aktivitätsperiodik bei Warmblütern. Z. Vgl. Physiol. **1968**, *58*, 364–380.
234. Erkert, H.G. Influence of Ambient Temperature on Circadian Rhythms in Colombian Owl Monkeys, *Aotus lemurinus griseimembra*. In *Primatology Today*; Akiyoshi Ehara, Ed.; Elsevier Science Publishers B.V.: Amsterdam, 1991; 435–438.
235. Underwood, H.; Steele, C.T.; Zivkovic, B. Circadian Organization and the Role of the Pineal in Birds. Microsc. Res. Tech. **2001**, *53*, 48–62.
236. Herzog, E.D.; Block, G.D. Keeping an Eye on Retinal Clocks. Chronobiol. Int. **1999**, *16*, 229–247.
237. Okano, T.; Fukada, Y. Photoreception and Circadian Clock System of the Chicken Pineal Gland. Microsc. Res. Tech. **2001**, *53*, 72–80.
238. Tosini, G. Melatonin Circadian Rhythm in the Retina of Mammals. Chronobiol. Int. **2000**, *17*, 599–612.
239. Jilge, B.; Hudson, R. Diversity and Development of Circadian Rhythms in the European Rabbit. Chronobiol. Int. **2001**, *18*, 1–26.
240. Shirakawa, T.; Honma, S.; Honma, K. Multiple Oscillators in the Suprachiasmatic Nucleus. Chronobiol. Int. **2001**, *18*, 371–387.
241. Michel, S.; Colwell, C.S. Cellular Communication and Coupling Within the Suprachiasmatic Nucleus. Chronobiol. Int. **2001**, *18*, 579–600.
242. Jagota, A.; de la Iglesia, H.O.; Schwartz, W.J. Morning and Evening Circadian Oscillations in the Suprachiasmatic Nucleus In Vitro. Nat. Neurosci. **2000**, *3*, 372–376.
243. De la Iglesia, H.O.; Meyer, J.; Carpino, A.J.; Schwartz, W.J. Antiphase Oscillation of the Left and Right Suprachiasmatic Nuclei. Science **2000**, *290*, 799–801.

244. Hoffmann, K. Splitting of the Circadian Rhythm as a Function of Light Intensity. In *Biochronometry*; Menaker, M., Ed.; National Academy of Sciences: Washington, 1967.
245. Damiola, F.; Le Minh, N.; Preitner, N.; Kornmann, B.; Fleury-Olela, F.; Schibler, U. Restricted Feeding Uncouples Circadian Oscillators in Peripheral Tissues from the Central Pacemaker in the Suprachiasmatic Nucleus. Genes Dev. **2000**, *14*, 2950–2961.
246. Stokkan, K.A.; Yamazaki, S.; Tei, H.; Sakaki, Y.; Menaker, M. Entrainment of the Circadian Clock in the Liver by Feeding. Science **2001**, *291*, 490–493.
247. Balsalobre, A.; Damiola, F.; Schibler, U. A Serum Shock Induces Circadian Gene Expression in Mammalian Tissue Culture Cells. Cell **1998**, *93*, 929–937.
248. Hastings, M.H.; Duffield, G.E.; Smith, E.J.D.; Maywood, E.S.; Ebling, F.J.P. Entrainment of the Circadian System of Mammals by Nonphotic Cues. Chronobiol. Int. **1998**, *15*, 225–245.
249. Allada, R.; Emery, P.; Takahashi, J.S.; Rosbash, M. Stopping Time: The Genetics of Fly and Mouse Circadian Clocks. Annu. Rev. Neurosci. **2001**, *24*, 1091–1119.
250. Lowrey, P.L.; Takahashi, J.S. Genetics of the Mammalian Circadian System. Photic Entrainment, Circadian Pacemaker Mechanisms, and Posttranslational Regulation. Annu. Rev. Genet. **2000**, *34*, 533–562.
251. King, D.P.; Takahashi, J.S. Molecular Genetics of Circadian Rhythms in Mammals. Annu. Rev. Neurosci. **2000**, *23*, 713–742.
252. Cermakian, N.; Sassone-Corsi, P. Multilevel Regulation of the Circadian Clock. Mol. Cell Biol. **2000**, *1*, 59–67.
253. Yu, W.; Nomura, M.; Ikeda, M. Interacting Feedback Loops Within the Mammalian Clock: BMAL1 Is Negatively Autoregulated and Upregulated by CRY1, CRY2 and PER2. Biochem. Biophys. Res. Commun. **2002**, *290*, 933–941.
254. Shigeyoshi, Y.; Taguchi, K.; Yamamoto, S.; Takekida, S.; Yan, L.; Tei, J.; Moriya, T.; Shibata, S.; Loros, J.J.; Dunlap, J.C.; Okamura, H. Light-Induced Resetting of a Mammalian Circadian Clock Is Associated with Rapid Induction of the *mPer1* Transcript. Cell **1997**, *91*, 1043–1053.
255. Tamaru, T.; Isojima, Y.; Yamada, T.; Okada, M.; Nagai, K.; Takamatsu, K. Light and Glutamate-Induced Degradation of the Circadian Oscillating Protein BMAL1 During the Mammalian Clock Resetting. J. Neurosci. **2000**, *20*, 7525–7530.
256. Mintz, E.M.; Marvel, C.L.; Gillespie, C.F.; Price, K.M.; Albers, H.E. Activation of NMDA Receptors in the Suprachiasmatic Nucleus Produces Light-Like Phase Shifts of the Circadian Clock In Vivo. J. Neurosci. **1999**, *19*, 5124–5130.
257. Ding, J.M.; Dong, C.; Weber, E.T.; Faiman, L.E.; Rea, M.A.; Gillette, M.U. Resetting the Biological Clock: Mediation of Nocturnal Circadian Shifts by Glutamate and NO. Science **1994**, *266*, 1713–1717.
258. Eide, E.J.; Virshup, D.M. Casein Kinase I: Another Cog in the Circadian Clockworks. Chronobiol. Int. **2001**, *18*, 389–398.
259. Lee, C.; Etchegaray, J.P.; Cagampang, F.R.; Loudon, A.S.I.; Reppert, S.M. Posttranslational Mechanisms Regulate the Mammalian Circadian Clock. Cell **2001**, *107*, 855–867.
260. Eide, E.J.; Vielhaber, E.L.; Hinz, W.A.; Virshup, D.M. The Circadian Regulatory Proteins BMAL1 and Cryptochromes Are Substrates of Casein Kinase Iε (CKIε). J. Biol. Chem. **2002**, *277*, 17248–17254.

261. Pennartz, C.M.A.; deJeu, M.T.G.; Schaap, J.; Geurtsen, A.M.S. Diurnal Modulation of Pacemaker Potentials and Calcium Currents in the Mammalian Circadian Clock. Nature **2002**, *416*, 286–290.
262. Colwell, C.S. Circadian Modulation of Calcium Levels in Cells in the Suprachiasmatic Nucleus. Eur. J. Neurosci. **2000**, *12*, 571–576.
263. Kiang, J.; Wu, Y.; Lin, M. Heat Treatment Induces an Increase in Intracellular Cyclic cAMP Content in Human Epidermoid A431 Cells. Biochem. J. **1991**, *276*, 683–689.
264. Kiang, J.; McClain, D. Effect of Heat Shock, $[Ca^{2+}]_i$ and cAMP on Inositol Trisphosphate in Human Epidermoid A431 Cells. Am. J. Physiol. **1993**, *264*, C1561–C1569.
265. Skrandies, S.; Bremer, B.; Pilatus, U.; Mayer, A.; Neuhaus-Steinmetz, U.; Rensing, L. Heat Shock- and Ethanol-Induced Ionic Changes in C6 Rat Glioma Cells Determined by NMR and Fluorescence Spectroscopy. Brain Res. **1997**, *746*, 220–230.
266. Andrews, G.K.; Harding, M.A.; Calvet, J.P.; Adamson, E.G. The Heat Shock Response in HeLa Cells Is Accompanied by Elevated Expression of the *c-fos* Proto-Oncogene. Mol. Cell Biol. **1987**, *7*, 3452–3458.
267. Elvert, R.; Kronfeld, N.; Dayan, T.; Haim, A.; Zisapel, N.; Heldmaier, G. Body Temperature Rhythms in Free Living Desert Spiny Mice (Acomys)—Its Relation to Activity Pattern. Biol. Rhythm Res. **1997**, *28*, 114.
268. Refinetti, R. Amplitude of the Daily Rhythm of Body Temperature in Eleven Mammalian Species. J. Therm. Biol. **1999**, *24*, 477–481.
269. Green, C.B.; Besharse, J.C.; Zatz, M. Tryptophan Hydroxylase mRNA Levels Are Regulated by the Circadian Clock, Temperature, and cAMP in Chick Pineal Cells. Brain Res. **1996**, *738*, 1–7.
270. Janik, D.; Mrosowsky, N. Nonphotically Induced Phase Shifts of Circadian Rhythms in the Golden Hamster: Activity Response Curves at Different Ambient Temperatures. Physiol. Behav. **1993**, *53*, 431–436.
271. Cassone, V.M.; Chesworth, M.J.; Armstrong, S.M. Entrainment of Rat Circadian Rhythms by Daily Injection of Melatonin Depends upon the Hypothalamic Suprachiasmatic Nuclei. Physiol. Behav. **1986**, *36*, 1105–1110.
272. Slotten, H.; Pitrosky, B.; Pevet, P. Entrainment of Rat Circadian Rhythms by Daily Administration of Melatonin. Influence of the Mode of Administration. Adv. Exp. Med. Biol. **1999**, *460*, 279–281.
273. Herxheimer, A.; Petrie, K.J. Melatonin for Preventing and Treating Jet Lag. Cochrane Database Syst. Rev. **2001**, *1*, CD001520.
274. Masana, M.I.; Dubocovich, M.L. Melatonin Receptor Signaling: Finding the Path Through the Dark. Sci. STKE **2001**, www.Stke.org/cgi/content/full/OC_Sigtrans; 2001/107/pe39.
275. Roy, D.; Belsham, D.D. Melatonin Receptor Activation Regulates GnRH Gene Expression and Secretion in GT1-7 GnRH Neurons. Signal Transduction Mechanisms. J. Biol. Chem. **2002**, *277*, 251–258.
276. Zemkova, H.; Vanecek, J. Dual Effect of Melatonin on Gonadotropin-releasing-hormone-induced Ca^{2+} Signaling in Neonatal Rat Gonadotropes. Neuroendocrinology **2001**, *74*, 262–269.
277. Sumova, A.; Vanecek, J. Melatonin Inhibits GnRH-Induced Increase of cFOS Immunoreactivity in Neonatal Rat Pituitary. J. Neuroendocrinol. **1997**, *9*, 135–139.

278. Lewy, A.J.; Bauer, V.K.; Ahmed, S.; Thomas, K.H.; Cutler, N.L.; Singer, C.M.; Moffit, M.T.; Sack, R.L. The Human Phase Response Curve (PRC) to Melatonin Is About 12 Hour out of Phase with the PRC to Light. Chronobiol. Int. **1998**, *15*, 71–83.
279. Redlin, U. Neural Basis and Biological Function of Masking by Light in Mammals: Suppression of Melatonin and Locomotor Activity. Chronobiol. Int. **2001**, *18*, 737–758.
280. Sharma, V.K.; Daan, S. Circadian Phase and Period Responses to Light Stimuli in Two Nocturnal Rodents. Chronobiol. Int. **2002**, *19*, 659–670.
281. Ruoff, P.; Vinsjevik, M.; Monnerjahn, C.; Rensing, L. The Goodwin Oscillator: On the Importance of Degradation Reactions in the Circadian Clock. J. Biol. Rhythms **1999**, *14*, 469–479.
282. Yatvin, M.B.; Cramp, W.A. Role of Cellular Membranes in Hyperthermia: Some Observations and Theories Reviewed. Int. J. Hyperthermia **1993**, *9*, 165–185.