Modeling the circadian clock: from molecular mechanism to physiological disorders

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Summary

Based on genetic and biochemical advances on the molecular mechanism of circadian rhythms, a computational model for the mammalian circadian clock is used to examine the dynamical bases of circadian-clock-related physiological disorders in humans. Entrainment by the light–dark cycle with a phase advance or a phase delay is associated with the Familial advanced sleep phase syndrome (FASPS) or the Delayed sleep phase syndrome (DSPS), respectively. Lack of entrainment corresponding to the occurrence of quasiperiodic oscillations with or without phase jump can be associated with the non-24 h sleep–wake syndrome. In the close vicinity of the entrainment domain, the model uncovers the possibility of infradian oscillations of very long period. Perturbation in the form of chronic jet lag, as used in mice, prevents entrainment of the circadian clock and results in chaotic or quasiperiodic oscillations. It is important to clarify the conditions for entrainment and for its failure because dysfunctions of the circadian clock may lead to physiological disorders, which pertain not only to the sleep–wake cycle but also to mood and cancer. *BioEssays* 30:590–600, 2008. © 2008 Wiley Periodicals, Inc.

Introduction

Circadian oscillations, which occur with a period of about 24 hours, play a key physiological role in the adaptation of living organisms to the alternation of day and night.[1] Experimental advances have largely unraveled the molecular bases of circadian rhythms in a number of organisms such as *Drosophila*,[2,3] *Neurospora*,[4] cyanobacteria,[5,6] plants[7] and mammals.[8–10] Adaptation to the periodic environment is mediated through the entrainment of circadian rhythms by light–dark (LD) cycles. Light acts by inducing degradation of the TIM protein in *Drosophila*,[11] and the expression of the genes *Frq* in *Neurospora*[12] and *Per* in mammals.[13] In all cases investigated so far, except in cyanobacteria,[6] it appears that autoregulatory negative feedback on gene expression plays a central role in the mechanism of circadian rhythmicity.[14–17] Based on available experimental data, detailed computational models have been considered for circadian rhythms in *Drosophila*,[18–20] *Neurospora*[20–22] and mammals.[23–26] These models show how the regulatory feedback loops in the circadian genetic network cooperate to produce sustained oscillations in continuous darkness. Incorporating the effect of light into these models accounts for phase shifts induced by light pulses and for the entrainment of the circadian clock by light–dark cycles.[19–26]

Recent studies indicate that dysfunctions of the circadian clock in humans are associated with physiological disorders of the sleep–wake cycle.[27–29] Thus, an advance or a delay of circadian rhythms is associated with the Familial advanced sleep phase syndrome (FASPS) or with the Delayed sleep phase syndrome (DSPS), respectively. Here, we show how a computational model for the mammalian circadian clock can be used to investigate the dynamical bases of circadian disorders. The model allows us to identify conditions in which circadian oscillations systematically fail to entrain to the LD cycle. This situation can be associated with the non-24 hour sleep–wake syndrome, where the sleep–wake cycle never settles to a fixed phase. Such a phenomenon is common in blind subjects but simulations indicate that it may also occur for dynamical reasons in sighted subjects. We use the model to clarify the mechanism that leads to the absence of entrainment, and to predict biological systemic approaches to restore normal circadian periodicity. The model also uncovers the possibility of progressive drifts of the phase, of very long period, when the circadian clock has an autonomous period close to 24 hours but fails to be entrained by the LD cycle.

Clarifying the dynamical behavior of the circadian clock in response to changes in our periodic environment is of import not only from a fundamental but also from a clinical point of view. Indeed, it becomes increasingly clear that the loss of circadian entrainment to the LD cycle is associated with a

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variety of physiological disorders pertaining not only to sleep[27–29] but also to mood[30] and cancer.[31–33]

Modeling endogenous circadian rhythms in mammals

In mammals, the mechanism of circadian rhythms relies on a genetic network involving positive and negative regulatory loops.[10] PER proteins and CRY proteins form a complex that indirectly represses the activation of the *Per* and *Cry* genes. Expression of these genes is indeed enhanced by the complex formed by the activators BMAL1 and CLOCK, products of the *Bmal1* and *Clock* genes. Binding of the PER–CRY complex to BMAL1–CLOCK prevents the expression of *Per* and *Cry* (see Fig. 1A). Moreover, *Bmal1* expression is subjected to negative autoregulation by BMAL1, via the product of the *Rev-Erbα* gene.[34] The PER–CRY complex enhances indirectly *Bmal1*

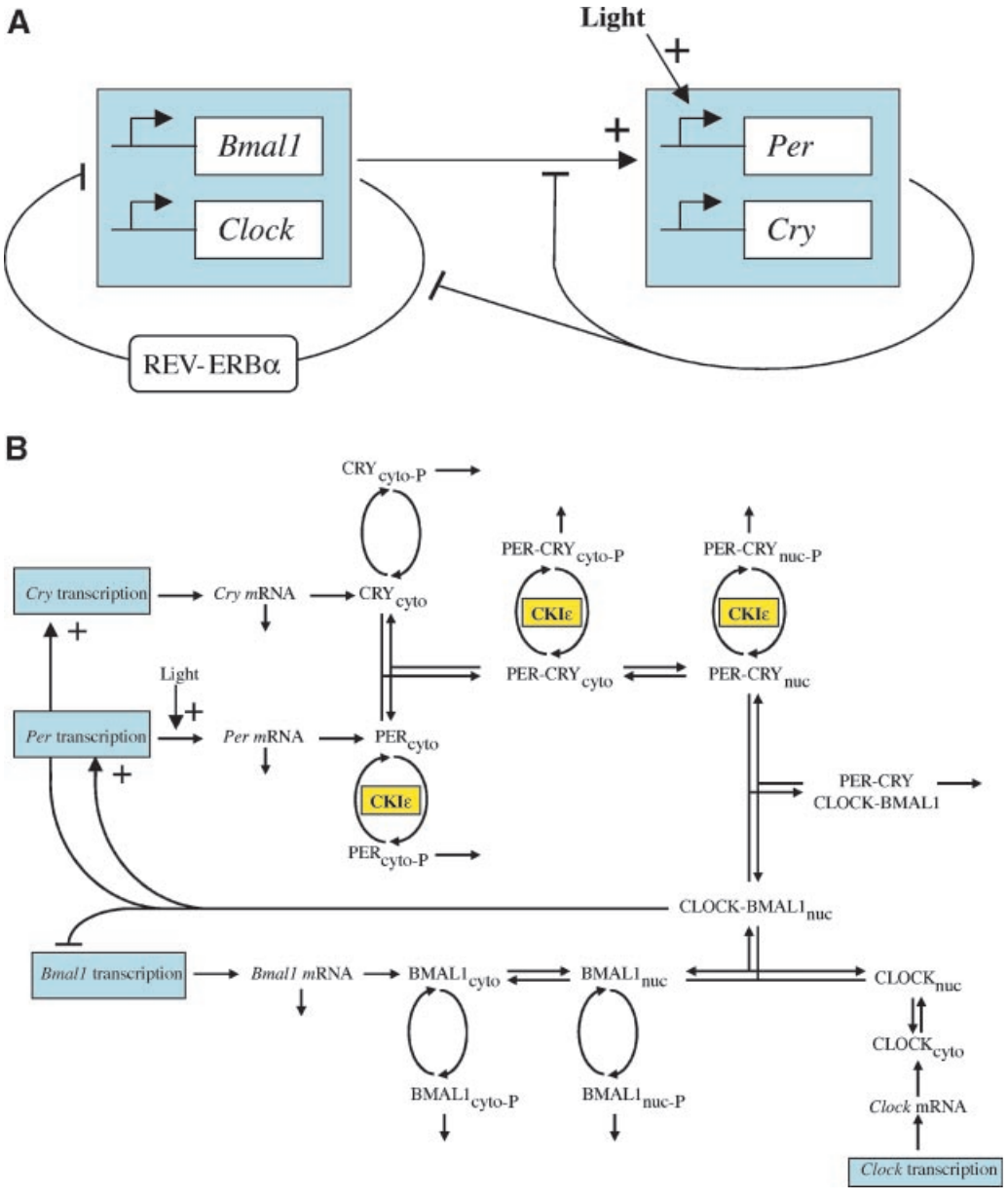

Figure 1. Model for circadian oscillations in mammals based on interlocked negative and positive regulations of the *Per, Cry, Bmal1, Clock and Rev-Erbα* genes by their protein products. **A:** Synthetic scheme of the regulatory interactions in the circadian clock gene network with the positive limb involving BMAL1–CLOCK and the negative limb involving PER–CRY. **B:** Model for the mammalian circadian clock used for investigating circadian disorders (see Ref. 23 for a detailed description).

expression by binding to BMAL1–CLOCK and thereby reducing the transcription of the *Rev-Erbα* gene.

Several computational models for the mammalian circadian clock have been proposed.[23–26] Such models throw light on the molecular mechanism of circadian oscillations, and can be used to investigate the origin of some physiological disorders related to dysfunctions of the circadian clock. To this end we will use a model of intermediate complexity (see Fig. 1B), which incorporates the regulatory effects exerted on gene expression by the PER, CRY, BMAL1 and CLOCK proteins, the regulation of these proteins by reversible phosphorylation, and the induction of *Per* expression by light.[23,24] In a light–dark cycle, the effect of light is expressed by the square-wave variation of the maximum rate of *Per* transcription (v_{sP}), which varies from a low value in the dark phase to a high value in the light phase (see Fig. 4A below). The model shows that, in conditions of continuous darkness (constant low value of v_{sP}), the regulatory interactions spontaneously produce rhythmic gene expression characterized by a circadian period. Moreover, the model accounts for entrainment by light–dark cycles, and for phase shifts induced by light pulses.

For simplicity, the model does not distinguish between the *Per1* and *Per2* genes, which are represented by a single *Per* gene; similarly *Cry1* and *Cry2* are represented by a single *Cry* gene. Moreover, as *Clock* mRNA and protein are constitutively expressed in high amounts,[16] we consider that the complex BMAL1–CLOCK only varies via BMAL1. We also treat the regulatory effect of BMAL1 on *Bmal1* expression as a direct, negative autoregulation without incorporating the role of *Rev-Erbα* (except in the case of Fig. 5E below). We previously showed that extending the model by considering explicitly the action of REV-ERBα in mediating the negative feedback by BMAL1 leads to similar conclusions.[23] (See refs. 23 and 24 for a detailed description of the molecular processes incorporated into the model and for a list of the kinetic equations governing its time evolution.).

Physiological disorders related to dysfunctions of the circadian system

Circadian rhythms have profound influences on human physiology. They control circadian variations in many hormone levels and in body temperature, as well as nutrition and the sleep–wake cycle. Mutations affecting the circadian clock may lead to physiological disorders, among which sleep disorders are of particular interest.[28,29] Thus, the familial advanced sleep phase syndrome (FASPS) is directly linked to an alteration of the circadian clock.[27] People affected by FASPS go to bed around 7:30 pm and wake up around 4:30 am, i.e. their sleep phase has a normal duration but is advanced by some 4 hours. Moreover, the autonomous period of the circadian clock is shortened by about one hour.[35] The FASPS syndrome is associated with hypophosphorylation of the human hPER2 protein by casein kinase 1ε, CK1ε.[27] The missense mutation affects the CKI-binding domain of hPER2. Recent data[36] highlight the role of another kinase that phosphorylates PER, CKIδ. Decreased activity of this kinase can also underlie the FASPS syndrome.[36] In the mirror case known as delayed sleep phase syndrome (DSPS), people go to bed and awake later than normal, while the duration of sleep is not affected.[37] As for the FASPS syndrome, a change in kinase activity could underlie DSPS.[38] The role of CK1ε appears, however, to be complex, because two distinct phosphorylations of PER by this kinase have been characterized, which have opposite effects on the period.[39,40]

While FASPS and DSPS correspond to a phase shift (delay or advance) of the sleep–wake pattern, other disorders correspond to a continuous drift in this pattern in the course of time. This is the case for the non-24 hour sleep–wake syndrome where the timing of the sleep phase changes every day (see Fig. 2 where various disorders of the sleep–wake cycle are schematized). The absence of entrainment can be related to the existence of a range of entrainment of circadian rhythms by the LD cycle. Outside the limits of entrainment the nonperiodic oscillatory behavior is often referred to as "relative coordination" (see, e.g. the book by Moore-Ede et al.[1] for a discussion of relative coordination and of the limits of entrainment, with reference to early work by von Holst, Aschoff and Pittendrigh). Finally, some irregular sleep–wake patterns are associated with neurological dysfunctions[41] or with alterations in hypocretin signaling in the case

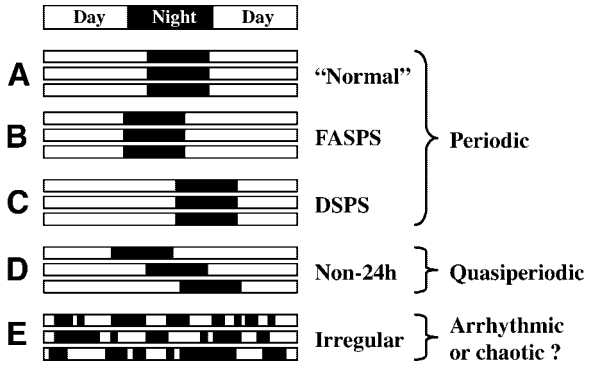

Figure 2. Schematic representation of sleep–wake cycle disorders. **A:** "Normal" circadian sleep pattern. **B:** Familial advanced sleep phase syndrome (FASPS): the duration of sleep is normal but sleep onset and offset are advanced by about 4 hours. **C:** Delayed sleep phase syndrome (DSPS): this syndrome is symmetrical to FASPS. **D:** Non-24 hour sleep–wake syndrome: the sleep–wake cycle is progressively advanced (or delayed) every day. **E:** Irregular sleep–wake patterns—such patterns may be associated with neurological disorders or narcolepsy, or with arrhythmicity or chaotic oscillations. White and black bars represent phases of activity and sleep, respectively.

of narcolepsy.[42,43] More or less irregular patterns could also arise from the lack of entrainment to the LD cycle, possibly in the form of quasiperiodic, arrhythmic or chaotic behavior (see Fig. 2). Quasiperiodic and chaotic oscillations are two types of irregular, aperiodic oscillatory behavior which differ by the fact that chaos displays a sensitivity to initial conditions, as illustrated in Fig. 5C below.

Modeling the advanced or delayed sleep phase syndromes

Given that alterations in PER phosphorylation are involved in the advanced sleep phase syndrome, we previously used the model for the mammalian circadian clock (Fig. 1B) to investigate the effect of parameter V_{phos}, which measures the maximum rate of PER phosphorylation by casein kinase Iε in this model.[23] We showed that the period of oscillations in DD rises, then decreases to a minimum, and increases again as V_{phos} increases. In the domain of entrainment, in a 12:12 LD cycle (12 hour of light followed by 12 hour of darkness), there exists a range of V_{phos} values such that when V_{phos} decreases, the autonomous period in DD is reduced and the phase in LD is advanced by a few hours, as observed in the case of FASPS.

To properly address the dynamical bases of FASPS we need, however, to extend the model to take into account the multiple phosphorylation of PER by CK1ε. A first phosphorylation of PER leads to increased protein degradation.[45] A second phosphorylation is associated with reduced nuclear clearance of the protein[44] and altered interaction with CLOCK–BMAL1 leading to enhanced *Per* transcription.[39] When we incorporate these two phosphorylations, the mammalian clock model can account for the two opposite effects described in the literature: decrease in period upon increasing the first phosphorylation by CK1ε, as observed for the *Tau* mutation,[45] and decrease in period and advance in phase upon decreasing the second phosphorylation of PER by CK1ε, as observed for FASPS.[39,44] These results will be presented in detail elsewhere.

Lack of entrainment and the non-24 hour sleep–wake syndrome

When the circadian clock operates under 12:12 LD conditions, for a given value of the light-controlled parameter (here, the maximum rate of *Per* expression), there is a domain of entrainment in which the system adopts a period of exactly 24 hours and the maxima or minima of the oscillations occur at a fixed phase. Outside the domain of entrainment, the clock free-runs and the phase of oscillations differs every day. The lack of entrainment during LD cycles can occur in two types of conditions (see Fig. 3A). Either the system operates outside the limits of entrainment, which generally extend over a few hours above and below the autonomous period in continuous darkness,[1,46] or no range of entrainment is found no matter the value of the control parameter.

The model shows that the latter situation can occur when the level of CRY protein is not sufficiently high.[23] Then, indeed, during the light phase, *Per* mRNA increases and, as a result, the level of PER protein rises. If CRY is not present in sufficient amount, free PER will accumulate because there is not enough CRY present to form a complex with PER. In such conditions, entrainment by the LD cycle fails to take place. Only when the maximum rate of *Cry* expression is sufficiently high can entrainment occur. This is illustrated by the diagram in Fig. 3A showing the region of entrainment by the LD cycle as a function of the rate of PER phosphorylation, V_{phos}, and of the rate of CRY synthesis measured by parameter k_{sC}. Below a critical value of k_{sC}, we do not find any range of V_{phos} for which entrainment by the LD cycle occurs.

To further characterize the loss of entrainability, we may fix the value of V_{phos} in Fig. 3A and determine the dynamical behavior as a function of k_{sC}. This amounts to cutting the entrainment surface by the vertical dashed line in Fig. 3A. Such a procedure yields a bifurcation diagram (Fig. 3B) where the variation of the period is plotted as a function of the rate of synthesis of the CRY protein, k_{sC}. The blue curve corresponds to the period in constant darkness (DD) while the red curve shows the period in continuous light (LL). The green dots represent the period—or, rather, the interval between two successive peaks of *Per* mRNA—in LD conditions. In this case, the system is either entrained exactly to 24 hours (horizontal green dotted line) when k_{sC} exceeds a critical value, or it fails to be entrained and the interval between peaks is different from 24 hours (green dots): this interval then varies within a range (vertical green dots) that remains in the envelope defined by the periods in DD or LD.

Entrainment and its failure are illustrated in Fig. 4A–F. On the left column, the oscillations of the *Per* mRNA (M_P) and non-phosphorylated cytosolic PER protein (P_C) are shown for three different values of the rate of CRY protein synthesis corresponding, respectively, to the values of k_{sC} indicated by points 1–3 in Fig. 3A and by arrows 1–3 in Fig. 3B. On the right column, the phase at which the peak of *Per* mRNA occurs is represented. Panel A shows the case of entrainment (arrow 1 in Fig. 3B) where the peak of the oscillations in *Per* mRNA always occurs at the same phase, i.e. in the middle of the light phase (B). Oscillations in C and E are of quasiperiodic nature. Although the system is subjected to a 12:12 LD cycle, oscillations fail to entrain to the 24 hour period but instead free-run with a slightly irregular amplitude and a period shorter or greater than 24 hours, depending on the value of k_{sC} (arrows 2 and 3 in Fig. 3B). Every day the phase of the oscillations is respectively advanced in panels C and D (where the free-running period is close to 21.5 hours) or delayed in E and F (where the free-running period is close to 25 hours). After several days, the phase of the peak of *Per* mRNA has moved through the whole 24 hour cycle. Quasiperiodic oscillations correspond to the modulation of the period,

amplitude and phase of the endogenous circadian rhythm by the external periodic signal provided by the LD cycle.

While the free-running period of the oscillations is generally close to 24 hours and varies in the envelope defined by the period in DD or LL (see Fig. 4B), the progressive shift of the phase is associated with a second, larger period of the order of tens of days or more. In Fig. 4C,E, we can clearly distinguish a daily peak of *Per* mRNA and a large oscillation over 9–10 days (C) or 24 days (E) of free PER protein (P_C). The reason why an infradian rhythm in PER develops can be related to the daily drift of the peak of *Per* mRNA synthesis through the D and L phases. When the peak of synthesis occurs in phase L, the amplitude of *Per* mRNA oscillations increases (because light induces *Per* expression) until it begins to decrease when the peak of synthesis occurs in phase D. The long period of infradian oscillations equals the time taken by the *Per* mRNA peak to cross the L and D phases.

As shown in Fig. 4C–F, the drift in phase associated with quasiperiodic oscillations corresponds to a daily advance or delay. Progressive delay is generally observed in blind subjects who free-run in LD, because their free-running period is often between 24 and 25 hours.[47] The simulations in Fig. 4D indicate that progressive advance is also possible if the autonomous period is shorter than 24 hours. Though less common, such cases have been reported in some blind subjects.[47]

Sometimes, quasiperiodic oscillations display a slowly varying drift every day followed by a rapid phase "jump" (Fig. 5A,B). Simulations of the model indicate that although the phase of the peak in *Per* mRNA never settles to a constant value, most of the time it is located in the L phase of the LD cycle. For example, in Fig. 5A,B, this peak falls in the L phase for about 18 consecutive days, then falls in the D phase during 2 consecutive days, before returning again to the L phase. The rapid passage of the *Per* mRNA peak through the D phase can be seen as a phase jump. Such jumps have been reported for a patient affected by a non-24 hour sleep–wake syndrome.[48]

Transition to chaotic oscillations

Besides periodic and quasiperiodic behavior, chaotic oscillations can also appear in LD. Then, the peak of *Per* mRNA never settles to one particular phase and oscillations are highly

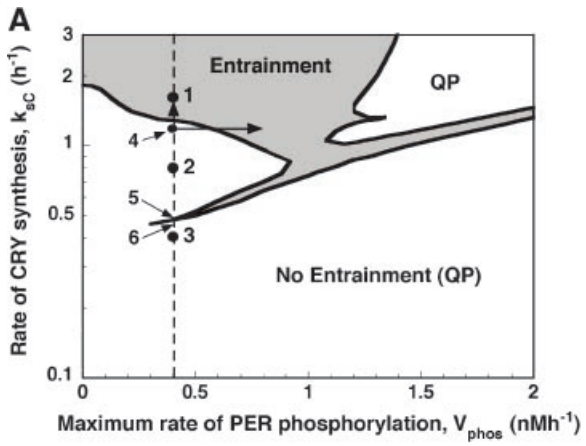

Figure 3. Domain of entrainment and of entrainment failure. **A:** Stability diagram established as a function of parameters V_{phos} (maximum rate of PER phosphorylation) and k_{sC} (rate of CRY protein synthesis), showing the different regions of periodic (entrainment) or quasiperiodic (no entrainment) oscillatory behavior in this parameter plane. The black dots marked 1, 2, and 3 refer to the three values of k_{sC} marked by arrows in Figure 3B and considered in Fig. 4A–B,C–D, and E–F, respectively. The dot marked 4 shows the starting point for circadian rhythm restoration in Fig. 6. Arrows marked 5 and 6 correspond to the conditions for long-term periodicity examined in Fig. 7, in panels A,B and C,D, respectively. The oblique narrow zone at the bottom of the entrainment domain corresponds to the case where the autonomous period in DD or LL is close to 24 hours. **B:** Bifurcation diagram showing the variation of the period as a function of k_{sC} in constant darkness (DD), constant light (LL), or in a 12:12 light–dark cycle (LD). The diagram corresponds to the cut by the vertical dashed line ($V_{phos} = 0.4$ nMh^{-1}) in Fig. 3A. The continuous lines correspond to the DD and LL conditions (blue and red lines, respectively), and green dots to oscillations in LD. In LD, the system is either entrained exactly to 24 hours (horizontal green dots on the right of the figure) or, below a critical value of k_{sC}, the system fails to be entrained and free-runs with a period different from 24 hours (green dots on the left). In the latter case, the interval between successive peaks of *Per* mRNA can vary between the values obtained in LL or DD (vertical green dots in the middle part of the figure). As in the following figures, the data are obtained by numerical integration of Eqs. 1–16 of the model for the mammalian clock.[23,24] Parameter values correspond to the basal set of values listed in Table 1 in Ref. 23. In LD conditions, as schematized in Fig. 4A, the maximum value of the rate of *Per* expression, v_{sp}, varies in a square-wave manner such that it remains at a constant low value of 1.5 nMh^{-1} during the 12 hour-long dark phase, and is raised up to the constant high value of 1.8 nMh^{-1} during the 12 hour-long light phase.

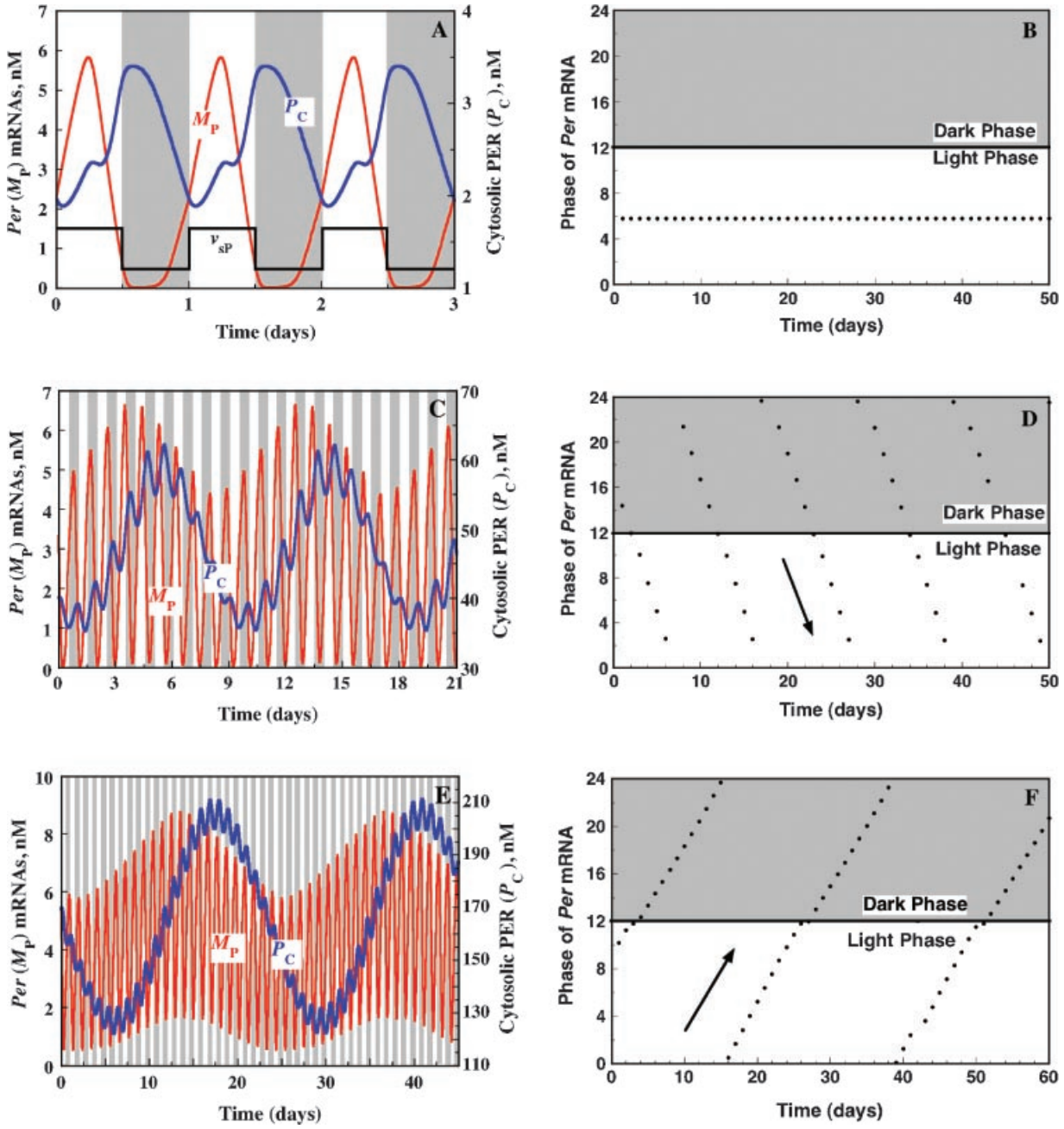

Figure 4. Entrainment or absence of entrainment by the 24 hour light–dark cycle. On the left column, the oscillations of *Per* mRNA (M_P) and unphosphorylated cytosolic PER protein (P_C) are shown for three different values of parameter k_{sC} (corresponding to points 1–3 in Fig. 3A and to the vertical arrows in Fig. 3B). Oscillations in **A** are of periodic, circadian nature, reflecting entrainment by the LD cycle, while in **C** and **E** they correspond to quasiperiodic oscillations, reflecting the absence of entrainment. On the right column, the phase of the peak of *Per* mRNA (dots) is represented as a function of time. When the system is periodic, the phase is locked to the same value (**B**) while in quasiperiodic conditions, it shifts every day either by an advance (**D**) or a delay (**F**). The square-wave variation of the rate of *Per* expression, v_{sP}, in LD is shown at the bottom of **A** (see text and legend to Fig. 3). The value of parameter k_{sC} is equal to 1.6, 0.8 or 0.4 h^{-1} for panels A–B, C–D, and E–F, respectively. Other parameter values are as in Fig. 3.

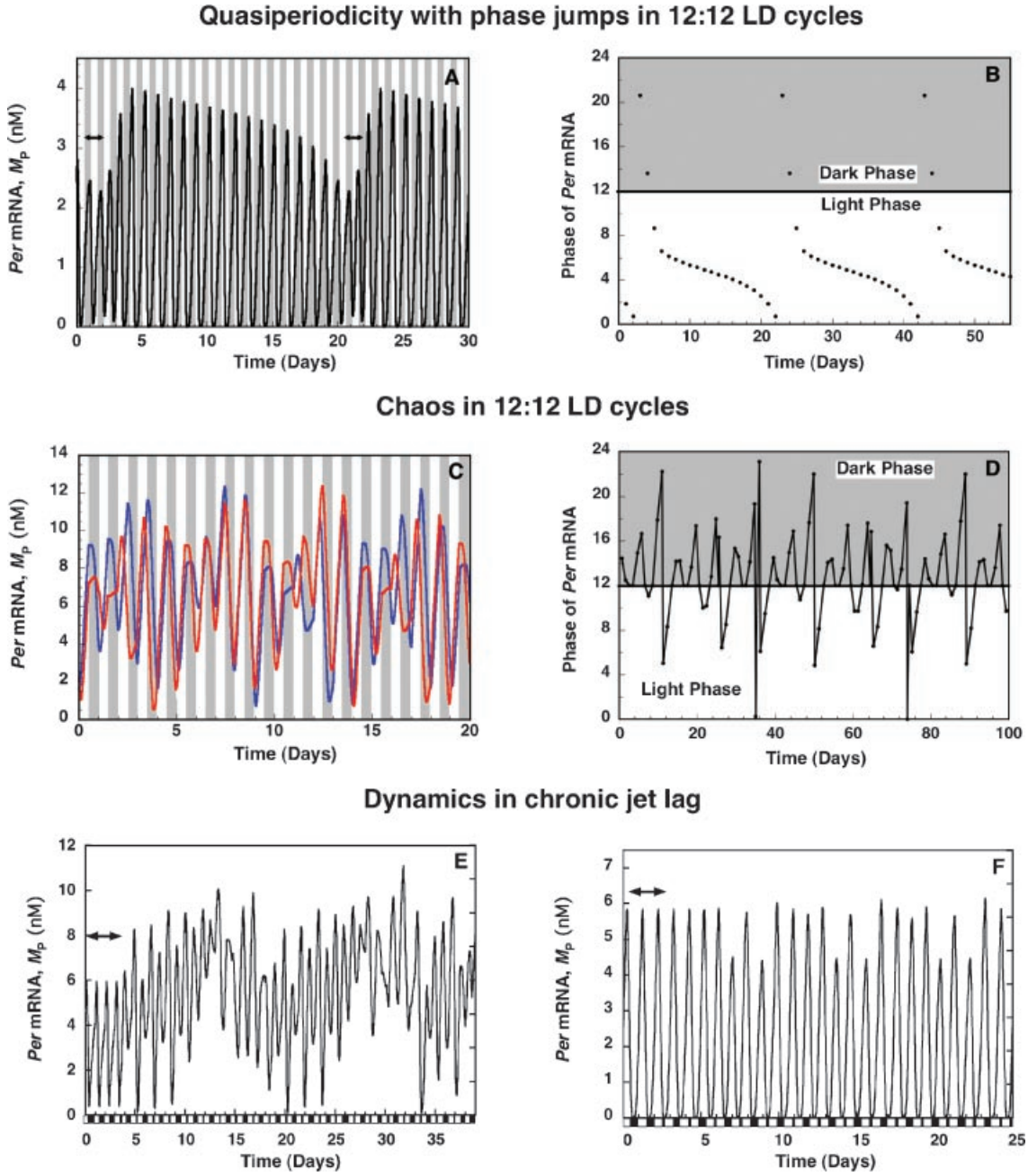

Figure 5. Complex oscillatory behavior outside the domain of entrainment. **A,B:** Quasiperiodic oscillations with phase jump in 12:12 LD cycles. **C,D:** Chaotic oscillations in 12:12 LD cycles. **E:** Chaos and **F:** quasiperiodic behavior in chronic jet lag. In **C**, the red and blue curves show the time evolution of *Per*mRNA starting from closely related initial conditions differing by the second decimal for this variable. The fact that the trajectories diverge in time demonstrates the sensitivity to initial conditions that is characteristic of chaotic oscillatory behavior. The phase pattern in **D** associated with these chaotic oscillations is highly irregular. In **E** and **F**, the first cycles (horizontal arrows) correspond to entrainment by a 12:12 LD cycle before chronic jet lag begins. Chronic jet lag is simulated by a phase advance of 8 hours every 2 days, i.e. one D phase out of two is reduced from 12 to 4 hours, while the L phase keeps a constant duration of 12 hours. Parameter values are as in Fig. 3 except for panels **A,B** where $v_{mP} = 1.45\ \mathrm{nMh^{-1}}$, and for panels **C,D** where $v_{sP} = 1.2\ \mathrm{nMh^{-1}}$ in dark phase and $1.9\ \mathrm{nMh^{-1}}$ in light phase, $v_{sC} = 1.3\ \mathrm{nMh^{-1}}$, $k_{sP} = 0.3\ \mathrm{h^{-1}}$, $k_{sC} = 0.4\ \mathrm{h^{-1}}$, $k_{sB} = 0.2\ \mathrm{h^{-1}}$, $V_{phos} = 1.1\ \mathrm{nMh^{-1}}$. The data are obtained as described in Fig. 3, except in panel **E** for which data are obtained by numerical integration of Eqs. 1–19 in ref. 23 for the parameter values listed there in Fig. 8, with $K_{IB} = 1$ nM and $v_{sP} = 2.4\ \mathrm{nMh^{-1}}$ in dark phase and $3.0\ \mathrm{nMh^{-1}}$ in light phase.

irregular (Fig. 5C). Here the phase shift alternates in an irregular manner between advances or delays (Fig. 5D). In contrast to quasiperiodic behavior, chaos is characterized by the sensitivity to initial conditions. As shown in Fig. 5C, a minute difference in the initial concentration of *Per* mRNA leads to very different patterns of oscillations after several cycles. Chaotic circadian oscillations resulting from coupling the circadian clock to the LD cycle have previously been analyzed in a theoretical model.[49] The study of a model for the *Drosophila* circadian clock has shown that the phenomenon might also occur in the form of autonomous chaos in continuous darkness.[50]

The question arises as to whether some irregular sleep–wake patterns might represent chaotic dynamics of the sort predicted by the model in Fig. 5C. Besides this possibility uncovered by the modeling approach, irregular sleep–wake patterns may originate from neurological disorders[41] or narcolepsy.[42,43] Arrhythmic behavior might also correspond to the operation of the circadian regulatory network in a non-oscillatory state. The source of irregular behavior may thus reside either in the circadian clock itself or on the path linking sleep to the circadian system.

Dynamics in chronic jet lag

Jet lag associated with rapid traveling across time zones represents one of the most common perturbations of circadian rhythmicity.[51] Many work patterns involve chronically shifting schedules. To study the pathophysiological implications of such perturbation of the circadian clock, an animal model for chronic jet lag has been developed. One of the most drastic schedules used in experiments in mice considers a phase advance of 8 hours every 2 days, i.e. one D phase out of two is reduced from 12 to 4 hours, while the L phase keeps a constant duration of 12 hours. Experiments indicate that endogenous circadian rhythms are lost and grafted tumors develop more rapidly in mice subjected to such chronic jet lag schedules.[32] The model for the mammalian circadian clock allows us to investigate the nature of the dynamical behavior of the circadian system subjected to chronic jet lag. Simulations of the model indicate that, in these conditions, the behavior becomes chaotic (Fig. 5E) or quasiperiodic (Fig. 5F). In either case, the circadian network ceases to oscillate periodically.

Restoring circadian rhythmicity: a systemic approach

If a physiological disorder such as the non-24 hour sleep–wake syndrome originates from lack of entrainment by the LD cycle, the question arises as to how to restore entrainment and circadian rhythmicity? Melatonin has been shown to be effective in restoring circadian rhythmicity in free-running, blind subjects.[52,53] The modeling approach suggests alternative ways to induce the return to circadian behavior, by acting directly on the clock machinery. Can a change in a single parameter be sufficient to switch from quasiperiodic to periodic oscillations? The bifurcation diagrams established in Fig. 3 indicate that oscillations are periodic above a threshold value of the maximum rate of CRY protein synthesis, k_{sC}, while below this threshold, CRY levels are such that quasiperiodic oscillations appear.

When the value of k_{sC} is below the threshold value, decreasing the level of PER restores rhythmicity. Because oscillations and entrainment are a systemic property, we may achieve this goal in many ways, e.g., by decreasing the level of *Per* mRNA, increasing the maximum rate of PER degradation, attenuating CLOCK–BMAL1 activation of *Per* expression, or decreasing the rate of synthesis of the protein. Altering parameters that control the levels of *Bmal1* mRNA and BMAL1 protein yields similar effects. One of the many ways to induce the transition from quasiperiodic to periodic circadian oscillations is illustrated in Fig. 6. The first part of the curve shows the quasiperiodic behavior for point 4 in Fig. 3A. The restoration of circadian oscillations in LD is shown to result from an increase (vertical arrow in Fig. 6) in the value of parameter V_{phos} (the transition corresponds to the horizontal arrow from point 4 in Fig. 3A). As indicated above, the transition can also be triggered by increasing other parameters such as the rates of CRY synthesis (vertical arrow from point 4 in Fig. 3A) or *Per* mRNA degradation.

Discussion

Adaptation of biological organisms to the periodically varying environment is the main function of circadian rhythms. In

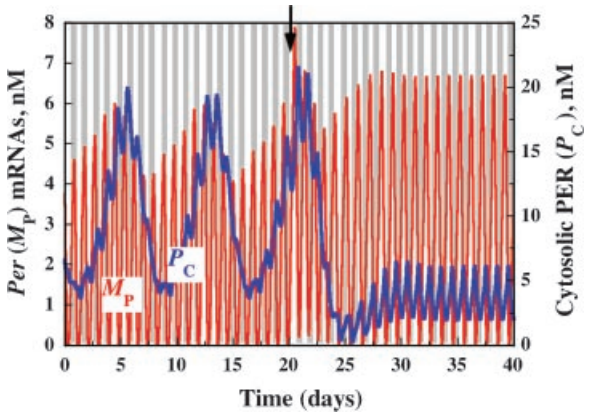

Figure 6. Restoration of circadian rhythmicity in the case of entrainment failure. Oscillations of *Per* mRNA (M_P) and unphosphorylated cytosolic PER protein (P_C) are shown in a 12:12 LD cycle. During the first 20 days, quasiperiodic behavior is observed (the situation corresponds to the value $k_{sC} = 1.2\ h^{-1}$ in Fig. 3B). Thereafter, restoration of circadian oscillations results from doubling the value of parameter V_{phos}, as schematized by the horizontal arrow starting from point 4 in Fig. 3A. Parameter values are as in Fig. 3.

humans, internal or external perturbation of the circadian clock can lead to physiological disorders, related in particular to the sleep–wake cycle.[27,32] The link between perturbation of the circadian clock at the molecular level and sleep–wake cycle syndromes was investigated here by means of a computational model for the mammalian circadian clock.[23] Besides accounting for the occurrence of autonomous circadian oscillations in continuous darkness and for entrainment by LD cycles,[23,24] computational models provide us with the unique opportunity to address not only the molecular mechanism of a key biological rhythm but also the dynamical bases of physiological disorders resulting from perturbations of the human circadian clock.[23,24,44,45] It becomes increasingly important to understand circadian dysfunctions because perturbations of the circadian clock are associated with disorders affecting not only the sleep–wake cycle but also mood[30] and cancer.[31–33]

In the Familial advanced sleep phase syndrome (FASPS), the phase of the sleep–wake cycle in LD is advanced by several hours, as a result of a decreased rate of PER phosphorylation.[27] To address the dynamical bases of FASPS, it is necessary to incorporate into the model the results from recent experimental studies, which show at least two sites of phosphorylation of PER by the kinase CKIε. We will report elsewhere how such an extended model allows us to recover the decrease in period and the phase advance associated with a decrease in PER phosphorylation by CKIε in FASPS,[39,44] as well as the effect of the decrease in period due to an increase in CKIε activity in the *Tau* mutation.[45] In *Drosophila*, mutations lowering the activity of the kinase Dbt (the equivalent of CKIε) may have opposite effects on the period.[54] Such observations could be associated with antagonistic effects similar to those observed in mammals. A further extension of the mammalian clock model is needed to

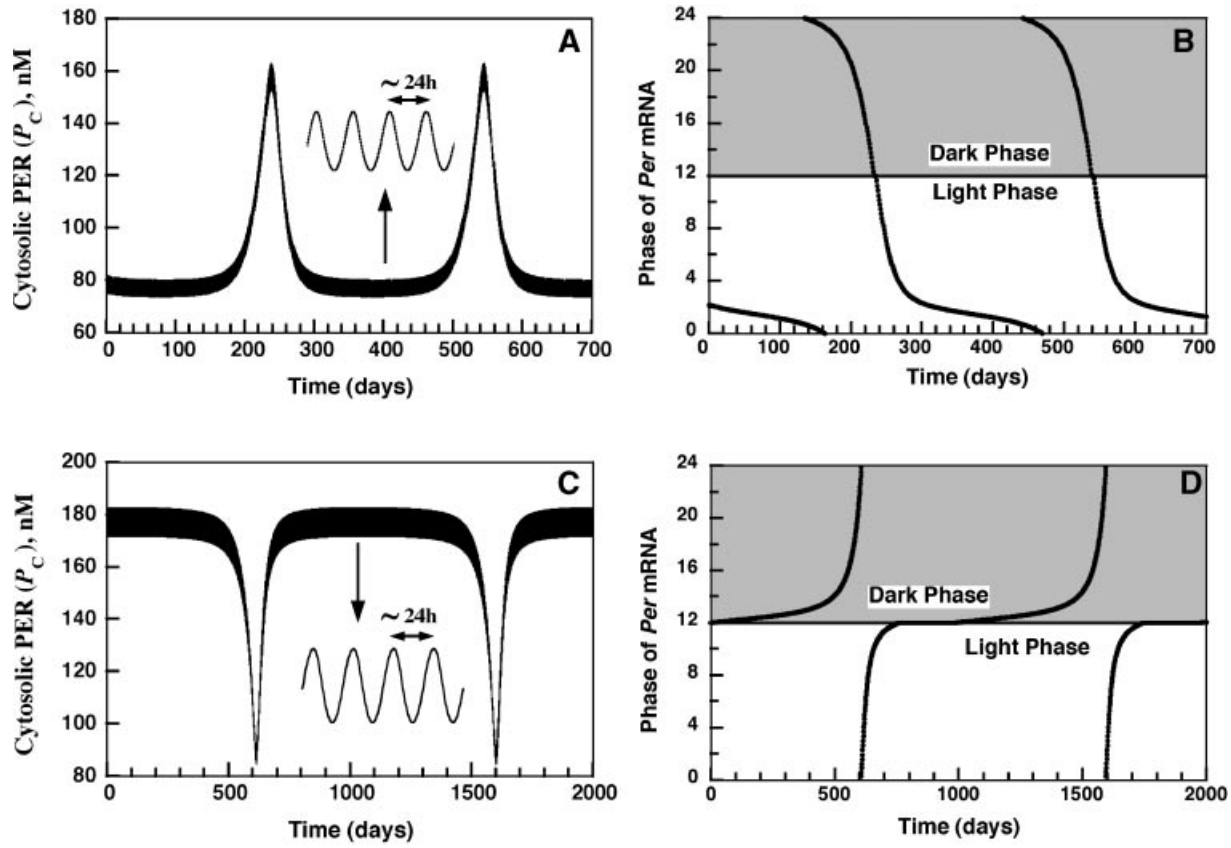

Figure 7. Prediction of very long periodic patterns outside the domain of entrainment. Quasiperiodic oscillations of very long period in LD occur in the model when the system is not entrained but free-runs with a period almost equal to 24 hours. The oscillations of unphosphorylated cytosolic PER protein (P_C) over extended periods of time are shown in **A** and **C** for two different values of k_{sC}. The corresponding variation of the phase of the peak of *Per* mRNA in the course of time is shown in **B** and **D**. The dark zones in **A** and **C** correspond to closely packed oscillations of about 24 hour period, as shown by enlargements. In panels **A,B** ($k_{sC} = 0.49\ h^{-1}$ corresponding to the location pointed by arrow 5 in Fig. 3A), the long period is close to 306 days while in **C,D** ($k_{sC} = 0.46\ h^{-1}$ corresponding to the location pointed by arrow 6 in Fig. 3A), the long period is close to 987 days (see text). Other parameter values are as in Fig. 3.

incorporate the phosphorylation by the kinase GSK3 of several clock proteins such as PER,[55] CRY,[56] and REV-ERBα.[57] The inhibition of GSK3 by lithium provides a link to explain how the latter compound is effective in treating mood disorders.[57]

We focused on disorders characterized by a lack of entrainment of the circadian clock by the LD cycle. While the non-24 hour sleep–wake syndrome is common in blind subjects who free-run in LD,[47] the model shows that the condition might also occur in sighted subjects who can perceive the LD cycle but fail to be entrained by it. Simulations suggest that the level of CRY is critical for entrainment. When the level of CRY remains too low, free PER builds up during successive light phases, as there is not enough CRY with which to form a complex; consequently, entrainment fails to occur (Figs. 3, 4). Lack of entrainment can nevertheless originate from other reasons than an insufficient level of CRY. Thus, numerical simulations show that quasiperiodic oscillations can also occur when the degradation of PER is too slow, so that the protein level builds up progressively from one light phase to the next until it starts to decrease again after days or weeks.

In the absence of entrainment in LD, the model shows that a period shorter than 24 hours leads to a phase advance every day (Fig. 4C,D) while periods longer than 24 hours cause a delay (Fig. 4E,F). Generally the drift is almost constant, as exemplified in Fig. 4 where the phase is either advanced by 2.5 hours every day (panels C,D) or delayed by 1 hour every day (panels E,F). In both cases, the progressive build up and subsequent decrease of PER is characterized by a long-term, infradian periodicity that prevents entrainment. The infradian period can be roughly computed by dividing 24 hours through the daily shift in period ΔT (in h/day), where $\Delta T = 24$ hours minus the mean period in LD (comprised between the autonomous period in LL or DD; see Fig. 3B). Thus, in Fig. 4C, the infradian period is close to $(24/2.5) = 9.6$ days, while in Fig. 4E it is close to $(24/1.0) = 24$ days.

The question arises as to what might occur in the absence of entrainment if the autonomous period is almost equal to 24 hours. The simulations of the model show that the infradian period could then reach values of up to hundreds of days or even years (Fig. 7). The phase of the clock might slowly drift and vary more abruptly at different stages of the long-period cycle. Rapid variations of the phase correspond to relatively steep increases (Fig. 7A) or decreases (Fig. 7C) in the level of a clock protein. Here again, depending on whether the free-running period is slightly below or above 24 hours, the shift in phase corresponds to an advance (see Fig. 7B, where the free-running period is 23.92 hours and the long-period is close to 306 days) or a delay (see Fig. 7D, where the free-running period is 24.02 hours and the long-period close to 987 days), respectively. The intriguing question remains open as to whether such long-period, cyclical changes predicted by the computational approach close to the domain of entrainment correspond to known disorders of the sleep–wake cycle.

The physiological disorders discussed above primarily pertain to the control exerted by the circadian clock on the sleep-wake cycle, which is also regulated by a homeostatic mechanism.[58] Beyond physiological disorders, the entrainment of circadian rhythms by the LD cycle bears, more generally, on the chronotype, which refers to the timing of sleep and wakefulness, determined by the phase of the circadian clock. The chronotype is known to vary among individuals, from "larks" to "owls", and according to age.[59] The dependence of chronotype on age and sex suggests[59] that hormones represent yet another factor that may markedly influence the pattern of entrainment of the human circadian clock.

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