Biological Rhythm Research
2001, Vol. 32, No. 2, pp. 285–298

0165-0424/01/3202-285$16.00

(Too Many) Mathematical Models of Circadian Clocks (?)

Martín A. Lema, Julián Echave and Diego A. Golombek

Universidad Nacional de Quilmes, Buenos Aires, Argentina

Abstract

Many mathematical models derived from the principles obtained from empirical observations in chronobiology have been proposed and explored. They cover several organisms and phenomena, and utilize quite different formal approaches. These models can be divided into the ones that intend to describe pacemaker core function, such the Goodwin-oscillator family, non-genetic approaches or purely mathematical (i.e., without clear biochemical correlations) models, and the ones that represent events depending on pacemaker activity, i.e., photoperiodic phenomena. We aim to illustrate the diversity of mathematical and methodological approaches to describe circadian systems and related matters.

Keywords: circadian, oscillators, mathematical models.

Introduction

Circadian clocks drive biological oscillations in biochemical, physiological, molecular and behavioral variables of all organisms, from cyanobacteria to mammals. The core of the clock mechanism seems to be a feedback loop of one or more molecular species that directly or indirectly affect their own genetic expression (Dunlap, 1999) and that can be entrained to environmental synchronizers or zeitgeber. Experimental approaches devoted to understand the nature of the molecular components of the clock have yielded important information regarding a common mechanism among organisms as varied as flies, fungi and mammals, suggesting a common ancestor of their circadian machinery. Indeed, this mechanistic similarity prompts for the design of theoretical models that may help to understand their basic properties and predict their intrinsic behavior, with the aid of mathematical models derived from the principles obtained from empirical observations, and that should be able to predict numerically the experimental data. We aim to review the diversity of mathematical and method-

Address correspondence to: Diego Golombek, Universidad Nacional de Quilmes, R. S. Peña 180, Bernal (B1876BXD), Pcia. Buenos Aires, Argentina. Fax: 54-11-4365-7132; E-mail: dgolombek@unq.edu.ar

ological approaches to the numerical aspects of circadian rhythms. We also hope to help the novice clock model maker by discussing some of the fundamental properties of these models in mathematical, biological and molecular terms.

Clock Modeling: An Overview

A mathematical model is a hypothesis expressed in the formal language of mathematics; it must summarize and be in accordance with the principles obtained from empirical observations, being able to explain and predict numerically the experimental data. The construction of a mathematical model and its numerical implementation can be the final stage of the assessment of every function and parameter of the system, or a first approach that fits the data, which is mostly the case for the circadian system(s). The value of these 'top-down' models is that, after a selection based on the comparison with experimental results, the fittest ones could give insights into undiscovered structures and functions.

Different mathematical models have been proposed recently to explain and describe circadian systems in general (Jewett & Kronauer, 1998; Vaz Nunes, 1998; Lema et al., 2000) and of specific organisms in particular (Benedito-Silva, 1997b; Pavlidis, 1981; Leloup & Goldbeter, 1998; Scheper et al., 1999a).

These models take into account recent information regarding molecular mechanisms responsible for circadian oscillations in cyanobacteria (Golden et al., 1998; Ishiura et al., 1998), fungi (Aronson et al., 1994; Crosthwaite et al., 1997; Merrow et al., 1997), flies (Darlington et al., 1998; Hunter-Ensor et al., 1996; Lee et al., 1996; Shigeyoshi et al., 1997) and mammals (Albrecht et al., 1997; Gekakis et al., 1998; Jin et al., 1999; Sangoram et al., 1998), among others.

The very early evidence of oscillations in a constant environment, damped or not, and of different free-running periods depending on the species, environmental parameters and rhythms analyzed, is enough to admit that these oscillations are not generated by a simple 'hourglass', slave of the environment. However, the presence of independent free-running rhythms (internal desynchronisation), anomalous responses to photoinduction experiments with variations in LD profile (with/without singular stimuli), among other examples, has required models where a central stable oscillator device interacts with an hourglass, a slave damped oscillator or another independent stable oscillator. Models of damping oscillators where the damping is dependent on model parameters so that it can show a profile that is stable, slightly damping, or deeply damping (which approaches it to a hourglass) oscillation have also been proposed (Benedito-Silva, 1997b; Vaz Nunes & Saunders, 1999).

The novice clock model maker will certainly find extremely useful a general panorama of the field such as Winfree's classical text (1987) or in Friesen et al. (1993). Of course, the formalities of circadian chronobiology were put forward by the work of Pittendrigh (1960, 1981, 1993) and of Pittendrigh and Daan (1976). Finally, the molecular state of the art to propose a model that pretends to describe the clock at a molecular level can be found in Dunlap (1999) and Goldbeter (1998), among others.

For modeling purposes, one of the first approaches and probably the most popular one is the use of limit cycles. A straightforward introduction to the use of them and other kinds of oscillators to model biological rhythms can be found in Glass and Mackey (1988), Lakin-Thomas (1995) and in Pavlidis (1981). Standard parameters (period, amplitude, phase, etc.) of experimental or numerical time-series data can be analyzed by several conventional methods (Benedito-Silva, 1997a; Morgan et al., 1992; Enright, 1981), but it should be noticed that other powerful approaches are arising (see Ortega et al., 1994; Van Dongen et al., 1999; Ruf, 1999).

Indeed, several mathematical models of biological rhythmic phenomena have been proposed (Benedito-Silva, 1997b): Aschoff and Wever (1976), Wever (1979), Kronauer et al. (1982), Enright (1980) and Eastman (1984) give examples of models whose mechanism is based on one or more oscillators; Borbély (1982), Daan and Beersma (1984) and Daan et al. (1984) present models whose mechanism is coupled to homeostatic properties; Beck (1980), Pittendrigh and Minis (1964), Pittendrigh (1966, 1972) developed models of photoperiodism.

With all this information in hand, it should be noticed that there has not been an effort towards converging the universe of independent models (validated by different methods, and typically intended to represent different species and phenomena) to a general model. Examples of partial exceptions to the previous statement, described next, are the work of Diez-Noguera (1994), who proposed a model that could make use of almost any of the limit cycle oscillators, Ruoff and Rensing (1996) who proposed an oscillator that pretends to be general and reproduce diverse phenomena observed in different organisms, or Lema et al. (2000), who proposed a general model aimed towards a common mechanism at a macro level, able to be implemented in diverse ways.

In the following section we aim to describe what we regard as representative examples of the conceptual and mathematical diversity of approaches to the modeling of circadian rhythms and related matters.

Models: Conceptual and Mathematical Diversity

1. The Goodwin family

The first molecular model of a biological oscillator is probably Goodwin's (1965), which includes a protein repressing the transcription of its own gene and thus causing sustained auto-oscillations. Nearly all the models that pretend to be a molecular description of the clock rest on a hypothesis based on this mechanism: a putative clock molecule (often a protein or proteins) that exerts retro-inhibition (when a time or concentration-dependent threshold is reached) on its own production.

1.1 Models restricted to Hill-type and linear equations

These models are built using only Hill-type enzymatic equations and first-order kinetic terms, with occasional additions of chemical equilibriums; this makes them more convincing from a realistic biochemical point of view.

The Goodwin model, studied by Ruoff and Rensing (1996): This model contains only three state variables: a mRNA, the protein it codes, and a product derived from or produced by this protein; this end-product, in turn, represses the mRNA synthesis. The authors (Ruoff et al., 1996; Ruoff et al., 1999a; Ruoff et al., 1999b) apply this oscillator and a slightly modified version to model circadian observations in different organisms, accounting for several phenomena like general circadian properties, mutant phenotypes, drug effects and temperature effects (temperature compensation, temperature entrainment, heat shock), showing that the model is firmly funded on molecular evidence and is able to display remarkable accordance with diverse experimental data.

Per-Tim model of Leloup and Goldbeter: This model has been thoroughly explored in many important aspects (behavior as a limit cycle, dependence on key factors, temperature compensation, chaos, biorhythmicity). Per is a *Drosophila* gene whose final product has nuclear localization where it directs the expression of other genes and itself. PER and TIM proteins oscillate in a circadian fashion, and their level of expression is subject to negative feedback of a complex between the two proteins. Homologs of these proteins have been found in mammals (Dunlap, 1999). Per is phosphorylated (Edery et al., 1994) prior to its entry to the nucleus; this could introduce an important delay. The first model (Goldbeter, 1995; Leloup & Goldbeter, 1997; Claude & Clairambault, 2000) was based only on PER dynamics. Recently, it has been improved according to experimental observations and molecular insights, incorporating the TIM protein into the dynamics (Leloup & Goldbeter, 1998, 1999; Leloup et al., 1999). Now that the interaction of these components with the cryptochrome protein has been described (Ceriani et al., 1999), a more complete model should be proposed.

The drawback is that this model looses generality while trying to describe a particular system, and still has to make many assumptions and guesses about the steps included and the parameter values, because of the limited information available.

1.2 Models that make use of other mathematical gizmos

These models make use of mathematical devices not related to classical empirical biochemistry. Their general drawback is that they are not so easy to understand for the unfamiliarized biologist; the behavior of a complex subjacent dynamics may depart from all-embracing terms (e.g., discontinuous functions or delay terms that stand for cumulative steps), and the validity of the equations may not support the whole relevant variable range (e.g., exponential functions standing for more complex non-linear terms). In contrast, their strength is the chance of simplifying the system and still explain its more relevant features even in absence of molecular evidence of the precise dynamics and biochemical data about the exact value of all the parameters.

The alternative hypothesis of Roenneberg and Merrow (1998): the authors argue that recent evidence is opposed to the usual mechanism depicted by a diagram where the input affects but is not affected by the oscillator, and the same for the oscillator and the output. What is more, the multiple features of the circadian system may be emergent characteristics of several independent cellular functions. These are very interesting arguments because many models are probably too simplistic and cannot mimic all the features of circadian rhythms.

The model they propose is something like two usual Goodwin oscillators (a protein exerts a retro-inhibition onto the synthesis of its own mRNA) that have opposite influences on each other. By rising the number of the model parameters, it is capable of displaying many features of circadian rhythms; the most prominent being temperature compensation, that probably arises from two equivalent systems having opposite effects on each other subjected to identical temperature coefficients (Ruoff, 1992; Hastings & Sweeney, 1957).

Recently, a variant of the previous model has been used in order to show that the metabolic feedback of the photosynthetic process could generate a circadian oscillation (Roennenberg & Merrow, 1999).

Delay models: A general model based on molecular data has recently been published (Scheper et al., 1999a, b), consisting of two coupled differential equations describing the kinetics of synthesis and degradation of a clock protein and of its mRNA. The innovation is that the protein kinetic equation depends on the value of the mRNA concentration a certain time before. The rationale behind this is that there are a number of factors that could give rise to an important delay between translation and the mature protein (post-translational modifications, transport across membranes, etc.).

Simplifying further, another model (Lema et al., 2000) propose a single time-delay differential kinetic equation that takes into account the expression of a clock protein, its degradation, and a time-delayed inhibition of this protein on its own expression. This allows reducing the number of parameters and variables, which facilitates analytical studies, while demonstrating that there is no need to explicitly account for the mRNA, whose role can be included in the delay.

Delay models propose a framework for the generation of circadian rhythms that is easy to understand in biological terms. The retroinhibition and delay terms stand for complex, not completely understood, and probably diverse phenomena, which makes these models useful at this particular moment when we begin to understand the molecular nature of the clock, but still lack many pieces and numbers. We may finally discover that the clock in different organisms operates by dissimilar mechanisms, but the model may still prove useful to understand all of them, if all of them share these proposed minimal features.

The more prominent disadvantages of delay models probably are that a fixed delay is not very realistic, and delay differential equations are not of widespread use and are difficult to solve analytically.

2. Non-genetic oscillators

CAM model of Blasius et al. (1997, 1998, 1999): Crassulacean acid metabolism (CAM) is a complement of classical photosynthesis (C3) that allows certain plants to concentrate CO_2 during the night (temporally fixed as malate), to avoid loss of water and photorespiration during the day. Strongly based upon both empirical evidence and mathematical analysis, the authors propose and validate a model for plant circadian oscillations that is based in the biochemical reactions of CO_2 fixation, production and utilization plus the transport mechanism of malate into and out of the plant cell vacuole.

This approach contrasts with all the other molecular models that are based on gene expression. It is a very solid and convincing model, and a huge log to the fire of the discussion about the poliphyletic origin of the circadian mechanism.

3. Abstract models

Cellular automata of Perazzo and Schuschny (1996): They propose a neural network to model an adaptive organism and simulate an evolutionary process. The hypothetical organism is thought to have sensor, motor and processing neurons whose connectivity pattern is progresively adapted with a genetic algorithm. Multiple individuals of this organism compete in a changing environment while they reproduce with mutations; their survival is decided by a fitness function calculated as a balance of numerical prizes or punishments received according to the 'behavior' displayed by the individual in reference to external conditions that vary during the day. No hypothesis over the exact circadian mechanism is proposed (nor is it relevant to this work). The authors explore the characteristics of the external stimulation, internal complexity and fitness function that could give rise to a circadian behavior. Computer simulation of evolutionary processes is a diverse emerging field that has not been fully exploited in connection with circadian rhythms.

Multioscillator model of Diez-Noguera (1994): It intends to explain develop-mental and hereditary features of rat motor activity. A nonfixed number of oscillators (located in separate cells of the nervous system) that exhibit stable limit cycles, with slightly different frequencies and have an asymmetric path shape (limit cycle) or trajectory velocity are intercommunicated by a network whose action is to reduce the differences among the instantaneous values of spatially neighbor oscillating units and impart external zeitgiving or feedback influences. Early in development the oscillators are on a free running regime and out of phase because the network is initially inactive; the network matures as the nervous system develops.

Although the model was originally proposed for a particular system, it may be generalized to other (multicellular) rhythms and species, because its characteristics do not rest in the particular set of equations defining the oscillators, but in the main functional properties of the elements included, especially the degree of communication among the oscillators.

This model and others (Acherman & Kunz, 1999) are interesting because they somehow make us raise our sight from the trees to see the forest. All the observed characteristics of circadian rhythms have been assessed in cultures, tissues or whole organisms; and many of them may be emerging properties of the interaction of a population of clocks. It remains to be explored if unicellular organisms (bacteria, yeast, algae) also display significant collective interactions or are better described by the 'lonely tree' models.

Nonrepresentational limit cycle models taken from natural sciences or abstract mathematics, like the Van der Pol oscillator or the brusselator, have often been borrowed to reproduce properties of the circadian clocks (Wever, 1972; Pavlidis, 1973; Kronauer et al., 1982; Lakin-Thomas et al., 1991; Ortega et al., 1992; Jewett & Kronauer, 1998; Jewett et al., 1999). These oscillators can certainly reach limit cycles, show entrainment and any other desired feature if refined and loaded with appropriate parameter values. But they were not originally meant to describe a circadian system; none of their relationships, functions or parameters has a physiological equivalent. They have the same value of a polynomial equation used to describe an unknown function: it can reproduce the accumulated data but it is not expected to have predictive capabilities in different ranges from those already observed and fitted, and does not really explain mechanistically the system under study. These models have the benefits of having been thoroughfully explored previously, therefore much work is saved; however, they could be replaced by other models in the cases where a growing number of known molecular insights ought to be accounted for.

The chaotic alternative: As stated previously, most models make use of a limit cycle oscillator. An interesting alternative hypothesis is presented by Lloyd and Lloyd (1993, 1994), regarding the clock as a controlled chaotic attractor. The authors claim that this model can account for the features usually demonstrated with limit cycles, but it could also explain arrhythmic states or the high variance in the period of mutants.

4. Modelling of other rhythmic phenomena

The general hypothesis is that there is a core clock ticking in the cells, which is used by subsequent slave systems to control the observable behavior of the organism. Under this assumption, we should, apart from having a fine model of the molecular clock, be able to model its control upon those secondary systems, as well as the secondary systems themselves.

Melatonin model of Brown et al. (1997): In mammals, the pineal hormone melatonin has an important role in the processing of environmental photic information, and the circadian pacemaker governs its levels. In humans the evidence is not as comprehensive as in animals, but melatonin is still regarded as a quantitative marker of the circadian pacemaker, and the temporal profile of the hormone is well character-

ized. The authors propose a fairly simple function for the pacemaker, as a hybrid of two exponential functions (the suprachiasmatic nuclei of the hypothalamus, primary mammal circadian pacemaker, acts upon the activity levels of N-acetyltransferase, the rate determining step of melatonin synthesis); and a straightforward model of the hormone plasmatic dynamics. Finally, what is more relevant to this discussion, the model is compared with human empirical data, even taking into account the assay-associated error. They obtain parameters for melatonin plasma dynamics and the underlying pacemaker. This is, in our opinion, what most clock models, that account for formal circadian properties, lack: an estimate of the model parameteres based upon a statistical analysis of experimental data.

Photoinduction model of Vaz Nunez (1998): This model seeks to explain how the time of the year, represented by the relative lengths of the photo- and scotophases, can influence insect development. Empirical evidence indicates that there is a critical night length for the photoinduction system; and relatively long and short nights seem to be assessed by different mechanisms, that even differ in their temperature sensitivity. Moreover, sometimes the mechanism seems to depend on a hourglass, sometimes on an oscillator.

The author proposes a model comprising two oscillators with different periods, not coupled to each other, and displaying variable damping. The onset of light during a particular phase of the oscillators generates a value that is stored in the 'counter', and long or short scotophases have opposite contributions to a sum, whose final value, after a number of periods, determines induction of diapause.

The oscillators used are based on a control system model developed by Gander and Lewis (1979). The concentration at a given time of a hypothetical chemical is a function of its immediately previous quantity, light intensity, and the difference between a reference value and the time delayed value of the chemical, so that we have, again, some sort of a delay model.

Thermoregulatory model of sleep control in humans by Nakao et al. (1995, 1999): The underlying hypothesis is that the non-rapid eye-movement sleep (NREM) is strongly affected by thermoregulatory feedback mechanisms. Certain warm-sensitive hypothalamic neurons, with a hypnogenic (sleep inducing) role, would be capable of amalgamate the thermoregulation and sleep control. The system also exhibits a 'heat memory', representing deviations from the temperature set by thermoregulation processes, being this difference produced by heat load and loss associated with sleep-wake cycles.

A primary 24 h sinusoidal oscillator and the heat memory control the 'set' body temperature. The dynamics of the effective body temperature (in the neurons) is a function of this set temperature and masking effects due to heat loss or gain during sleep or wake, respectively. Integration over the past values of the difference between the body temperature and the set temperature gives the heat memory (with more recent values having the largest weight on the integration). Finally, the sleepiness is controlled by another sinusoidal oscillator (phase shifted to the first one), the heat

memory and the set and body temperature. The value of the sleepiness determines (via thresholds) the sleep-wake cycles that in turn affect (via two separate functions for each state) the masking effects on the body temperature, closing the circle.

The authors promise to refine the model including non-entrained situations and account for the effects of light perception, which will require describing the oscillator dynamics instead of caricaturing it with sinusoidal functions, and incorporating melatonin rhythms. For further comments on this model and the work of Borbély (1982), read Dijk and Kronauer (1999).

Conclusions

A plethora of models of circadian clocks can be found in the literature. They have been intended to represent different species and phenomena (suprachiasmatic nuclei activity, mice locomotor activity, insect pupal eclosion, etc.) and have been validated in a dissimilar fashion (simulating plausible time series data and PRCs, emulating temperature compensation, fitting them to experimental data, etc.). These models are appropriate to describe dissimilar aspects (entrainment, photoinduction, evolution) and represent the circadian phenomena with different degrees of biological realism. Now it is about time to establish a model that can do all of that.

This new brave model would have a set of equations to describe the core clock, with simple and general equations, to make easier the interpretation, mathematical analysis, application to different species and parameter estimation. All of the parameters should be readily translatable to biological or physiological equivalent terms. To validate this stage, the time plot of at least one of the molecular species invoked must be quantifiable in experimental assays. Maybe the model that is closest to this end is the one of Ruoff and Rensing (1996). On the other hand, the price in complexity of such a ‘supermodel’ could happen to be very high, so a compromise between range and usefulness may be necessary.

To explore more complex circadian behavior (locomotor activity, pupae eclotion, hormone profiles, etc.), a second stage in the model would be necessary, with the same requierements as above. The entire model must be validated by prediction and experimental verification, and, if possible, applied to practical aspects of chornobiology. Such a model could even shed some light into the origins of circadian rhythms and how they evolved from non-autonomously rhythmic biochemical systems, by evolution simulation on a computer.

So, are these models useful for anything? It is true that many models have not been completely developed up to the point that they can be used to represent real-life situations. However, on their behalf, it should be said that there is a strong feedback interaction between modeling biological data and experimental design, in such a way that successful models could become an extremely useful tool to gather more data, which could in turn generate new models. And so on.

Acknowledgements

This work was supported by the Universidad Nacional de Quilmes, ANPCyT and Fundación Antorchas. JE and DAG are members of CONICET. We are grateful to Dr. A. Diez-Noguera for his useful comments on the manuscript. Martín Lema is a fellow of the Scientific Research Commission of Buenos Aires (CIC).

References

Achermann P, Kunz H (1999): Modelling circadian rhythm generation in the suprachiasmatic nucleus with locally coupled self-sustained oscillators: phase shifts and phase response curves. *J Biol Rhytms 14*: 460–468.

Albrecht U, Sun ZS, Eichele G, Lee CC (1997): A differential response of two putative mammalian circadian regulators, mPer1 and mPer2, to light. *Cell 91*: 1055–1064.

Aronson BD, Johnson KA, Loros JJ, Dunlap JC (1994): Negative feedback defining a circadian clock: autoregulation of the clock gene frequency. *Science 263*: 1578–1584.

Aschoff J, Wever R (1976): Human circadian rhythms; a multioscillatory system. *Federation Proc 35*: 2326–2332.

Beck SD (1980): *Insect photoperiodism, 2nd Ed*. New York, Academic Press.

Benedito-Silva AA (1997a): Aspectos metodológicos de la cronobiología. In: Marques N, Menna-Barreto L, Golombek D, eds., *Cronobiología: principios y aplicaciones*. Buenos Aires, Editorial Universitaria de Buenos Aires, pp. 239–261.

Benedito-Silva AA (1997b): El modelado de los ritmos biológicos. In: Marques N, Menna-Barreto L, Golombek D, eds., *Cronobiología: principios y aplicaciones*. Buenos Aires, Editorial Universitaria de Buenos Aires, pp. 111–125.

Blasius B, Neff R, Beck F, Lüttge U (1997): A model for photosynthetic oscillations in crassulacean acid metabolism. *J Theor Biol 184*: 345–351.

Blasius B, Neff R, Beck F, Lüttge U (1998): Oscillatory model of crassulacean acid metabolism: structural analysis and stability boundaries with a discrete histeresis switch. *Pl Cell Env 21*: 775–784.

Blasius B, Neff R, Beck F, Lüttge U (1999): Oscillatory model of crassulacean acid metabolism with a dynamic hysteresis switch. *Proc R Soc Lond 266*: 93–101.

Borbély AA (1982): A two process model of sleep regulation. *Human Neurobiology 1*: 195–204.

Brown EN, Choe Y, Shanaham TL, Czeisler CA (1997): A mathematical model of diurnal variations in human plasma melatonin levels. *Am J Physiol 272*: E506–E516.

Ceriani MF, Darlington TK, Staknis D, Mas P, Petti AA, Weitz CJ, Kay SA (1999): Light-dependent sequestration of timeless by cryptochrome. *Science 285*: 553–556.

Claude D, Clairambault J (2000): Period shift induction by intermittent stimulation in a Drosophila model of Per protein oscillations. *Chronobiol Int 17*: 1–14.

Crosthwaite SK, Dunlap JC, Loros JJ (1997): Neurospora wc-1 and wc-2: transcription, photoresponses, and the origins of circadian rhythmicity. *Science 276*: 763–769.

Daan S, Beersma DGM (1984): Circadian gating of human sleep-wake cycles. In: Moore-Ede MC, Czeisler CA, eds., *Mathematical Models of the circadian sleep-wake cycle*. New York, Raven Press.

Daan S, Beersma DGM, Borbély AA (1984): Timing of human sleep: recovery process gated by a circadian pacemaker. *Am J Phisiol 246*: R161–R178.

Darlington TK, Wager-Smith K, Ceriani MF, Staknis D, Gekakis N, Steeves TDL, Weitz CJ, Takahashi JS, Kay SA (1998): Closing the circadian loop: clock-induced transcription of its own inhibitors per and tim. *Science 280*: 1599–1603.

Diez-Noguera A (1994): A functional model of the circadian system based on the degree of intercommunication in a complex system. *Am J Physiol 36*: R1118–R1135.

Dijk D-J, Kronauer RE (1999): Commentary: models of sleep regulation: Successes and continuing challenges. *J Biol Rhytms 14*: 569–573.

Dunlap JC (1999): Molecular bases for circadian clocks. *Cell 96*: 271–290.

Eastman C (1984): Are separate temperature and activity oscillators necessary to explain the phenomena of human circadian rhythms? In: Moore-Ede MC, Czeisler CA, eds., *Mathematical Models of the circadian sleep-wake cycle*. New York, Press Raven.

Edery I, Zwiebel LJ, Dembiska ME, Rosbash M (1994): Temporal phosphorylation of the Drosophila period protein. *Proc Nat Acad Sci USA 91*: 2260–2264.

Enright JT (1980): The timing of sleep and wakefulness. In: *Studies in Brain function, vol 3*. Berlin, Springer-Verlag.

Enright JT (1981): Data Analysis. In: Aschoff J, ed., *Handbook of behavioural neurobiology vol. 4: Biological Rhythms*. New York, Plenum Press, pp. 41–54.

Friesen WO, Block GD, Hocker CG (1993): Formal approaches to understanding biological oscillators. *Annu Rev Pysio 55*: 661–681.

Gander PH, Lewis RD (1979): The circadian locomotor activity rhythm of Hemideina thoracica (Orthoptera): A feedback model for the underlying clock oscillation. *Int J Chronobiol. 6*: 263–280.

Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, Takahashi JS, Weitz CJ (1998): Role of the clock protein in the mammalian circadian mechanism. *Science 280*: 1564–1569.

Glass L, Mackey MC (1988): *From clocks to chaos*. Princeton, Princeton University Press.

Goldbeter A (1995): A model for circadian oscillations in the *Drosophila* period protein (PER). *Proc R Soc Lond B 261*: 319–324.

Goldbeter A (1998): Les bases moléculaires des rythmes biologiques. Chimie Nouvelle *16*: 1871–1878.

Golden SS, Johnson CH, Kondo T (1998): The cyanobacterial circadian system: a clock apart. *Curr Opin Microbiol 1*: 669–673.

Goodwin BC (1965): Oscillatory behavior in enzymatic control processes. *Adv in Enzyme regul 3*: 425–438.

Hastings JW, Sweeney BM (1957): On the mechanism of temperature independence in a biological clock. *Proc Natl Acad Sci USA 43*: 804–811.

Hunter-Ensor M, Ousley A, Sehgal A (1996): Regulation of the Drosophila protein timeless suggests a mechanism for resetting the circadian clock by light. *Cell 84*: 677–685.

Ishiura M, Kutsuna S, Aoki S, Iwasaki H, Andersson CR, Tanabe A, Golden SS, Johnson CH, Kondo T (1998): Expression of a gene cluster kaiABC as a circadian feedback process in cyanobacteria. *Science 281*: 1519–1523.

Jewett ME, Forger DB, Kronauer RE (1999): Revised limit cycle oscillator model of human circadian pacemaker. *J Biol Rhytms 14*: 493–499.

Jewett ME, Kronauer RE (1998): Refinement of a limit cycle oscillator model of the effects of light on the human circadian pacemaker. *J Theor Biol 192*: 455–465.

Jin X, Shearman L, Weaver D, Zylka M, DeVries G, Reppert S (1999): A molecular mechanism regulating output from the suprachiasmatic circadian clock. *Cell 96*: 57–68.

Kronauer RE, Czeisler CA, Pilato SF, Moore-Ede MC, Weitzman CD (1982): Mathematical model of the human circadian system with two interacting oscillators. *Am J Physiol 242*: R13–R17.

Lakin-Thomas PL (1995): A beginner's guide to limit cycles, their uses and abuses. *Biol Rhy Res 26(2)*: 216–232.

Lakin-Thomas PL, Brody S, Coté GG (1991): Amplitude model for the effects of mutations and temperature on period and phase resetting of the Neurospora circadian oscillator. *J Biol Rhythms 6*: 281–297.

Lee C, Parikh V, Itsukachi T, Bae K, Edery I (1996): Resetting the Drosophila clock by photic regulation of PER and a PER-TIM complex. *Neuron 21*: 857–867.

Leloup JC, Goldbeter A (1997): Temperature compensation of circadian rhythms: control of the period in a model for circadian oscillations of the PER protein in Drosophila. *Chronobiol Int 14*: 511–520.

Leloup JC, Goldbeter A (1998): A model for circadian rhythms in Drosophila incorporating the formation of a complex between per and tim proteins. *J Biol Rhythms 13*: 70–87.

Leloup JC, Goldbeter A (1999): Chaos and birhytmicity in a model for circadian oscillations of the PER and TIM proteins in Drosophila. *J Theor Biol 198*: 445–459.

Leloup JC, Gonze D, Goldbeter A (1999): Limit cycle models for circadian rhytms based on transcriptional regulation in Drosophila and Neurospora. *J Biol Rhytms 14*: 433–448.

Lema M, Golombek DA, Echave J (2000): A simple delay model system of the circadian pacemaker. *J Theor Biol 204*: 565–573.

Lloyd AL, Lloyd D (1993): Hypotesis: the central oscillator of the circadian clock is a controlled chaotic attractor. *Biosystems 29*: 77–85.

Lloyd D, Lloyd AL (1994): Hypothesis: a controlled chaotic attractor constitutes the central oscillator of the circadian clock. *Biochem Soc Trans 22*: 322S.

Merrow MW, Garceau NY, Dunlap JC (1997): Dissection of a circadian oscillation into discrete domains. *Proc Nat Acad Sci USA 94*: 3877–3882.

Morgan E, Díez-Noguera A, Minors D (1992): Methods of rhythm analysis in a biological time-series. *Journal of Interdisciplinary Cycle Research 23*: 199–201.

Nakao M, McGinty D, Szymusiak R, Yamamoto M (1995): A termoregulatory model of sleep control. *Jpn J Physiol 45*: 291–309.

Nakao M, McGinty D, Szymusiak R, Yamamoto M (1999): Termoregulatory model of sleep control: losing the heat memory. *J Biol Rhytms 14*: 547–556.

Ortega GJ, Golombek DA, Otero D, Romanelli L, Cardinali DP (1992): Effect of zeitgeber intensity reduction on a simulated dual-oscillator human circadian system: classical and dynamic analysis. *Chronobiology International 9*: 137–146.

Ortega GJ, Romanelli L, Golombek DA (1994): Statistical and dynamical analysis of circadian rhythms. *J Theor Biol 169*: 15–21

Pavlidis T (1973): *Biological oscillators: their mathematical analysis*. New York, Academic Press.

Pavlidis T (1981): Mathematical models. In: Aschoff J, ed., *Handbook of behavioural neurobiology vol. 4: Biological Rhythms*. New York, Plenum Press, pp. 41–54.

Perazzo RP, Schuschny A (1996): An adaptive Boolean automation to model circadian cycles. *Int J Neural Syst 7*: 83–99.

Pittendrigh CS (1960): Circadian rhythms and the circadian organization of living systems. *Cold Spring Harb Symp Quant Biol 25*: 159–184.

Pittendrigh CS (1966): The circadian oscillation in *Drosophila pseudoobscura* pupae: A model for the photoperiodic clock. *Z Pflanzenphysiol 54*: 275–307.

Pittendrigh CS (1972): Circadian surfaces and the diversity of possible roles of circadian organization in photoperiodic induction. *Proc Natl Acad Sci USA 69*: 2734–2737.

Pittendrigh CS (1981): Circadian systems: Entrainment. In: Aschoff J, ed., *Handbook of behavioral neurobiology. Vol IV: Biological Rhythms*. New York, Plenum Press, pp. 95–124.

Pittendrigh CS (1993): Temporal organization: reflections of a Darwinian clock-watcher. *Annu Rev Physiol 55*: 16–54

Pittendrigh CS, Daan S (1976): A functional analysis of circadian pacemakers in nocturnal rodents. IV: Entrainment: Pacemaker as clock. *J Comp Physiol A 106*: 291–331.

Pittendrigh CS, Minis DH (1964): The entrainment of circadian oscillations by light and their role as photoperiodic clocks. *Amer Nat 98*: 261–294.

Roenneberg T, Merrow M (1998): Molecular circadian oscillators: an alternative hypothesis. *J Biol Rhythms 13*: 167–179.

Roenneberg T, Merrow M (1999): Circadian systems and metabolism. *J Biol Rhytms 14*: 449–459.

Ruf T (1999): The Lomb-Scargle periodogram in biological rhythm research: analysis of incomplete and unequally spaced time-series. *Biol Rhy Res 30*: 178–201.

Ruoff P (1992): Introducing temperature-compensation in any reaction kinetic oscillator model. *J Interdiscipl Cycle Res 23*: 92–99.

Ruoff P, Mohsenzadeh S, Rensing L (1996): Circadian rhythms and protein turnover: the effect of temperature on the period lengths of clock mutants simulated by the Goodwin oscillator. *Naturwissenschaften 83*: 514–517.

Ruoff P, Rensing L (1996): The Temperature-Compensated Goodwin Model Simulates Many Circadian Clock Properties. *J Theor Biol 179*: 275–285.

Ruoff P, Vinsjevik M, Mohsenzadeh S, Rensing L (1999a): The Goodwin model: simulating the effect of cycloheximide and heat shock on the sporulation rhythm of Neurospora crassa. *J Theor Biol 196*: 483–494.

Ruoff P, Vinsjevik M, Monnerjahn C, Rensing L (1999b): The Goodwin oscillator: on the importance of degradation reactions in the circadian clock. *J Biol Rhytms 14*: 469–479.

Sangoram AM, Saez L, Antoch MP, Gekakis N, Staknis D, Whiteley A, Fruechte EM, Vitaterna MH, Shimomura K, King DP, Young MW, Weitz CJ, Takahashi JS (1998): Mammalian circadian autoregulatory loop: a timeless ortholog and mPer1 interact and negatively regulate clock-bmal1-induced transcription. *Neuron 21*: 1101–1113.

Scheper TO, Klinkenberg D, Pelt JV, Pennartz C (1999b): A model of molecular circadian clocks: multiple mechanisms for phase shifting and a requirement for strong nonlinear interactions. *J Biol Rhythms 14(3)*: 213–220.

Scheper TO, Klinkenberg D, Pennartz C, Pelt JV (1999a): A mathematical model for the intracellular circadian rhythm generator. *J Neurosci 19*: 40–47.

Shigeyoshi Y, Taguchi K, Yamamoto S, Takeida S, Yan L, Tei H, Moriya T, Shibata S, Loros JJ, Dunlap JC, Okamura H (1997): Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer transcript. *Cell 91*: 1043–1053.

Van Dongen HPA, Olofsen E, VanHartevelt JH, Kruyt EW (1999): A procedure of multiple period searching in unequally spaced time-series with the Lomb-Scargle method. *Biol Rhy Res 30*: 149–177.

Vaz Nunes M (1998): A double circadian oscillator model for quantitative photoperiodic time measurement in insect and mites. *J Theor Biol 194*: 299–311.

Vaz Nunes M, Saunders D (1999): Photoperiodic time measurement in insects: a review of clock models. *J Biol Rhythms 14*: 84–104.

Wever RA (1972): Virtual synchronization towards the limits of the range of entrainment. *J Theor Biol 36*: 119–132.

Wever RA (1979): *The circadian system of man: Results of experiments under temporal isolation*. Berlin, Springer-Verlag.

Winfree AT (1987): *The timing of biological clocks.* New York, Scientific American Books.