

Computational Modeling and Mechanistic Explanation: Understanding Circadian Rhythms

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Computational modeling and research on mechanisms both play important roles in the attempts to understand phenomena in the cognitive sciences and in the life sciences generally. What is the connection between them? Often they seem to have little to do with one another. Sometimes this is due to the state of development of empirical inquiries. When little is known about the particulars of the mechanism responsible for a phenomenon, as in the case for many cognitive phenomena (problem solving, language use, etc.), modeling proceeds with little information about the actual parts and operations within the brain responsible for them. The models often serve as preliminary guesses as to the mechanism. In these cases modeling is often the only way to discover anything about the mechanism (whether a certain kind of mechanism might be required to account for the phenomena or is a plausible candidate to do so). But in other domains empirical research has succeeded in providing considerable information about the parts, operations, and organization of the responsible mechanism. Models are not needed to launch the inquiry but serve different roles. One is simply to assess whether the parts and operations identified are adequate to explain the phenomena. Another is to identify which parts of the mechanism serve to explain a given aspect of the phenomenon. A third role is to discover gaps in the current knowledge of the mechanisms and make suggests for further empirical research. A further set of uses arises when researchers need to understand not just the operations within a given mechanism but also between mechanisms. The complexity of such processes raises the prospect that one set of operations will disrupt others and modeling is required to ascertain what will be the overall behavior.

In this paper I focus on computational modeling of the oscillators responsible for circadian rhythms, the approximately 24 rhythms that are exhibited in a wide range of physiological functions (e.g., body temperature), behavioral phenomena (e.g., locomotor activity), and even cognitive activities (as exhibited in, e.g., reaction times in performing cognitive tasks). The ability of organisms to keep time, even when deprived of external cues such as exposure to sunlight, has fascinated investigators since ancient times, but figuring out how they do so proved immensely challenging. Systematic study of these phenomena only began in the middle of the 20th century as biologists from a number of specialties (evolution, genetics, biochemistry, physiology, etc.) began to create a distinct field of chronobiology.¹ Research in this period focused on delineating the phenomenon to be explained: detailing features of circadian rhythms (e.g., how they change with environmental stimuli) and measuring them. Since circadian

¹ Chronobiology concerns itself not just with circadian rhythms but a wide range of rhythms ranging from those with periods of only seconds or minutes to those with periods of months and years. The former are often referred to as *ultradian* and the later as *infradian*.

phenomena are exhibited in all organisms in which measurement has been possible, including single-celled cyanobacteria, it seemed likely that the critical oscillator was intracellular. Yet, as of 1995 only two genes and their related proteins, *per* in *Drosophila* and *frq* in *Neurospora* had been identified. In the ensuing decade, however, a host of genes and proteins involved in the clock mechanisms of different species were discovered. Like a child who takes apart a mechanical clock, researchers suddenly had in front of them a host of clockworks. The challenge now was to explain how they all worked together as a mechanism to produce the phenomenon previously characterized.²

Attempts to develop mathematical models of circadian phenomena developed simultaneous with their systematic delineation. But as components of the mechanisms were identified, modelers began to incorporate them into their models so as to understand whether and how mechanisms with such components could produce rhythms. These models will be the focus of my discussion, although I will also briefly characterize approaches to modeling circadian rhythms that do not focus on the components of the mechanism. I will limit my discussion to models of circadian rhythms in *Drosophila* and mammals, although extensive research has also been done on plants, bacteria, and fungi. One of the aspects of circadian rhythm generation in vertebrates that has attracted significant attention is that while individual cells seem to generate their own rhythms, the periods of these rhythms vary considerably and must be synchronized to produce a reliable time signal to the rest of the organism. Thus, in addition to modeling efforts directed at the intracellular mechanism I will examine models of synchronization of oscillation between cells. In addition, the population of cells in the SCN is not homogeneous and different regions oscillate somewhat out of synchrony with each other. There are also populations of circadian oscillators in other organs of the body whose oscillations are coordinated with those of the SCN (usually lagging considerably behind the oscillation in the SCN). The disruption of the coordination of these populations of oscillators is manifest in phenomena such as jet lag and one exciting domain of inquiry has been to model interactions between different populations of oscillators and how they become disrupted. Before examining the modeling of these phenomena, though, I need to say a bit more about mechanistic explanation and the distinctive challenges it poses for computational modeling.

1. Mechanisms and Mechanistic Explanation

Many philosophical presentations of cognitive science (and other sciences) continue to focus on laws as the explanatory vehicle. Laws are commonly construed as universal generalizations that have a modal status—they identify not just what has happened when particular conditions are met, but what must happen under those conditions. But cognitive scientists, and indeed life scientists generally, seldom identify laws and when they do (in psychology, typically referring to them as *effects*), they generally serve to characterize the phenomenon to be explained (Cummins, 2000). When they advance explanations, life scientists commonly seek to identify the mechanism responsible for the phenomenon of interest. Recently, a number of philosophers whose focus has been largely on biology have attempted to characterize what scientists mean by a mechanism and

² One of the things that often happens in research on mechanism is that one's characterization of the phenomena itself changes (Bechtel & Richardson, 1993, characterized this as reconstituting the phenomenon). I will not be able to focus on this here, but it is one of the possible results of modeling to reveal different aspects of the phenomena not previously discovered and thereby change the explanatory target.

how they go about developing and evaluating mechanistic explanations. My own account is that “a mechanism is a structure performing a function in virtue of its component parts, component operations, and their organization” (Bechtel & Abrahamsen, 2005; for related accounts, see Bechtel & Richardson, 1993; Glennan, 2002, 1996; Machamer, Darden, & Craver, 2000; Thagard, 2006).

The appeal to mechanisms to explain phenomena is in part a legacy of the mechanistic philosophy of the 17th century when Descartes and later Boyle appealed to such features as the shapes and movement of the corpuscles comprising objects to explain their behavior. But mechanism took on a life of its own as biology developed. Many investigators attempting to explain various behaviors or properties of living systems sought to decompose organisms into their components and to explain their biological properties in terms of those components. This tradition of research was opposed by other biologists who contended that the specifically biological features of living things could not be explained from their physical constitution (alone) and that biology could go no further than to identify the vital properties or processes characteristic of life. Those who pursued the mechanistic project, though, developed powerful techniques for decomposing living systems into their parts and determining the operations they performed. Beginning in the 19th century they started to identify the parts chemically and characterize the operations as chemical reactions, often promoted by a catalyst or enzyme (Bechtel, 2006). In the second half of the 20th century tools for identifying genes and the proteins for which they coded supplemented the project. In the course of these inquiries biologists significantly expanded the set of properties of parts to which they could appeal beyond shape and motion to which Descartes had appealed (although these have remained surprisingly important—the catalytic capacities of enzymes typically depend upon their shape and the manner in which their components move), but have maintained his vision of explaining biological processes by identifying the relevant mechanisms.

Accounts of mechanistic explanation always allude to the importance of how the components are organized, but this has been the least developed aspect of both philosophical accounts of mechanistic explanation and indeed of mechanistic science. Although mechanistic explanation depends upon identifying components whose operations do not produce the phenomenon themselves but only in collaboration with other parts performing different operations (otherwise there is no explanatory gain), generally only the simplest way of linking the components are envisaged (e.g., linear strings as in assembly lines). But, as biological theorists from Bernard on have recognized, it is the distinctive organization in biological mechanisms that enable them to exhibit the properties of living organisms. First negative feedback and later positive feedback and self-organizing cycles have enriched the ability of biologists to understand distinctive features of living systems (Bechtel, 2007). But these modes of organization often result in complex dynamical behavior, including oscillations, which cannot typically be predicted without computational modeling.

2. Modeling and Mechanism in Cognitive Science

The term *model* is used in a wide variety of way. Since it is not the mechanism itself operating in the world that is invoked in the explanation, but an account of it, any mechanistic explanation must appeal to a model. The range of things counted as models includes scale models (Waskan,

2006), conceptual models (often presented in diagrams as well as in linguistic descriptions), and mathematical and computational models. In this paper I am primarily concerned with mathematical and computational models which propose quantitative relations between parameters and variables and ways in which these change over time. There are different functions such models play. Often they serve to identify relationships within the phenomenon to be explained but make no attempt to characterize the mechanism. On other occasions they are intended to characterize the operations within the mechanism responsible for the phenomenon. The first of these uses of mathematical models is illustrated by much of the research in mathematical psychology.

Although philosophers have paid little attention to the field of mathematical psychology, it has been a thriving part of psychology during the same period as the emergence of cognitive psychology and has even earlier roots in fields such as psychophysics. Pioneers in psychophysics, such as Weber and Fechner, set out to quantify the relations between sensory stimuli and perceptual experience. Weber's law (E. H. Weber, 1834) identified a constant relationship between the intensity of a stimulus I and the minimum increment above I that was noticeable (ΔI —the *just noticeable difference*)

$$\frac{\Delta I}{I} = k.$$

Fechner (1860) added an assumption of cumulativity to Weber's law to obtain a logarithmic function relating intensity of sensation and the ratio of the intensity of the stimulus to a threshold

$$\Psi = c \log \frac{I}{I_0}.$$

In the mid-20th century Stevens (1957) proposed an alternative power law relation. What is important for purposes of this paper is that these exemplars and much other work in mathematical psychology (e.g., Ebbinghaus's forgetting curve and Estes' and Bush & Mosteller's Markov models of learning) establish relations between variables characterizing the phenomenon—they are not describing a mechanism (Abrahamsen & Bechtel, 2006).

In some cases, however, the mathematical relations exhibited in the phenomena are presented as evidence for or against mechanisms that might explain the phenomena. Thus, when Sternberg (1966) found that the reaction time to decide whether items were on a recently studied list depended only on the set size s of the items studied

$$RT = 392.7 + 37.9s$$

and was independent of whether the test item was on the list or not, he used this as evidence that items stored in short-term memory were exhaustively serially scanned before a person made a decision. Likewise, Sheppard and Metzler's (1971) used the finding that the reaction time for deciding whether one figure was a rotated version of another depended upon the degree of rotation to argue for an analog mental process comparable to actually rotating a figure (Cooper & Shepard, 1984). Even in these cases, though, the mathematical model characterizes relations between variables describing the phenomena, not variables describing parts and operations within the mechanism.

In contrast, much of the computational modeling that is most familiar to philosophers has been focused on the mechanism responsible for the phenomenon. Here a second contrast is needed concerning the ways in which models relate to mechanisms and the understanding of the

mechanism responsible for the phenomenon of interest. In some cases, models precede empirical attempts to decompose the mechanism into its parts and operations. The modelers will propose parts and characterize how they operate, but these proposals are not grounded in empirical decompositions of the system but are hypothesized to account for the phenomenon (I will refer to this as the *modeling-first approach*). In other cases, the models are developed on the basis of extensive research decomposing the system into parts and operations (I refer to this as the *decomposition-first approach*). The equations in the model then describe the known operations performed by these parts and their relations to one another and attempt to show how the system would behave.

The modeling-first approach is well illustrated in both symbolic and connectionist modeling in cognitive science. Each modeling style advances a conception of the architecture of the cognitive mechanisms responsible for the phenomena. The symbolic approach conceives of the system as consisting of representational vehicles that are manipulated in ways that correspond to inferential relations whereas the connectionist approach conceives of the system as consisting of neuron-like units that take on activation values and excite or inhibit each other depending on the weights on the connections linking them.

Both in the 1960s, when the two architectures were first being developed, and in the 1980s, when connectionism was reinvigorated (largely as a result of the discovery of algorithms such as back-propagation to change connection weights throughout the system so as to enable the system to learn to produce desired outputs), there were acrimonious debates about the appropriateness of invoking one or the other architecture to model cognitive activities. Defenders of the symbolic approach (Fodor & Pylyshyn, 1988) appealed to features of cognitive activity, such as productivity and systematicity, and argued that only a symbolic system, that is, a system with a compositional syntax, could produce these features. Connectionists have pursued a variety of strategies to address this type of objection, and have focused on other features of cognitive systems, such as their ability to learn and their robustness to damage, and argued that they are best explained with a network type architecture (Bechtel & Abrahamsen, 2002).

For the most part, those advancing either style of model did not focus on the relation between the parts and operations specified in the model and the parts and operations within the brain. It was assumed that the operations in the model would be realized by brain activity, but exactly how was left unspecified. Connectionists often characterized their modeling as “brain-style” since the units and connections in networks shared some characteristics with neurons and their processes, but many connectionists disowned the claim that units in their model were to represent individual neurons (Rolls & Treves, 1998, model of the hippocampus is an important exception). Both symbolic and connectionist models were evaluated not by whether they correctly characterized the parts and operations in the brain but by their ability to account for the phenomena to be explained. Thus, the controversy initiated by Rumelhart and McClelland’s (1986) connectionist model of learning the past-tense of English verbs (Pinker & Prince, 1988; Plunkett & Marchman, 1993) focused on whether the model or its successors could account for data about how children actually learn the past-tense (e.g., the input they receive and the kinds of errors they make). Essentially, the argument concerned whether a single connectionist-style mechanism involving gradual changes to the weights between units could account for the phenomena of learning, or

whether two mechanisms were required, one generating regular forms by applying a formal rule (add *ed* to a base structure) and the other recovery of irregular forms from a memory store.³

The decomposition-first approach, in contrast, develops a model based on information generated through experiments about the parts of the mechanism and the operations they perform and what can be gleaned about how these are organized in space and time. I will be illustrating this approach below. The difference between these approaches is revealed not only in how the models are developed but in the functions they serve.⁴ At a first pass, computational modeling in both cases seems to serve the same function—establishing that the parts and operations proposed would be sufficient to generate the phenomenon of interest. But depending on whether the model is grounded on empirical research into the parts and operations, the response to inevitable ways in which the model fails to capture the phenomena will differ. In the modeling-first approach, the main consideration is the type of architecture employed. There is a great deal of freedom to revise specific assumptions in the model as long as the modeler does not radically change the architecture. In the decomposition-first approach, the failure to account for the phenomenon is evidence that something has been missed or incorrectly characterized—either there are additional parts and operations that need to be identified, the account of the operations by known parts revised, or researchers must find ways in which the parts interact that haven't been identified. Researchers are not free just to build new operations into the model—they must procure data that such operations are occurring within the mechanism. (A given modeler starting from a decomposition-first perspective might tinker with the model, introducing new components or organization to see if that would account for the phenomena prior to initiating a search for empirical evidence for the components or organization. This would represent a hybrid of the two approaches, but the vindication of the model still requires empirical support for the parts and operations.)

My strategy in the remainder of this paper is to illustrate the different styles and uses of computational modeling in the decomposition first approach by focusing on computational models they have been employed in the study of circadian rhythms. This is a rich field in which to explore these relations between mechanisms and models as there has both been extensive research on the mechanisms responsible for circadian rhythms and many modeling endeavors.

3. Circadian Phenomena and Modeling Projects

While the awareness that organisms coordinate various of their activities with the time of day has been recognized since ancient times, and investigators often suspected that the organisms

³ For the current state of the debate, see McClelland and Patterson (2002b; , 2002a) and Pinker and Ullman (2002b; , 2002a).

⁴ The distinction I am advancing is not sharp as there are plausible intermediate cases. Sometimes a mathematical model is inspired by ideas about the actual structure of the mechanism that might be performing the phenomena without a strong commitment to the correctness of those ideas. Thus, in developing their highly influential mathematical representation of the action potential, Hodgkin and Huxley (1952) speculated about the types of physical processes that might be involved in the fluxes of sodium and potassium in and out of the cell. These ideas show up in the coefficients of the equation they used to characterize the action potential. See Weber (2005) and Craver (2007) and numerous conferences exchanges between them for illuminating discussion of whether the Hodgkin and Huxley model constitutes a mechanistic explanation.

themselves kept time (as opposed to responding to signals in the environment), the demonstration that this was the case required developing experimental procedures for keeping organisms in constant stimulus conditions (e.g., constant darkness without changes in temperature, as could be achieved in caves) and monitoring their activities. The fact that organisms still maintained regular rhythms that were about, but not exactly, 24 hours led to them being characterized as circadian (*circa*-about + *diem*-day). Two other features of this rhythmic behavior came to be identified as constituting the phenomenon of interest—the ability of the rhythms to be entrained to external cues (Zeitgebers), which is crucial for responding to different day lengths during different seasons (or for those of us who travel across time zones), and the ability to maintain time in different temperature conditions (which usually cause chemical reactions to proceed at different speeds). There is a long legacy of circadian research devoted to providing detailed quantified accounts of circadian phenomena (for useful overviews, see Refinetti, 2006; Dunlap, Loros, & DeCoursey, 2004).

The idea quickly developed that if organisms can keep time, there must be a part in them that does the time keeping (a clock). Performing lesion studies in mice, Stephan and Zucker (1972) and Moore and Eichler (1972) identified the suprachiasmatic nucleus (SCN), a structure in the anterior hypothalamus consisting of approximately 16,000-20,000 cells (8,000-10,000 on each side of the brain), as the locus where damage resulted in arrhythmic behavior. Moore (1973) also demonstrated, using radioactive tracers, a pathway from the eyes to the SCN through which a signal about the presence of light could serve to entrain the clock. Further evidence that the SCN was *the* clock in mammals was provided by finding rhythms in electrical activity even when the SCN was isolated from the organism (Inouye & Kawamura, 1979) and that shortened rhythms could be generated in golden hamsters by transplanting the SCN from a mutant with shorter than usual rhythms into a host whose SCN had been removed (Ralph, Foster, Davis, & Menaker, 1990). A few years later Welsh, Logothetis, Meister, & Reppert (1995), by extracting SCN tissue onto multielectrode arrays, determined that individual neurons in the SCN maintained rhythms, albeit the periods different considerably between individual neurons.

Locating the clock in the SCN, or clocks in individual SCN neurons, only reveals where the mechanism is located—it doesn't explain how it works. That requires identifying a number of parts that each perform different operations but that work together to generate a 24 hour rhythm. Although the first genes involved in the clock, *period* in *Drosophila* and *frequency* in *Neurospora*, were discovered in the 1970s, the research that led to an understanding of the basic clock mechanisms in many different species only developed in the 1990s. In the following section I will describe this research and the modeling efforts that went along with it. As I will show, different modelers pursued different strategies for modeling the mechanism as it was being discovered. Moreover, not all research or modeling was focused on the level of intracellular mechanisms. The variability Welsh et al. had noted indicated that there is synchronization between neurons within the SCN. The fact that the oscillator within SCN cells can be entrained by light and other Zeitgebers and that the SCN can affect behavior generated elsewhere in the organism require linkages between the SCN and other parts of the organism. After reviewing the research and modeling of intracellular oscillators, I will more briefly examine modeling of intercellular synchronization and entrainment of the SCN by signals from the retina.

4. Discovering and Modeling the Intracellular Oscillator

As soon as a consensus emerged that circadian rhythms were endogenously controlled, researchers began to think in terms of them in terms of oscillators discussed in physics. One of the simplest mathematical models of oscillatory phenomena was developed by Balthasar van der Pol (1920), a Dutch electrical engineer to characterize limit cycles in electrical circuits involving vacuum tubes. The evolution of the cycles he proposed is characterized by a second order differential equation:

$$\frac{d^2x}{dt^2} + \mu(x^2 - 1)\frac{dx}{dt} + x = 0$$

Depending on the values of x and μ , this equation can approximate a linear differential equation or describes a harmonic oscillator, but for larger values of the two parameters it generates a limit cycle. Accordingly, circadian theorists such as Wever (1972) employed it to model a circadian oscillator. To model circadian rhythms, two state variables, amplitude (x_1) and phase (x_2), and two equations are employed to describe the changes in the system:

$$\begin{aligned}\frac{dx_1}{dt} &= \alpha[x_2 + \varepsilon(x_1 - \beta x_1^3)] \\ \frac{dx_2}{dt} &= -\alpha x_1\end{aligned}$$

In modeling circadian phenomena, α is set to $\pi/12$ to make the period of oscillation close to 24 hours. β is commonly set to $4/3$ and the parameter ε , referred to as the *stiffness* since it determines how quickly solutions converge to a steady state, is set to a low value between 0.1 and 0.25. These models do not make any effort to describe the components of the oscillator but to describe its overall behavior. They continue to be used for a variety of purposes where detail concerning internal operations is not needed (see below). Jewett, Forger and Kronauer (1999) for example, used it in their study of entrainment of circadian rhythms by light-dark cycle and phase shifts after light pulses. [BRING IN Forger and Kronauer (2002).] Since these models are not attempts to describe the oscillator mechanism, though, I will not focus on them further here.

Even before the first components of the circadian oscillator were discovered, a hypothesis about its operation emerged. Brian Goodwin (1963) was inspired to explore the dynamics of a system with feedback in light of Jacob and Monod's (1961) operon model of gene regulation. In the account of the operon, gene products bind to promoter or inhibitor sites on the DNA, inhibiting or releasing the gene from inhibition. Goodwin began to explore the idea that a feedback loop between gene products and the gene itself might create an oscillatory process and proposed the model shown in Figure 1. In this model, Reaction 1 (R1) generates an mRNA (X) which, in Reaction 2, is translated into a protein Y. In Reaction 3, Y catalyzes production of product Z, which inhibits Reaction 1. Three additional reactions (R4, R5, and R6) remove some of each of the three constituents. Goodwin characterized each of these reactions by differential equations:

$$\begin{aligned}\frac{dX}{dt} &= \frac{k_1}{Z^n + 1} - k_4 X \\ \frac{dY}{dt} &= k_2 X - k_5 Y \\ \frac{dZ}{dt} &= k_3 Y - k_6 Z\end{aligned}$$

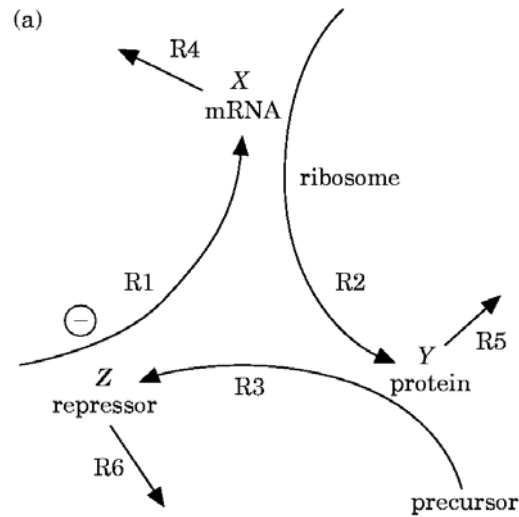


Figure 1. The Goodwin oscillator as developed as a model of circadian rhythms by Gonze et al.

The critical component of the model for generating oscillations is the non-linear term in the denominator of the first equation, n . This is known as the Hill coefficient and represents the number of molecules that must cooperate to achieve repression. Using analog simulations, Goodwin had reported oscillatory behavior with values of n as low as 2 or 3, but shortly afterwards Griffith (1968) showed that undamped oscillations would only occur when $n > 9$, a value too high to be realistic.⁵

The Goodwin oscillator has directly or indirectly inspired many efforts to model circadian rhythms once a feedback process like that Goodwin envisaged was identified as central to the circadian oscillator. The challenge posed by the unrealistically high Hill coefficient has been dealt with in several ways. One strategy is to introduce non-linearity into the degradation terms. Thus, Gonze, Bernard, Waltermann, Kramer, and Herzog (2005) characterize each of the degradation steps in terms of Michaelis-Merton kinetics, for example replacing $k_4 X$ in the first equation with $v_4 \frac{X}{k_4 + X}$. The result was that limit cycles were obtained with a Hill coefficient of

4, judged to be more biologically plausible. Some of these models, such as Gonze et al.'s to which I will return in the next section, remain at the abstract level of Goodwin's initial proposal while others inspired by Goodwin attempt to incorporate the details of the mechanism as revealed in empirical research.

*Modeling the Basic per-*PER* Cycle in *Drosophila**

The first key component of the circadian clock in vertebrates was discovered in *Drosophila* in research that involved inducing mutations chemically and screening for mutants which showed

⁵ Griffith concluded "the present work must be regarded as casting serious doubt on the possibility that negative feedback from a product of a single gene can ever give rise in practice to undamped oscillations in the concentrations of cellular constituents. It has not, however, discussed at all the question of whether such oscillations might arise in systems in which two or more genes are inductively or repressively coupled."

aberrant circadian rhythms. Konopka and Benzer (1971) found a locus where mutations resulted in rhythms with shortened or lengthened periods or in loss of rhythms altogether, and they designated the responsible gene *period* (*per*). Once *per* had been sequenced in the 1980s researchers discovered that its mRNA and its protein, PER, both cycled in neurons that seemed to be the pacemakers for circadian rhythms in flies with PER lagging about six hours behind the mRNA. Hardin, Hall, and Rosbash (1990) proposed a mechanism involving a negative feedback loop. First *per* was transcribed into an mRNA that was transported to the cytoplasm and translated into a protein, PER.⁶ Subsequently, PER was transported back into the nucleus where it ended up suppressing *per* transcription and translation (see Figure 2). Although PER lacked the region (domain) needed to bind to DNA and function as a transcription factor itself, the fact that it was homologous to two other proteins (the group is known as the PAS group for the first three proteins identified) that were known transcription factors suggested that somehow, perhaps by forming a compound with one of these transcription factors or a yet unknown intermediate, PER did suppress its own transcription and translation. This proposal received further support when Ederly, Rutila, and Rosbash (1994) discovered that the molecular mass of PER also changed through the day, indicating phosphorylation and dephosphorylation figured in the process and could affect both the degradation of PER and its transport into the nucleus

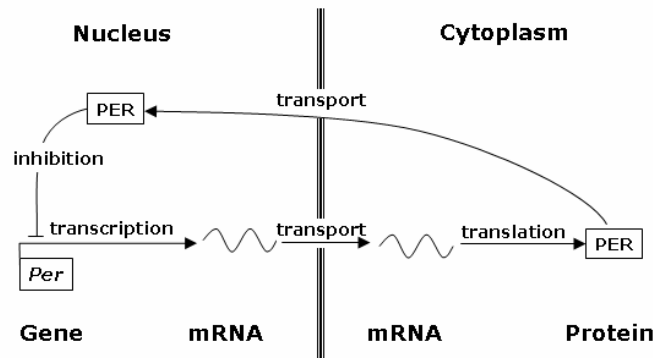


Figure 1. Basic process whereby the transcription and translation of the *per* gene results in a protein that inhibits the transcription of the gene.

Although the conceptual model shown in Figure 2 suggests that it might generate regular oscillations, demonstrating that it actually could requires computational modeling. Inspired by the Goodwin oscillator and the empirical discoveries captured in Figure 2, Albert Goldbeter (1995) created a computational model employing five kinetic equations to describe (1) transcription of *per* into its mRNA, (2) transport of *per* mRNA into the cytoplasm as well as the degradation of mRNA, (3) the synthesis of PER at a rate proportional to the level of *per* mRNA, (4) reversible phosphorylation and degradation of PER, and (5) transport of PER into the nucleus and inhibition of transcription of *per* (Figure 3). The equation describing formation of *per* mRNA is particularly important to the operation of the model:

$$\frac{dM}{dt} = v_s \left(\frac{K_1^n}{K_1^n + P_N^n} - v_m \frac{M}{K_m + M} \right)$$

⁶ Gene and protein names are commonly abbreviated to three letters. Protein names are written in uppercase. In *Drosophila*, gene names are written in lowercase italics. In mammals, the first letter of the gene name is usually campitalized.

As in the Goodwin model, n is the Hill coefficient, which represents the degree of cooperativity involved in repressing formation of *per* mRNA (M). By supplying appropriate parameter values, Goldbeter showed in numerical simulations that the steady state condition becomes unstable and gives rise to limit cycle oscillations in the concentrations of PER of the sort observed in *Drosophila* (Figure 4). He focused in particular on the parameter v_d , the maximum rate at which PER is degraded and found that between two critical values, the period of oscillation varied between 19.3 hours and 64 hours (the precise effect of v_d also depended on the value of other parameters, such as k_s , the rate of protein synthesis). Since mutants with shorter or longer periods were what Konopka used in identifying *per* as a clock gene, Goldbeter saw the ability of his model to generate alterations in period length from one specific parameter as pointing to a possible explanation of the variation—it was due to genetically induced variation in the rate of PER breakdown. Thus, Goldbeter’s model served both to show that the proposed feedback involving *per* could generate the oscillatory phenomenon and that perturbations of the model could result in behavior corresponding to mutant organisms.

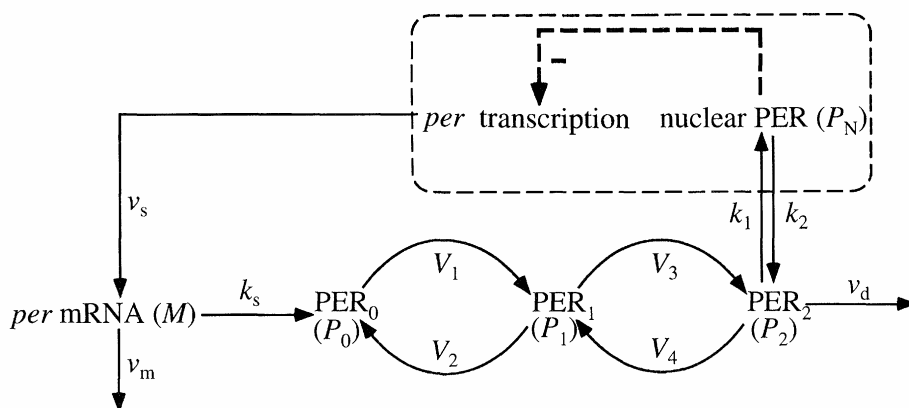


Figure 3: Goldbeter’s PER Oscillator. For each reaction the maximum rate (v or V) or the constant for transport into or out of the nucleus are shown. Each of the reactions is also governed by the Michaelis constant, not shown.

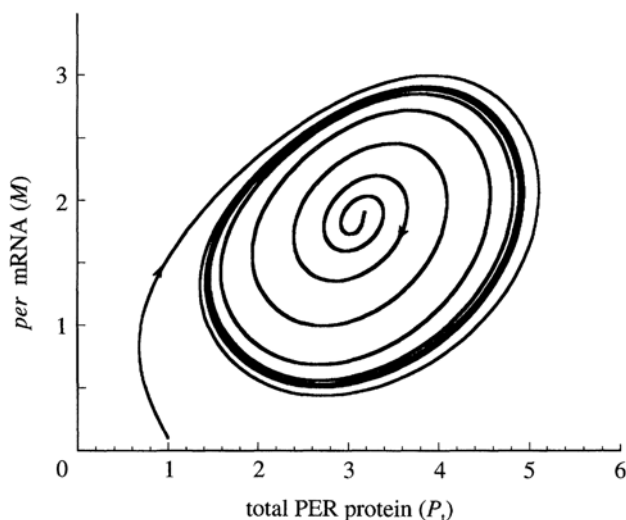


Figure 4. Limit cycle generated by Goldbeter’s model

Modeling More Components of the Drosophila Oscillator

Goldbeter's first model did not account for the ability of light to entrain the oscillation, which turned out to be appropriate since in *Drosophila* light does not directly affect PER, but rather acts to degrade another protein, TIM, that was discovered during the same timeframe as he was developing his model. Shortly after the discovery of TIM several additional clock proteins were discovered raising questions of how each of them fit together into the oscillator mechanism and what role and how important each was to the proper circadian functioning of the oscillator. Different modelers pursued different strategies at this point: Goldbeter continued to focus on PER and added its newly discovered dance partner TIM to his models while Paul Smolen and his collaborators focused on the additional components, which figured in cycles of their own, to determine whether they were pertinent to explaining circadian oscillations.

Like PER, TIM was discovered by a search for clock mutants. Sehgal, Price, Man, and Young (1994) produced mutants that manifested no rhythmic behavior from a gene located on another chromosome and named it *timeless (tim)*. In these mutants, PER did not enter the nucleus and was unstable in the cytoplasm (Vosshall, Price, Sehgal, Saez, & Young, 1994). Unlike PER, TIM turned out to be broken down when flies were exposed to light (Hunter-Ensor, Ousley, & Sehgal, 1996), pointing to a mechanism whereby light exposure might entrain the oscillator. After the discovery of *tim* and the effect of light on TIM, Goldbeter, in collaboration with Jean-Christophe Leloup (Leloup & Goldbeter, 1998; see also Tyson, Hong, Thron, & Novak, 1999), extended his model. The extended model required 10 differential equations and created limit cycles comparable to those of the PER only model. By varying parameter values, Leloup and Goldbeter sought to understand the relation between phosphorylation of PER and TIM, the dimerization process, and the degree of cooperativity required. They showed that with greater cooperativity, the range of other parameters in which oscillations could be maintained was much broader, and that requiring phosphorylation and dimerization both extended these ranges. In exploring these conditions in the model Leloup and Goldbeter were going beyond simply showing that the mechanism might suffice for generating circadian rhythms and beginning to use the model to explore how the mechanism might respond in differing conditions.

To account for the effect of light, Leloup and Goldbeter focused on v_{dT} , which represents the maximum rate of TIM degradation. They doubled it to characterize its increased degradation during the light phase. This produced a more precipitous drop in TIM levels than when the parameter was left fixed to reflect continuous darkness, but the overall effects were minor. Leloup and Goldbeter found that the results corresponded closely to the results of empirical investigations. Leloup and Goldbeter also explored the effects of a variety of parameter settings, and found some that produced two stable oscillatory regimes with different periods for the same set of parameter values, a phenomenon known as birhythmicity, as well as chaotic oscillations. Although commenting "It probably is too early to speculate on their possible physiological significance, particularly in view of the reduced range of parameter values in which they occur," they nonetheless propose that this might account for the phenomenon of rhythm splitting observed empirically by Pittendrigh (1960) upon changes in environmental conditions. Pittendrigh thought this pointed to multiple oscillators, but Leloup and Goldbeter suggest it may be due to the same oscillator in different cells responding differently. (In a further elaboration of

the model which I will not discuss here (Leloup & Goldbeter, 1997, showed how temperature compensation could be accounted for.)

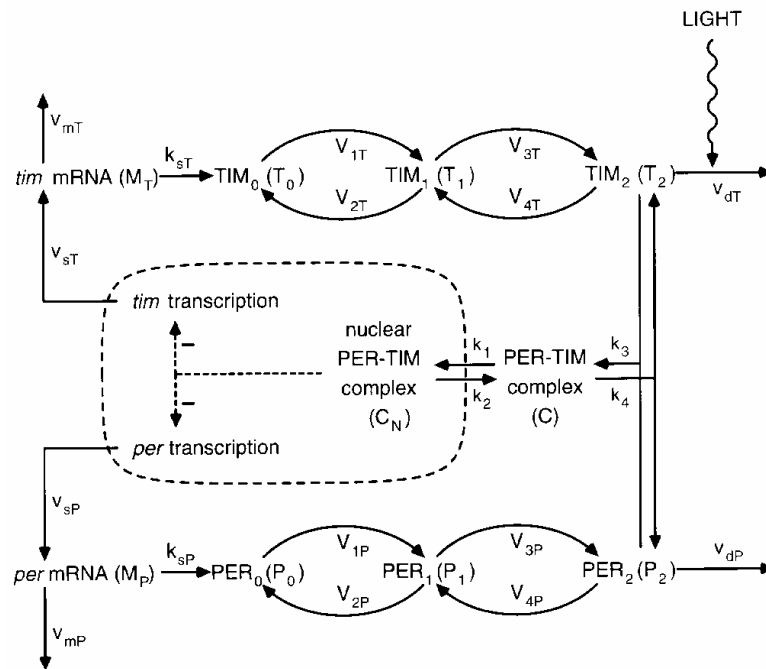


Figure 5. Leloup and Goldbeter's Model Incorporating TIM

In rapid order, many additional clock genes and proteins were discovered, some of which involved additional cycles. Goldbeter's modeling suggested that the feedback loops involving PER and TIM were sufficient for explaining circadian oscillations, but the existence of other components raised questions as to their importance. Of particular importance were two proteins, CLOCK and CYCLE. In the early 1990s no clock genes had been identified in mammals, and to rectify this, Takahashi, Pinto, & Vitaterna (1994) proceeded like Konopka, applying mutagens and screening for circadian abnormalities. They found one that exhibited a long circadian rhythm and named it *Clock* (for Circadian Locomotor Output Cycles Kaput). A few years later, Darlington et al. (1998) found a homolog in *Drosophila* (*dclock*). Unlike PER or TIM, CLOCK contains a region that enables it to bind to the promoter on *per*. CLOCK was also found to have a dimerization partner, named CYCLE (despite the fact it doesn't cycle) in *Drosophila* (in mammals BMAL1 performs this function). Darlington et al. therefore proposed that CLOCK together with its dimerization partner promote the transcription of *per* and *tim* and that when the PER:TIM dimer enters the nucleus, it somehow inhibits CLOCK from performing this function.

The CLOCK:CYCLE dimer not only excites the transcription of *per* and *tim* but also represses *clock* transcription. This results in a positive feedback loop: an increase in *clock* transcription results in increased *per* and *tim* transcription, and the resulting PER and TIM serve to bind to the CLOCK:CYCLE dimer, thereby releasing *clock* from its own inhibition. This raised the question of how the positive feedback related to the negative feedback: did it "cancel out" the negative feedback? Or was it crucial to maintaining oscillations, as Hastings (2000) proposes? To address these questions, Smolen, Baxter, & Byrne (2001) made the loop involving CLOCK and CYCLE the focus of their model. Rather than representing the processes of transporting PER and TIM

between the nucleus and the cytoplasm, they simply included delay factors between changes in CLOCK concentrations and changes in PER and CLOCK generation. Their model generated oscillations in total PER (in their model they did not distinguish PER and TIM but combined them into one variable PER) and CLOCK concentrations with a period of 23.6 hours, a result that was robust over substantial variation in the different parameters. To model the effects of light they enhanced the degradation of PER, which served to release CLOCK from its bound state.

Since Smolen et al.'s model focused on the role of CLOCK, they investigated whether it was essential to maintain rhythmicity. Even when CLOCK levels were made constant, oscillations were maintained, and they remained robust to substantial changes in the other parameters. Entrainment was also not affected. The parameter that was critical for maintaining rhythmicity was the delay factor for the influence of CLOCK on PER generation. This suggested that the positive feedback loop was not critical to the generation of circadian rhythms; rather, the circadian oscillations were due solely or primarily to the negative feedback loops involving PER and TIM (which were only abstractly represented by the delay factor in Smolen et al.'s model).

The modeling discussed in this second case primarily served two functions. The first was to show that rhythmic behavior of the right sort would result from the basic mechanism shown in Figure 2 and that the feedback loops involving some of the other clock components, especially CLOCK, were not central to the generation of rhythms. It is worth noting that the demonstration Smolen et al.'s model conducted with their model would have been very difficult to perform in real cells. This is also true of some of the other explorations I have noted in which parameter values were modified to determine their effects. In principle any of these alterations could have been performed experimentally, but it is far easier to set the value of a parameter in the model than to isolate and modify a component of the mechanism.

Modeling the mammalian oscillator

Starting with CLOCK, which as I noted above was first discovered in mammals, circadian researchers quickly found many additional clock genes. Mammals possess three homologues of PER, at least two of which seem to be involved in the core clock mechanism. Although TIM is found in mammals, it is not clear it plays a role in the central oscillator. Rather, CRYPTOCHROME (CRY), a blue-light receptor which in *Drosophila* served in light entrainment by expediting the breakdown of TIM, replaced TIM as the dimerization partner of PER in mammals. Light entrainment occurs through a different set of operations—melanopsin serves as the photoreceptor and acts by promoting *Per* transcription. In response to these discoveries, Leloup and Goldbeter (2003; , 2004) modified their *Drosophila* model, employing 16 kinetic equations and using “semiarbitrary” parameter values “in the physiological range” for the 45 parameters in these equations. Their objective was “to account for the effects of the various genes that control circadian clocks” (2004, p. 542). In addition to equations representing the regulatory effect on gene expression by PER, CRY, BMAL1, and CLOCK, their model included equations characterizing the reversible phosphorylation that led to the breakdown of these proteins and the induction by light of *Per* expression.

Their simulation generated the basic phenomenon—limit cycle oscillations in continuous darkness, with BMAL1 mRNA oscillating in opposite phase to the mRNA of *Per* and *Cry*. Leloup and Goldbeter note “Sustained oscillations only occur in an appropriate range of parameter values. Outside this range, rhythmic behavior disappears and the system evolves toward a stable steady state; such an evolution is often accompanied by damped oscillations” (p. 7052). These damped oscillations, though, can be entrained through periodic (sine-wave) variations in specific parameters such as the one specifying maximum rate of *Per* expression. They used a square-wave oscillation of the same parameter to model exposure to a light-dark cycle (high constant value in light, low constant value in dark) and were thereby able to simulate the light entrainment of the oscillator.

In addition to accounting for the light entrainment by increasing the rate of *Per* transcription, they found that slight changes to parameters such as the maximum rate of PER phosphorylation moved the peak in *Per* mRNA with respect to the onset of light by several hours. Goldbeter proposes that this “lability could explain why the phase of circadian oscillations in mammals varies in peripheral tissues with respect to the phase of the central pacemaker located in the suprachiasmatic nuclei within the hypothalamus” (Goldbeter, 2002, p. 243). He also suggested that the model not only accounts for the phenomenon of normal circadian rhythms but can also help explicate pathological conditions such as advanced sleep phase syndrome and delayed sleep phase syndrome and conditions under which entrainment by light fails. For example, they found that if CRY levels are too low in their simulation, free PER continues to accumulate over cycles since there is insufficient CRY with which to dimerize, and PER is no longer able to entrain the oscillator. They proposed that this may explain the phenomenon of patients lacking a 24 hour wake-sleep cycle. Likewise, the familial advanced sleep-wake cycle syndrome (or Advanced Sleep Phase Syndrome, in which patients sleep in the early evening and wake in the middle of the night) could be accounted for by altering parameters affecting PER phosphorylation.

One interesting result of Leloup and Goldbeter’s exploration with their model is that even though when they removed the negative feedback loop involving PER and CRY (by setting the rate of PER synthesis to 0), oscillations disappeared as expected, they could be restored by altering other parameters characterizing other parts of the mechanism, such as the degree of cooperatively of repression BMAL1 on *Bmal1*. The period of the restored oscillations, however, is much shorter (19.8 hours). They consider the possibility that this oscillation is concealed by the PER oscillations, or that it is damped and only maintained by extended light pulses. On the other hand, eliminating the feedback of BMAL1 upon its own synthesis does not stop oscillation of BMAL1 since it can still arise via the PER:CRY negative feedback loop involving BMAL1, which Leloup and Goldbeter interpret as supported by the preserved circadian oscillations in mice lacking REV-ERB α , an intermediate in the feedback of BMAL1 on its own synthesis (Preitner et al., 2002).

In their 2004 paper Leloup and Goldbeter performed a sensitivity analysis designed to examine how various parameter settings affected model performance. The model was particularly sensitive to the parameters governing the translation of *Bmal1* and the degradation of BMAL1. In addition they found that in entrainment in a light-dark environment, the parameters affecting CRY heavily influenced the phase of oscillations.

The three models of the intracellular oscillator have brought out three important aspects of modeling in the context of mechanistic research. On the one hand, the models showed that the right sort of phenomena would indeed result from the component parts and operations that had been discovered and described in the empirical research. Second, they allowed investigators to ask which part of the mechanism was centrally important to explaining the main phenomenon of interest. Third, they permitted modifications of the basic model that suggest explanations for a variety of the phenomena, including pathologies, associated with circadian rhythms. Exploration of models by altering parameter values in models is a common practice in modeling research. What is important here is that when the models themselves are motivated by empirical research decomposing the mechanism, the explorations with the model suggest a more link to the responsible mechanism. Knowledge from real-world experiments can thus limit the choice of manipulations in the model. On the other hand, the simulation results identify possible explanations that can be explored more in experiments on the real system.

5. Modeling Synchronization Between Cellular Oscillators

Studies in the 1990s that demonstrated that individual cells in the mammalian SCN oscillated also revealed significant differences in the oscillations of individual neurons. Welsh, Logothetis, Meister, and Reppert (1995; see also Honma, Nakamura, Shirakawa, & Honma, 2004) cultured SCN cells on a microelectrode array (allowing recording from multiple cells that were still connected by functioning synapses) and found that their period ranged from 21.25 to 26.25 hours, with a standard deviation of 1.2 hours. When spatial relations between SCN neurons are maintained in explants, the variability between cells was much reduced, indicating that individual neurons were being synchronized (Herzog, Aton, Numano, Sakaki, & Tei, 2004). Evidence points to vasoactive intestinal polypeptide (VIP) as the synchronizing agent (Aton, Colwell, Harmar, Waschek, & Herzog, 2005).

Synchronizing oscillators is a non-trivial problem as the dynamics can easily get very complex, producing toroidal oscillations, deterministic chaos, or coexistence of multiple attractors (Grebogi, Ott, & Yorke, 1987). In an attempt to understand how synchronization is achieved among neurons in the SCN, Gonze, Bernard, Waltermann, Kramer, and Herzog (2005) developed a model of how release of VIP might achieve synchronization. (For a model using a van der Pol oscillator to study synchronization, see Kunz & Achermann, 2003; for an earlier model using variables within a gene-expression oscillator that explored couplings involving different variables, see Ueda, Hirose, & Iino, 2002.)

They started with the modified Goodwin oscillator described above, and added equations for the induction of VIP (V_i) synthesis by clock genes (X_i) in each cell i and the generation of a mean field (F) or average concentration of V . Terms were also added to the equation for the change in activity of the clock gene (X_i) by the mean field (F) and light (L), resulting in the following set of equations:

$$\begin{aligned}\frac{dX_i}{dt} &= v_1 \frac{k_1^n}{Z_i^n + k_1^n} - v_4 \frac{X_i}{k_4 + X_i} + v_c \frac{KF}{K_c + KF} + L \\ \frac{dY_i}{dt} &= k_2 X_i - v_5 \frac{Y_i}{k_5 + Y_i} \\ \frac{dZ_i}{dt} &= k_3 Y_i - v_6 \frac{Z_i}{k_6 + Z_i} \\ \frac{dV_i}{dt} &= k_7 X_i - v_8 \frac{V_i}{k_8 + V_i} \\ F &= \frac{1}{N} \sum_{i=1}^N V_i\end{aligned}$$

Gonze et al. modeled a population of 1000 cells, each of whose behavior was specified by these equations. To establish a base in which cells were not synchronized, they employed a random rescaling of the rate constants; the result was that, with K set to 0, the periods exhibited a normal distribution with a mean of 23.5 hours and a S.D. of 1.17 hours (Figure 6A and 6B). When K was set to 0.5, to simulate the release of VIP, all oscillators became synchronized to a period of 26.5 hours (Figure 6C and 6D).

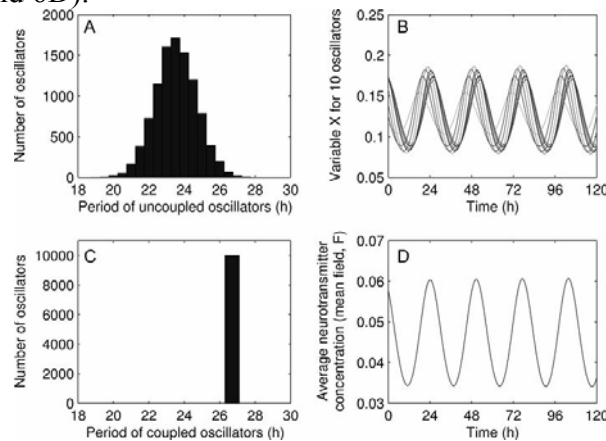


Figure 6. Periodicity with $K=0$ (A) and $K=0.5$ (C) and synchronization with $K=0$ (B) and $K=0.5$ (D)

They then conducted a variety of more detailed studies in an effort to better understand the synchronization process. Examining the case of just two oscillators, for example, they found that some coupling strengths led to a cessation or dampening of oscillations, and that such dampening is fostered by fixing the mean field parameter F to 0. Gonze et al. suggest that synchronization is achieved in the SCN by dampening individual oscillations but employing the oscillations in F to generate synchronized behavior. Of particular interest was the relation between coupling and the oscillatory period—the way in which coupling (or light entrainment) is achieved via an effect on X has the effect of increasing the period. Gonze et al. suggest that if the account in the model is correct, real SCN neurons require a means to compensate if coupled oscillators are to maintain the same period as individual oscillators.

Finally, the model was able to mimic the effect of administering tetrodotoxin (TTX), a Na^+ channel inhibitor, to the SCN, which has the effect of temporarily desynchronizing cells. Once

TTX is removed, though, cells resume resynchronize to the previous phase (Yamaguchi et al., 2003). By setting parameter K to 0 for 200 hours, and then restoring it to 0.5, Gonze et al. mimicked this effect.

By using the Goodwin oscillator, Gonze et al. did not focus on the detail of the mechanism underlying oscillations in individual cells. They also did not take into account the specific pathway via which VIP affects *per* transcription (VIP binds to the VPAC₂ receptor site and its effect on *per* is mediated by Ca²⁺ and CREB phosphorylation). Given the complexity of the interactions involved, these could readily be imagined to alter the synchronization process. To consider the possible impact of these details on the synchronization process, To, Henson, Herzog, and Doyle (2007) adapted the Leloup and Goldbeter model for the mammalian oscillator discussed above, and modified it to incorporate the communication between cells. The Leloup and Goldbeter model employed 16 ordinary differential equations, which To et al. supplemented with one ordinary differential equation describing the concentrations of phosphorylation of CREB (leading to its breakdown) and five algebraic equations to describe the extent of VPAC2 saturation, the intracellular calcium concentration, the extent of CREB activation, the maximum transcription rate of *Per* mRNA, and the VIP release rate (see Figure 7). A function based on CREB activation and phosphorylation altered one parameter of the original Leloup and Goldbeter model, v_{SP} , which had the effect of altering the phase, period, and amplitude of individual cells.

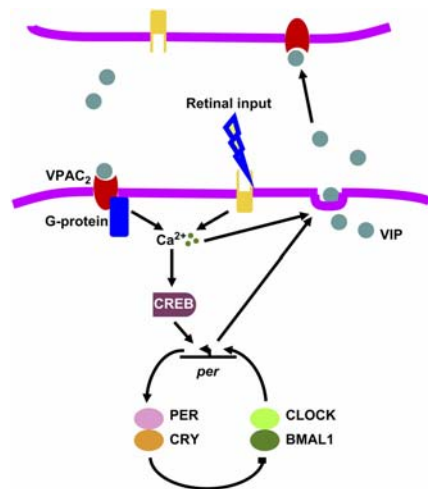


Figure 7.

In their simulation, To et al. used 400 cells situated on a 20 x 20 grid (one of the equations employed a weighting factor to capture the diffusion rate between cells depending on their location on the grid). They also randomly perturbed the parameter v_{SP0} (basal transcription rate of *Per* mRNA) so that only approximately 40% of the cells oscillated in the absence of VIP (in the actual SCN only 30% of cells are capable of sustaining oscillations on their own), and randomly perturbed 8 kinetic parameters in the Leloup and Goldbeter model that affected creation of dimers and their transport to create greater diversity in the free-running periods. The synchronization was more gradual than in the Gonze et al. simulation, but after 3 cycles (days) over 90% of the cells on the grid were synchronized. Like Gonze et al., To et al. found that synchronization also resulted in somewhat lengthening the period of oscillation. Unlike Gonze

et. al., who suggested this pointed to the need for some compensatory mechanism, To et al. propose that this result comports well with the empirical finding that in mice lacking VIP and VPAC₂ receptors, the oscillation periods are shortened. When VIP signaling was blocked (by setting the parameter determining VPAC₂ receptor saturation to 0, approximately 60% of neurons lost rhythmicity after two cycles and the intrinsic oscillators that continued to oscillate lost synchrony, replicating the empirical findings of Alton et al. Conversely, when VIP pulses (achieved by setting the VPAC₂ receptor saturation to maximum for 3 hours) in simulations in which VIP release had been set to 0, to mimic the application of pulses of VPAC₂ agonists to VIP deficient mice, 70% of cells exhibited synchronized rhythms.

To et al. also simulated entrainment to a 12L:12D cycle by setting the parameter representing Ca²⁺ to its maximum value and setting VPAC₂ receptor saturation to maximum for 12 hour intervals. More cells synchronized than in constant darkness, and the overall rhythm was more coherent. Exposure to constant bright light is known to abolish circadian rhythms in locomotor activity and synchrony diminishes between SCN neurons even though they continue to oscillate (Ohta, Yamazaki, & McMahon, 2005). To et al. found similar results in their model—all neurons were rhythmic but desynchronized.

Although synchronization between oscillators can give rise to complex dynamics very different than found in circadian rhythms, these two simulations suggested that relatively straight forward processes of peptide release and uptake would produce the sort of synchronization found in SCN neurons. Moreover, the models suggested rather direct ways to account for the entrainment properties of light and explain phenomena associated with different light regimes.

6. Modeling Interactions between Populations of SCN Cells

I conclude with two models that advance intriguing new suggestions about how mechanisms involving as their components different populations of cells in the SCN operate in entrainment to light and in adjusting to large changes in the time of daylight, as in international travel. In the models discussed in the previous section, the SCN is conceived of as an homogenous collection of cells.⁷ But researchers have long noted anatomical differences within the SCN; the challenge, to which these models respond, is to figure out what is the functional significance of these differences. It is worth noting that in considering relations between populations of cells, we are now moving up to a third level of organization. The first involved intracellular processes within SCN cells, the second synchronization between SCN cells. The models I now consider treat populations as themselves parts in a yet higher-level mechanism involving multiple populations.

The most basic anatomical division distinguishes the ventrolateral or core region from the dorsomedial or shell. The areas generate different neuropeptide output signals. It is the core cells that express (VIP). They also express substance P and gastrin-releasing peptide. In contrast, the shell cells express vasopressin (Moore, Speh, & Leak, 2002). Within the core is a dense collection of calbindin D28K (CalB) cells (Silver, LeSauter, Tresco, & Lehman, 1996). When these cells are destroyed and the remainder of the SCN neurons are not altered, overt physiological and behavioral rhythms are eliminated (LeSauter & Silver, 1999). Yet, these

⁷ Gonze et al. did a further simulation to account for the differences between regions within the SCN by having only some of the cells in a given simulation express VIP and found they still achieved overall synchronization.

calbindin cells do not themselves oscillate either in neuronal firing or in *Per* gene expression. Rather, PER1 and PER2 are synthesized in them in response to photic stimulation (Hamada, LeSauter, Venuti, & Silver, 2001; see also Shirakawa, Honma, & Honma, 2001).

These peculiar facts about the CalB cells raise the question of what operation they could be performing. Antle, Foley, Foley, and Silver (2003) offered the following hypothesis:

SCN cells in the CalB subregion act as a gate that relays photic resetting information to oscillator cells located in the SCN shell, thereby providing the daily signal that synchronizes the oscillators in the rhythmic region of the SCN. The fact that the destruction of these nonrhythmic cells leads to arrhythmicity indicates that they may be essential for overt rhythmicity (p. 340).

To evaluate this proposal, they developed a model. Since they were not concerned with the details of the genetic and biochemical processes within neurons, they started with van der Pol oscillators. To these they add a gate with two states, open and closed. When in the open state, the gate can be activated by a photic stimulation or by the overall output of the ensemble of oscillators. When the gate is activated, it adjusts the phases of the individual oscillators. Using polar coordinates, Antle et al. parameterized the equations for the van der Pol oscillator as follows:

$$\frac{dr}{dt} = -\omega r \cos(\theta)^2 [-1 + r \cos(\theta)^2]$$

$$\frac{d\theta}{dt} = \omega [-1 + \varepsilon r^2 \cos(\theta)^3]$$

where θ is the angle and r the radius, ω the frequency of the oscillator and ε the “stiffness” that affects how closely the oscillator mimics a cosine curve. Antle modeled the SCN as a population of 1000 such oscillators whose outputs are summed. Unless all the oscillators have precisely the same period, they will gradually move out of phase with each other (that is, their θ values will vary), and the summed output will decay to 0.

In their model, the resetting process was simulated by adding or subtracting to each oscillator’s θ value depending on how far it was from the null value to bring it back toward the null value. Such resetting would only occur when the gate was open. With a low threshold for the gate even in a desynchronized network the gate will occasionally be exceeded by chance, and at that point the θ s for the various oscillators will be reset and the network will self-organize into synchronous oscillations. With a somewhat higher threshold, synchronized oscillations can be maintained, but if the units are desynchronized, they are very unlikely to self-organize into a synchronized state. Since the gate cells were intended to represent the calbindin cells, Antle et al. viewed their model as providing a possible explanation as to why loss of these cells eliminates rhythmicity despite the fact that they do not themselves oscillate—they are responsible for allowing the propagation of the signal that allows the other SCN core cells to synchronize. Since the gate cells are assumed by Antle et al. both to process photic inputs and ensemble output, they consider the possibility that there may be an interaction—the endogenous input may modulate the threshold which a photic input must provide to activate the gate. Finally, Antle et al. suggest that assigning a gating function to the calbindin cells explains the inability of peripheral oscillators (those in tissues like liver, the lungs, or skeletal muscle) to maintain coherent oscillations without input from the SCN.

One of the most frustrating aspects of entraining circadian rhythms to new light schedules is the slowness of response, resulting in jetlag when humans travel across multiple time zones. This raises the question of why clock resetting is delayed. Although there is evidence that cells in the SCN can entrain to the new time zone within one cycle, the effects generally continue for many days. Moreover, sometimes the body seems to overcompensate—advancing or delaying circadian rhythms more than required for the time zones traversed (there is evidence that the SCN does as well). The most common explanation is that peripheral oscillators (those in the liver, etc.) are slower to entrain than those in the SCN. Leise and Siegelmann (2006) offer a different hypothesis: “The symptoms of jet lag may partly be due to this transient desynchrony, triggered by abrupt shifts of the LD cycle that cause large changes in the relative phases of the components of the circadian system as they scramble to entrain to the new schedule” (p. 315). That is, they propose that the normal relations between populations of oscillators is delicate and the radically altered input conditions can lead to complex dynamical relations between the different populations of oscillators

To support this hypothesis, Leise and Siegelmann developed a model that was grounded in results of empirical research. Several inquiries showed that multiple components to the SCN can oscillate out of phase with each other (Nagano et al., 2003; Nakamura, Yamazaki, Takasu, Mishima, & Block, 2005; Albus, Vansteensel, Michel, Block, & Meijer, 2005). In rats the ventrolateral or core SCN has the greatest input of retinal fibers. On the other hand, Nakamura et al. found that *Per* expression began first in the shell or dorsomedial SCN around 2 hours after lights on (ZT2), reaching a maximum at ZT 7.8, whereas it reached a peak in the ventrolateral or core SCN at ZT 8.6. Nakamura et al. experimented with exposing rats to both a six hour delay (equivalent of traveling westward, achieved by delaying the turning off of light by 6 hours and creating an 18 hour day) and a six hour advance (corresponding to traveling eastward, achieved by turning lights on 6 hours early, creating a 6 hour night). They found that on the first full cycle after a six hour delay (corresponding to the day after travel), a greater delay was exhibited in the lateral (both ventral and dorsal) SCN than in the medial (lateral and dorsal) regions (the ventrolateral SCN exhibited a 5.5 hour delay, whereas the dorsolateral SCN only showed a 4 hour delay). After three days, though, the relative phase differences had returned to the pre-delay levels. A much more complicated arrangement occurred after a six hour advance. After one day the ventrolateral SCN had shifted significantly more than the dorsomedial (or ventromedial) SCN, resulting in an inversion of the normal order of with the cycles. In fact, ventrolateral SCN had advanced more than 9 hours, a significant overshoot of the 6 hour change in the light schedule. By day three the overshoot had reduced, while the dorsomedial had advanced nearly 6 hours, resulting in virtually no phase differences between them. By day 6 the normal phase relation between ventrolateral and dorsomedial were restored, although both were advanced beyond the 6 hours.

Like Antle et al., Leise and Siegelmann abstracted from the biochemical details and used a model of the oscillators that employed just two equations that characterized the oscillation of only one protein:

$$\frac{dx}{dt} = \frac{r_x}{1+Y^2} - q_x X$$

$$\frac{dY}{dt} = r_Y X^2 (t - t_{lag}) - q_Y Y$$

Although the equations were first employed by (Scheper, Klinkenberg, van Pelt, & Pennartz, 1999) to model the relation between BMAL1 mRNA and protein in the mammalian oscillator, Leise and Siegelmann insisted on an abstract interpretation of the two state variables they use to track changes in the oscillator. Initially they modeled the ventrolateral (core) oscillator alone. With appropriate parameter values supplied, the model exhibits approximately 24 hour oscillations. Entrainment is achieved by reducing the value of r_Y when light is available. With either a six hour advance or a ten hour delay, the limit cycle was restored with an initial overshoot of two hours (see Figure 8).

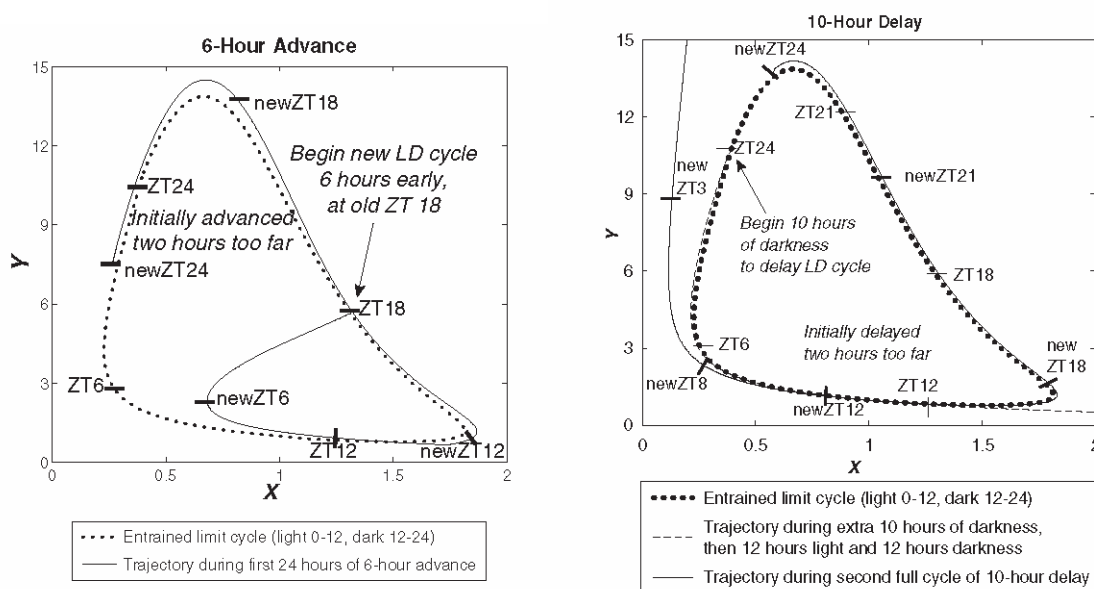


Figure 8. Affects on the limit cycle in the Leise and Siegelmann oscillator as a result of a 6-hour advance (A) or a 10-hour delay

In the next step they connected multiple oscillators. The model of the ventrolateral or core oscillator (Master pacemaker in the model) was coupled to the Intermediate component (representing cells in the shell or dorsomedial SCN) by having the Y-production in the Intermediate component increase (by increasing r_Y) when the value of X in the Master pacemaker exceeds a threshold (0.3 of its maximum value). The Intermediate component was then connected to four Peripheral components representing the oscillators in peripheral organs so that when the X value (or, for #3 and #4, the Y value) of the Intermediate component exceeds a set threshold (different for each oscillator), the r_X value for the peripheral oscillator is increased. The various components and their key parameters are shown in Figure 9. The differing initial r_X values for the peripheral oscillators determine whether their oscillations are self-sustaining or damped.

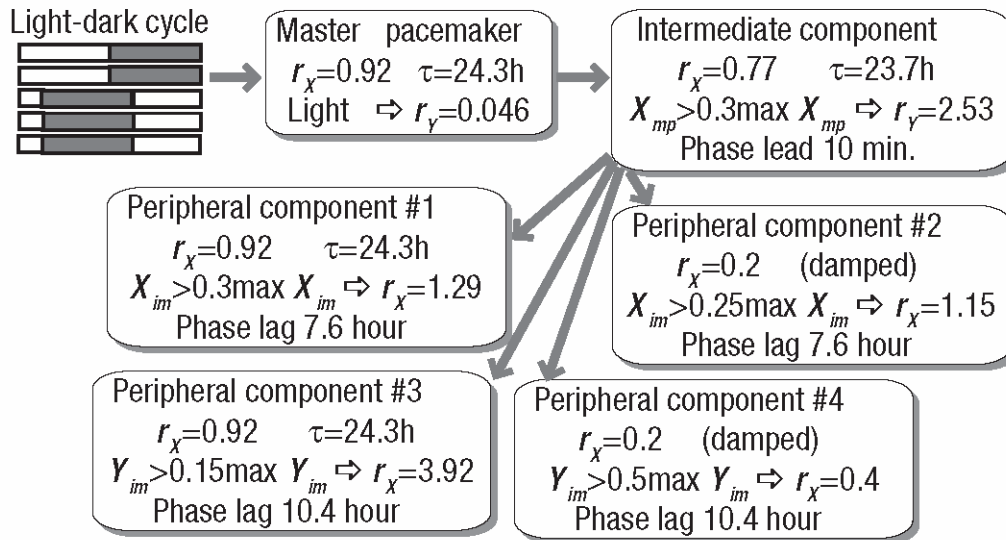


Figure 9. The various components in Leise and Siegelmann’s model and their parameter values.

In Leise and Siegelmann’s simulations, the Master pacemaker reset within two days, except in cases of large advances or delays, in which an additional one to three days was required for it to relax back from an initial overshoot. The other components responded more slowly and exhibited different distinctive patterns of resetting (involving delays and overshoots). For example, as seen in Figure 10A, the self-sustained peripheral oscillator responded to a six hour advance as it would for an 18 hour delay and took a prolonged period to reset (a phenomenon found in body temperature and ion secretion in humans with advances of 6-9 hours). With the same delay, the intermediate component appears to have reset after six days, but then advances further and requires several days to settle into the proper phase. Leise and Siegelmann have, thus, shown how coupled populations of oscillators can be perturbed by radical changes in exposure to light in ways that correspond to features of jetlag.

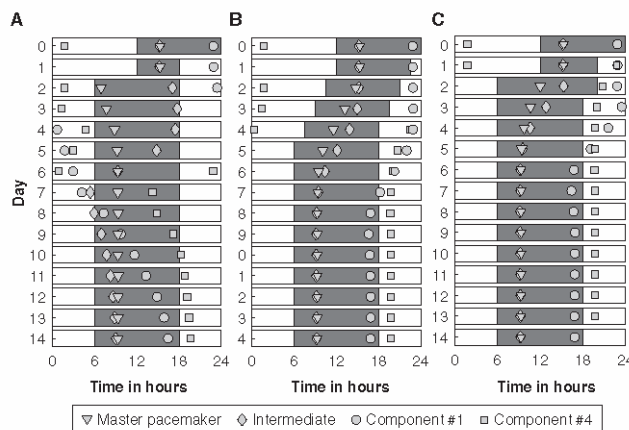


Figure 10. Changes in components of the Leise and Siegelmann model in response to advances in the onset of light of 6 hours in one day (A), four advances of 1.5 hours (B), and an advance of four hours followed by two hours (C).

As we have seen repeatedly, developing a computational model that can account for the basic phenomena puts one in position to explore further perturbations of the model. Leise and Siegelmann thus explored the consequences of more gradual light advances and found that in fact a four-hour advance followed by a two-hour advance produced a smoother resetting (Figure 10C) than even a gradual shift of 1.5 hours per day (Figure 10B). These results suggest possible ways to ameliorate the severe jetlag exhibit especially by traveling eastward six or more time zones.

7. Conclusions

While mechanistic research has developed a powerful array of tools for studying the behavior of mechanisms and for decomposing them into their parts and operations, it does not have as highly developed resources for studying the organization of mechanisms and for understanding how the operations of the parts are organized and orchestrated to produce the phenomena of interest. Often mechanistic researchers have relied on their ability to imagine the parts in operation and how one affects others. However, when the causal order departs from linear sequences and when the components interact with each other in non-linear ways, this approach often fails. Accordingly, one of the major contributions of computational modeling has been to determine whether the components will interact as anticipated, or exhibit phenomena such as instabilities that were not anticipated. The various computational models I have presented have illustrated this use of computational modeling in the study of circadian rhythms.

The models by Goldbeter and Leloup and Goldbeter of the primary cycles involving PER and TIM in *Drosophila* and PER and CRY in mammals revealed what was expected—that these loops were adequate to generate the basic features of the phenomena manifested in the central oscillators in these organisms. Given the number of parameters available in these models, it is to some degree not surprising that the modelers could produce the phenomenon of interest in their models. Yet, it does provide an important kind of support to the empirical research by showing that there are plausible parameter settings that generate the appropriate oscillatory patterns. The model by Smolen et al., which focused on the additional operations within the central that were discovered shortly thereafter, such as the positive feedback loop involved CLOCK, indicated that they were not critical to the oscillations that represented the timekeeping function in these neurons. The ability in a model to show that particular operation are not crucial to producing the phenomena serves to make it clear which operations are explanatorily important. (When Leloup and Goldbeter then extended their work to the mammalian case, they were also able to show how parameters in their model could be altered to illustrate some of the most common pathologies involving circadian rhythms.)

In the case of synchronizing oscillators either in a population of cells or between populations, modeling assumes additional significance since it is not intuitively clear how the component operations will interact with each other. The first such model I considered, that of Gonze et al., showed that VIP could in principle synchronize oscillators, but left out many factors, including information about the internal operations within the oscillator and the fact that dispersal of VIP disperses gradually over distances, that could affect the ability to synchronize into a stable oscillation. It was thus important to incorporate these factors to ensure that they did not prevent synchronization, as To et al. did. As I noted, these models not only were able to capture

important features of synchronization, but allowed for the exploration of changes in parameter values that exceed what has been possible experimentally.

In effect, the first set of simulations focused on a single level of organization whereas the second considered two levels of organization—oscillations within cells and synchronization between cells in a population. But there are also different populations of oscillators, both within the SCN proper and between it and peripheral organs. This introduces a third level of organization. With coordination required between levels of organization, greater potential for complex dynamics arise. This is illustrated in the last two simulations I considered. The first by Antle et al. suggests how a subpopulation of cells in the SCN that does not itself oscillate may play a causal role in the synchronization and that how it operates may determine whether a set of oscillators may self-organize into synchronous firing. The second, by Leise and Siegelmann, considers the interactions between two populations within the SCN and various peripheral oscillators and provides suggestive clues as to how the phenomenon of jetlag might result from desynchronizing relations between oscillators. Here the complexity of the interactions far exceeds what can be represented through mental simulation and reveals the critical importance of computational modeling to understanding mechanisms.

One of the major differences between the various models I discussed in the detail at which they represent the mechanism. Both highly abstract models using the van der Pol oscillator and highly detailed models incorporating the details of the genetics and biochemistry have important roles to play. To some degree, it is natural to abstract from the details within the lower level oscillator when modeling interactions between oscillators or populations of oscillators. Abstracting away from detail not taken to be relevant to a particular aspect of the phenomenon allows researchers to focus on those operations taken to be most central. But the potential that these higher-level operations could affect those within oscillators makes it also important to develop models that bring in details at lower levels. Given the different aspects of mechanisms that need to be understood, there are needs for different styles of modeling.

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