To appear in C. A. Hooker, *Philosophy of Complex Systems. Handbook of the Philosophy of Science, Volume 10.* Elsevier.

Complex Biological Mechanisms: Cyclic, Oscillatory, and Autonomous

William Bechtel
Department of Philosophy and Interdisciplinary Programs
in Science Studies and Cognitive Science
University of California, San Diego

Adele Abrahamsen
Center for Research in Language
University of California, San Diego

1. Introduction

The mechanistic perspective has dominated biological disciplines such as biochemistry, physiology, cell and molecular biology, and neuroscience, especially during the 20th century. The primary strategy is reductionist: organisms are to be decomposed into component parts and operations at multiple levels. Researchers adopting this perspective have generated an enormous body of information about the mechanisms of life at scales ranging from the whole organism down to genetic and other molecular operations.

Repeatedly, though, critics have emerged to challenge the mechanistic project. In the 18th and 19th centuries vitalists complained that mechanistic approaches to biology could not explain some of the important features of organisms. Xavier Bichat (1805) famously declared that organisms "resist death"—that is, owing to a distinctive, inherent ability to maintain themselves (vital force), living systems during their lifespan manage to foil the physical forces that threaten their viability. Although no longer embracing the label "vitalist," 20th century opponents alleged that mechanistic sciences failed to recognize that organisms are wholes, that is, organized systems with capacities very different from those of their constituent parts.[1]

In the past these opponents lacked research techniques and tools that could explain rather than just denote the phenomena that seemed to escape mechanistic explanation. The recent application of mathematical tools for analyzing network structures and complex dynamical interactions has opened such systemic properties to analysis, and this project of complex systems modeling has begun to take root in the small but important subdisciplines of systems biology and computational biology. Certain advocates of complex systems models align with the earlier critics of mechanism, presenting their proposals as supplanting the mechanistic program. This is misguided. The tools of complex systems modeling provide a needed extension of, but do not supplant, substantive accounts of mechanisms. To adapt a turn of phrase from Kant, dynamical models without mechanistic grounding are empty, while mechanistic models without complex dynamics are blind.

[1] Many of these critics appealed to *emergent phenomena* in arguing that wholes are not just the sum of their parts, but the notion of emergence has been difficult to explicate and to insulate from concerns of spooky metaphysics. For a particularly clear discussion of emergence, see Boogerd, Bruggeman, Richardson, Stephan, and Westerhoff (2005).

The central thrust of the mechanistic approach is to account for a target phenomenon by identifying, to a first approximation, the component parts, operations, and organization of the responsible mechanism. Ironically, given their centrality in the life sciences, mechanisms and mechanistic explanations were not much discussed by 20th century philosophers of science. Their legacy is the deductive-nomological framework and its focus on laws as the primary explanatory vehicle; for them, a scientific observation is explained by formally deriving it from laws and initial conditions. More recently a number of philosophers, focusing primarily on biology, have sought to characterize mechanistic explanations and to differentiate them from deductive-nomological explanations. Although the vocabulary sometimes differs, the key elements of a basic mechanistic explanation are (1) the identification of the working parts of the mechanism, (2) the determination of the operations they perform, and (3) an account of how the parts and operations are organized so that, under specific contextual conditions, the mechanism realizes the phenomenon of interest.[2]

An organism—even a mere virus or amoeba—comprises numerous biological mechanisms. The first step in investigating each mechanism is to identify some of its component parts and operations (a step often carried out in different disciplines at different times for parts vs. operations). These components are important because they both make possible and limit what can be accomplished in the larger system. The use of lipids as building blocks of membranes, of proteins as catalysts, and of phosphate bonds for storage of energy determine many of the fundamental characteristics of organisms and provide the resources for them to maintain themselves. That chromosomes are composed of DNA, with its double helix structure, "immediately suggests a possible copying mechanism" (the pithy final remark by Watson & Crick, 1953). Beyond this, the nature of the bonds between nucleotides creates the possibility of complex editing, so that different proteins can be synthesized at different times from a single DNA sequence. These are just a few examples of what can be gained by identifying and investigating specific components; each has characteristics that are important for understanding the processes that maintain life. The opposite strategy—attempting to theorize about organisms without consideration of their actual building blocks—can lead to empty models, exhibiting interesting properties but not actually characterizing the organisms of this world.

Identification of component parts and operations is thus seen to be a crucial first step. The focus of this paper, though, is the implications of complex systems modeling for mechanistic explanation in biology and our understanding of it. These implications are substantial. The nonlinear and non-equilibrium nature of the interacting operations within organisms often is downplayed in initial proposals of how the parts and operations are organized so as to comprise a mechanism, but they are critical to the orchestration of operations that is required for the mechanism to perform its task. Moreover, the operations performed by the parts, and even the very identity of these parts, are affected by their interactions with other parts. Consequently, the characterization generated in other, typically simpler, contexts may have to be revised as researchers come to understand the dynamical interactions occurring within organisms (Boogerd, et al., , 2005). Openness to such recharacterization of parts and operations fortunately lies within the mechanistic framework—as does recharacterization of their organization, if that

[2] (Bechtel & Richardson, 1993; Bechtel, 2006; Craver, 2007; Darden, 2006; Machamer, Darden, & Craver, 2000; Thagard, 2006). For more on our own construal of mechanistic explanation and how it differs from nomological explanation, see Bechtel and Abrahamsen (2005).

framework is appropriately extended. Consider that mechanistic research often begins with an extremely simple conception of organization. The components are thought to operate largely independently, with each feeding the product of its internal operations to another component that has limited if any impact on the earlier component. Simon (1980) spoke of such systems as *nearly decomposable*. Numerous systems that he cites do fit that description, but biological mechanisms properly conceived generally do not. Increased recognition of their complexity has prompted inquiry into previously neglected temporal dynamics and the implications for our understanding of how operations are orchestrated in real time.

In brief, we are claiming that mechanistic research has resources for self-correction sufficient to encompass complex dynamics—there is no need to choose between mechanistic and complexity-theoretic approaches. When researchers extend the basic mechanistic program to seriously address the orchestration of operations in real time, dynamical systems and complexity theory offer relevant new tools. To flesh this out, we examine the discovery processes that led from certain mechanistic accounts with relatively simple organization to later accounts that recognized the complex dynamics characteristic of biological systems. We begin by describing how biologists came to recognize the ubiquity of cyclic organization in biology, focusing primarily on biochemistry. We then address the dynamics of such systems. In some, values of variables fluctuate irregularly (perhaps randomly) when repeatedly measured over time. Others—of greatest interest here—produce oscillations approximating the periodicity of a harmonic oscillator, such as a pendulum. Systems producing regular changes of this kind (e.g., in the concentration of a metabolite across minutes, or in alertness across hours) are referred to as *biological oscillators*. Even when there are nontrivial variations in period and amplitude, powerful tools for analysis can be brought to bear by treating such systems as oscillators. It should be mentioned, finally, that a few biologists (e.g., Skarda & Freeman, 1987) have proposed models incorporating *chaos* (dynamics that are highly irregular, but deterministic) to explain certain biological phenomena.

The full range of dynamics should hold interest and relevance to biologists, more so than steady-state accounts, and available tools for characterizing these dynamics include mathematical modeling with differential equations and (from dynamical systems theory) limit cycles, bifurcations, chaotic regimes, and more. We are gradually moving beyond the era in which biological oscillations were concealed by such practices as focusing on the mean concentration of the product of a biochemical reaction rather than retaining the pattern of values over time. While still in the minority, there is a growing community of researchers whose questions, procedures, data, and analytic techniques are directed to discovering and characterizing biological oscillations.

There is much to be gained from enhanced attention to cyclic organization and the resulting dynamics, especially oscillations. Equally important, though, is to ask what cyclic organization and oscillatory dynamics do for the organism. The short answer is that they provide invaluable resources for controlling and orchestrating biological operations. As to why such resources are so crucial, it has been suggested that organisms most fundamentally are systems far from equilibrium that must maintain themselves as such or die: *autonomous systems* in the lexicon of the theorists offering this characterization.[3] Autonomous systems are continuously active,

[3] (Ruiz-Mirazo, Peretó, & Moreno, 2004; Bickhard, 2000; Christensen & Hooker, 2000; Collier & Hooker, 1999).

constantly carrying out operations necessary to their self-maintenance. But different operations can be inconsistent and even inimitable to each other. For example (as detailed later), organisms use metabolic operations to mine energy from foodstuffs taken in from the environment, and some of these are inconsistent with operations of protein synthesis. Some means of orchestration is therefore necessary. In human engineering this most often involves external controllers, but a more elegant solution is internal cycles that interact to produce coupled oscillations. There is evidence that the ubiquity of this design in organisms figures crucially in their ability to regulate and maintain themselves.

In confronting these three features of biological mechanisms—cyclic organization, oscillatory activity, and autonomy—researchers are moving towards what we call *dynamic mechanistic explanation*. This approach significantly extends and refocuses the philosophical account of mechanism. It retains the basic mechanistic commitment to identifying parts, operations, and simple organization, but gives equal attention to determining how the activity of mechanisms built from such parts and operations is orchestrated in real time. The result is a novel framework that integrates the mechanistic philosophy of science that arose in the 1990s with the previously independent movement to understand complex systems and their dynamics. In a final section we briefly discuss the challenges in integrating mechanistic and dynamical or complexity theoretic perspectives and address broader implications.

2. From Sequential to Cyclic Organization

Humans typically conceive of causal operations as involving one entity acting on another—a rock damages a car by hitting its windshield, or one molecule catalyzes a reaction that changes another molecule (e.g., by oxidizing it or adding a phosphate group to it). Note that often there are changes to the entity taken to be the cause as well as to the one affected—the rock might split when it hits the car—but this tends to be minimized as we typically conceptualize change. Moreover, once multiple steps are involved, we tend to conceptualize them as occurring sequentially. Human manufacturing focuses on adding one component at a time to a partially constructed object (as in an assembly line) and usually presumes that the already installed components are not altered in the process.

These predilections for simple organization were clearly manifest in research on alcoholic fermentation, the biochemical process essential to brewers that transforms glucose into alcohol and carbon dioxide. The chemical composition of glucose ($C_6H_{12}O_6$ in modern symbolism) and alcohol (ethanol, C_2H_5OH) was known by the early 19th century, when it was assumed that fermentation was an ordinary chemical reaction. The discovery in the 1830s of yeast and its role in fermentation raised the question of whether or not fermentation was a process carried out only in whole living cells. Pasteur vigorously advocated this position and also established that fermentation occurs only in anaerobic conditions. Compelling evidence that living cells were not required finally came in 1897, when Buchner produced fermentation in extracts made by grinding and filtering yeast cells. Since these chemical soups contained a great variety of molecules as well as subcellular organelles, Buchner's success gave rise to a new question: what component(s) of cells, retained in the cell-free extracts, might be responsible for fermentation?

Buchner's answer illustrates a common initial move in explaining a phenomenon: attribute it to a single component when a more complex, multi-component mechanism is actually responsible. Accordingly, Buchner suggested that a hypothetical enzyme he named *zymase*, acting on glucose, accounted for fermentation. (By then enzymes had been characterized as chemical catalysts within cells and the suffix *–ase* used to designate them.) Other investigators, however, posited that fermentation involved multiple reactions, each catalyzed by a different enzyme, and gathered evidence pointing to various possible intermediates. Over the next thirty years they pieced together reactions involving phosphorylations, dephosphorylations, and oxidations, as well as internal reorganizations and the splitting of a six-carbon molecule into two three-carbon ones. The same reactions (except for the final one in which pyruvate is converted to alcohol) were responsible for aerobic and anaerobic glycolysis.[4] Figure 1 illustrates how the biochemists who uncovered this glycolytic pathway conceptualized it as a sequence of reactions—the simplest possible temporal organization scheme. The involvement of ATP and NAD also received minimalist treatment, as side reactions appended to the linear backbone.

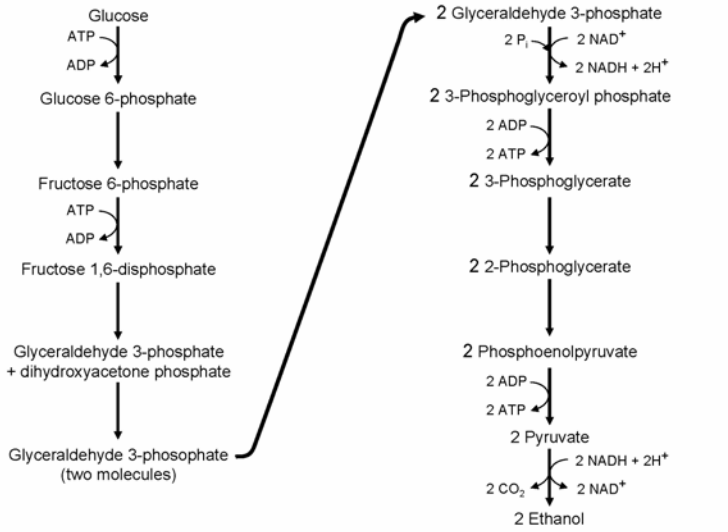


Figure 1. Glycolysis is represented as a sequence of chemical reactions.

In the context of oxidative metabolism (which requires aerobic conditions), pyruvate is not converted to ethanol but rather is taken up by another system of reactions to be further catabolized to water and carbon dioxide. Researchers focused on this system pursued the same strategy as for glycolysis, seeking to identify a sequence of molecular intermediates between an initial substrate and a final product. As before, each intermediate was assumed to be the product of one reaction and substrate of the next so as to fill in the sequence. Following upon Wieland's characterization of oxidative reactions as involving the removal and transfer of pairs of hydrogen atoms either to oxygen or to another hydrogen acceptor, Thunberg (1920) proposed a sequence of reactions, some involving oxidations, that led from succinic acid to acetic acid (with pyruvic acid as an intermediate rather than as an incoming product of glycolysis due to fragmentary knowledge of both pathways at this time):

[4] See Bechtel, 2006, chapter 3, for a review of these advances in biochemistry.

Succinic acid → fumaric acid → malic acid → oxaloacetic acid → pyruvic acid → acetic acid

At this point Thunberg confronted a problem, since removal of two hydrogen atoms from acetic acid would not yield a known chemical compound. His solution was to propose that two molecules of acetic acid would combine; in the process each would surrender a hydrogen atom, yielding succinic acid. Necessity thus led Thunberg to close the sequence of reactions for which he had direct evidence into a cycle, but the implications were profound: a cyclic system of reactions helps resupply its own initial substrate. As it turned out, the first three reactions and the general claim of cyclic organization survived the test of time, but it was not until a landmark publication by Krebs and Johnson (1937) that a good, though still incomplete, account of this metabolic pathway was achieved. Figure 2 compares these two proposals. It can be seen that the initial substrate – the one replenished at each turn of the cycle when an internal product reacts with an externally supplied product of glycolysis – in fact is citrate (citric acid), not succinic acid as in Thunberg's proposal.[5]

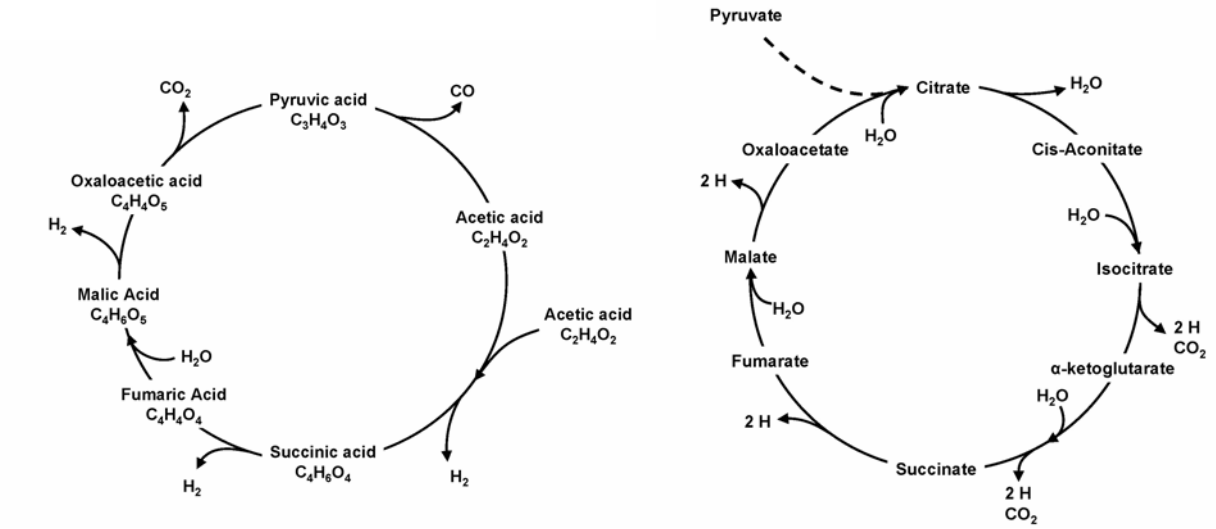


Figure 2. Two accounts of a key pathway of oxidative metabolism that recognized its cyclic organization. On the right is an early version of the Krebs cycle that was essentially correct, though incomplete. Its cyclic organization had been anticipated by Thunberg (1920), as shown on the left, but his conjecture that the crucial reaction produced succinic acid from acetic acid proved incorrect.

Krebs had come to this project primed to find a cyclic solution—most directly by his own success in working out the ornithine cycle with Hanseleit in 1932. Though such cycles were born of chemical necessity, he took an interest in their functional significance and organization. Krebs (1946-8) proposed that they actually consisted of two levels of cycles. The outer, metabolic cycle

[5] The Krebs diagram lacks some important reactions, some discovered later and some detailing that the pairs of hydrogen atoms (2 H) were used to convert two molecules of NAD^+ to NADH or (in one reaction) FAD to $FADH_2$. It also masks debates regarding the precise role of citric acid that led to multiple names: citric acid cycle, tricarboxylic acid cycle, and simply Krebs cycle. The diagram does reflect a mid-century switch in reference from *succinic acid* to *succinate*, *citric acid* to *citrate*, etc. Both the Thunberg and Krebs diagrams must be understood as snapshots in what was a dynamic research area.

repeatedly regenerates an initial substrate by means of a series of intermediate reactions, as shown in Figure 2 for the Krebs cycle and its initial substrate, citrate. Each of these reactions, though, depends upon an enzyme cycle that is simpler in that it involves different forms of the same enzyme rather than a series of intermediates. He notes (p. 92) that metabolic cycles are "complex mechanisms which can be resolved into a chain of enzyme cycles" whereas enzyme cycles "cannot be further resolved into smaller cycles." Figure 3 shows how Krebs envisioned this as a cycle of cycles. Taking the enzyme cycle at the upper left as an example, the relevant substrate (malate) first binds to the enzyme (malic dehydrogenase), forming an "enzyme substrate complex"–the first step in the oxidation reaction achieved by this cycle. The enzyme takes two hydrogen atoms from malate and sends the product (oxaloacetate) to the next enzyme cycle (to the right), itself temporarily taking the form of dihydro malic dehydrogenase. The extra hydrogen atoms then combine with available oxygen to form water, leaving malic dehydrogenase free to begin the next turn of this cycle by again accepting a molecule of malate (sent from the preceding enzyme cycle as the product of a reaction with fumarate). The outer loop of metabolites (in which malate is an intermediate between fumarate and oxaloacetate, for example) is "on another plane of the chemical organisation of living matter" (p. 92) than the enzyme loops that create it. Krebs claimed that such complexly organized metabolic cycles are distinctive of life (in contrast to enzyme cycles, which are organized identically to inanimate catalytic cycles), and he was intrigued by how they enabled organisms to maintain themselves.

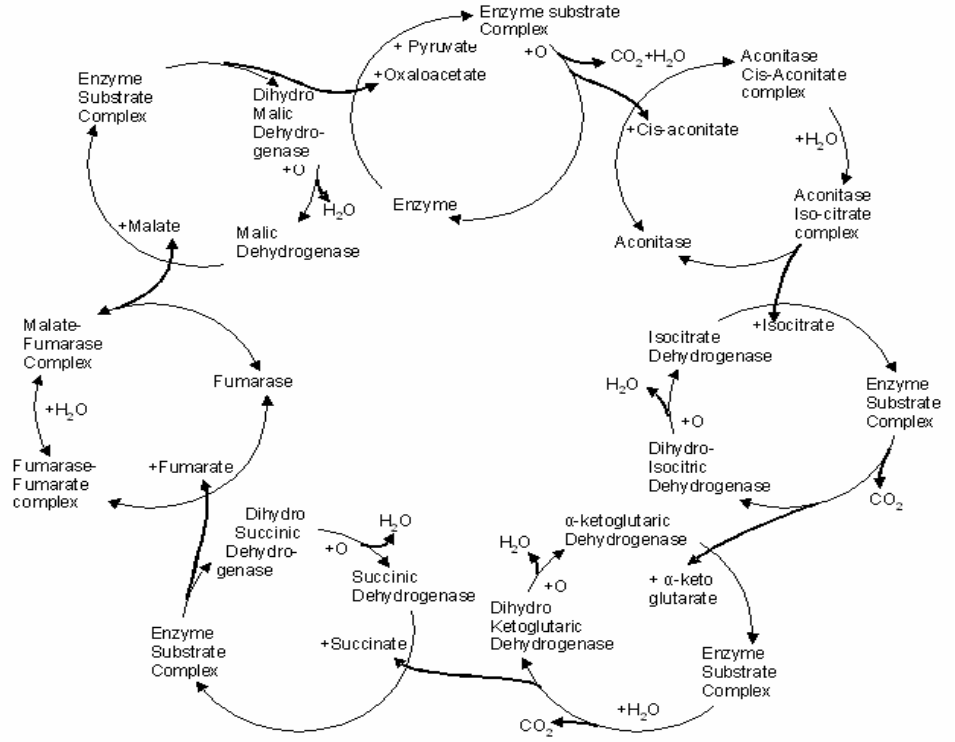


Figure 3. Krebs' (1946-48) characterization of the Krebs cycle as a cycle of cycles. (Note that citrate was omitted because its status as the initial substrate was temporarily in doubt.)

In the end, Krebs hinted at deeper reasons for cyclic organization than restoration of an initial state.[6] Nonetheless, a similar idea was pursued in much greater depth by the Hungarian chemist Tibor Gánti (1975), who sought to characterize the simplest chemical system that might exhibit the basic features of life. Like Maturana and Varela (1980), Gánti emphasized the need for such a system to maintain itself and identified cyclic organization as enabling a system, after it carries out a process, to be in the requisite state to perform the process again. This is true not just of biological systems but also of motors and other machines of human design. Gánti thought cycles were especially crucial for living organisms, though, because they must regularly recruit matter and energy from their environment and use it to build themselves (while expelling what they do not use as waste). Thus, he adopted an abstract characterization of the Krebs cycle as the core of his metabolic system and combined it with a limiting membrane (itself made by that system) that regulated the accumulation of metabolites. Together they constituted "a super-system" that could exhibit the fundamental biological properties of self-maintenance, growth, and reproduction.

Krebs anticipated a greater role for cycles as biochemists advanced their research: "Even if the specific meaning of cycles is still a puzzle, the fact that many processes have been found to be cycles suggests, as a working hypothesis, that other mechanisms as yet unknown might be cycles" (p. 98). He was right that the count of known cycles would increase, but might have found disappointing the limited pursuit of explanation. Attention to cyclic organization is discouraged even by notational conventions; serial sequences of reactions (as shown for glycolysis in Figure 1) are convenient, but also reflect and reinforce an essentially linear conceptual framework. Figure 4 conveys the limitations of a linear framework by comparing an abbreviated version of Figure 1 (left) to a rediagrammed version that reveals considerable cyclic organization (right). The simplest cycle is obtained by connecting the side-loop in which NAD^+ is reduced in the oxidation of glyceraldehyde-3-phosphate to the one in which NADH is oxidized in the reduction of pyruvate to alcohol. This illustrates how the hydrogen captured in the reduction reaction is thereby available downstream for consumption in the oxidation reaction (with NAD as carrier).

[6]In particular, Figure 3 includes two kinds of reactions: irreversible (single-headed arrows) and reversible (bidirectional arrows); in consequence, the overall cycle of reactions is irreversible. Krebs conjectured that the inclusion of reversible reactions lent flexibility to the irreversible cycle in the face of changing requirements.

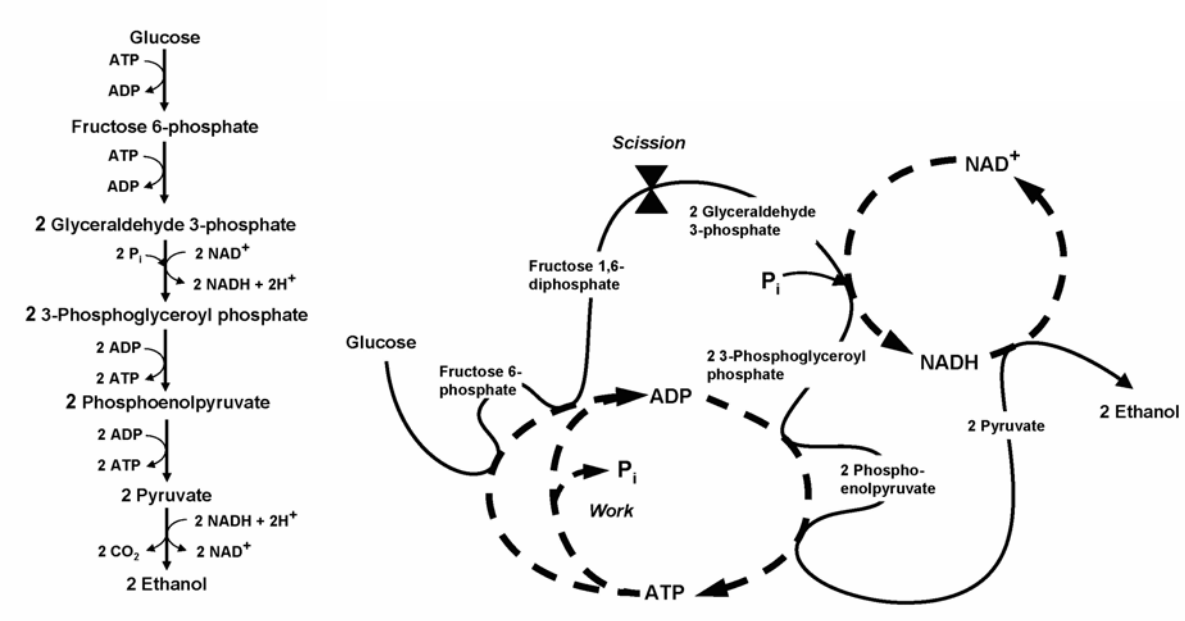


Figure 4. In contrast to the linear schematization in Figure 1 (repeated here on the left, but showing only those reactions involving NAD or ATP), the glycolytic process is re-represented on the right by closing the loops involving NAD and ATP.

The ADP-ATP cycle is a bit more challenging to understand, in part because consumption of the energy stored in ATP's third phosphate bond (PO_4 or simply P_i) occurs earlier in glycolysis than the reactions that capture and store energy in such bonds. (No trick is involved; the ATP → ADP reactions take advantage of the supply of ATP in the milieu from earlier cycles of glycolysis or from other systems of reactions.) Moreover, the diagram is jigged to accommodate the fact that four different glycolytic reactions are involved (two consumption and two storage). But the net result is that twice as much ATP is produced than consumed. The key to understanding this is the scission of what had been a single molecule into two molecules. The phosphorylation reactions that consume energy from ATP precede the scission and the dephosphorylation reactions that store energy in ATP follow it, thereby involving twice as many molecules. This makes two ATP molecules available to re-enter the glycolytic pathway (one phosphorylating the glucose molecule and one the product of that reaction, fructose 6-phosphate) and leaves two additional ATP molecules available for other work (e.g., protein synthesis).

In brief, changing notation away from a linear conception helps us appreciate the crucial role of cyclically organized processes. Figure 4 shows how the cycles involving NAD and ATP integrate the catabolic reactions of glycolysis into a coherent system, and hints at the dynamism of that system. Moreover, though not specifically diagrammed here, such cycles link glycolysis to other biochemical systems. ATP is used for protein synthesis and numerous other energy-consuming tasks, for example, and NADH gets shuttled into the mitochondrial matrix where it links to oxidative metabolism—especially to the electron transport chain, which uses hydrogen (electrons) carried by NADH from glycolysis and the Krebs cycle to power oxidative phosphorylation (an especially efficient conversion of ADP to ATP). This gives our

biochemistry the character of Watts and Strogratz's (1998) *small worlds*: networks in which most links are between local components but a few more distant links serve to integrate the overall network. The overall metabolic system can be regarded as a small-world network. Each pathway has a series of local links, e.g., the sequential backbone of reactions in glycolysis, but the pathways are connected to each other by more distant links—especially those involving the NAD cycle. We will return to consider the role this might play in coordinating and regulating operations within the cell, after first considering oscillatory phenomena.

3. Recognizing and Explaining Oscillatory Phenomena

The discovery of cyclic organization seems fairly straightforward: a sequence of reactions is found to close into a loop (the Krebs cycle) and/or to snake its way through reversible cycles that bring it into contact with other systems (a way of viewing the side reactions of glycolysis and the Krebs cycle). However, in nature such loops give rise to complex temporal dynamics. Investigators who move beyond identifying operations and sequences to consider how they unfold in real time find a wealth of phenomena to be explored. In particular, there has been an emerging awareness of the importance of oscillations in biological systems. Many oscillatory phenomena, such as the rhythmic flashing of fireflies and the beating of hearts, are obvious to the unaided senses. Others were not discovered until scientific instruments and research techniques were appropriately deployed by an attentive investigator. For example, neural oscillations have attracted substantial interest and novel proposals (see Buzsáki, 2006, for discussion). It appears that oscillations are quite widespread in the biological world, from the intracellular chemical level all the way to the ecological level.

Despite this, biochemists and numerous other biological researchers have traditionally proceeded in a manner that blinds them to oscillations. Giving little thought to potential regularities across time that might be functional for the process of interest, but giving much thought to minimizing fluctuations regarded as noise in the data, they use preparations and techniques intended to create a steady-state system in close to equilibrium conditions. Moreover, focusing on summary measures such as mean and standard deviation conceals the dynamics of variation across time. Finding and explaining oscillations requires a major shift in thinking. We discuss three telling cases in which scientists have advanced evidence for oscillatory phenomena, identified the responsible mechanism, and investigated its characteristics as a biological oscillator. The first two cases involve *ultradian* oscillators (those with periods substantially shorter than 24 hours) and the third involves circadian oscillators (those with a period of approximately 24 hours):

(a) ultradian oscillations in the glycolytic pathway discussed above;
(b) ultradian oscillations separating glycolytic metabolism (during which DNA replication and protein synthesis occur) from oxidative metabolism;
(c) circadian oscillations that coordinate the physiological processes and behavior of most organisms with the day-night oscillation in the environment.

In each case, once an oscillator was identified and characterized, the key question of its biological significance had to be addressed. The three cases are discussed in order from least to most satisfactory answers at our current level of knowledge: glycolytic oscillations with regular periodicity have not even been conclusively shown to occur under physiologically realistic conditions, whereas there is strong evidence that some circadian oscillators subserve important

biological functions. (Perhaps coincidentally, cases (a) to (c) also are ordered from shortest to longest period of oscillation.)

Glycolytic oscillations

Glycolysis provides a potent first example of the discovery and explanation of oscillations in what traditionally had been approached as a steady-state system. The initial discovery stemmed from Britton Chance's pioneering efforts to quantify biochemical processes. Working in Chance's laboratory, Amal Ghosh produced glycolysis by the usual method (adding the substrate, glucose, to suspensions of extracts from baker's yeast, which provide the necessary glycolytic enzymes). When he used Chance's spectrophotometric techniques to more closely examine the dynamics of the reaction sequence, he found that the concentration of NADH oscillated with a period of about 1 minute (Chance, Estabrook, & Ghosh, 1964). The oscillations dampened rapidly, but Hess, Brand, and Pye (1966) developed a preparation in which oscillations of NADH continued for up to 22 hours. Within a few years, further tests revealed that the other reactants in glycolysis also showed periodic oscillations in their concentrations (Hess, Boiteux, & Krüger, 1969). Moreover, neighboring reactants in the glycolytic pathway oscillated together (i.e., in phase), whereas those on opposite sides of two major reactions were opposed (i.e., 180^o out of phase). The idealized graphical representation in Figure 5 shows that each reactant could be assigned to one of just four oscillatory patterns differing in relative phase, and that the phase offset ($\Delta\alpha$) for the top versus bottom inverse pairs varied with conditions—here, 70^o. By referring back to Figure 1, it can be seen where each subset of reactants resides in the glycolytic pathway—the first step in achieving a dynamic mechanistic explanation of the oscillatory phenomena.

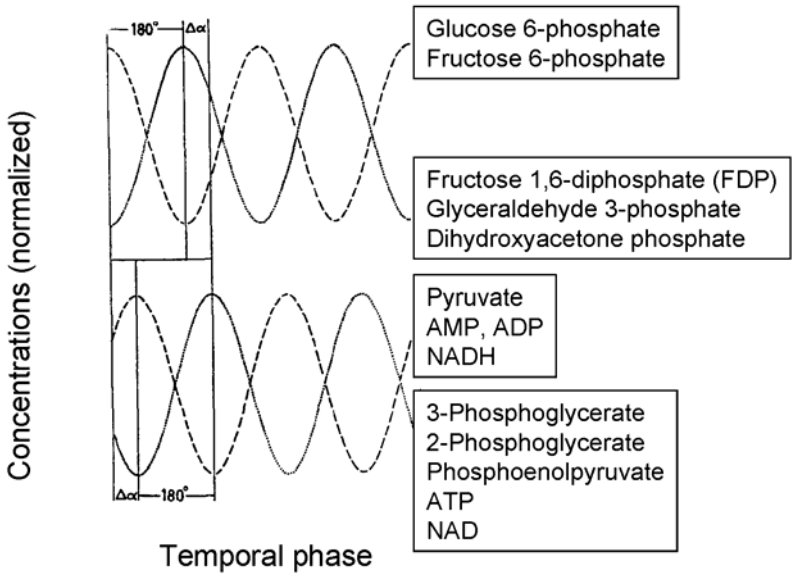


Figure 5. Idealized relative phases in the glycolytic oscillator. When each reactant's normalized concentration over time is plotted, they fall into four phase groups as

shown. See text for description. (Adapted from Hess, Boiteux, & Krüger, 1969, Figure 8.)

The next step requires zooming in on the third reaction in Figure 1, in which the transfer of a phosphate group from ATP converts F6P to FDP and ATP to ADP. The fact that the first two sets of reactants in Figure 5 straddle this reaction with a phase offset of 180^o points to the enzyme involved, phosphofructokinase (PFK), as the critical factor in the oscillation (Hess, Boiteux, & Krüger, 1969). PFK is an allosteric enzyme—that is, an enzyme with binding sites not only for its substrate but also for other molecules that modulate its activity When these modulators are at high concentrations they more readily bind to PFK and hence have a greater effect, which is stimulatory for some modulators and inhibitory for others (Monod, Wyman, & Changeux, 1966). It turns out that PFK is stimulated in this way by both products of the reaction it catalyzes (FDP and ADP) and also by AMP (made from ADP by removal of one of its two phosphate groups). Their binding to PFK serves a regulatory function, causing the entire glycolytic process to run faster—thus increasing the concentrations of NADH (immediately downstream) and ATP (further downstream). But high concentrations of ATP inhibit the reaction, contributing to its regulation by putting a long-range negative feedback loop into contention against the short-range positive feedback loops.

The times at which each loop has its maximum effect alternate, influenced in part by depletion and resupply of the substrate (F6P) and of the reactants in those loops. The interactions are quite complex, but some sense of the dynamics can be obtained by focusing on individual reactants. For example, concentrations of FDP (a product of the reaction) would tend to rise as the reaction runs faster (due in part to its own positive feedback loop) and then level off and fall as the reaction runs slower (due in part to inhibition from the ATP that had become plentiful when the reaction ran fast), then level off and rise again, and so forth. Concentrations of F6P (the substrate) would show the inverse pattern—its supply gets depleted as the reaction runs fast, which makes the reaction run slower, which allows the supply to build back up. As a final example, ATP's inhibitory effect results in less ATP being produced, which leads to less inhibition, which leads to more ATP, and so forth. In short, the feedback loops and other processes that deplete or resupply reactants dynamically interact. The net effect is a periodic oscillation in the rate of the reaction and, due to its regulatory role, in the overall rate of glycolysis. This results in measurable oscillations in the concentrations of the various reactants in the glycolytic pathway—with phase offsets of 0^o, 70^o, or 180^o between different reactants (Figure 5) depending on where they fall in the pathway and the feedback loops.

We have qualitatively described the mechanism creating the oscillations, but equations specifying its operations quantitatively are necessary to account for period and amplitude of the oscillation and the conditions under which it will occur. Already in 1964 Joseph Higgins, working with Chance, published a computational model of glycolytic oscillation. It focused on the reaction catalyzed by PFK and just three of the factors known to influence it: availability of its substrate (F6P), positive feedback from the product (FDP), and removal of the product (Higgins, 1964, p. 994). He succeeded in finding parameter value ranges in which concentrations of F6P and FDP oscillated with a phase offset close to 180^o. Shortly thereafter Sel'Kov (1968)

argued that Higgins' model failed to generate a limit cycle[7] using physiologically realistic parameter values, and advanced an alternative model that, most importantly, included the inhibitory ATP loop. With the primary source of tension now between this long-range negative feedback loop and the short-range positive feedback loops, the dynamics of this model much more closely resembled the known dynamics of the (in vitro) reaction. Thus, the PFK-catalyzed reaction alone could carry much of the explanatory burden for glycolytic oscillations, as shown also by Goldbeter & Leferver (1972) in a model comprising seven differential equations. Nonetheless, a different approach, constructing equations for all the reaction steps in glycolysis (involving 57 differential equations), was pursued by Garfinkel, Frenkel and Garfinkel. Notably, it included the other allosteric enzyme-catalyzed reaction in the glycolytic pathway, that in which ATP is resynthesized in the conversion of phosphoenolpyruvate to pyruvate. Not coincidentally, that is the reaction straddled by the third and fourth sets of reactants in Figure 5 which, like the first two sets, exhibit a phase offset of 180^{o}. But this reaction was not a major contributor to the overall rate of glycolysis. Including it (along with all the other reactions) yielded an account that, while more precise, was unwieldy.

None of these modelers was satisfied merely to find equations and parameter values that produced the targeted phenomena; instead, they coordinated mechanistic and dynamical systems approaches to explanation in drawing out the significance of their mathematical models. On the mechanistic side, the variables and parameters in their equations were anchored to specific parts and operations in a specific mechanistic account of glycolysis—not to global properties of the glycolytic pathway. On the dynamic side, in their pursuit of a deeper understanding of biological oscillations they used to good advantage tools for analysis of complex systems, such as limit cycles and bifurcations. We cannot develop that here, but a good early source is Gurel (1975). In the 1980s and 1990s these applications were extended in a variety of ways. Hess (1997) provides a review of modeling and empirical work on cell-free glycolysis that reveals a full range of dynamic phenomena, from steady state to periodic to chaotic.

One extension involved the dynamics of coupled oscillators. It is a striking fact that when Huygens mounted several pendulums on the same non-rigid wall, they synchronized their oscillations. This required that an energetic product of at least one of these oscillators perturbs the oscillation of the others. Hence, when Chance et al. (1973) found that large populations of cells tended to synchronize their glycolytic oscillations, it raised the question of what cell product was responsible. More than 20 years later, Richard, Teusink, Hemker, Dam & Westerhoff (1996) determined that concentrations of acetaldehyde secreted into the extracellular milieu from individual cells oscillated at the same frequency as the glycolytic oscillations, and that adding acetaldehyde to a preparation could shift the phase. This pointed to it as the synchronizing agent between independent oscillating cells.

To return to the point with which we opened this section, glycolytic oscillations provide an excellent first example of how investigators came to appreciate that cyclic organization can give

[7] To show that the two reactants oscillate out of phase, it is sufficient to plot each across time as in Figure 5. Equivalently, the concentrations of F6P and FDP can be plotted against each other (one point per timestep); this yields a repeating loop specific to the range of concentrations and their phase offset. To show that this loop is a limit cycle, it must also be the case that if the initial pair of values (F6P, FDP) lie off the loop, or if the system is perturbed, subsequent values follow a trajectory that brings them onto the loop.

rise to unexpected temporal patterns. This involved two adjustments in how they construed glycolysis. First, since glycolysis is a continuous process, the default assumption had been that concentrations of the reactants would hold steady or change gradually with conditions, but in fact they oscillate with a period of approximately one minute. Second, glycolysis is not a purely linear pathway, but crucially involves cycles. This was illustrated very schematically in Figure 4, but it turned out that the ATP/ADP and NADH/NAD^+ cycles as shown there were only part of the story. Computational modeling indicated that the key cycles were positive and negative feedback loops modulating the allosteric PFK-catalyzed reaction that converts F6P to FDP. To underscore this crucial point: it was not just that ATP obtained late in the pathway could supply a reaction early in the pathway (on the next turn of the cycle), but also that in so doing, ATP binds to PFK so as to inhibit it.

Britton Chance was particularly attracted to the glycolytic oscillator because he foresaw that it might be the basis for explaining the ability of organisms to endogenously keep time so as to produce behaviors at the appropriate time of day (circadian rhythms). Chance was to be doubly foiled in finding any confirmation for what he envisioned, however. First, as we discuss below, more recent research seeking a mechanistic explanation of circadian rhythms has pointed to gene expression, not glycolysis. Second, it proved difficult to get evidence that glycolytic oscillations occur under physiological conditions (even in whole yeast cells) and hence that they have functional significance in organisms.[8] But other oscillatory processes, with periods only slightly longer than those of glycolytic oscillators, appear to be important to physiological processes as they clearly do occur under physiological conditions and are demonstrably employed in regulating cellular processes. We turn next to these.

Other Ultradian Oscillations

In addition to glycolytic oscillations with a periodicity of one minute, researchers were finding other ultradian oscillations in the biochemical processes within cells. (Rapp, 1979, provides an atlas of oscillators discovered through the 1970s.) We will focus on findings of an oscillation in the overall metabolic cycle (i.e., alternations between glycolysis and oxidative metabolism) and the important claim that this oscillation is coupled both to the cell division cycle (based on measurements of the timing of DNA replication) and to gene expression (based on measurements of the timing of DNA transcription or protein synthesis). It has been easier to demonstrate such

[8] In the 1970s there were a variety of proposals as to the functional significance of the glycolytic oscillator. It was thought, for example, that it might drive rhythmic contractions in slime molds or account for slow wave oscillations in insulin secreting β-cells (via a decrease in potassium conductance attributed to GAP dehydrogenase and phosphoglycerate kinase). Given the failure to find compelling evidence for any of these proposals, research on glycolytic oscillations declined after the 1970s. However, a new round of research was undertaken by Hans Westerhoff and his colleagues in the 1990s, spurred by the development of techniques that permitted measurement of metabolite concentrations in whole cells. Danø, Sørensen, & Hynne (1999), for example, found a way to make measurements while continuously providing glucose and cyanide (to suppress oxidative metabolism) and removing waste, They determined that a stable attractor gave way to a limit cycle as the flow of substrate increased and that, if perturbed, the reactions showed a spiraling return to the unstable attractor—characteristics of a Hopf bifurcation. Richard, Teusink, Westerhoff, and van Dam (1993) found that some of the metabolites generated after FDP—glyceraldehyde-3-phosphate (GAP), dihydroxyacetone phosphate, and phosphoenolpyruvate—either did not oscillate or did so with much smaller amplitudes. This suggested to them that the NADH oscillations were due, not to the reaction catalyzed by PFK, but rather to oscillations in the Gibbs energy of ATP hydrolysis (with the coupling achieved by GAP dehydrogenase and phosphoglycerate kinase).

oscillations than to get consensus on their timing. The first reports regarding the metabolic cycle involved periods of just a few minutes, whereas those for protein synthesis were closer to one hour. But in brewer's yeast (*Saccharomyces cerevisiae*) grown under aerobic, glucose-restricted conditions, Satroutdinov, Kuriyama, and Kobayashi (1992) found a 40-minute metabolic cycle with oscillations in numerous reactants. Ethanol, for example, accumulated during the glycolytic (anaerobic) phase and was re-assimilated during the oxidative (aerobic) phase, whereas several other reactants showed the opposite oscillation (a phase offset of 180^o). As in the case of the much faster-running glycolytic oscillator, these oscillations were synchronized across cells via the action of diffusible substances such as acetaldehyde and H_2S (Sohn, Murray, & Kuriyama, 2000). This became a model system for David Lloyd and Douglas Murray, who found oscillations in concentrations of a host of reactants, including NAD and NADP, glutathione, ethanol, acetaldehyde, acetic acid, H_2S, and residual O_2 (the oxygen that remains dissolved in the media after the organisms have drawn what they need). Lloyd and Murray (2005, p. 376) referred to this suite of oscillations as the *ultradian metronome*:

> We propose that the 40-min oscillation percolates not only throughout the cellular network, including organelles, transcriptome, metabolome and proteome, but also throughout the entire population of organisms. This oscillatory state is not an exceptional curiosity found only in a peculiar system but, rather, a universal trait that is necessary for the maintenance of the robust metabolic auto-dynamic state characteristic of normally healthy cells.

Lloyd and Murray (2007) reported oscillations of this kind in cells from a variety of organisms (though with some variations from the 40-minute period) and found them to be coupled both to the cell division cycle and to gene expression. Specifically, the glycolytic phase of the metabolic cycle (the peak period for the reduced forms NADH and NADPH) coincides with DNA replication and transcription, whereas the oxidative metabolism phase (the peak period for the oxidized forms $NADH^+$ and $NADPH^+$) coincides with the parts of those cycles in which DNA is intact. Lloyd and Murray (2007) also proposed a candidate mechanism for the coupling.
At its core is a reduction-oxidation (redox) cycle that constructs and breaks down disulfide bridges between two molecules of glutathione (or other thiols or small proteins). The resulting 40-minute period is robust through a wide range of temperature fluctuations, a phenomenon known as temperature compensation (Murray, Roller, Kuriyama, & Lloyd, 2001).[9] Most important, this redox cycle mediates the couplings of interest. It links to metabolic pathways via NAD and NADP. That it links to DNA transcription (initiating gene expression) is evidenced by their examination of the transcripts of 5329 genes: 650 were maximally expressed during the oxidative phase and 4679 during the reductive phase (Murray, Beckmann, & Kitano, 2007).[10] It links to DNA replication probabilistically: on any given oscillation only a subset of the cells

[9] Lloyd (2006) proposed that the central role of sulfur both in the synchronizing of rhythms between cells via H_2S and the building of disulfide bridges in the intracellular maintenance of the cycle could be a remnant of the origin of eukaryotic cells through a sulfur syntrophy between a-Proteobacterim and an archaeobacterial sulfide-producing host. These are progenitors of today's photosynthetic green sulfur bacteria that oxidized H_2S (either photosynthetically or using O_2) and basal Archeon, which reduced sulfur to H_2S. Such a proposal for the origin of modern mitochondria is advocated by Searcy (2003).

[10] The first suggestion of oscillations in gene expression stemmed from Soviet research Vsevolod Brodsky (1975, 2000). Using UV-cytophotometry and microinterferometry to measure, among other things, RNA content, protein content, and amino acid incorporation into proteins, he identified oscillations ranging from 20 to 120 minutes, which he referred to as *circahoralian rhythms*.

initiate DNA replication (for cell division), but over about 8 hours all cells will replicate their DNA. We will return to the potential significance of this coupling in section 4.

One last finding bears mention. Tu & McKnight (2006) reported oscillations in the same systems, and the same couplings, but with periodicity of approximately 4 to 5 hours rather than 40 minutes—a disconcerting discrepancy that has not yet been resolved. One possibility is that each 4-5 hour oscillation contains within it (at a lower level) a number of 40-minute oscillations.

Circadian Oscillations

We conclude this discussion of oscillations with perhaps the best known class of oscillatory phenomena in biology: circadian rhythms (*circa* = about + *dies* = day). A variety of physiological and behavioral manifestations have been reported from ancient times, apparently in nearly all life forms (e.g., body temperature in animals and the folding of leaves in plants; cave dwelling organisms are the most likely exception). Circadian rhythms did not become a focus of experimental investigation in biology until the work of Colin Pittendrigh (1960) and his contemporaries. The initial focus was to rigorously establish that the rhythms in animals were endogenously controlled by recording them after eliminating Zeitgebers (exogenous cues, such as daily light and temperature cycles). Experiments in caves showed that oscillations indeed were maintained under these conditions, albeit with periodicity varying somewhat from 24 hours. The first explanatory challenge was to find the endogenous mechanism(s) responsible for maintaining an approximately 24-hour rhythm. The second challenge was to find out how any such endogenous mechanism could be entrained by Zeitgebers so as to stay in synchrony with local day-night cycles, especially as they varied across seasons of the year, and how they could remain constant over a wide range of temperatures.

Both challenges sent researchers down to molecular biology to find answers. In the search for a molecular mechanism that could oscillate with an approximately 24-hour period, the first clue came from Konopka and Benzer (1971). They identified a gene in *Drosophila* for which mutations resulted in shortened or lengthened rhythms or arrhythmic behavior, which they named *period* (*per*). The cloning of *per* in the 1980s led to the discovery that concentrations of its mRNA and protein oscillate in cells: specifically, *per*-mRNA peaks at the beginning of the night and the protein it codes for, PER, peaks about 6 hours later. Hardin, Hall, and Rosbash (1990) proposed a mechanism with a feedback loop to explain these phenomena, as shown schematically in Figure 6. First, transcription of the *per* gene generates *per* mRNA in the nucleus. These macromolecules are transported to the cytoplasm, where they are translated by ribosomes into molecules of the corresponding protein PER. After several hours PER molecules are transported back into the nucleus, where they suppress further transcription of *per*. This decreases the rate of synthesis of PER and hence also its transport into the nucleus. As the PER already present in the nucleus is broken down, *per* is released from inhibition and a new turn of the cycle begins. The elegant design of this mechanism has turned out to be applicable in a variety of other contexts. The general labels and pathway in Figure 6 (gene → mRNA and so forth) therefore qualify as a *mechanism schema*, in the terminology introduced by Machamer, Darden & Craver (2000). In this particular context, however, some important parts and operations in the mechanism were still unknown; for example, it was not understood how PER

could suppress *per* transcription since PER molecules lack the necessary region for binding to DNA.

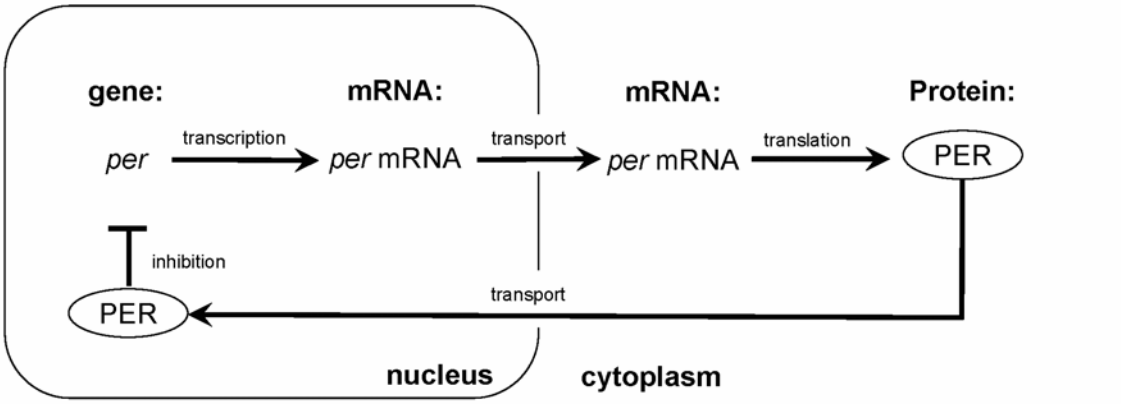
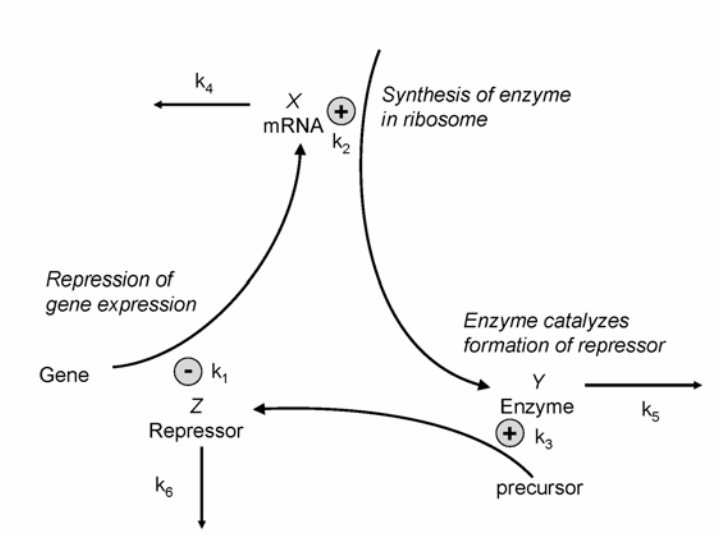


Figure 6. Hardin, Hall, and Rosbash's (1990) mechanism for circadian oscillations in *Drosophila*. Expression of the gene *per* (transcription, transport and translation) produces the protein, PER, which is transported back into the nucleus. There PER inhibits further transcription of *per*. As this nuclear PER breaks down, *per* is released from inhibition and a new turn of the cycle begins.

Given the complexity of the interactions, mathematical modeling is needed to determine whether such a mechanism is actually capable of generating oscillations. Already in the 1960s, just as oscillatory phenomena were being discovered in living systems, Brian Goodwin (1965) offered an initial proposal. Inspired by the *operon* gene control mechanism proposed by Jacob and Monod (1961), he developed a system of equations that characterized a generalized version of that mechanism (Figure 7). Here two kinds of proteins collaborate to inhibit gene expression: (1) an enzyme, and (2) the product of a reaction catalyzed by that enzyme, which as a repressor molecule directly inhibits gene expression. The critical parameter for determining whether oscillations occur is n (also known as the Hill coefficient), which specifies the minimum number of interacting molecules needed to inhibit expression of the gene. Carrying out simulations on an analogue computer, Goodwin concluded that oscillations would arise with n equal to two or three. But subsequent simulations by Griffith (1968) determined that oscillations occurred only with $n > 9$, a condition that was deemed biologically unrealistic. However, if nonlinearities were introduced elsewhere (e.g., in the subtracted terms representing the removal of the various substrates from the system), it was possible to obtain oscillations with more realistic values of n. Accordingly, Goldbetter (1995b) developed his own initial model of the *Drosophila* circadian oscillator by modifying the Goodwin oscillator. By capturing the operations in the circadian mechanism shown in Figure 6 in a system of differential equations adapted from those in Figure 7, he achieved a 24-hour oscillation in concentrations of *per* mRNA and PER. Plotting these against each other over multiple cycles and conditions revealed a limit cycle (i.e., the two periodic oscillations with their particular phase offset acted as an attractor).



$$\frac{dX}{dt} = \frac{k_1}{Z^n + 1} - k_4 X$$

$$\frac{dY}{dt} = k_2 X - k_5 Y$$

$$\frac{dZ}{dt} = k_3 Y - k_6 Z$$

Figure 7. The Goodwin oscillator. The curved arrows specify that a gene, when not repressed, is translated into mRNA *(X)*, which is transcribed into an enzyme *(Y)* in the ribosome, which then catalyzes the formation of the repressor protein *(Z)*. The repressor then slows down the process that created it. The straight arrows indicate that the mRNA, enzyme and repressor molecules gradually break down. The rate of each operation is specified by a parameter (k_1, k_2, . . . k_6).. The differential equations on the right give the rates at which concentrations of *X*, *Y*, and *Z* change over time *(t)*.

In the subsequent decade many additional components of the intracellular circadian oscillator in *Drosophila* were discovered and it was established that the oscillator in mammals utilizes homologues of many of the same components, albeit with some salient differences. The crucial cells for maintaining circadian rhythms in mammals had been localized in the 1970s to the suprachiasmatic nucleus (SCN), a midbrain structure of approximately 10,000 neurons, in each hemisphere, located just above the optic chiasm. Using neonatal rat cells cultured on a microelectrode array, Welsh, Logothetis, Meister, and Reppert (1995) established that individual SCN neurons in culture sustained oscillations with a period of approximately 24 hours, though with a large standard deviation (1.2 hours). The considerable variability eventually prompted great interest, since circadian behavior in organisms is far more precise. After showing much less variability in measurements of period length for running wheel behavior in mice and for SCN slices compared with dispersed neurons, Herzog, Aton, Numano, Sakaki, and Tei (2004, p. 39) concluded: “Taken together, these results indicate that cell-cell interactions within the SCN synchronize SCN cells to each other and narrow the range of free-running periods expressed behaviorally.” The same team subsequently advanced evidence that vasoactive intestinal protein (VIP) was the key synchronizing agent. SCN has two regions (core and shell), and a subset of cells in the core that release VIP are the only SCN cells that maintain sustained oscillations. It now appears that cells in the SCN shell are dependent on the VIP releasing cells for both continued oscillation and synchrony. Synchronizing of oscillators is known to be tricky and can often result in toroidal oscillations, deterministic chaos, or the coexistence of multiple attractors (Grebogi, Ott, & Yorke, 1987). A variety of simulations in the last few years have demonstrated that release of VIP is capable, at least in the models, of sustaining oscillations and producing synchronization. Moreover, using biologically plausible parameter values, Bernard, Gonze,

Čajavec, Herzel, and Kramer (2007) have replicated the empirical finding that shell oscillators tend to oscillate approximately 40 minutes ahead of core oscillators.

The problems of synchronization loom larger, however, when one considers responses to external inputs from Zeitgebers that are radically out of synchrony with the internal oscillators. The resulting disruptions are something human travelers experience when they cross multiple time zones. The effects of jetlag show up not just in sleep, but in a wide range of behavioral and physiological variables. Researchers learned that such variables are directly influenced by peripheral clocks in bodily organs and brain regions. Originally it appeared that these peripheral oscillators could not sustain oscillations when cut off from the SCN, so they were regarded as "slaves." However, there is now evidence that they can sustain their own oscillations but rely on complex couplings with the SCN for synchronization (Welsh, Yoo, Liu, Takahashi, & Kay, 2004). Recent simulations of relations between the SCN core and shell and between the shell and peripheral oscillators reveal complex responses when the system is perturbed by a six-hour change in day-light cycles (comparable to those experienced by travelers flying between North America and Europe). Empirical studies revealed that although cells in the SCN shell usually exhibit peaks in PER prior to those in the core, after a six hour light advance this order was reversed. Moreover, both advanced more than six hours (overshot the target adjustment) and it took several days to restore normal synchronization (Nakamura, Yamazaki, Takasu, Mishima, & Block, 2005). In simulating the response of coupled oscillators representing both core and shell SCN oscillators and those in peripheral organs, Leise and Siegelmann (2006) found a very complex dynamical pattern including overshoots like those of the actual SCN oscillators. They also were successful in simulating the large number of cycles required before the peripheral oscillators returned to a normal relation to the SCN. (For further discussion of the roles of computational modeling and experimental research in achieving dynamic mechanistic explanations of circadian rhythms, see Bechtel & Abrahamsen, in press.)

4. Cyclic Organization and Oscillations as Features of Autonomous Systems

One might treat the prevalence of cyclic organization and of oscillatory dynamics in living systems as simply accidents of the way biological systems happened to develop. But in fact both are of fundamental significance. One of the important features of living organisms is that they are systems far from thermodynamic equilibrium; to maintain themselves as such they must recruit matter and free energy from their environments and deploy them in the construction and repair of their own components. Insofar as such systems determine their own future existence by their success in constructing and maintaining themselves, they are referred to as *autonomous systems* (note 3). Ruiz-Mirazo and Moreno (2004) characterize basic autonomy in terms of

> the capacity of a system to *manage* the flow of matter and energy through it so that it can, at the same time, regulate, modify, and control: (i) internal self-constructive processes and (ii) processes of exchange with the environment. Thus, the system must be able to generate and regenerate all the constraints—including part of its boundary conditions—that define it as such, together with its own particular way of interacting with the environment (p. 240).

An autonomous system is, of necessity, an active system—it must continually perform operations to maintain itself in a non-equilibrium relation with its environment. It contrasts with

reactive systems that primarily respond to their environment. As Goodwin describes, the reactive perspective has been assumed in much biological research: "The traditional view of the cell as a biochemical system is that molecular populations move towards steady-state levels determined by the environment, and that when a steady state is reached the system maintains itself by a constant flow of intermediates. This view regards the cell as a passive system which changes state only in response to environmental stimuli" (Goodwin, 1965, p. 425). Goodwin went on to show through simple models of feedback between reactions with nonlinear kinetics that spontaneous rhythmic activity was to be expected in cells and proposed: "This intrinsic rhythmic activity represents a type of biological energy which cells and organisms can use for organizing in time the staggering complexity of biochemical processes which make up living systems, thus achieving coherence and order in these activities. The interactions of nonlinear oscillators, illustrated in this paper, provide a dynamic basis for this self-organizing property of oscillating cellular control circuits" (p. 436).

To build and maintain themselves, living organisms require at their core a metabolic system that captures energy and builds the basic constituents of the organism itself. It also requires the management of a boundary so that substances needed by the system are admitted and waste products are expelled. These represent two of the three components of Gánti's (1975, 2003) proposal for a chemoton—a minimal system that manifests the basic features of living systems. Gánti's third component is a control system, which he implements through a mechanism for building polymers loosely inspired by DNA. Although such a component can play an important role in controlling a system (Griesemer & Szathmáry, 2008), considerable regulation can be achieved through cyclic organization and oscillatory processes without an external control system. It is an open question whether these are sufficient to realize all of the fundamental regulatory processes of life, or alternatively, whether entities comparable to genes are required to regulate even basic metabolism and movement of substances to and from the chemoton.[11]

The claim is that a particular biological cycle, regardless of whether it produces oscillations, provides a vehicle for regulating a system so that individual operations are performed at the time needed. One way to see this is to look at one of the feedback loops in glycolysis on its own, rather than in the usual competitive context known to produce oscillations. In particular, consider the negative loop in which high concentrations of ATP inhibit the PFK catalyzed reaction. This ensures that glucose will not be metabolized unless energy is needed for other cell activities. Thus, even a single cycle enables at least some regulation of an important metabolic process.

[11] One argument for the claim that something like genes are needed for effective control is that in all known organisms metabolic reactions and control over boundaries are achieved by complex proteins, and we have no account of how these structures could reliably develop in simple chemical systems via self-organization alone. Moreno (personal communication, September 2008) contends that in order to realize effective control, the controller must be at least partly dynamically decoupled from the system controlled. Before concluding that this is correct, though, we should explore further how much regulation can be achieved through interactions such as those that give rise to limit cycles that can be employed to segregate reactions in time (discussed below). At some point, living systems did begin to rely on structures such as genes as partially decoupled controllers. Clearly a significant consequence of relying on genes as control elements is that their stability enables them to be inherited and thereby provide the heritability needed in evolutionary processes including natural selection. It is important to note that genes, as well as the polymers generated in Gánti's chemoton, are static entities that do nothing on their own. Other components, including the apparatus for transcribing DNA into mRNA, editing the mRNA, and translating mRNA into proteins must also be inherited. Even if partly decoupled from what it controls, the actual control system is itself a dynamic system, not a static element.

When a mechanism's temporal dynamics do result in oscillations, these too can be used to regulate other mechanisms by coupling their activity. Goodwin (1963) proposed that oscillators provided a means of temporally segregating incompatible cellular events.[12] Recall that DNA replication and most transcription of genes occurs during the glycolytic phase, when oxygen consumption is low. Both DNA replication and transcription involve opening up the double helix structure and exposing the nucleic acids, which can be damaged by exposure to oxygen. Thus, by limiting these activities to periods when oxygen levels are low, DNA is protected (Lloyd & Murray, 2006; Tu & McKnight, 2006).

Circadian oscillations provide an even stronger illustration of the idea that oscillatory processes provide a means of segregating incompatible operations. A clear example is found in the cyanobacterium, *Synechococcus elongates*. The enzyme nitrogenase, critical for nitrogen fixation, is destroyed by oxygen, which the organism produces during photosynthesis. Its circadian oscillator ensures that nitrogen fixation and photosynthesis occur at different times, with photosynthesis proceeding during daylight hours, when the required sunlight is most likely to be available, and nitrogen fixation at night, when no oxygen is being produced.

Circadian oscillations also perform another control role: enabling physiological and behavioural processes to occur at optimal times of day. Examples include sleep during the night (for diurnal animals) sleep during the day (for nocturnal animals), and food foraging when prey are available. It might seem sufficient to rely on environmental cues for many of these activities, but appropriate performance often requires preparation before the environment cue would be available.

5. Conclusion: Implications for Mechanistic Science

Mechanistic research has been extremely successful in identifying the parts, operations, and basic organization of a vast range of biological mechanisms. It has been less successful, though, in understanding the implications of various forms of organization—especially the temporal dynamics that orchestrate the functioning of biological mechanisms. Biochemists' basic strategy has been to put together linear sequences of reactions, moving to cyclic organization (e.g., the Krebs cycle) only as necessary. By focusing on near-equilibrium steady-state conditions and summary statistics (e.g., mean concentration of a metabolite), traditionally biologists have screened themselves off from oscillatory phenomena. We have suggested that the resulting mechanistic accounts are blind to crucial dynamics of the systems they target. The new mechanistic philosophy of science has tended to parallel biology in this respect, emphasizing the discovery of component parts and operations and simple organizational schemes, and providing little systematic attention to orchestration.

[12] Spatial differentiation of organelles is another way to obtain such segregation. Enzymes involved in breaking down cellular constituents, for example, are segregated in the lysosome so that they operate only on materials that have been transported for that purpose into that organelle. Temporal segregation can achieve the same purpose.

Although explanation in biology remains primarily mechanistic, small clusters of investigators have confronted the complex dynamics that serve to orchestrate the functioning of biological mechanisms. We have described a few of these endeavors, focusing especially on explorations of the oscillatory dynamics that can result from some common types of cyclic organization (for other exemplars, see Goldbeter, 1995a; Noble, 2006; Buzsáki, 2006; Ellner & Guckenheimer, 2006). All of the cases we discussed were grounded in accounts of parts, operations, and organization of a particular biological mechanism but added concepts and tools of mathematical modeling and dynamical systems. Hence, they well exemplify the project of dynamic mechanistic explanation that we have endorsed.

Dynamic mechanistic explanation stands in contrast not only to purely mechanistic explanation but also to theoretical inquiries that emphasize complex dynamics in living systems conceived abstractly—at best neglecting but in some cases explicitly rejecting the mechanistic project. Artificial life research, for example, is conducted on a plane removed from research on actual biological mechanisms. While accounts oriented purely to complexity or dynamics can make unique and valuable contributions, they provide no understanding of how the dynamic relations are actually realized in living systems if they do not get anchored to component parts and operations of actual mechanisms. That is, they are empty. We contend that complexity and dynamical systems theory find their best use as tools in a more integrated endeavor.

Some theoretical biologists (e.g., Kauffman, 2000) have not only preferred to work on an abstract plane, but also aspired to achieve a unified, law-based theoretical framework. In the spirit of cybernetics and general systems theory, they direct themselves to the big picture that seems to be neglected in reductionistic inquiry. Again, this endeavor has produced some ingenious and valuable directions for further inquiry, but does not in itself achieve the integration we regard as crucial. The most promising contributions for near-term integration probably come not from comprehensive systems, but from particular proposed principles of organization: self-organization through positive feedback in non-equilibrium conditions, small-world organization, scale-free networks (Barabási & Bonabeau, 2003), and so forth.

A characteristic feature of modern biology is its particularity. Biochemical pathways, while showing common patterns across phyla, also reveal substantial differences that matter to the functioning of particular organisms. The same is true of circadian oscillators (Bechtel, in press). The resulting extrapolation from studied models to other systems is very different from the generalization achieved by universal quantifiers in laws. Researchers do not know in advance which features change and which remain constant (or nearly so) when extrapolating, and must be prepared to modify specific parts and operations in mechanisms as they move to new instances. The same is likely to apply to the tools of complex systems analysis. That is, the general understanding of how small-worlds phenomena emerge from a few long-range connections in networks primarily constituted of short-range connections will need to be adapted given the particular long-range connections found in a given system. Complex systems analyses provide a rich toolkit for appreciating organization and orchestration of operations in biological mechanisms, and invoking these tools can illuminate how these mechanisms generate the rich phenomena biology seeks to explain. This will not, however, obviate the need to understand the particularity of any given mechanism.

Thus, we see an immediate future in which dynamic mechanistic researchers in biology will continue to offer piecemeal, context-specific accounts, even as they stretch them to incorporate dynamics. Systems biologists and philosophers of science can, and should, add insight and perspective while remaining grounded by examining a spectrum of these accounts. Such generalizations as we extract will be works in progress, with frequently modified contextual and other limitations, and will not readily be systematized—including those regarding cycles, oscillations and autonomy.

Acknowledgements: We thank Clifford Hooker, Xabier Barandiaran, Michael Grinfeld, Alvaro Moreno, and Kepa Ruiz-Mirazo for very helpful comments on an earlier version of this chapter.

References

Barabási, A.-L., & Bonabeau, E. (2003). Scale-free networks. *Scientific American*(May), 50-59.

Bechtel, W. (2006). *Discovering cell mechanisms: The creation of modern cell biology*. Cambridge: Cambridge University Press.

Bechtel, W. (in press). Generalization and discovery through conserved mechanisms: Cross species research on circadian oscillators. *Philosophy of Science*.

Bechtel, W., & Abrahamsen, A. (2005). Explanation: A mechanist alternative. *Studies in History and Philosophy of Biological and Biomedical Sciences, 36*, 421-441.

Bechtel, W., & Abrahamsen, A. (in press). Dynamic mechanistic explanation: Computational modeling of circadian rhythms as an exemplar for cognitive science. *Studies in History and Philosophy of Science Part A*.

Bechtel, W., & Richardson, R. C. (1993). *Discovering complexity: Decomposition and localization as strategies in scientific research*. Princeton, NJ: Princeton University Press.

Bernard, S., Gonze, D., Čajavec, B., Herzel, H., & Kramer, A. (2007). Synchronization-induced rhythmicity of circadian oscillators in the suprachiasmatic nucleus. *PLoS Computational Biology, 3*(4), e68.

Bichat, X. (1805). *Recherches Physiologiques sur la Vie et la Mort* (3rd ed.). Paris: Machant.

Bickhard, M. H. (2000). Autonomy, function and representation. *Communication and Cognition - Artificial Intelligence, 17*, 111-131.

Boogerd, F. C., Bruggeman, F. J., Richardson, R. C., Stephan, A., & Westerhoff, H. V. (2005). Emergence and its place in nature: A case study of biochemical networks. *Synthese, 145*, 131-164.

Brodsky, V. Y. (1975). Protein synthesis rhythm. *Journal of Theoretical Biology, 55*(1), 167-200.

Brodsky, V. Y. (2000). Circahoralian (ultradian) cellular rhythms: Initial studies and some results. *Russian Journal of Developmental Biology, 31*, 345-353.

Buzsáki, G. (2006). *Rhythms of the brain*. Oxford: Oxford University Press.

Chance, B., Estabrook, R. W., & Ghosh, A. (1964). Damped sinusoidal oscillations of cytoplasmic reduced pyridine nucleotide in yeast cells. *Proceedings of the National Academy of Sciences, 51*(6), 1244-1251.

Chance, B., Williamson, G., Lee, J., Mela, L., DeVault, D., Ghosh, A. K., et al. (1973). Synchronisation phenomena in oscillation of yeast cells and isolated mitochondria. In B.

Chance, E. K. Pye, A. K. Ghosh & B. Hess (Eds.), *Biological and biochemical oscillations* (pp. 285-300). London: Academic.

Christensen, W. D., & Hooker, C. A. (2000). Autonomy and the emergence of intelligence: Organised interactive construction. *Communication and Cognition - Artificial Intelligence, 17*, 133-157.

Collier, J. D., & Hooker, C. A. (1999). Complexly organised dynamical systems. *Open Systems and Information Dynamics, 6*, 241-302.

Craver, C. (2007). *Explaining the brain: What a science of the mind-brain could be*. New York: Oxford University Press.

Danø, S., Sørensen, P. G., & Hynne, F. (1999). Sustained oscillations in living cells. *Nature, 402*(6759), 320.

Darden, L. (2006). *Reasoning in biological discoveries: Essays on mechanisms, interfield relations, and anomaly resolution*. Cambridge: Cambridge University Press.

Ellner, S. P., & Guckenheimer, J. (2006). *Dynamic models in biology*. Princeton, NJ: Princeton University Press.

Gánti, T. (1975). Organization of chemical reactions into dividing and metabolizing units: The chemotons. *BioSystems, 7*, 15-21.

Gánti, T. (2003). *The principles of life*. New York: Oxford.

Goldbeter, A. (1995a). *Biochemical oscillations and cellular rhythms: The molecular bases of periodic and chaotic behaviour*. Cambridge: Cambridge University Press.

Goldbeter, A. (1995b). A model for circadian oscillations in the *Drosophila* Period protein (PER). *Proceedings of the Royal Society of London. B: Biological Sciences, 261*(1362), 319-324.

Goldbeter, A., & Lefever, R. (1972). Dissipative structures for an allosteric model application to glycolytic oscillations. *Biophysics Journal, 12*(10), 1302-1315.

Goodwin, B. C. (1963). *Temporal organization in cells; a dynamic theory of cellular control processes*. New York: Academic Press.

Goodwin, B. C. (1965). Oscillatory behavior in enzymatic control processes. *Advances in Enzyme Regulation, 3*, 425-428.

Grebogi, C., Ott, E., & Yorke, J. A. (1987). Chaos, strange attractors, and fractal basin boundaries in nonlinear dynamics. *Science, 238*(4827), 632-638.

Griesemer, J. R., & Szathmáry, E. (2008). Gánti's chemoton model and life criteria. In S. Rasmussen, L. Chen, N. Packard, M. Bedau, D. Deamer, P. Stadler & D. Krakauer (Eds.), *Protocells: Bridging nonliving and living matter*. Cambridge, MA: MIT Press.

Griffith, J. S. (1968). Mathematics of cellular control processes I. Negative feedback to one gene. *Journal of Theoretical Biology, 20*(2), 202-208.

Gurel, O. (1975). Limit cycles and bifurcations in biochemical dynamics. *Biosystems, 7*(1), 83-91.

Hardin, P. E., Hall, J. C., & Rosbash, M. (1990). Feedback of the *Drosophila period* gene product on circadian cycling of its messenger RNA levels. *Nature, 343*(6258), 536-540.

Herzog, E. D., Aton, S. J., Numano, R., Sakaki, Y., & Tei, H. (2004). Temporal precision in the mammalian circadian system: A reliable clock from less reliable neurons. *Journal of Biological Rhythms, 19*(1), 35-46.

Hess, B. (1997). Periodic patterns in biochemical reactions. *Quarterly Reviews of Biophysics, 30*(2), 121-176.

Hess, B., Boiteux, A., & Krüger, J. (1969). Cooperation of glycolytic enzymes. *Advances in Enzyme Regulation, 7*, 149-167.

Hess, B., Brand, K., & Pye, K. (1966). Continuous oscillations in a cell-free extract of *S. carlsbergensis*. *Biochemical and Biophysical Research Communications, 23*(1), 102-108.

Higgins, J. (1964). A chemical mechanism for oscillation of glycolytic intermediates in yeast cells. *Proceedings of the National Academy of Sciences, 51*(6), 989-994.

Jacob, F., & Monod, J. (1961). Genetic regulatory systems in the synthesis of proteins. *Journal of Molecular Biology, 3*, 318-356.

Kauffman, S. A. (2000). *Investigations*. Oxford: Oxford University Press.

Konopka, R. J., & Benzer, S. (1971). Clock mutants of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences (USA), 89*, 2112-2116.

Krebs, H. A. (1946-8). Cyclic Processes in Living Matter. *Enzymologia, 12*, 88-100.

Krebs, H. A., & Johnson, W. A. (1937). The role of citric acid in intermediate metabolism in animal tissues. *Enzymologia, 4*, 148-156.

Leise, T., & Siegelmann, H. (2006). Dynamics of a multistage circadian system. *Journal of Biological Rhythms, 21*(4), 314-323.

Lloyd, D. (2006). Hydrogen sulfide: clandestine microbial messenger? *Trends in Microbiology, 14*(10), 456-462.

Lloyd, D., & Murray, D. B. (2005). Ultradian metronome: timekeeper for orchestration of cellular coherence. *Trends in Biochemical Sciences, 30*(7), 373-377.

Lloyd, D., & Murray, D. B. (2006). The temporal architecture of eukaryotic growth. *FEBS Letters, 580*(12), 2830-2835.

Lloyd, D., & Murray, D. B. (2007). Redox rhythmicity: clocks at the core of temporal coherence. *BioEssays, 29*(5), 465-473.

Machamer, P., Darden, L., & Craver, C. (2000). Thinking about mechanisms. *Philosophy of Science, 67*, 1-25.

Maturana, H. R., & Varela, F. J. (1980). Autopoiesis: The organization of the living. In H. R. Maturana & F. J. Varela (Eds.), *Autopoiesis and cognition: The realization of the living* (pp. 59-138). Dordrecht: D. Reidel.

Monod, J., Wyman, J., & Changeux, J.-P. (1966). On the nature of allosteric transitions: A plausible model. *Journal of Molecular Biology, 12*, 88-118.

Murray, D. B., Beckmann, M., & Kitano, H. (2007). Regulation of yeast oscillatory dynamics. *Proceedings of the National Academy of Sciences, 104*(7), 2241-2246.

Murray, D. B., Roller, S., Kuriyama, H., & Lloyd, D. (2001). Clock control of ultradian respiratory oscillation found during yeast continuous culture. *Journal of Bacteriology, 183*(24), 7253-7259.

Nakamura, W., Yamazaki, S., Takasu, N. N., Mishima, K., & Block, G. D. (2005). Differential response of *Period 1* expression within the suprachiasmatic nucleus. *Journal of Neuroscience, 25*(23), 5481-5487.

Noble, D. (2006). *The music of life: Biology beyond the genome*. New York: Oxford.

Pittendrigh, C. S. (1960). Circadian rhythms and the circadian organization of living systems. *Cold Spring Harbor Symposia on Quantitative Biology, 25*, 159-184.

Rapp, P. E. (1979). An atlas of cellular oscillators. *Journal of Experimental Biology, 81*(1), 281-306.

Richard, P., Teusink, B., Hemker, M. B., Dam, K. v., & Westerhoff, H. V. (1996). Sustained oscillations in free-energy state and hexose phosphates in yeast. *Yeast, 12*(8), 731-740.

Richard, P., Teusink, B., Westerhoff, H. V., & van Dam, K. (1993). Around the growth phase transition S. cerevisiae's make-up favours sustained oscillations of intracellular metabolites. *FEBS Letters, 318*(1), 80-82.

Ruiz-Mirazo, K., & Moreno, A. (2004). Basic autonomy as a fundamental step in the synthesis of life. *Artificial Life, 10*, 235-259.

Ruiz-Mirazo, K., Peretó, J., & Moreno, A. (2004). A universal definition of life: Autonomy and open-ended evolution. *Origins of Life and Evolution of the Biosphere, 34*, 323-346.

Satroutdinov, A. D., Kuriyama, H., & Kobayashi, H. (1992). Oscillatory metabolism of *Saccharomyces cerevisiae* in continuous culture. *FEMS Microbiology Letters, 98*(1-3), 261-267.

Searcy, D. G. (2003). Metabolic integration during the evolutionary origin of mitochondria. *Cell Res, 13*(4), 229-238.

Sel'Kov, E. E. (1968). Self-oscillations in glycolysis. 1. A simple kinetic model. *European Journal of Biochemistry, 4*(1), 79-86.

Simon, H. A. (1980). *The sciences of the artificial. Second Edition.* Cambridge, MA: MIT Press.

Skarda, C. A., & Freeman, W. J. (1987). How brains make chaos to make sense of the world. *Behavioral and Brain Sciences, 10*, 161-195.

Sohn, H.-Y., Murray, D. B., & Kuriyama, H. (2000). Ultradian oscillation of *Saccharomyces cerevisiae* during aerobic continuous culture: hydrogen sulphide mediates population synchrony. *Yeast, 16*(13), 1185-1190.

Thagard, P. (2006). *Hot thought: Mechanisms and applications of emotional cognition*. Cambridge, MA: MIT Press.

Thunberg, T. L. (1920). Zur Kenntnis des intermediären Stoffwechsels und der dabei wirksamen Enzyme. *Skandinavisches Archiv für Physiologie, 40*, 9-91.

Tu, B. P., & McKnight, S. L. (2006). Metabolic cycles as an underlying basis of biological oscillations. *Nature Reviews Molecular Cell Biology, 7*(9), 696-701.

Watson, J. D., & Crick, F. H. C. (1953). Molecular structure of nucleic acids. *Nature, 171*, 737-738.

Watts, D., & Strogratz, S. (1998). Collective dynamics of small worlds. *Nature, 393*(440-442).

Welsh, D. K., Logothetis, D. E., Meister, M., & Reppert, S. M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron, 14*(4), 697-706.

Welsh, D. K., Yoo, S.-H., Liu, A. C., Takahashi, J. S., & Kay, S. A. (2004). Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Current Biology, 14*(24), 2289-2295.