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Complex Biological Mechanisms: Cyclic, Oscillatory, and Autonomous

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1. Introduction

The mechanistic perspective has dominated biological disciplines such as biochemistry, physiology, cell and molecular biology, and neuroscience, especially during the 20th century. Moreover, the mechanistic approach has generated an enormous body of information about how living systems function from the genetic and molecular level to the level of the whole organism. But repeatedly critics have emerged to challenge the mechanistic project. Throughout the 18th and 19th century vitalists complained that mechanistic approaches to biology could not explain some of the important features of living systems such as the fact, celebrated by Xavier Bichat (1805), that they "resist death"—that is, they resist the forces in the non-living world that would annihilate them. Although no longer adopting the label "vitalist," 20th century opponents of the reductionist approach to decomposing living systems into their parts and operations alleged that mechanistic sciences failed to recognize that living organisms are wholes, organized systems, with capacities that were very different from their constituent parts.[1] In the past these critics 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 enabled biologists to analyze such systemic properties. In some cases the advocates of complex systems models align with the earlier critics of mechanism and present their proposals as supplanting the mechanistic program. This is misguided. The tools of complex systems modeling provide a needed complement to, but do not supplant, mechanistic research. To paraphrase Kant, dynamical models without mechanistic grounding are empty, while mechanistic models without complex dynamics are blind.

The central contribution of mechanistic science has been to identify and describe, to a first approximation, the components parts and operations that account for the various phenomena exhibited in living systems.[2] The constituents of living systems are important because they both

[1] These critics often appealed to the emergence of phenomena from organized wholes, but the notion of *emergence* has been difficult to explicate and often raises concerns of spooky metaphysics. For a particularly clear discussion of emergence, see Boogerd, Bruggeman, Richardson, Stephan, and Westerhoff (2005).

[2] Ironically, given their centrality in the life sciences, mechanisms and mechanistic explanations were not much discussed by 20th century philosophers of science, who focused instead on laws as the explanatory vehicle. Recently, though, a number of philosophers, focusing primarily on biology, have sought to characterize mechanisms and to identify ways in which mechanistic explanations differ from deductive-nomological explanations that seek to derive statements of phenomena from laws and initial conditions. On the accounts of mechanistic explanation, the key elements 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 and orchestrated so that, under specific contextual conditions, the mechanism realizes the phenomenon of interest (Bechtel & Richardson, 1993; Bechtel, 2006; Craver, 2007; Darden, 2006; Machamer, Darden, & Craver, 2000; Thagard, 2006). For a discussion of some of the central differences between nomological and mechanistic explanation, see Bechtel and Abrahamsen (2005).

make possible and limit what can be accomplished in organisms. The use of proteins as catalysts, of lipids as the basis of membranes, and of phosphates bonds for storage of energy determine many of the fundamental characteristics of living beings and provide the resources for organisms to maintain themselves. The double helix structure of DNA rendered it capable of being copied and passed to the next generation, as Watson and Crick (1953) recognized in the conclusion of their famous paper announcing its discovery. As well, though, the nature of the bonds between nucleotides creates the possibility of complex editing that enables many different proteins to be generated from a single DNA sequence. These specific features of the constituents in living organisms are important for understanding the processes that maintain life. The fact that these constituents are often affected by the features of the systems in which they are incorporated may require revising the first approximation account mechanistic science provides of these constituents, but attempting to theorize about life without consideration of the actual building blocks of living systems can lead to empty models—models that exhibit interesting properties but do not correctly describe the organisms of this world.

The focus of this paper is on the implications of complex systems modeling for mechanistic biology. These implications are substantial: the non-linear and non-equilibrium nature of the interactions within living organisms often are downplayed in initial proposals of how the parts and operations are organized into whole mechanisms, but they are critical to the ability of these mechanisms to realize the phenomena of life. Moreover, both the operations the parts perform and even the very identity of the parts are affected by these interactions. Consequently, the characterization generated in other, typically simpler, contexts may have to be revised as researchers come to understand the dynamical interactions occurring in organisms. But the openness to such recharacterization of parts and operations lies within the mechanistic framework. A central, but often unheralded, feature of mechanisms is their organization, both spatial and temporal. Mechanistic research often begins with an extremely simple conception of organization—the components are thought to operate largely autonomously, with each feeding its product to another component that has limited impact on the earlier component (Simon, 1980). But biological mechanisms seldom exhibit this kind of purely feedforward organization. The discovery that they are not prompts the quest to understand the implications of more complex modes of organization for the phenomena under investigation.

To appreciate the self-corrective character of mechanistic research, I examine the discovery process that lead from mechanistic accounts with relatively simple organization to ones that recognize the complex dynamics characteristic of biological systems. I begin by describing how biologists came to recognize the ubiquity of cyclic organization in biology, focusing primarily on biochemistry where the Krebs cycle came to be regarded as the central component of basic metabolic systems. Cyclic organization need not produce complex dynamics, but when the responses of the components are non-linear and the system functions far from equilibrium, cyclic organization often gives rise to dynamical behavior such as oscillations. Since many of the techniques used in mechanistic research, such as focusing on reaction means, conceal the oscillatory character of many biological processes, they were little noted until the mid-20th century and still are often ignored. But oscillatory phenomena, like cycles, are ubiquitous in biology and their repeated discovery has prompted investigations of the organization in mechanisms that give rise to them.

Equally important, though, is to understand how organisms utilize cycles and oscillations. While these design features are sometimes dismissed as uninteresting byproducts of the way organisms are constituted, they also provide valuable resources for orchestrating operations within living organisms. To appreciate the importance of such control, it is helpful to focus on the sort of systems biological organisms are—fundamentally, they are systems far from equilibrium that either succeed in maintaining themselves in such a state or disappear. To maintain themselves, organisms must recruit matter and energy and deploy them in their self-maintenance. They are therefore characterized by various theorists as autonomous systems (Ruiz-Mirazo, Peretó, & Moreno, 2004; Bickhard, 2000; Christiansen & Hooker, 2000; Collier & Hooker, 1999). An important feature of autonomous systems is that they are intrinsically active—carrying out operations necessary to their self-maintenance. These operations, though, may not be consistent with one another and many are in fact inimical to other operations. Accordingly, these processes must be orchestrated so as not to interfere with each other. In human engineering such orchestration is often achieved by building external controllers, but cyclic organization and oscillatory systems provide ways for systems to regulate their own processes and there is evidence that their ubiquity in living systems figures crucially in enabling living systems to maintain themselves.

In confronting these three features of biological mechanisms—cyclic organization, endogenous oscillations, and autonomy—mechanistic research must transform itself from a nearly exclusive focus on discovering parts and operations to a perspective that also embraces and utilizes the tools for analyzing the mechanisms built from such parts and operations as complex dynamical systems. I end with more general consideration of the character of modified mechanistic research program. One characteristic feature of modern biochemistry, cell and molecular biology, and physiology, is the lack of global unifying principles. Rather, one finds a plethora of specific accounts for specific phenomena. An aspiration of some advocates of complexity and systems theorizing is the development of a unifying theoretical framework that addresses emergent coherent global constraints (Kauffman, 2000). Such aspirations may be unsatisfiable and biology may need to continue with its piecemeal, context-dependent approach. But, to return to the paraphrase of Kant, if mechanism is not to be blind, it needs to draw upon the resources of complex systems theory in understanding the congeries of mechanisms that are biological systems.

2. From Sequential to Cyclic Organization

Humans typically conceive of causal operations as involving one entity acting on another—one car runs into another, damaging it, or one molecule catalyzes a reaction that changes another (e.g., by oxidizing it or adding a phosphate group to it). As the example of a car crash should remind us, there are often changes to the entity taken to be the cause as well as to the one affected, but these tend 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 the partially constructed object (as in the assembly line), and usually assumes that the already installed components are not altered in the process. A similar perspective has often been adopted when seeking to understand biological systems.

These predilections for simple organization were clearly manifest in research on fermentation, the biochemical reaction that transforms glucose into alcohol and carbon dioxide. The chemical composition of glucose ($C_6H_{12}O_6$ in modern symbolism) and alcohol (C_2H_5OH) were ascertained by the beginning of the 19th century, when it was assumed that fermentation was an ordinary chemical reaction. The discovery of the involvement of yeast in the 1830s raised the question of whether or not it was a process carried out only in whole living cells, a view vigorously defended by Pasteur. Compelling evidence that living cells were not required finally came in 1897 when Buchner produced fermentation in extracts in which all whole cells had been eliminated. Buchner's success gave rise to a new question: what inside cells was responsible for fermentation? Buchner's answer illustrates a common strategy—propose that a single component, an enzyme, within the cell was responsible. He named this enzyme, to which he assigned the name *zymase* (by then enzymes had been characterized as chemical catalysts within cells and the suffix *–ase* used to designate them). Other investigators, however, recognized that the reaction required more than one step, and a variety of evidence pointed to possible intermediates. Over the next thirty years researchers pieced together a sequence of reactions (see left side of Figure 1) involving phosphorylations, dephosphorylations, and oxidations, as well as internal reorganizations and splitting of a six-carbon compound into two three-carbon ones (see Bechtel, 2006, chapter 3, for a review of these advances in biochemistry).

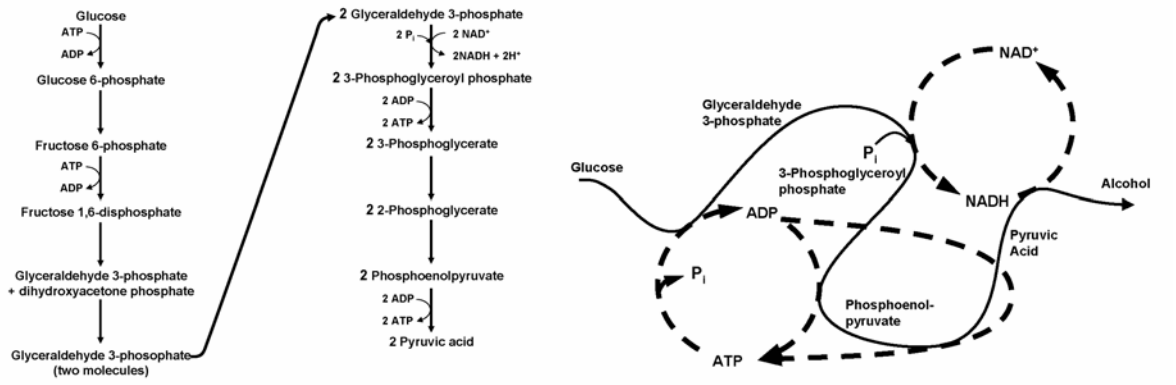


Figure 1. On left, the chemical reactions in glycolysis represented in a sequential manner. On the right, the glycolytic process re-represented by closing the loops involving NAD and ATP.

Fermentation is only a first step in the overall metabolic processing of glucose; when molecular oxygen is available, organisms commonly further catabolize the pyruvic acid to water and additional carbon dioxide. Researchers attempting to understand these further steps pursued a similar strategy of trying to identify a sequence of intermediates. Following upon Wieland's characterization of oxidation reactions as involving the transfer of pairs of hydrogen atoms either to oxygen or to another hydrogen acceptor, Thunberg (1920) proposed a sequence of reactions, some involving oxidations, leading from succinic acid to acetic acid

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

At this point he confronted a problem since removal of two hydrogen atoms from acetic acid would not yield a known chemical compound. Thunberg's solution to this problem was to propose that two molecules of acetic acid would combine and in the process each would surrender a hydrogen atom, thereby yielding succinic acid. Necessity led Thunberg to propose a

cycle of reactions, and the cycle he proposed is in many ways similar to the tricarboxylic cycle (also known as the citric acid or Krebs cycle) that Krebs and Johnson (1937) proposed a decade and a half later (Figure 2 compares the two cycles).

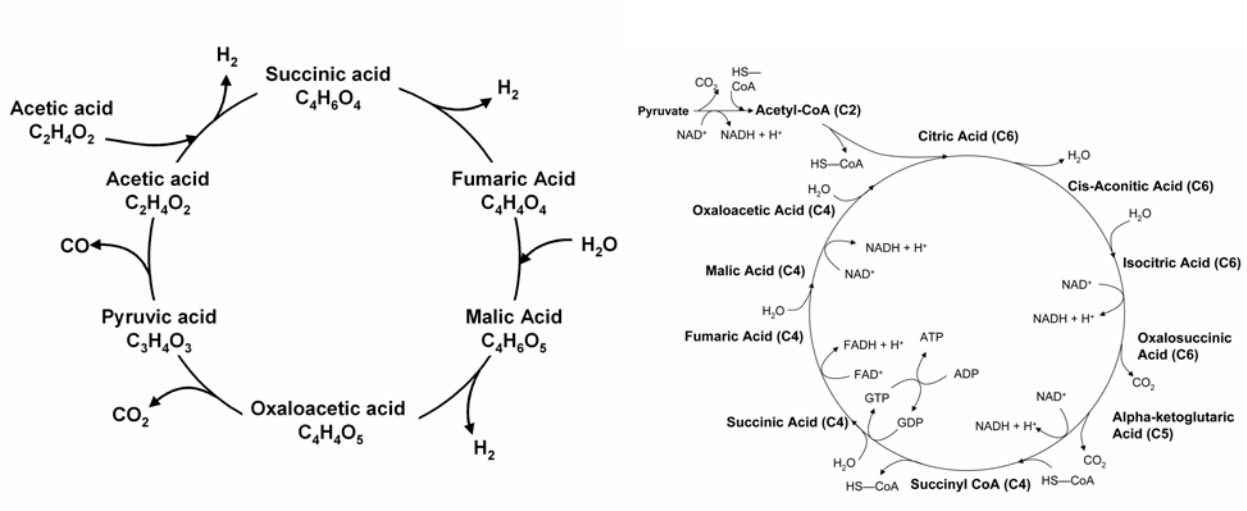


Figure 2. Cycle of oxidative metabolism proposed by Thunberg (1920) on left and that proposed by Krebs and Johnson (1937) on right.

Although the Krebs cycle, like the ornithine cycle which Krebs and Henseleit (1932) had discovered earlier, were born of chemical necessity, Krebs took an interest in the functional significance of cycles. Krebs (1946-8) proposed that what he called metabolic cycles (such as the Krebs cycle) actually consisted of two levels of cycles. At the lower level, enzyme cycles involved the successive oxidation and reduction of a cofactor that serves to transport pairs of hydrogen atoms or electrons from one metabolite to a subsequent receiver. One of the metabolites generated in each of these cycles is further processed in a subsequent cycle, and the resulting sequence of cycles form a closed loop "on another plane of chemical organization of living matter" (p. 92). He claimed that such metabolic cycles (Figure 3 presents his portrayal of the Krebs cycle as such a metabolic cycle), consisting of cycles of cycles, are distinctive of life. He further proposes that they play an important function in enabling organisms to maintain themselves. Even though some of the reactions in an enzyme cycle are reversible (they can proceed in either direction depending upon the concentrations of the substrates), at least one in each cycle is not, making the whole cycle irreversible. This makes the sequence of reactions involving these cycles itself irreversible. Krebs emphasizes that metabolic cycles have the advantage of automatically returning to their starting point and so "are less likely to upset the 'equilibrium' in living matter than non-cyclic reactions" (p. 97). Krebs appeals to Frederick Gowland Hopkins' (1913) notion of a dynamic equilibrium that organisms maintain in the face of continuing change—change necessitated in part by the fact that the reactions within them are irreversible.[3]

[3] Krebs notes that the irreversibility of component reactions requires that a different set of reactions is required to reconstitute a metabolite than to catabolize it, and invokes as an example the different reaction pathways used to store glucose as glycogen and to reconstitute glucose from glycogen. He does not, however, draw attention to the requirement of cycles for an input of new substrate or energy (in the form of a transfer of a phosphate bond from ATP).

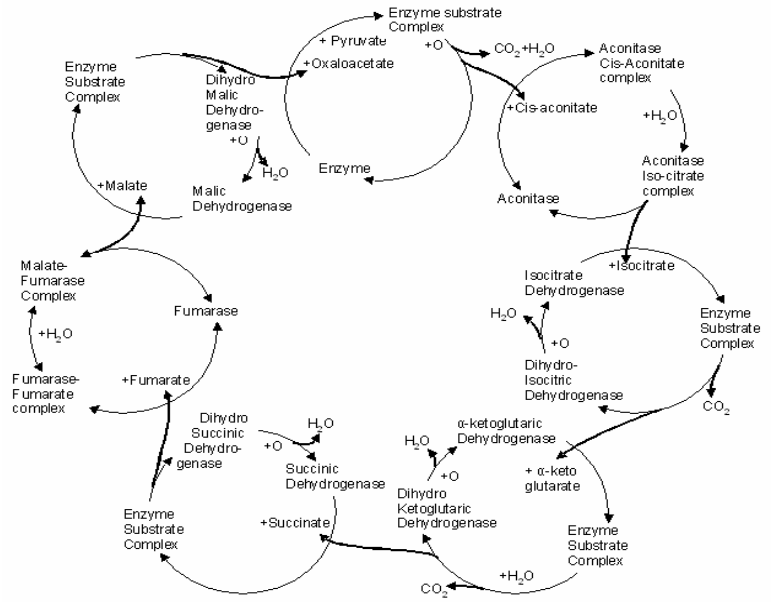


Figure 3. Kreb's (1946-48) characterization of the Kreb's cycle as consisting of a cycle of cycles.

In the end, Krebs raised some doubts about whether restoring the mechanism to its original state does capture the reason for the widespread reliance on cycles in biology. Nonetheless, a similar idea was developed by the Hungarian chemist Tibor Gánti (1975), who sought to characterize the simplest chemical system that exhibits 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 an operation, to be in the requisite state to perform the operation again. This is true not just of biological systems but of motors and other human-designed machines human design. It is especially important 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 that, when combined with a limiting membrane (itself made by that system) that regulated the accumulation of metabolites, constituted "a super-system" that could exhibit the fundamental biological properties of self-maintenance, growth, and reproduction. (In Gánti's account, the system also reproduces since the increase in the volume required by the materials is slower than required to completely occupy the space created by the membrane, which then collapses against itself and breaks into two super-systems.)

Krebs raised the prospect that many more metabolic processes in living systems might consist of cycles: "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). In one respect Krebs' working hypothesis has not been supported: although many metabolic pathways have been identified in biochemistry, most are represented as serial sequences of reactions in the same manner as fermentation in the left-hand side of Figure 1. However, if one attends to the loops on the left side in which either ATP enters and ADP leaves the pathway, or vice versa (as in the

right side of Figure 1), fermentation itself appears to involve cycles. Likewise, there is a cycle involving the step in which NAD is reduced in the oxidation of glyceraldehyde-3-phosphate, and the step in which NADH is again oxidized in the reduction of pyruvic acid to alcohol. In fact, reactions involving ATP, NAD^+, and $NADP^+$, etc. serve to link reactions in many biochemical pathways, giving metabolic systems the character of Watts and Strogratz's (1998) small worlds. These are networks in which most links are between local components but where a few more distant connections serve to integrate the overall networks. I will return to consider the roles this might play in coordinating and regulating operations within the cell, after first considering oscillatory phenomena.

3. Recognizing and Explaining Oscillatory Phenomena

The move from sequential to cyclic organization of metabolic reactions involves a seemingly simple alteration—discovering how the sequence of operations closes into a loop. However, when one shifts from an a-temporal perspective to consideration of how the processes change in time, we can appreciate how the alteration gives rise to a complex dynamical pattern. Of particular interest is the fact that activity in such systems can oscillate, proceeding through a sequence of states until the original state is restored. The pendulum provides an example of a simple physical oscillator. Many biological oscillatory processes, such as the rhythmic flashing of fireflies and the beating of hearts, are obvious without sophisticated techniques. Others, such as neural oscillations, have attracted substantial interest and novel proposals from a number of investigators (Buzsáki, 2006). Oscillatory processes, however, are ubiquitous at all levels in the biological world, from the intracellular chemical level all the way to the ecological level. Wherever researchers have developed appropriate techniques to look for them, biological systems have been shown to exhibit oscillations. Despite this, much biological research is conducted in a manner that blinds the researchers from ongoing oscillations. For example, many of the techniques employed to study biological systems focus on steady-state conditions and so tend to obscure the fact that oscillations are occurring. In addition, researchers often create preparations for studying systems that are close to equilibrium conditions and report means or averages, thereby missing the oscillations. Identifying and understanding the significance of these oscillations requires adopting a different perspective on the system under study. I discuss three telling examples in which oscillations have been discovered: (a) oscillations in the glycolytic pathway discussed above, (b) an ultradian oscillator separating oxidative phases in cells from reductive phases during which protein synthesis occurs, and (c) circadian rhythms that coordinate the behavior of most organism with the day-light oscillation in the environment.

Glycolytic oscillations

Oscillations involving fermentation provide a potent first example. Amal Ghosh was working in the laboratory of Britton Chance, who had pioneered the use of spectrophotometric techniques to quantify biochemical processes by measuring concentrations of NAD^+ and NADH. Ghosh discovered periodic oscillations with a period of about 1 minute in the concentrations of NADH upon the addition of glucose to suspensions of extracts from bakers' yeast or upon the inhibition of aerobic metabolism (Chance, Estabrook, & Ghosh, 1964; for a somewhat earlier report, see

Duysens & Amesz, 1957).[4] Although oscillations in the first studies dampened rapidly, Hess, Brand, and Pye (1966) developed a preparation in which oscillations continued for up to 22 hours. Examination of glycolytic intermediates revealed that fructose 1,6-diphosphate (FDP) and subsequent intermediates oscillate together 70° out of phase with NADH while fructose 6-phosphate (F6P) and earlier intermediates shown in the left side of Figure 1 oscillated together 180° out of phase with NADH (Figure 4). The fact that the switchover occurred at the reaction catalyzed by phosphofructokinase (PFK) pointed to it as the critical enzyme in the oscillation. PFK catalyzes the transfer of a phosphate group (PO_4) from ATP (leaving ADP or, if two phosphates are transferred, AMP) to F6P to generate FDP (Hess, Boiteux, & Krüger, 1969). A similar phase offset was found later in the reaction pathway between phosphoenolpyruvate and pyruvic acid, one of the reactions at which ATP was resynthesized.

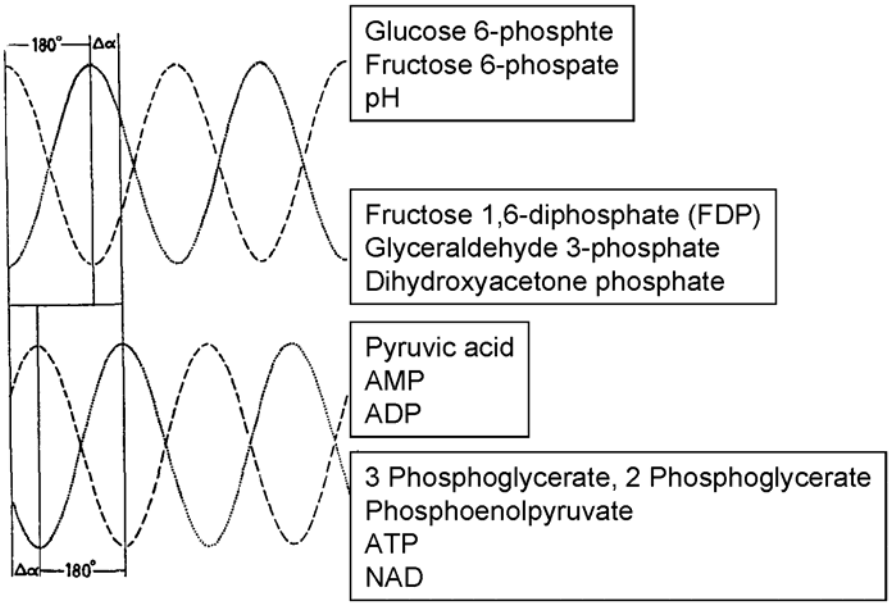


Figure 4. Phase shifts between the oscillation of different substrates in the glycolytic oscillator. Adapted from Hess, 1971, Figure 1.

The way in which this oscillation is initiated is relatively easy to understand. PFK is an allosteric enzyme—an enzyme in which the binding of substrates at different locations on the enzyme alters its ability to catalyze the reaction (Monod, Wyman, & Changeux, 1966). PFK's catalytic activity is enhanced by one of its inputs, F6P, and two of its products, ADP and AMP. Thus, whenever F6P is available, the positive feedback from the ADP or AMP produced increases the rate of the reaction. But the other input, ATP, is inhibitory. As the reaction proceeds, it initially begins to deplete available ATP, thereby reducing this inhibition (allowing the positive feedback to increase the rate of the reaction). As the reaction runs faster, more NADH is generated downstream. But the downstream processes also result in the generation of even more ATP (glycolysis is important to bioenergetics since it generates 2 molecules of ATP for each one that

[4] In the same period NADH oscillations were observed in a soluble enzyme system with horseradish peroxidase (Yamazaki, Yokota, & Nakajima, 1965). A decade earlier Wilson and Calvin (1955) identified oscillations in CO_2 levels in photosynthesis.

is consumed). The increased concentration of ATP now inhibits the reaction. As a result, less NADH is generated and the NADH previously generated is oxidized in other reactions; consequently, less ATP is produced and and that already produced is dephosphorylated in other reactions to yield more ADP. This releases PFK from its negative feedback and allows the positive feedback from F6P to again speed up the reaction. Over time, however, the available F6P is consumed, and this will result in dampening the oscillation unless a new supply of glucose is provided.

I have described the mechanism creating the oscillation qualitatively, but to determine the period and amplitude of the oscillation and the conditions under which it will occur, it is necessary to construct equations that incorporate information about the rates of the various reactions and employ them in a computational model. Already in 1964 Joseph Higgins, working with Chance, published a computational model of glycolytic oscillation based on three features of the behavior of PFK: "the feed-in of a substrate through a first-order (or enzymatic reaction), a product-activated enzyme step, and an enzymatic step for removing this product" (Higgins, 1964, p. 994). Shortly thereafter Sel'Kov (1968) proposed a model using substrate inhibition and product activation that captured many of the phenomena that had been demonstrated experimentally. These models showed that the basic behavior could be explained by focusing on one reaction step, but a different approach of constructing equations for all the reaction steps in glycolysis (involving 57 differential equations) was pursued by David Garfinkel and his collaborators (Garfinkel, Frenkel, & Garfinkel, 1968; Achs & Garfinkel, 1968). While this model could yield a more precise account of the phenomena, it was less useful to understanding what gave rise to the oscillations. (For a simpler model using only seven differential equations, see Goldbeter & Lefever, 1972.) Concepts developed for the analysis of complex systems, such as limit cycles and bifurcations, began to be applied in the analysis of biological oscillations, such as the glycolytic oscillator (Gurel, 1975). In the 1980s and 1990s these were extended in a variety of ways; see Hess (1997) for a description of modeling and empirical work on cell-free glycolysis that reveals a full range of dynamic behaviors, from steady state to periodic and quasi-periodic to chaotic behavior.

One of the striking features of pendulums that are mounted on the same wall is that they synchronize their oscillations. This requires that a product produced by at least one of the oscillators perturbs the oscillation of the other. Already in early research, Chance et al. (1973) had found that in large populations of cells glycolytic oscillations within each tended to synchronize with those in others, raising the question of the identity of the responsible product. Richard, Teusink, Hemker, Dam, & Westerhoff (1996) determined that concentrations of extracellular acetaldehyde oscillated at the same frequency as the gylcolytic oscillations and that adding acetaldehyde could shift the phase. This pointed to it as the synchronizing agent between independent oscillating cells.

Glycolytic oscillations reveal how a relatively small departure from sequential organization can give rise to an unexpected complex temporal pattern—an oscillation in concentrations of substrates rather than a steady flow of substrates through the reaction pathway. The glycolytic oscillator was discovered at roughly the same time as several other experimental and conceptual developments that served to situate thinking about such dynamical processes. Boris Belousov (1959), investigating a possible inorganic analog of the Krebs cycle, discovered that a mixture of

potassium bromate, ceric sulfate, propanedioic acid and citric acid in dilute sulfuric acid generated a reaction in which the concentrations of cerium^{4+} and cerium^{3+} ions oscillated. This was due to cerium^{3+} being oxidized by bromate and the resulting cerium^{4+} ions being reduced by propanedioic acid. After Zhabotinsky (1964) subjected the reaction to further examination, it came to be know as the Belousov-Zhabotinsky (B-Z) reaction and has become a prototype of how non-equilibrium conditions can generate chemical oscillations in systems with non-linear reaction processes. Around the same time Ilya Prigogine (1968) began his investigation of open systems that exchange matter and energy with their environment and are far from thermodynamic equilibrium. He characterized them as dissipative systems in which symmetry is spontaneously broken and complex structures emerge on their own (Nicolis & Prigogine, 1977). The B-Z reaction, glycolytic oscillations, and metabolic reactions in living systems generally, involve open systems far from equilibrium and it is these features, combined with the cyclic organization, that generate the oscillatory phenomena.

Britton Chance was particularly attracted to the possible significance of 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. Although circadian (*circa* = about + *dies* = day) rhythms had been observed in many plant and animal behaviors for centuries, their endogenous control had only recently been rigorously established experimentally by Colin Pittendrigh (1960) and other pioneers in circadian research. Chance, however, was to be doubly foiled in this vision. First, as I discuss below, research on circadian rhythms in most organisms has pointed to a different oscillatory mechanism involving inhibitory feedback on gene expression. Second, it is not clear that glycolytic oscillations occur, even in yeast cells, under physiological conditions, and hence that they have functional significance in organisms.[5] 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 employed in regulating cellular processes.

Other Ultradian Oscillations

At the same time as oscillations in glycolysis were discovered, researchers were also finding a variety of other oscillations in cell functions, including oxidative metabolism (in the mitochondrion) and protein synthesis. (Rapp, 1979, provides an atlas of oscillators discovered

[5] In the 1970s there were a variety of proposals as to the functional significance of the glycolytic oscillator—it was thought it might drive rhythmic contractions in slime molds, account for slow wave oscillations in insulin secreting β-cells (via a decrease in potassium conductance as a result of operation of glyceraldehyde-3-phosphate dehydrogenase (GDH) and phosphoglycerate kinase (PGK)), and molluscan burster cells. As a result of the failure to find a compelling account of his functional significance, research on glycolytic oscillations declined after the 1970s, although a new round of research was pursued by Hans Westerhoff and his colleagues in the 1990s, spurred by the development of new techniques that permitted measurements of metabolite concentrations from whole cells. Danø, Sørensen, & Hynne (1999), for example, developed a means for continuous provision of glucose and cyanide (to suppress oxidative metabolism) and removal of waste and determined that a stable attractor gave way to a limit cycle as the flow of substrate increased and that, if perturbed, the reactions revealed a spiraling return to the unstable attractor, characteristics of a Hopf bifurcation. Richard, Teusink, Westerhoff, and van Dam (1993) found that some of the substrates generated after FDP— glyceraldehyde-3-phospate (GAP), dihydroxyacetone, phosphoenolpyruvate, and pyruvate—either did not oscillate or with much smaller amplitudes, suggesting that PFK was not, in the end, responsible for NADH oscillations. Rather, they attributed it to oscillations in the Gibbs energy of ATP hydrolysis, with the coupling achieved by GAP dehydrogenase and phosphoglycerate kinase (PGK).

through the 1970s.) The first reports of mitochondrial oscillations involved periods of just a few minutes, whereas the periods for protein synthesis were closer to one hour. But in the brewer's yeast *Saccharomyces cerevisiae* grown under aerobic conditions, Satroutdinov, Kuriyama, and Kobayashi (1992) found a 40-minute metabolic cycle. In its fermentative phase concentrations increased for ethanol and ATP and decreased for NADH, pyruvate and acetate; this was followed by a respiratory phase during which ethanol was re-assimilated, glycogen increased, NADH, pyruvate, and acetate reached a maximum, and ATP decreased. Moreover, the oscillation was found in individual cells and synchronized across the population. This became a model system for David Lloyd and Douglas Murphy, who identified cycling in concentrations of a host of metabolites, including residual O_2 (the O_2 that remains in the media after the organisms have drawn what they need), NAD and NADP, glutathione, ethanol, acetaldehyde, acetic acid, and H_2S. Sohn, Murray, and Kuriyama (2000) provided evidence that synchronization across individual cells is achieved via the action of diffusible substances such as acetaldehyde and H_2S. Having found similar cycles, albeit with different periods, in a variety of different organisms, Lloyd and Murray (2005) have referred to it 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 & Murray, 2005, p. 376).

Lloyd and Murray also proposed a mechanism for this oscillation. At its core is a redox cycle involving NADH and NADPH, which are oxidized by GSH reductase, thereby reducing glutathione or other thiols or small proteins (e.g. thioredoxin and glutaredoxin) and creating protein disulfide bridges. These disulfide bridges are in turn reoxidized by oxygen ions, free radicals, or peroxides (collectively referred to as *reactive oxygen species* or *ROS*) that are produced as normal products of oxygen metabolism as well as by environmental stress. This period is robust through a wide range of temperature fluctuations, a phenomenon known as temperature compensation (Murray, Roller, Kuriyama, & Lloyd, 2001).[6]

Working from a very different direction using UV-cytophotometry and microinterferometry in retinal cells (and subsequently in glandular cells, blastomeres, and bacteria), Soviet researcher Vsevolod Brodsky found that a large number of cell properties exhibited oscillations, including dry weight, protein content, amino acid incorporation into proteins, number of polysomes, and RNA content. Over a variety of species the period of these oscillation ranged from 20 to 120 minutes, leading Brodsky (1975; Brodsky, 1998, 2000) to refer to these as cirahoralian rhythms. Mano (1977) found that in sea urchin eggs, protein synthesis was synchronized with increased concentrations of reduced glutathione (hence, during the reductive phase of the metabolic cycle). Murray and his colleagues (Klevecz, Bolen, Forrest, & Murray, 2004) linked this cycle in gene expression to the redox cycle they had found in yeast. First, DNA replication begins at the

[6] Lloyd (2006) proposes 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 synthrophy between a-Proteobacteria, progenitors of today's photosynthetic green sulfur bacteria that oxidize H_2S (either photosynthetically or using O_2), and basal Archeon, which reduced sulfur to H_2S (a proposal for the origin of modern mitochondria advocated by Searcy, 2003).

transition from the oxidative phase (when NAD^+ and $NADP^+$ concentrations are heightened) to the reductive phase (when NADH and NADPH predominate). Of the 5329 genes whose transcripts they examined, 650 were maximally expressed during the oxidative phase and 4679 during the reductive phase (Murray, Beckmann, & Kitano, 2007). Murray and his collaborators also found that events in the cell cycle, including DNA replication and chromosome segregation are tied to the ultradian oscillations, beginning at the transition between the oxidative and reductive phases. On any given oscillation only a few cells initiate DNA replication, but over about 8 hours all cells will replicate their DNA. I will return to the potential significance of this coupling below.

Circadian Oscillations

I conclude this discussion of oscillations with perhaps the best known class of oscillatory phenomena in biology—circadian rhythms. Although various physiological and behavioral manifestations of circadian rhythms (such as the folding of leaves in plants and body temperature in animals) were reported from ancient to modern times, extensive research on them only began in the mid-20^{th} century. Initially this research focused on establishing that the rhythms in animals were endogenously controlled by recording them after eliminating exogenous cues, such as daily light and temperature cycles. This research demonstrated that the rhythms were endogenously controlled but also found their periodicity varied somewhat from 24 hours (hence the name *circa*dian). This made salient the further question of how endogenous rhythms could be entrained by Zeitgebers (external cues such as light and daily fluctuations in temperature) so as to stay in synchrony with local day-night cycles and, moreover, to adjust to seasonal variations in such cycles). An important discovery during this period was that circadian rhythms are temperature compensated. That is, even though there may be transient fluctuations when an organism is subjected to an environment with a different temperature range, a rhythm soon re-establishes with nearly the same periodicity as before. Explaining this presented a substantial challenge since most biochemical reactions are highly temperature sensitive—their rate approximately doubles with a 10^o C increase in temperature.

Having characterized the phenomenon of circadian rhythms, the challenge circadian biologists faced was discovering a molecular mechanism that could generate them endogenously. The first clue was Konopka and Benzer's (1971) identification of a gene in *Drosophila* in which mutations produced shortened or lengthened rhythms or arrhythmic behavior. Once the gene they named *period* (*per*) was cloned in the 1980s, it was discovered that the concentrations of the corresponding mRNA and protein (PER) oscillated in cells, with the mRNA reaching a maximum at the beginning of the night and protein levels reaching a maximum about 6 hours later. Hardin, Hall, and Rosbash (1990) proposed a feedback loop to explain this (shown schematically in Figure 5): transcription of the *per* gene resulted in increased *per* mRNA. These macromolecules are transported to the cytoplasm where they are translated by the ribosomes into the corresponding protein PER. PER molecules in turn are transported back into the nucleus, where they suppressed further transcription of *per*. The resulting decline in PER synthesis resulted in less PER being transported into the nucleus, thereby releasing *per* from this inhibition. At the time many parts and operations in the mechanism were still unknown; for example, it was not understood how PER could suppress *per* transcription since PER lacked the necessary domain for binding to DNA.

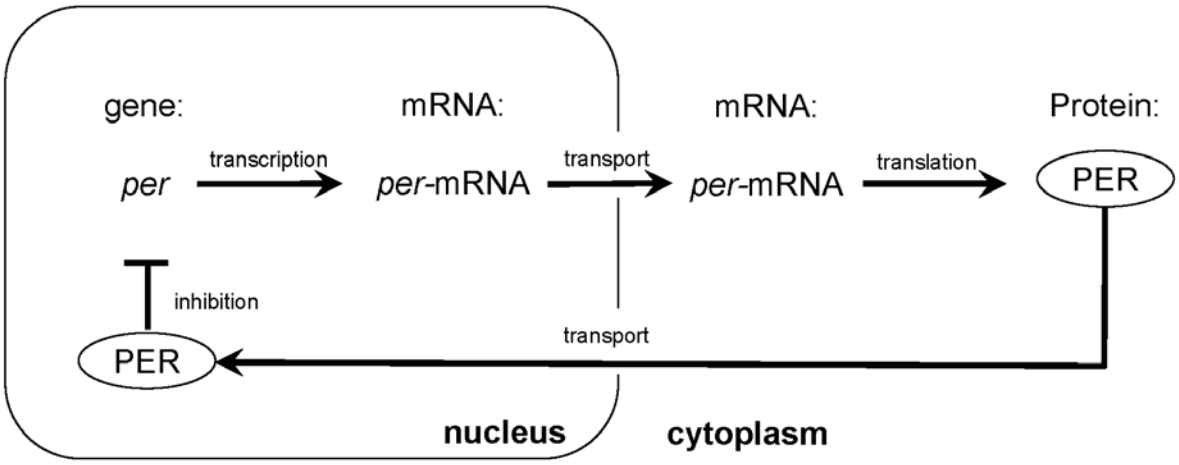
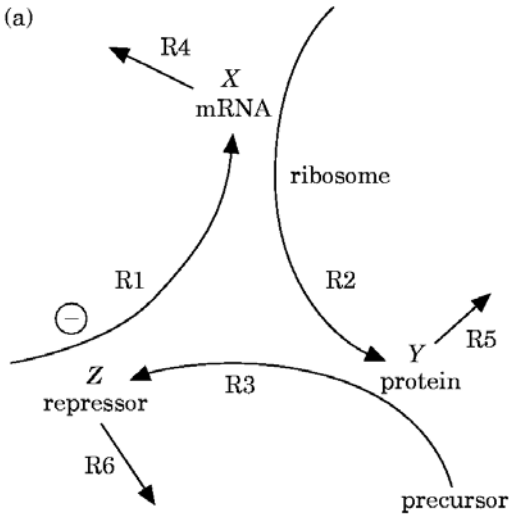


Figure 5. The proposed mechanism for circadian oscillations involving the protein, PER, translated from the gene *per* feeding back to inhibit *per*'s transcription.

The sketch above of how a mechanism such as this might operate is suggestive, but given the complexity of the interactions, mathematical analysis and modeling is needed to determine whether it is actually capable of generating oscillations. Already in the 1960s, just as biological oscillators were being discovered, Brian Goodwin (1965) modeled a possible oscillatory mechanism. His focus was on the gene control mechanism identified by Jacob and Monod (1961), in which proteins serve to inhibit gene expression, and he developed equations to characterize the process (Figure 6). The critical parameter for determining whether oscillations occur is *n*, which is known as the Hill coefficient and describes the number of interacting molecules needed to inhibit the expression of the gene. On the basis of his 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 only occurred when $n > 9$, a condition that was biologically unrealistic. However, if non-linearities 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 lower values of *n*. Accordingly, Goldbetter (1995b) developed his first model of the *Drosophila* circadian oscillator by modifying the Goodwin oscillator. He developed differential equations to describe the different operations in Figure 5 and showed that, if appropriate parameter values were supplied it could generate 24 hour oscillations. The system behaved as a limit cycle: if the simulation were started with initial values or perturbations that resulted in faster or slower oscillations, it would gradually return to the cycle.



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

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

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

Figure 6. The Goodwin oscillator in which a metabolite Z, generated by a reaction involving a protein Y synthesized from mRNA X, represses the formation of X.

In the subsequent decade many additional components of the intracellular 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. In mammals the crucial cells for maintaining circadian rhythms had been localized in the 1970s to the suprachiasmatic nucleus (SCN), a nucleus of approximately 10,000 neurons on each side of the brain, situated 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 sustained oscillations of approximately 24 hours, although with considerable variability (the standard deviation was 1.2 hours). The large degree of variability eventually prompted great interest, since circadian behavior in organisms is far more precise than the variation found in individual SCN neurons. After showing much smaller variation in the oscillations in running wheel behavior in mice and in 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. VIP is only released by a subset of SCN cells in the core region, and only these cells 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 producing toroidal oscillations, deterministic chaos, or the coexistence of multiple attractors (Grebogi, Ott, & Yorke, 1987). A variety of models developed in the last few years have demonstrated that release of VIP is capable of sustaining oscillations and producing synchronization, including the result that shell oscillators tend to oscillate approximately 40 minutes ahead of core oscillators, with biologically plausible parameters (Bernard, Gonze, ÄŒajavec, Herzel, & Kramer, 2007).

The problems of synchronization, however, loom larger when one considers responses to external inputs from Zeitgebers that are radically out of sync with the internal oscillators. The resulting disruptions are something human travelers experience when they cross multiple time

zones. The effects of jetlag show up in a wide range of behavioral and physiological factors, not just sleep. Various behaviors and physiological activities are directly influenced by peripheral clocks found in different regions of the brain and organs of the body. These peripheral oscillators cannot sustain oscillations when they are cut off from the SCN and so were initially assumed to be directly controlled by the SCN and were referred to as slaves. However, there is increasing evidence that the dynamics of the peripheral oscillators is locally determined and that there are complex coupling processes linking them their oscillations to those of SCN cells. Recent simulations of relations between the two different regions of the SCN and peripheral oscillators reveal complex responses when the system is perturbed by a six-hour change in day-light cycles comparable to those travelers experience going 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, their order was reversed, and both advanced more than the 6 hours. It took several days to restore normal synchronization (Nakamura, Yamazaki, Takasu, Mishima, & Block, 2005). In simulating the response of connected oscillators representing both core and shell SCN oscillators and those in peripheral organs, Leise and Siegelmann (2006) found a very complex dynamical pattern, involving overshoots by the SCN oscillators. In the simulations many cycles were required before the peripheral oscillators returned to a normal relation to the SCN.

4. Cyclic Organization and Oscillations as Features of Autonomous Systems

One might treat the prevalence of cyclic organization and oscillatory dynamics in living systems as simply accidents of the way biological systems happened to develop and of little fundamental interest. But in fact they are both of great significance in the context of living systems. One of the important features of living organisms is that they are systems far from thermodynamic equilibrium and 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 by theorists such as Varela (1979), Ruiz-Mirazo and Moreno (2004), and Collier and Hooker (1999). Ruiz-Mirazo and Moreno 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 non-linear 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 non-linear oscillators, illustrated in this paper, provide a dynamic basis for this self-organizing property of oscillating cellular control circuits" (p. 436).

To be systems that 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 system that builds polymers. Although such a component, motivated by the role DNA plays in living organisms, can play an important role in controlling a system (Griesemer & Szathmáry, 2008), it is far from the most basic kind of control a system such as a chemoton requires.[7] More fundamental is to regulate the different metabolic processes required and the entry to and egress from the chemoton over the boundary. It is here that cyclic organization and oscillatory processes assume fundamental roles for they provide vehicles for control.

The role of cyclic organization in control is fairly easy to understand since a cycle provides a vehicle for feedback, which can be either negative or positive. In glycolysis, for example, if ADP levels are low, as they would be when most ADP had already been converted to ATP and little had been utilized in energy demanding operations, the steps in glycolysis that require ADP will be slowed. As well, due the allosteric nature of PFK discussed above, the high concentrations of ATP will inhibit the early reactions in glycolysis. These steps insure that glucose will not be metabolized unless there is need for energy for other cell activities, and will instead be converted to glycogen for storage. When, on the other hand, the energy stored in the phosphate bond is needed and ATP is broken down to ADP, the increased concentration of ADP will enable glycolysis to proceed and generate additional ATP.

Although it is not clear whether glycolytic oscillations occur under normal physiological conditions in living organisms or what control function they might play, both the oxidation-reduction oscillator and the circadian oscillator clearly play control functions. Goodwin (1963) proposed that oscillators provided a means of temporarily segregating imcompatible cellular events. Spatial differentiation of organelles is one 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 into the lysosome for that purpose. Temporal segregation can achieve the same purpose. The fact that DNA replication begins at the end of the oxidative respiratory phase when oxygen consumption decreases, and ends at the end

[7] One motivation for focusing on genes as control elements is that they appear to be the primary units of organisms that can be inherited and thereby figure in evolutionary processes including natural selection. But genes, and the polymers generated in Gánti's system, are static entities that do not do anything 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. The actual control system is a dynamic system, not a static element.

of the reductive phase, minimizes the oxidative damage to highly sensitive nucleic acid, a sensitivity that is enhanced when the double helix is opened (Lloyd & Murray, 2006).

The idea that oscillatory processes provide a means of segregating incompatible operations is also seen with circadian oscillations. Circadian oscillations appear to be present in nearly all life forms (with cave dwelling organisms the most likely exception). A clear example of segregating incompatible operations is found cyanobacteria, *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 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—sleep during the night (diurnal animals) or day (nocturnal animals), seek food at appropriate times, etc. It might seem sufficient to rely on environmental cues for many of these activities, but in many cases appropriate performance 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 and operations of a vast range of biological mechanisms. It has been less successful, though, in identifying ways in which these mechanisms are organized and the implications of various forms of organization to their normal functioning. Biochemists tried to force reactions into a linear organization and only considered cycles, such as the Kreb's cycle, when they failed to find a suitable sequential relation between reactions. By focusing on near-equilibrium steady-state conditions and averaging results, biologists have generally screened themselves off from oscillatory phenomena. Accounts of mechanisms and mechanistic explanation in philosophy of science have paralleled biology, emphasizing the discovery of component parts and operations and, while acknowledging the importance of organization, providing little detail about how biologists do or could understand organization or appreciate its implications.

Although still peripheral to much mechanistic research in biology, small clusters of researchers in various fields of biology have confronted the complex dynamics that result from the cyclic organization and the oscillatory character of many biological phenomena, exploring ways in which they serve to orchestrate the functioning of biological mechanisms (Goldbeter, 1995a). I have described a few of these endeavors. Sometimes researchers who emphasize the complex dynamics that can arise in living systems seem to be rejecting the mechanistic project. That is, however, mistaken as accounts of dynamics that ignore what is understood of the component parts and operations of mechanism are empty and provide no understanding of how the dynamic relations are actually established in living systems. Thus, as in other fields of biology, once the phenomenon of endogenously controlled circadian oscillations was delineated, researchers sought the mechanism responsible for it. But there is an important difference between this research and more traditional mechanistic investigation, as the focus is on understanding the dynamic relations resulting from the cyclic and non-linear nature of the processes in a non-

equilibrium system. Understanding how cyclic organization and non-linear operations give rise to complex dynamical relations (such as regular oscillators) requires more than identifying parts and operations—it requires developing the appropriate mathematical techniques to model the operation of the mechanism. Without this, the mechanistic account is blind.

Some theorists (Kauffman, 2000; Kauffman & Clayton, 2005) hold out the hope that scientists may soon discover new general laws that characterize the dynamical processes found in living systems. In the spirit of cybernetics and general systems theory, the hope is that these principles will provide the unity that reductionistic inquiry failed to produce. Recent investigations have, in fact, illuminated important general principles of organization such as self-organization through positive feedback in non-equilibrium conditions, small-world organization, and scale-free networks (Barabási & Bonabeau, 2003). But 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, forthcoming). 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—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, but this will not obviate the need to understand the particularity of any given mechanism.

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