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Using Computational Models to Discover and Understand Mechanisms

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Abstract

Areas of biology such as cell and molecular biology have been dominated by research directed at constructing mechanistic explanations that identify parts and operations that when organized appropriately are responsible for the various phenomena they investigate. Increasingly the mechanisms hypothesized involve non-sequential organization of non-linear operations and so exceed the ability of researchers to mentally rehearse their behavior. Accordingly, scientists rely on tools of computational modeling and dynamical systems theory in advancing dynamic mechanistic explanations. Using circadian rhythm research as an exemplar, this paper explores the variety of roles computational modeling is playing. They serve not just to determine whether the mechanism will produce the desired behavior, but in the discovery process of hypothesizing mechanisms and in understanding why proposed mechanisms behave as they do.

1. Introduction

Many areas of biology (physiology, cell and molecular biology, etc.) have been slow to embrace computational modeling (fields such as population genetics and neuroscience being exceptions). The focus of research has been on developing and applying experimental techniques to develop accounts of mechanisms thought to be responsible for phenomena such as gene expression, metabolism, and cell division. Accounts of mechanistic explanation advanced by philosophers (Bechtel & Richardson, 1993/2010; Bechtel & Abrahamsen, 2005; Machamer, Darden, & Craver, 2000) have emphasized the importance of characterizing parts and operations that, when organized appropriately, are able to generate the phenomena of interest. Little, though, has been said about how researchers connect their understanding of parts and operations with the phenomenon to be explained. When they hypothesize mechanisms with parts are organized in relatively simple ways, researchers are able to rely on mentally rehearsing the operations proposed to evaluate whether they could generate the phenomenon. For example, by imagining the execution of each successive step in the textbook description of protein synthesis one can imagine how a polypeptide chain is generated that matches the sequence of codons on the DNA. Mental operations mirror the causal operations proposed to operate in nature. Computational modeling was not needed to understand how these mechanisms (which are the focus of what I refer to as *basic mechanistic explanations*), could account for the phenomenon.

In the late 20th century the mechanisms proposed in fields such as cell and molecular biology became more complicated as more parts and operations were identified. This

alone, however, was not sufficient to lead biologists to turn to computational modeling, as even with lots of parts, they could still rehearse the succession of operations mentally to determine whether the proposed mechanisms sufficed to generate the phenomenon. Rather, the need for computational models became more serious with the development of accounts of mechanisms in which the operations were no longer organized sequentially but featured multiple feedback loops. The need became even greater when the operations appealed to could only be described by non-linear mathematical equations. Humans, including scientists, perform poorly in predicting the behavior of non-linear processes and keeping track of multiple interactions due to feedback loops, especially if they operate on multiple time-scales. To overcome these limits, scientists often develop mathematical characterizations of the different operations and employ these in computational models that simulate how hypothesized mechanisms will behave. Mechanisms whose behavior can only be accounted for with computational modeling and analytic tools such as those offered in dynamical systems theory no longer count as basic mechanistic explanations but fall in the hybrid category of dynamic mechanistic explanations (Bechtel & Abrahamsen, 2010, 2012). The goal of this paper is to elaborate on the roles computational models play in dynamic mechanistic accounts.

In this paper my main focus is on mechanistic explanations (explanations in which proposed mechanisms play a central role).[1] Evaluating explanations often involves predicting (or retrodicting) what would happen if the explanation were correct). The most basic use of computational modeling in biology involves prediction—predicting whether a proposed complex mechanism would produce the phenomena for which it is posited as an explanation. I will introduce this role for computational modeling in section 2. Much of scientific research, however, is involved not in evaluating, but in discovering explanations (proposing possible mechanisms). The very factors that made it important to employ computational models to determine how a proposed mechanism would behave have also led researchers to employ them in the discovery process. In this paper I will illustrate such use of computational modeling in proposing a mechanism (section 3) and in proposing how a given mechanism might be embedded in another (section 4). A common, although not a necessary, goal of explanation is to provide understanding—making it intelligible to us why the proposed mechanism would account for the phenomenon. As proposed mechanisms become complicated and complex, one can predict how a mechanism will behave through computational modeling without understanding why it will behave that way. Acquiring understanding often requires abstracting from the details of a mechanism to uncover basic design principles it employs (Levy & Bechtel, 2013; Green, Levy, & Bechtel, 2014). In section 5, I discuss the use of computational modeling in identifying the basic design principles that enable a mechanism to generate a phenomenon.

To illustrate these different roles of computational modeling, I will focus on modeling in chronobiology, the field of biology devoted to understanding circadian and other biological

[1] Mechanistic explanations are a species of explanation and there are other types of explanation scientists employ. Even in the fields of biology I discuss, explanations sometimes take the form of historical analyses or network analyses (which situate the responsible mechanism in a larger context).

rhythms. Circadian rhythms are endogenously generated oscillations that are entrainable to the light/dark cycle of the local environment and regulate a wide range of physiological and behavioral activities by restricting expression of specific genes to particular times of day. Research in the early 20th century demonstrated that these rhythms were endogenous by showing that in the absence of cues from the environment, organisms would exhibit rhythms slightly different than 24 hours (hence, the rhythms were named *circadian* from *circa*, about, and *dies*, day). Much of the pioneering research in the later decades of the 20th century on the mechanism responsible for circadian rhythms was conducted on animals (especially fruit flies and mice), but circadian rhythms have also been demonstrated and studied in plants, fungi, and cyanobacteria. I begin with research on animal circadian rhythms as that makes apparent the conditions under which computational modeling first became important but also how it followed on the articulation of proposed mechanisms. In subsequent sections I turn to research on circadian rhythms in cyanobacteria where modeling has played a central role in the discovery of the responsible mechanism and in understanding how it produces the phenomenon.

2. Modeling to Establish a Proposed Mechanism's Sufficiency

The proposal of a mechanism for circadian rhythms in animals built on experimental findings that suggested the nature of the mechanism. Through a screen of mutant fruit flies for disrupted or altered circadian rhythms, Konopka and Benzer (1971), discovered strains of flies that exhibited shortened or lengthened rhythms or were arrhythmic. They traced the mutation in all strains to a single gene they named *period* (*per*). Discovering how the gene figured in generating circadian rhythms had to await the development of cloning techniques. In pioneering research applying these techniques to circadian rhythms, Hardin, Hall, and Rosbash (1990) determined that concentrations of both *per* mRNA and the protein PER oscillated with a period of about 24 hours, and that the phase of peak PER concentration lagged about four hours behind that of *per* mRNA. This led them to propose a mechanism involving a negative feedback loop (known as a transcription-translation feedback loop or TTFL) in which, after it is synthesized, PER is transported back into the nucleus where it inhibits (by a then unknown process) the transcription of *per* (see Figure 1).

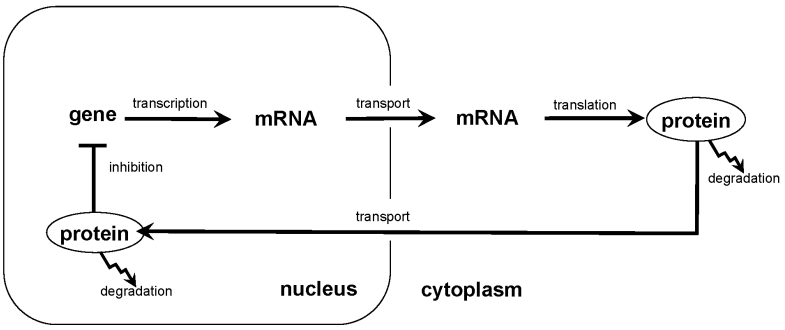

Figure 1. The transcription-translation feedback loop (TTFL) proposed to explain circadian rhythms in fruit flies. See text for details.

The idea of oscillations resulting from negative feedback was familiar in engineering and it is relatively easy to rehearse mentally the operations of the mechanism to understand how it could give rise to the oscillations in the concentrations of *per* mRNA and PER: When concentrations of PER are low, the gene *per* is not inhibited and it is transcribed into *per* mRNA, which is then translated into PER. This causes concentrations of PER to increase, but as they do so, more is transported back into the nucleus where it inhibits the process of transcription. As less PER is synthesized and what there is is broken down, concentrations of PER drop again. But as they do so, the inhibition on *per* transcription is reduced and more PER is once again synthesized. Although such an account reveals how this mechanism could generate oscillations, it is compatible both with the oscillations gradually dampening and the mechanism settling into a steady state where the transcription and translation of PER is just sufficient to match its degradation and with the oscillations being sustained indefinitely, which is required if the oscillation in PER concentration is to explain circadian rhythms. Determining which happens requires tracking quantitatively the concentrations of PER. This is made even more difficult because sustained oscillations require at least one non-linear operation, which are very challenging to keep track of in mental rehearsal.

Five years after the TTFL explanation was first proposed, Goldbeter (1995) produced a computational model to show that the proposed feedback mechanism would not dampen but would exhibit sustained oscillations. The basic structure of his model had in fact been introduced several decades earlier by Goodwin (1963), who drew upon then recent discovery by Jacob and Monod of the feedback mechanism involved in regulating bacterial genes responsible for enzymes needed to metabolize different sugars. The key to generating sustained oscillations in his model was the inhibitory process, which Goodwin modeled using equation (1). The first term on the right side of the equation characterizes the effect of reduced transcription on the concentration of mRNA (X) as a result of the number of molecules n of the inhibitor Z that must act together (the second term reflects the decay of mRNA dependent on its concentration).

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

For Goodwin's model to exhibit sustained oscillations, n had to be greater than 9, recognized as biologically implausible. When he revived Goodwin's model, Goldbeter modified it; Figure 2a shows how the equation in his model that includes the crucial non-linearity relates to parts of the TTFL mechanism advanced by Hardin et al. This modification produced sustained oscillations with biologically plausible values for n as well as the other parameters. To show that the mechanism could generate sustained oscillations and be robust against noise, Goldbeter plotted the results of simulations in phase space, as in Figure 2b. Here the amount of *per* mRNA and PER protein are plotted on the two axes and the change in concentrations over time from two starting points are shown along the lines with arrows. If the system were to settle into a steady state all the trajectories would end in a point, known as a point attractor. The fact that they asymptote on the dark oval indicates that the system will continue to oscillate—the oval represents a *limit cycle*.

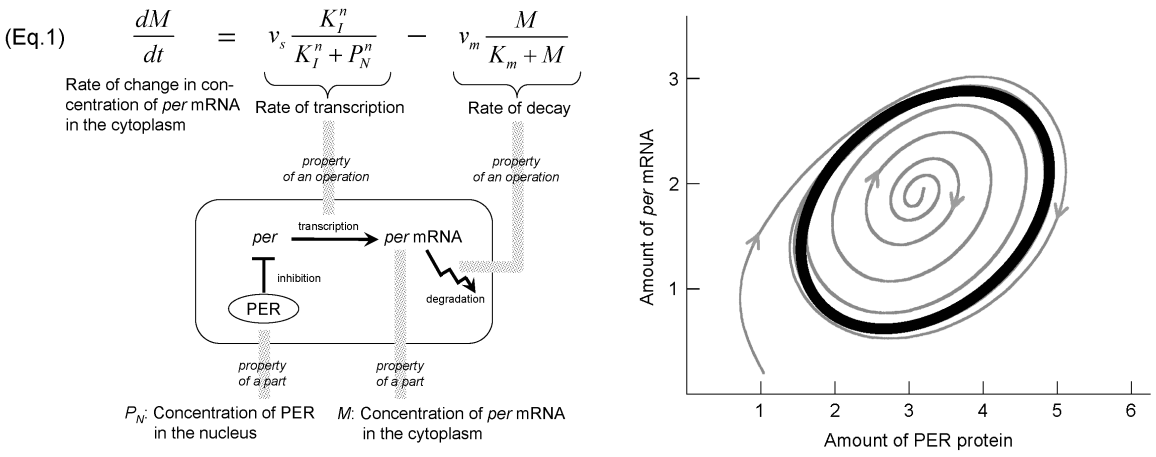

Figure 2. A. A schematic representation of how the first equation in Goldbeter's model relates to the parts and operations in the TTFL. B. Two trajectories of the simulation, plotted in state space, approach the limit cycle, indicating that the TTFL mechanism will generate sustained oscillations.

About the time Goldbeter was developing this computational model, circadian researchers were discovering several other genes and proteins that are involved in the circadian clock in fruit flies and mice. Rather than just one feedback loop, multiple feedback relations were proposed, including a positive feedback loop. As a result of these discoveries, the proposed mechanism was becoming much more complicated and it was possible that the additional parts and operations that were being identified and fitted into the proposed mechanism would impair its ability to sustain oscillations. As a result, over the years Goldbeter continued to develop models incorporating new components and operations for both the fruit fly and mouse circadian clocks. These showed that the more complicated mechanisms would continue to sustain oscillations (Leloup & Goldbeter, 1998, 2003, 2004). He also demonstrated that these models could simulate a wider range of phenomena such as entrainment by light and, when modified appropriately, could replicate known circadian pathologies.

Goldbeter's models illustrate a basic yet crucial role computational models play in mechanistic explanations—showing that a posited mechanism is in fact sufficient to generate the phenomenon. As a result of non-sequential organization and non-linear operations in the proposed TTFL mechanism, researchers could not determine by mentally simulating the mechanism whether it would generate sustained oscillations. While such modeling performs a crucial function, it is largely parasitic on the research that gave rise to the proposal of the mechanism. Given that they had developed this proposal on the basis of empirical evidence of parts and the operations they performed in the biological system and that through mental simulation they could show that the mechanism could oscillate, experimental researchers expected a positive result. The models went beyond what experimental researchers could establish through mental simulation in demonstrating that the oscillations would be sustained.

3. Modeling to Formulate a Mechanistic Hypothesis

In the case of circadian rhythms in cyanobacteria, modeling has often played an important role in the endeavors to formulate the accounts of the mechanisms themselves. Although initially researchers were dubious that organisms that live less than a day would maintain circadian rhythms, Kondo, Strayer, Kulkarni, Taylor, Ishiura, Golden, and Johnson (1993) demonstrated, using a luciferase knock-in that enabled easy tracking of gene expression, that cyanobacteria do maintain such rhythms. Subsequent research demonstrated that individual bacteria pass on phase information to their descendants. In fact, almost all genes in cyanobacteria are regulated in a circadian fashion so as to have maximum expression around dawn or dusk. Soon after the existence of circadian rhythms was established, three crucial genes (*kaiA*, *kaiB*, *kaiC*) were identified through screens of mutants with altered rhythms (Ishiura, Kutsuna, Aoki, Iwasaki, Andersson, Tanabe, Golden, Johnson, & Kondo, 1998). Researchers tended to interpret these studies in light of the TTFL mechanisms that had been advanced by that time not just for fruit flies and mice, but also for plants and fungi. Some researchers, however, began to focus on the proteins themselves. KaiC was found to bind ATP and to both autophosphorylate and autodephosphorylate. KaiA was determined to increase the rate of KaiC autophosphorylation, while the addition of KaiB to KaiC and KaiA reduced the rate of phosphorylation and, if KaiC were already phosphorylated, facilitated dephosphorylation. The cycle of phosphorylation and dephosphorylation of KaiC itself takes 24 hours, leading Ditty, Williams, and Golden (2003, p. 524) to conclude that these activities involving KaiC "are central to the timekeeping ability of the Kai oscillator." Nonetheless, the dominance of the TTFL perspective was sufficiently great that it was a surprise to many when Nakajima, Imai, Ito, Nishiwaki, Murayama, Iwasaki, Oyama, and Kondo (2005) demonstrated circadian rhythms in a preparation involving only the Kai proteins and ATP. This indicated that a TTFL is not required and that the cycle of phosphorylation and dephosphorylation of KaiC is the central component of the cyanobacterial clock mechanism.

Once the TTFL mechanism was shown not to be essential to generating circadian rhythms and that periodic phosphorylation and dephosphorylation of KaiC sufficed, the challenge researchers faced was to identify mechanistic operations that could control the successive phosphorylation and dephosphorylation of KaiC. Two research groups proposed different operations, and in both cases computational models played a fundamental role in their projects.

The first group, Mori, Williams, Byrne, Qin, Egli, McHaourab, Stewart, and Johnson (2007), took up this challenge, first acquiring new data through electron microscopy studies and then invoking computational modeling to fit the data into a mechanistic proposal. The EM studies provided a means of assessing how much KaiC was unbound or bound either to KaiA or to KaiA and KaiB at seven time periods during the day. KaiC typically occurs as a hexamer (a compound formed from six monomers), but it is the individual monomers that become phosphorylated. At all times some KaiC monomers are bound to Kai A or KaiB, but the percentage of unbound KaiC and KaiC bound with KaiA and KaiB changes across the course of the day. The challenge was to develop a hypothesis about the type of mechanism that would control whether KaiC bound with KaiB or KaiA. Mori et al. put forward their

proposal in the diagram reproduced in Figure 3A in which KaiC is portrayed as cycling between the states denoted α, β, χ, and δ. A key feature of the diagram is that the χ and δ states are distinguished from those earlier by showing KaiC in dark blue and labeled KaiC*. This distinction between these states was crucial since on their account the same total phosphorylation levels are obtained both during the phosphorylation and dephosphorylation process. Without such a change in state, the mechanism would quickly settle to mean levels of KaiC phosphorylation. To demonstrate that the state change proposed in the diagram could generate the phenomenon, Mori et al. constructed a computational model.[2]

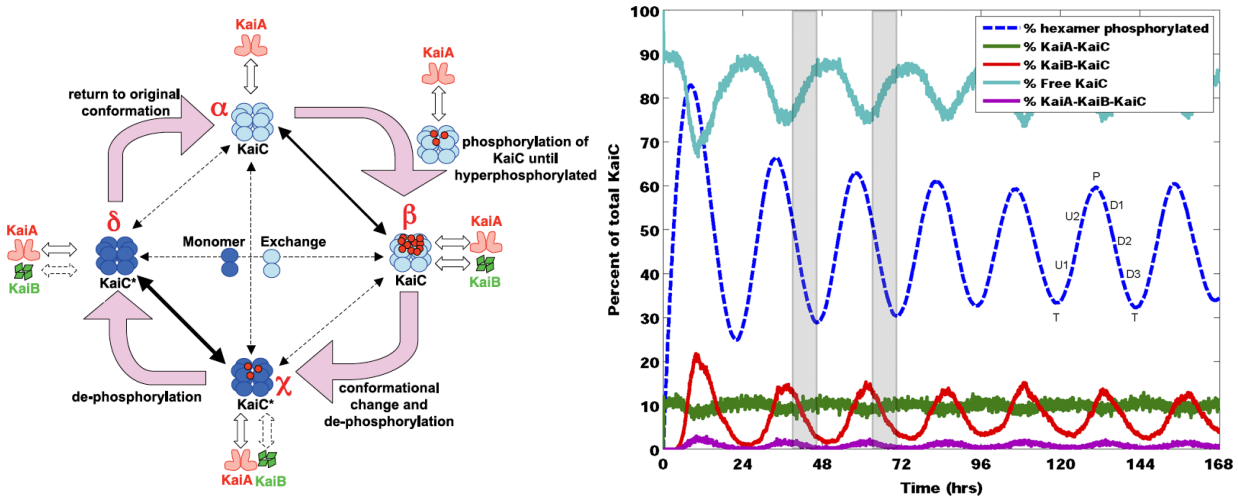

Figure 3. A. Diagram Mori et al. (2007) used to construct their mathematical model in which KaiC advances through four states, being phosphorylated and dephosphorylated as a result of interactions with KaiA and KaiB and changing its own state to KaiC*. B. Simulation results showing oscillation in percentage of phosphorylation of KaiC hexamers and of the amount of KaiC unbound or bound to KaiA, KaiB, and KaiA and KaiB.

Mori et al.'s computational model treated this state change as stochastic so that, with a specified probability, a molecule of KaiA would bind with a KaiC monomer, which would increase the likelihood of phosphorylation during the period when it is bound. Figure 3B presents the results of the simulation, which closely fit the results of the EM studies. The percentage of phosphorylated Kai C oscillates through the seven simulated days in phase with the oscillation of KaiB-KaiC (KaiB binds to KaiC preferentially when KaiC is phosphorylated). The state change employed in the model, however, introduced a variable in need of physical interpretation. The authors proposed that the value KaiC* represented a changed conformation of KaiC, but they offered no direct evidence for such a conformation change. The evidence was limited to the fact that the computational model incorporating

[2] Jones and Wolkenhauer (2012) have investigated the process of using diagrams of mechanisms in which variables and parameters are noted as the bases for computational modeling, referring to them as *locality aids*.

the conformation change could reproduce the phenomenon. (Another feature of Mori et al.'s model is the exchange of monomers between hexamers, which had recently been discovered and hypothesized as functioning to synchronize phosphorylation in different hexamers. The simulation appeared to support this proposal since when hexamer exchange was turned off, the percentage of phosphorylated KaiC flattened as would be expected when oscillators are not synchronized).

Rust, Markson, Lane, Fisher, and O'Shea (2007) pursued a different hypothesis to explain the progression through phosphorylation states that grew out of a different type of empirical evidence. Whereas most researchers had treated phosphorylation at two different sites, serine 431 (S431) and threonine 432 (T432) on the KaiC monomer, as equivalent, Rust et al. investigated the timing of phosphorylation at each site alone and at both sites at once. Notably, the peak of phosphorylation at just T432 preceded phosphorylation at both sites, which preceded phosphorylation at just S431. Accordingly, Rust et al. developed an alternative diagrammatic representation (Figure 4a) and computational model in which Kai C is assumed to be transformed sequentially into different phosphoforms (U=unphosphorylated, T=phosphorylation at T432 alone, ST=phosphorylation at both T432 and S431, and S=phosphorylation at S431 alone).

Based on these results and other data about the reactions promoted by different phosphoforms (e.g., KaiB only induces dephosphorylation when levels of the S phosphoform are high), Rust et al. developed a computational model in which the amount of active KaiA alters the rate constants for the transformation between phosphoforms and each S phosphoform monomer inactivates one KaiA molecule. (The first equation shown in Figure 4b shows how the rate constants for each of the transitions between phosphoforms (*X* and *Y* are variables for phosphoforms) depends on the concentration of active KaiA (A), which, as indicated in the second equation, is reduced by S. They used the actual data procured for each reaction in isolation to calibrate parameters in the model. The model generated the sustained oscillations shown in Figure 4c in which the peaks of the various phosphoforms occur in the order T, ST, and S.

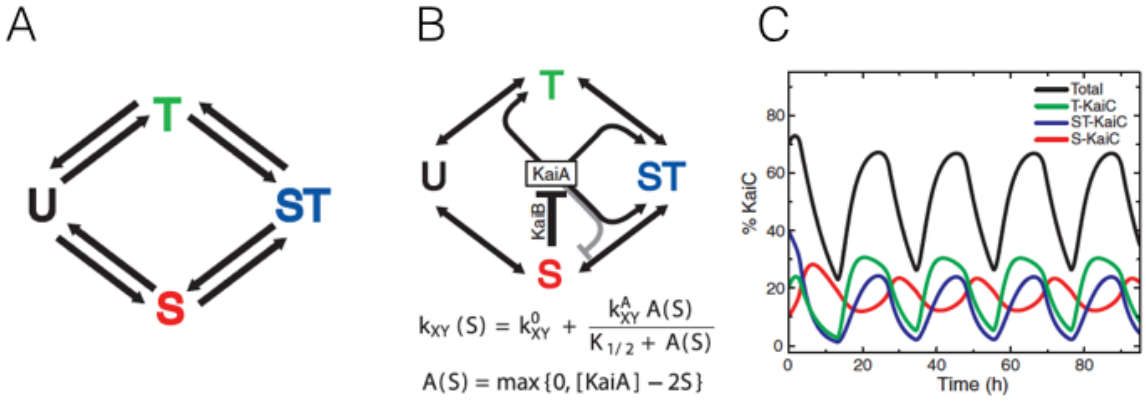

Figure 4. A. The steps of phosphorylation and dephosphorylation between the U (unphosphorylated) phosphoform of Kai C and those involving phosphorylation at the threonine (T), serine (S) and both loci (ST). B. Rust et al.'s proposed mechanism with the key equations used in their computational model. C. The results of the

simulation showing oscillations in each phosphoforms with peaks in the order T, ST, and S.

The two examples discussed in this section demonstrate how computational modeling can figure in the development of mechanistic hypotheses. In Mori et al.'s case, the development of the model pointed to the need to assume an alternative conformation of KaiC once it is fully phosphorylated in order to explain the direction of progress through the cycle. Rust et al. advanced their alternative hypothesis in which the binding of KaiB to KaiA and KaiC as concentrations of the S phosphoform increases regulates the rates of the various phosphorylation and dephosphorylation reactions. The interaction between constructing the model and the empirical investigations is apparent in the process of identifying the rate constants through fitting the parameters to the data and in developing the crucial non-linear equation relating these constants to concentration of active KaiA. In both of these examples, the modeling played a central role in the formulation of the proposed mechanistic account; it did not merely serve to demonstrate that an already advanced model could generate the phenomenon. Subsequent research has vindicated versions of each proposal—phosphorylation occurs in the order identified by Rust and there is evidence that alternative folding of the KaiC protein in part regulates its binding with KaiA and KaiB (Chang, Cohen, Phong, Myers, Kim, Tseng, Lin, Zhang, Boyd, Lee, Kang, Lee, Li, Britt, Rust, Golden, & LiWang, 2015).

4. Modeling to Determine How One Mechanism Fits within Another

Although the demonstration of circadian oscillation with just the three Kai proteins and ATP showed that transcription and translation of the *kai* genes is not a necessary part of the mechanism, it is still the case that transcription of the *kai* genes is regulated by the phosphorylation oscillator. As a result, there is still a functioning TTFL, raising the question of what role it might play in generating circadian rhythms. Several researchers have proposed that it might function to stabilize the oscillations generated by the phosphorylation oscillator. Teng, Mukherji, Moffitt, de Buyl, and O'Shea (2013) have provided experimental support for this hypothesis: when cyanobacteria are placed in constant light so as to eliminate external cues to entrain the oscillators, the individual mutant bacteria in which the transcription-translation loop is impaired become desynchronized after four days. Teng et al. amended Rust et al.'s model to incorporate synthesis and degradation of KaiC and a feedback process in which KaiC represses its own synthesis and showed that with the TTFL the oscillators remain much more synchronized than without it.

Accepting the importance of the TTFL raised a new question—how is the phosphorylation oscillator linked to the TTFL. SasA was implicated in this relation. SasA had first been identified as a sensor kinase in cyanobacteria in 1993 and later in the decade was shown to interact with KaiC and to be important in maintaining circadian rhythms. Shortly after their research group had succeeded in reconstituting a circadian oscillator with the three Kai proteins and ATP, Takai, Nakajima, Oyama, Kito, Sugita, Sugita, Kondo, and Iwasaki (2006) identified RpaA as a response regulator capable of binding DNA and proposed that it linked SasA to DNA transcription. But precisely how SasA and RpaA should be understood as

integrated into the post-translational oscillator was not clear. Moreover, there was evidence that, at different phosphorylation stages, KaiC functioned both to increase and decrease expression of the *kaiB* and *kaiC* genes. (The *kaiB* and *kaiC* genes are located next to each other and share a common promoter site; hence, one often sees references to *kiaBC*.) I contrast the efforts of two research groups that both attempted to show which state of KaiC phosphorylation (referred to as the *phosphoform*) was linked to *kaiBC* expression. Computational models figured in the efforts of both research groups, but in different ways. The first group relied on testing the predictions from multiple computational models to find which one best fit time series data that had previously been collected. The second used a more traditional experimental manipulation to determine how KaiC phosphoforms related to *kaiBC* expression, but then invoked a computational model to demonstrate the fit of the resulting proposed mechanism with time-series data.

In the first study, Hertel, Brettschneider, and Axmann (2013) turned to computational modeling to determine which phosphoforms of KaiC were responsible for controlling *kaiBC* expression. Their strategy was to construct one computational models for each likely pattern of connection between phosphoforms and gene expression (32 different models) and then rule out those that failed to correctly account for time-series data that had been collected by Murayama, Oyama, and Kondo (2008) on the amount of *kaiBC* mRNA, unphosphorylated KiaC, and total phosphorylated Kai C generated in wild-type and *kai*-gene knockouts under constant light. Panels A and B of Figure 5 each show one of the proposed patterns of connection. In Panel A, for example, the D phosphoform form (Hertel et al. use D rather than ST for the doubly-phosphorylated state) from the pool of KaiC hexamers acts positively on *kaiBC* transcription and the T phosphoform from the same pool acts as an inhibitor. As seen in the graph which compares the computational model to actual data about *kaiBC* mRNA, unphosphorylated KaiC, and phosphorylated KaiC, this pattern of connection failed to fit the data (specifically, it reversed the phase of the oscillation of unphosphorylated KaiC and unphosphorylated KaiC). In contrast, the pattern shown in 5B not only fit this data but was also the only model that fit all of the data Hertel et al. considered. They fleshed out this pattern of connection in Figure 5C in which the excitatory pathway from the T and D phosphoform to the *kaiBC* promoter is modulated by SasA and RpaA while the inhibitory pathway from the U phosphoform to the promoter is modulated by RpaB.

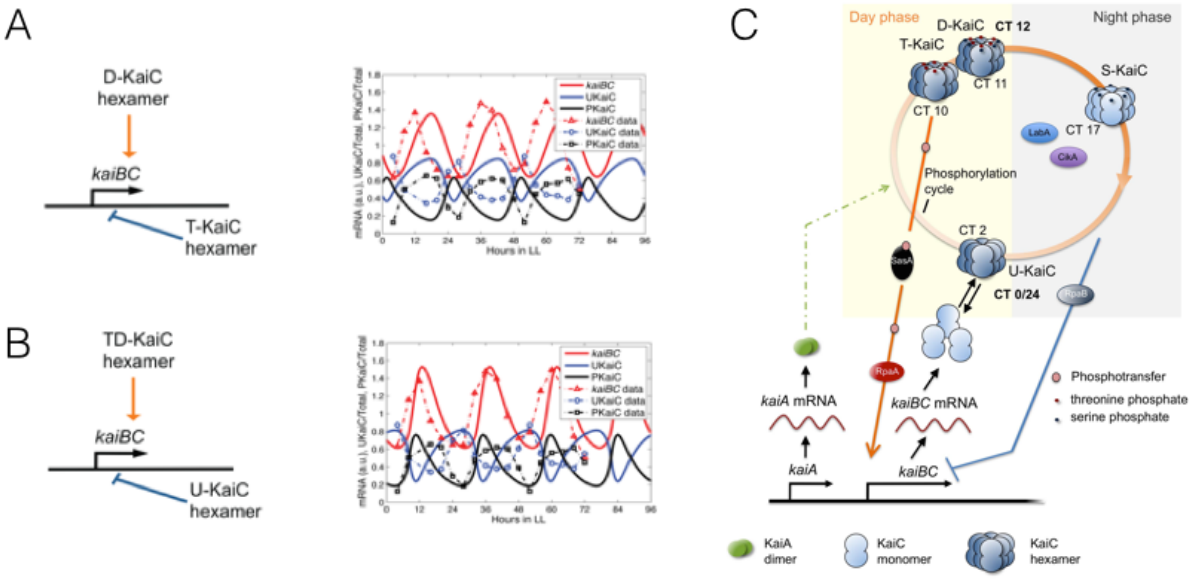

Figure 5. A and B. Two of the patterns of connection developed by Hertel et al. (2013) together with the comparison of simulation data using the model with actual data. The one shown in panel A reverses the phase of two of the molecules while that shown in panel B. fits the data reasonably well. In C the model from B, which fit all the data Hertel et al. considered, is shown in relations to the *kaiBC* genes, which it is proposed to regulate via SasA, RpaA, and RbaB.

In the same year, Paddock, Boyd, Adin, and Golden (2013) experimentally manipulated the effect of different phosphoforms, rather than doing so only in a computational simulation, and arrived at a different proposal for the mechanism. They created molecules (mimetics) to mimic each of the phosphoforms of KaiC and a luciferase reporter to detect transcription of *kaiBC* and a second gene, *purF*, that oscillates out of phase with *kaiBC*. They found that the S-phosphoform generated maximally inhibited transcription of *kaiBC* and enhanced transcription of *purF*. Moreover, they demonstrated that *kaiC* knockouts had effects opposite those of *rpaA* knockouts, leading them to conclude there were two pathways that competed with each other. They proposed that the RpaA pathway is activated (i.e., Rpa-A is phosphorylated) by an earlier phosphoform (Paddock et al. do not say which) and when it is dominant, serves to block any inhibition from the S-phosphoform and to excite *kaiBC* transcription. When the S-phosphoform is dominant, *kaiBC* transcription is inhibited, but *purF* is excited. Paddock et al. offer a simple mathematical equation that, when combined with experimental data about the time-dependence of the S phosphoform and the phosphorylated state of RpaA, could be used to simulate both the oscillation in gene expression and the effects of the mimetics of the T- and S- phosphoforms.

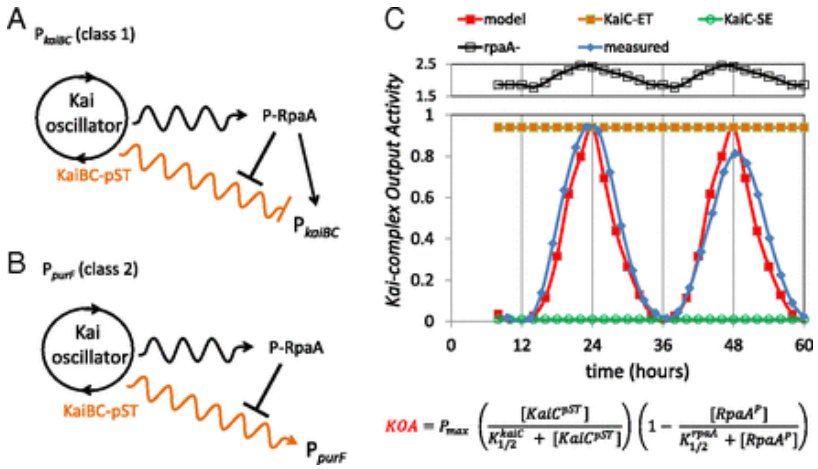

Figure 6. A and B. Paddock et al.'s proposed mechanism for regulating (A) *kaiBC* and (B) *purF* in cyanobacteria involving a pathway through RpaA and a second originating from the S-phosphoform of KaiC (labeled KaiBC-pST). C. The mathematical equation used in their model to determine Kai-complex Output Activity (*KOA*) is shown at the bottom. Above it is a plot indicating the fit of the mathematical model to the measured data.

Hertel et al. and Paddock et al. illustrate two different strategies for determining how one mechanism, involving KaiC phosphorylation, fits within another, involving the synthesis of KaiB and KaiC. One group employed computational modeling to winnow proposed connections whereas the other employed a more direct experimental procedure and relied on a mathematical model to evaluate the proposal supported by the experimental results. Interestingly, the account of the relation between the mechanisms advanced by Paddock et al. is quite similar to the connection pattern that fared second best in Hertel et al.'s competition. Yet there are differences between them, and conceivably Paddock et al.'s proposal would have won Hertel et al.'s competition. One thing this reveals is that Hertel's et al.'s strategy can only eliminate models that are actually considered. It is also conceivable that if Paddock et al.'s proposal were evaluated against the additional time-series data used in Hertel et al.'s study it would have exhibited inadequacies. What the two studies do reveal are different ways in which computational modeling can be invoked in trying to determine how one mechanism fits within another.

5. Modeling to Extract Design Principles

As proposed mechanisms become complicated and complex, researchers may be able to determine that the mechanism would generate a phenomenon without understanding why it would do so. Given the desire not just to explain but also to understand, researchers want to know what it is about the mechanism that is actually responsible for generating the phenomenon. They seek the design principles instantiated in the mechanism. To discover these, researchers sometimes find it useful to abstract from detail and model as few parts and operations of the mechanism as they can while still generating the phenomena and probe the resulting model to determine the parameter values required to produce the phenomenon. Inspired by the discovery of the phosphorylation-dephosphorylation

oscillator in cyanobacteria, Jolley, Ode, and Ueda (2012) investigated further and found that the simplest phosphorylation-dephosphorylation mechanism that could generate sustained circadian oscillations involved phosphorylation and dephosphorylation at at least two sites. They developed the model shown in Figure 7 which employs 16 parameters ($k_1 \ldots k_8$ are rate parameters specifying the number of substrate molecules converted to product molecules in a given reaction per enzyme per minute and $K_{m1} \ldots K_{m8}$ are binding parameters that determine the substrate concentrations at which the reaction reaches half its maximum rate). Since the model generated sustained oscillations only with some parameter values, they searched for parameter values that did generate sustained oscillations. They found that only ~0.1% of the parameter values they checked do so, but they identified more than one million of these and made them the focus of further analysis.

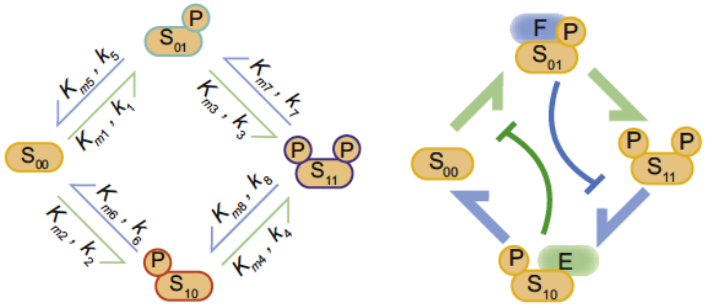

Figure 7. A. Schema of the model used by Jolley et al. to determine which parameter sets would generate oscillations. B. Representation of the two motifs realized in most of the realizations that generated oscillations. See text for details.

Jolley et al. determined that most of the parameter values that generated sustained rhythms implemented two motifs. One, indicated by the arrows around the parameter in Figure 7b, involves sets of rate parameters in which the parameters for the clockwise reactions are higher than for those in the opposite direction. The second, indicated by the two arrows crossing though the middle of the diamond, involve phosphoforms inhibiting reactions two steps beyond by binding (sequestering) the enzyme needed for both reactions at the first site until that reaction is complete. To support their claim that it is the implementation of these motifs that produce the oscillations, the authors showed that constraining the search of parameters to ones that fit the motifs significantly enhanced the probability of finding parameters that will generate sustained oscillations.

To further enhance their understanding of how the motifs create sustained oscillations, the researchers developed a probabilistic version of the model in which both motifs were present in multiple individual oscillators that together constituted a population. Since the model was probabilistic, the oscillators exhibited variability, especially with respect to the percentage of time an individual simulated molecule resided in the S_{10} or S_{01} phosphorylation states, but the population remained highly synchronized overall. In particular, the transitions $S_{00} \rightarrow S_{01}$ and $S_{11} \rightarrow S_{10}$ are synchronized across simulated oscillators, suggesting that sequestration is in fact synchronizing the oscillation of individual molecules. They inferred that it was the motif of higher values of the parameters for forward reactions that generated oscillations and the motif producing sequestering that

resulted in synchronization. This differentiation of roles was supported by simulations in which, when all the rate parameters were set to the same value, oscillation was largely lost, but when the binding parameters were set equal, individual molecules continued to oscillate, but the population was desynchronized. Once elucidated, these motifs are simple enough that one can mentally rehearse their effects (e.g., greater parameter values for forward reactions than reverse reactions will lead each to proceed in the forward direction so that one can follow the reactions around the diamond). Appealing to them allows one to understand the behavior of the full mechanism.

Unlike the models discussed in section 3 above, Jolley et al.'s model did not build in specific details hypothesized by the researchers, but rather used the search of parameter space to determine what motifs would produce the phenomenon. Nonetheless, the authors see their results as consilient with those of modelers such as Rust: "The model of Rust et al. (2007) describes a two-site system similar to ours, in which the two phosphorylation states of the substrate (KaiC) have distinct roles. . . . Similarities to our model exist in the general principles of kinetic bias (i.e., T432 is phosphorylated more quickly than S431) and synchronization by feedforward inhibition, although the molecular details are quite different" (p. S9). The point of modeling, for them, is different than for Rust: whereas Rust et al. were concerned to identify parts and operations of the actual mechanism, Jolley et al. view the motifs they identify as design principles that explain why mechanisms of the type that are found in cyanobacteria but also elsewhere generate sustained oscillations. These design principles are simple enough that they enable researchers to understand why the various mechanisms that employ them generate oscillations.

6. Conclusions

Accounts of dynamic mechanistic explanation emphasize the need to complement traditional mechanistic approaches with computational modeling and principles from dynamical systems theory in the case of proposed mechanisms that feature non-sequential organization and non-linear operations. Here I have focused on four specific roles of computational modeling in explanations of such systems. The most straightforward is to demonstrate that the mechanism proposed could generate the phenomenon. Hypothesized mechanisms with more than a little non-sequential organization and non-linear interactions exceed the ability of researchers to determine how they will behave by mental rehearsal alone, and reliance on computational modeling is required to predict how the proposed mechanism will actually behave. This role corresponds to the use of derivation in more traditional nomological accounts of explanation; although mechanisms are not represented as laws, in both cases one is predicting (or retrodicting) what will happen. These predictions can then be used to explain the phenomenon and evaluate the correctness of the law or mechanism.

Two other uses of models involve the phase of discovery. Some philosophers of science denied that philosophical analysis could say anything informative about the discovery process, but research on mechanisms has revealed a variety of experimental strategies researchers use to develop mechanistic accounts (Craver & Darden, 2013; Bechtel & Richardson, 1993/2010; Bechtel, 2006). The cases discussed in section 3 reveal

complementary discovery strategies involving the combination of diagrams and computational models based on them. In these cases, computational modeling allowed researchers to advance proposals for additional operations to explain how a mechanism involving phosphorylation and dephosphorylation of a molecule could produce sustained oscillations. Mori et al. showed that a change of value of a state variable would account for the change from phosphorylation to dephosphorylation whereas Rust et al. developed a proposal centered on their empirical discovery of phosphorylation at two loci. To characterize the operations that responded to the successive phosphorylation at these two sites, they advanced a computational model and fit the parameters for each operation to the data derived from isolated systems. In both cases, the modeling figured in the discovery process—it was not simply a check that an already proposed mechanism could generate the phenomenon.

A second use in discovery is illustrated in the research fitting one mechanism, the phosphorylation-dephosphorylation oscillator, into a larger-scale one, the TTFL that is still present in cyanobacteria. Hertel et al. deployed a strategy of creating 32 models characterizing different proposals for connecting the mechanisms and evaluating each against different sets of available data. Only one accounted for all data sets. Such a strategy of winnowing models, however, is only successful if all candidate mechanisms are considered, and the study by Paddock et al. identified new experimental evidence from which these researchers constructed a variant on the candidate from the Hertel et al. study that came in second. Paddock et al. employed a computational model to evaluate their proposed mechanism, showing that it could provide a close fit to the empirically observed oscillation. Since the researchers did not know of each others' pursuits, neither analyzed the specific model Paddock et al. advanced against the datasets employed by Hertel et al. The fact that different discovery strategies generated different outcomes reinforces the idea that no discovery strategy is guaranteed to find the correct mechanism. Nonetheless, the Hertel et al. strategy illustrates a potential role for computational modeling in constraining the space of possible mechanisms.

The final role for computational models I identified is to determine the design features that enable a complicated or complex mechanism to produce the phenomenon. When the details of the mechanism are complicated, a detailed computational model may show that a mechanism could produce the phenomenon, but not provide understanding of how it does so. To gain such understanding, researchers often abstract from details and focus on what they take to be the mechanism's core components. What Jolley et al. added to this was to investigate, using a computational model of the simplified mechanism, which parameter values suffice to generate the phenomenon. They then analyzed those that did to identify two basic motifs that are realized in successful parameter sets. Each motif revealed a design principle and the researchers were able to make intelligible how the two together could generate sustained oscillations. In further testing they sought to demonstrate that these design principles contribute in the manner they suggested and that they are instantiated in other computational models of the cyanobacterial oscillator that were more closely based on empirical findings.

Dynamical mechanistic explanations differ from basic mechanistic explanations in employing computational modeling in central roles. My strategy has been to show, through the analysis of actual cases, different roles computational modeling plays in predicting the effects of a complex mechanism, discovering a mechanism that could generate the phenomenon, and in understanding the design principles realized in a complex mechanism. These are surely not the only roles, but they suffice to show how computational modeling employed in dynamic mechanistic explanations can play a number of roles in evaluating, discovering, and understanding the mechanisms proposed to explain biological phenomena.

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