Modeling/Experimenting?

The Synthetic Strategy in the Circadian Clock Research

Abstract:

In which respects do modeling and experimenting resemble or differ from each other? We explore this question through studying in detail the combinational modeling strategy in the study of the circadian clock. In this area of synthetic biology scientists triangulate experiments on model organisms and mathematical models with a new type of model—a synthetic model. We argue that this combinational strategy is due to the characteristic constraints of the three aforementioned epistemic activities that make them *complementary* with respect to each other. These constraints are closely linked to the question of materiality. In the case of synthetic biology, materiality clearly matters: it provides the very rationale of synthetic modeling. Consequently, although modeling and experimenting share some common features, there are also significant differences between them and they perform different roles in actual scientific practices.

1. Introduction

In philosophical discussion, models have been located between theories and experiments, often as some sort of go-betweens facilitating the points of contact between the two. Although the relationship between models and theories may seem closer than the one between models and experimentation, there is a growing body of literature that focuses on the similarities (and differences) between modeling and experimentation. The central questions of this discussion have concerned the common characteristics shared by modeling and experimenting as well as the ways in which the inferences licensed by them are justified.

In this paper we will study the different stands taken in the discussion on modeling and experimentation through examining the modeling practice of the circadian clock research in synthetic biology. Circadian clock research studies the day and night rhythms of organisms. In this area experiments on model organisms, mathematical models (and their simulations), and synthetic models are being closely triangulated. Moreover, there is often no division of labor in synthetic biology laboratories: the same scientists typically engage in experimentation as well as in mathematical and synthetic modeling. Consequently, one might expect that there are good reasons why synthetic biologists proceed in such a combinational manner. We will argue that this is due to the characteristic *constraints* of each of the aforementioned activities that

make them *complementary* with respect to each other. In particular, we wish to show that there is a gap between modeling and experimentation that, in the field of synthetic biology, gave rise to a new type of model, the synthetic model, built from genetic material using a mathematical model as a blueprint. As such it is a hybrid entity that shares characteristics of both experiments and models, serving to make clearer the differences between the two.

Considering the constraints of synthetic models vis-à-vis those of mathematical models and experiments also provides a novel perspective on the discussion concerning the role of materiality in modeling and experimentation. We show how the "same materiality" or the "same stuff" was clearly relevant for the epistemic functioning of synthetic models, but only in combination with a carefully engineered theoretically preconceived mechanism. To exemplify a synthetic model we will examine more closely the *Repressilator* (Elowitz and Leibler 2000), which was one of the first hybrid models of its kind. Before going into synthetic modeling and the combinational modeling practice of synthetic biology, we will review some relevant philosophical discussion on the relationship of modeling and experimentation.

2. Modeling vs. Experimenting

The philosophical discussion of the relationship of modeling and experimentation has concentrated on which grounds, if any, the two activities can be clearly distinguished from

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each other, and whether the inferences made to real target systems are more direct in the case of experimentation than in modeling. As regards the similarity between modeling and experimentation, there are many ways to cash it out. Firstly, one can consider them as largely analogous operations. Both in modeling and in experimentation one aims to *seal off* the influence of other causal factors in order to study how a causal factor operates on its own. Whereas in experimentation this sealing off happens through experimental controls, modelers use various techniques, such as abstraction, idealization, and omission as vehicles of isolation (see, e.g., Cartwright 1999; Mäki 2005). Consequently, a theoretical model, too, can be considered as an outcome of the *method of isolation*: in modeling a set of elements is theoretically removed from the influence of other elements through the use of a variety of unrealistic assumptions (Mäki 1992).

Although the idea of seeing both experimentation and modeling as instances of the method of isolation seems intuitively plausible, one specific property of mathematical modeling does not easily fit into this picture. Idealizing assumptions are often driven by the requirements of tractability rather than those of isolation. The model assumptions do not merely neutralize the effect of the other causal factors but rather construct the modeled situation in such a way that it can be conveniently mathematically modeled, making it often unclear which assumptions are crucial for the results, or whether the results are dependent on the specific mathematical construction of the model (see, e.g., Cartwright 1999; Morrison 2008). This feature of mathematical models is further enhanced by their use of general, cross-

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disciplinary computational templates that are, in the modeling process, adjusted to fit the field

of application (Humphreys 2004; Knuuttila and Loettgers 2012). Such templates are often

transferred from other disciplines, as in the case of the circadian clock research, where many

models, formal methods and related concepts originate from physics and engineering (e.g., the

concepts of oscillator, non-conservative system, feedback mechanism, and noise—see below).

There is also reason to doubt whether the method of isolation succeeds in capturing the *actual

modeling heuristic* since models are often constructed by depicting hypothetical systems

instead of being abstracted from real world systems in any straightforward manner (Weisberg

2007).

Even if one does not want to endorse the idea that models are results of isolation, this

insight implies another respect in which modeling and experimentation may resemble each

other. In experimentation some real world target system is intervened on. Likewise in

modeling one typically intervenes on the model system, although in the context of

mathematical modeling one usually talks about the manipulation of a model (Morgan 2003).

Thus the second sense in which models and experiments may resemble each other is due to the

fact that in both modeling and experimentation one seeks to *intervene on* a system in the light

of the results of this intervention.[1] But the question is how deep this resemblance really cuts.

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[1] Apart from the arguments concerning isolation and intervention, a third motivation for claiming that the

practices of modeling and experimentation are similar to each other invokes the fact that both simulationists and

experimentalists produce data and are dealing with data analysis and error management (see Winsberg 2003 and

Barberousse et al. 2009 for somewhat divergent views on this matter)

Two issues in particular have sparked discussion: the supposed target systems of simulations versus experiments and the role of materiality they incorporate. In neither of these questions has any consensus emerged among the philosophers of science—although the discussion itself has provided some novel insights.

As regards the target systems of simulations vis-à-vis experiments a common intuition seems to be that whereas in experimentation one intervenes on the real target system of interest, in modeling one merely interacts with a model system (e.g., Gilbert and Troitzsch 1999; Barberousse et al. 2009). Yet a closer examination has assured several philosophers that these intuitions may be deceptive. Winsberg (2009) argues that both "experiments and simulations have objects on the one hand and targets on the other, and that, in each case, one has to argue that the object is suitable for studying the target" (579; see also Guala 2002). Thus both experimentation and modeling/simulation display features of surrogate reasoning (Swoyer 1991), which is visible for instance in the experimentation on model organisms instead of the actual organisms of interest. Consequently the relationship of a model or experiment to its respective target need not distinguish the two activities from each other. Peschard (2012) disagrees, however. She focuses on what the kinds of "target systems" simulation and experimentation pick, respectively, in cases where they are used *in tandem* in scientific research. According to her the target system of the simulation is the system represented by the manipulated model: "it is the system simulation is designed to produce information about" (see also Giere 2009 for a similar view). In the case of experimentation,

the target system is the experimental system, for example the model organism. The experimental results concern the interventions on the model organism, e.g. rats, although the eventual *epistemic motivation* might be to gain information on the influence of a certain drug in humans.

Peschard's argument may seem not, at least at first sight, to fit fictional models whose target systems are the hypothetical model systems themselves in an analogy to the case of model organisms. This fictional feature of modeling has even been made the distinguishing mark of model-based theoretical strategy in science (Godfrey-Smith 2006; Weisberg 2007). Yet, on Peschard's account one can treat fictional models as experimental systems, in which case they boil down to experiments. As a consequence the distinction between what is an experiment and what is a simulation/model is made on pragmatic grounds, that is, how they are used and on what they are supposed to give knowledge about. A mathematical model is an experiment if it is manipulated to give us information about the model itself. It is a model when it is used to give information of another system through representing. Yet, at the level of scientific practice we nevertheless distinguish model systems from experimental systems, although borderline cases exist. The question that needs to be addressed is this: are we not usually interacting with *different kinds of scientific objects* when simulating or experimenting? At this point, the issue of materiality starts to emerge as the inevitable next puzzle.

As with the question concerning target systems, when it comes to materiality, the verdict is still out regarding its roles in modeling and experimentation. Whereas simulations appear as

non-material to some, for others the similarity of simulations to experiments is importantly related to their physicality or materiality. Obviously, analog simulations involving the use of scale models or other specially designed physical artifacts, for example, qualify as material things. But what about computer simulations—are they non-material things as Morgan (2003) suggests? Many philosophers of science seem to agree that the fact that computer simulations are implemented on a concrete device and thus involve physical processes when run on it, provides them with a material status (see, e.g., Humphreys 1994; Barberousse et al. 2009; Parker 2009). Yet the opinion is divided on what epistemic role this materiality plays. In particular, does materiality play the same role in justifying the inferences concerning target systems in the case of simulations as in the case of experiments?

The answer given by Norton and Suppe (2001) is most straightforward: since programmed computers are real systems, observations of them can give "new knowledge of the world" precisely as experiments do. But the challenge is then to show, how this can be the case given that computer simulations and the real world target systems are very different kinds of things. According Norton and Suppe simulations "embed" theoretical models in programmed computers through "lumped models": a simulation model is a lumped model embedded into a programmed computer. However, for the simulation to give knowledge of the real world targets it is mimicking, some rather stringent mapping relations have to hold between the model of the data, the modeled physical system itself, the base model, and the lumped model. Yet the burgeoning literature on simulation has so far established that the

relationship between the base model and what Norton and Suppe call the lumped model is far from straightforward.[2] Furthermore, what seems potentially even more problematic to Norton and Suppe is the critique presented by Barberousse et al. (2009). They argue that in most simulations the computer's physical states cannot be said to realize the lumped model, that is, the lumped model and the programmed computer qua physical system need not share a common structure. This seems to pull the rug from under the idea that "the computation's being a physical process itself should explain the 'mimicry' relation" between simulation and its real world target (ibid., 566).

Indeed, the *right kind of* materiality has been claimed to be the distinguishing mark of experiments and even the reason for their epistemic superiority to simulations. Either, it has been claimed that computer simulations are basically non-material (Morgan 2003, see above), or that the relationship between a simulation and its target is abstract, whereas the relationship between an experimental system and its target is grounded in the same material being governed by same kinds of causes (Guala 2002). The crucial difference between modeling and experimentation, according to this latter view, is that whereas in simulation one experiments with a (formal) representation of the target system, in experimentation the experimental and target systems are made of the "same stuff". This difference also explains,

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[2] The recent discussants on simulation models have stressed that the discretized programmed equations of the simulation model stand in no straightforward relationship to the equations of the basic theoretical model (e.g., Winsberg 2003; Lenhard 2007; Barberousse et al. 2009; Parker 2009).

according to Morgan and Guala, why experiments have more epistemic leverage than simulations. For example, anomalous experimental findings are more likely to incur change in our theoretical commitments than unexpected results from simulations.

Despite the intuitive appeal of the importance of the "same" materiality, it has been contested on different grounds. Morrison (2009) points out that even in the experimental contexts the causal connection with the physical systems of interest is often established via models (see, however, Giere 2009 for a counter-argument). Consequently, according to her, materiality provides no unequivocal epistemic standard that distinguishes simulation outputs from experimental results. Parker (2009) attacks directly the alleged significance of the "same stuff". She interprets the "same stuff" to mean for instance the same fluid and points out that in traditional laboratory experiments on fluid phenomena many other things such as the depth of the fluid and the size, shape, roughness and the movement of any container holding it may matter. This leads her to suggest that it is the "relevant similarities" that matter for the justified inferences about the phenomena that seems, once again, to make experimentation and modeling similar to each other.

In trying to establish to which extent modeling and experimentation really function in the same way—or deliver the same kinds of results—we will in the next sections take an excursion into the emerging field of synthetic biology. We focus, in particular, on synthetic modeling. The raison d'être of synthetic models, we suggest, is due to their hybrid theoretical-cum-material nature, which provides an interesting and revealing case regarding the

characteristics of modeling and experimentation. The right kind of materiality gives synthetic models their epistemic leverage—including also a carefully crafted causal mechanism.

3. The Combinational Modeling Strategy of Synthetic Biology

Synthetic biology is a relatively novel and highly interdisciplinary field. It is located at the interface of engineering, physics, biology, chemistry and mathematics. The research practice in this field is a combination of methods, concepts, tools, and theories from those fields. In synthetic biology itself one can distinguish between two main research branches: an engineering branch focusing on the engineering of novel biological components / systems and a basic science branch using synthetic models to gain understanding on the basic design principles underlying specific biological functions, such as the circadian clock regulating day and night rhythms in organisms.

In our study we are focusing on the latter branch of synthetic biology. One of the defining strategies of the basic science approach is the *combinational use of mathematical models, model organisms, and synthetic models*. The basic idea of this combinational modeling strategy is shown in figure 1, which is taken from a review article on synthetic biology by Sprinzak and Elowitz (2005). The two authors call this approach "the synthetic biology paradigm". As the diagram suggests, the results gained from each of the three different epistemic activities inform the other ones.
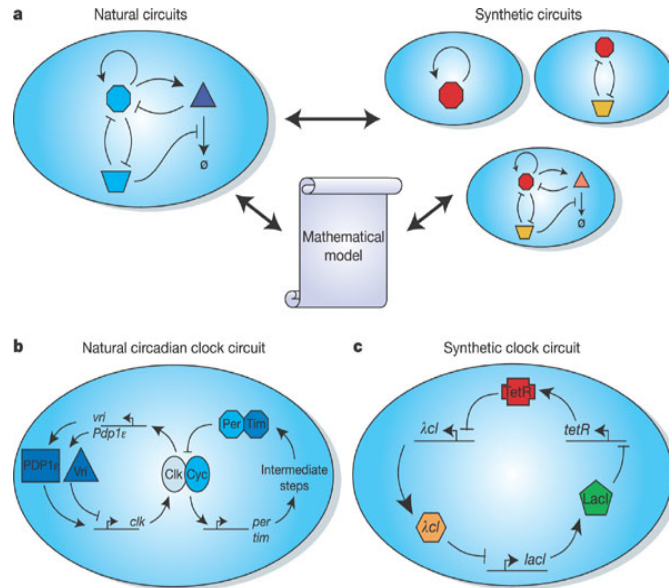
Figure 1. The "synthetic biology paradigm" according to Sprinzak and Elowitz (2005). The upper part of the diagram depicts the combinational modeling strategy and the lower part compares a natural gene regulatory network and a synthetic one.

Why do researches make use of a combinational modeling strategy in studying organizational principles in biology? A clue can be found from the lower part of the diagram. The left hand side of the diagram depicts our present understanding of the "natural gene regulatory circuit" of the circadian clock of *Drosophila* (fruit fly) consisting of interacting genes and proteins and the right-hand side a synthetic model of the circadian clock, the *Repressilator*, introduced by Elowitz and Leibler (2000). The diagram indicates the two main differences between the natural and synthetic system:

1. The natural system exhibits a much higher degree of complexity than the synthetic system.

2. The synthetic circuit has been designed by using different genes and proteins.

Consequently, synthetic models have the advantage of being less complex than model organisms. On the other hand, in comparison with mathematical models they are of the "same materiality" as their biological counterparts, gene regulatory networks. That is, they consist of the same kind of interacting biochemical components and are embedded in a natural cell environment. This "same materiality" is crucial for the epistemic value of synthetic modeling. It means that synthetic models are expected to operate in the same way as biological systems. As we will argue, the synthetic strategy rose as a response to the constraints of mathematical modeling on the one hand, and experimentation with model organisms on the other hand. There seemed to remain a gap between what could be shown mathematically and what was established experimentally. In particular, the experiments could not give any conclusive answer as to whether the hypothetical mechanisms that were suggested by mathematical models could be implemented as molecular mechanisms capable of physically generating oscillatory phenomena like the circadian rhythm. This question was probed by constructing synthetic models on the basis of mathematical models and their simulations. In what follows, we will discuss in more detail the specific constraints of each of the three epistemic activities:

mathematical modeling, experimentation and synthetic modeling. We start from mathematical modeling, which actually predated the experimental work in this area.

3.1. Mathematical modeling

One of the earliest and most influential mathematical models of circadian rhythm was introduced by Brian Goodwin in his book *Temporal Organization in Cells* (1963). This book is an example of an attempt to apply concepts from engineering and physics to biology. Inspired by Jacob and Monod's (1961) operon model of gene regulation Goodwin explored the mechanism underlying the temporal organization in biological systems, such as circadian rhythms, in terms of a negative feedback system. Another source of inspiration for him was the work of the physicist Edward Kerner (1957). Kerner had tried to formulate a statistical mechanics for the Lotka-Volterra model, which then prompted Goodwin to attempt to introduce a statistical mechanics for biological systems. These aims created the partly competing constraints on the design of Goodwin's model both shaping its actual formulation and the way it was supposed to be understood. A third constraint was due to the limitations of the mathematical tools for dealing with non-linear dynamics. These three constraints are different in character. The first constraint was largely *conceptual* but it had mathematical implications: the idea of a negative feedback mechanism that was borrowed from engineering provided the conceptual framework for Goodwin's work. It guided his conception of the

possible mechanism and its mathematical form. The second constraint, due primarily to the

attempt to formulate a general theory according to the example provided by physics, was

based on the assumption that biological systems should follow the basic laws of statistical

mechanics. Goodwin described his approach in the following way:

> The procedure of the present study is to discover conservation laws or invariants for a
>
> particular class of biochemical control systems, to construct a statistical mechanics for
>
> such a system, and to investigate the macroscopic behaviour of the system in terms of
>
> variables of state analogous to those of physics: energy, temperature, entropy, free
>
> energy, etc. What will emerge from the programme is a set of concepts which are
>
> strictly biological in content […] although there is a formal mathematical relationship
>
> because we are using the same analytical constructions as are used in classical physics.
>
> (Goodwin 1963, 7)

This procedure provided a course of action but also imposed particular constraints such as the

restriction to conservative systems although biological systems are non-conservative as they

exchange energy with the environment (see below). We call this particular constraint deriving

from the goal of formulating statistical mechanics for biological systems a *fundamental* theory

constraint. The third set of constraints was *mathematical* in character and due to the nature of

the mathematical methods, equations, and computational templates used in the formulation of

the model. Because of the non-linearity introduced by the negative feedback loop, the tractability of the equations of the model posed a serious challenge.

The three types of constraints, conceptual, fundamental, and mathematical provide the theoretical toolbox available for modeling a specific system. But as a hammer reaches its limits when used as a screwdriver, both engineering concepts and the ideal of statistical mechanical explanation proved problematical when used in the context of biological systems. Indeed, in later systems and synthetic biology the aim for a statistical mechanics for biological systems was replaced by the search for possible *design principles* in biological systems. The concept of the negative feedback mechanism was preserved, functioning as a cornerstone for subsequent research, but there was still some uneasiness about it that eventually motivated the construction of synthetic models.

The toolbox-related constraints should be distinguished from the constraints more directly related to the biological systems to be modeled. These constraints are due to the enormously complex behavior of biological systems, which, as we will show, plays an important, yet different role in mathematical modeling, experimentation, and synthetic modeling, respectively. Obviously the tool-related constraints are not static. Mathematical and computational methods develop and theoretical concepts can change their meaning especially in interdisciplinary research contexts such as synthetic biology (see below). Furthermore, the constraints are interdependent and the task of a modeler consists in finding the right balance between them.

We will exemplify these points by taking a closer look into the Goodwin model. The basic structure of the network underlying the molecular mechanism of Goodwin's model of temporal organization is represented in the following diagram:
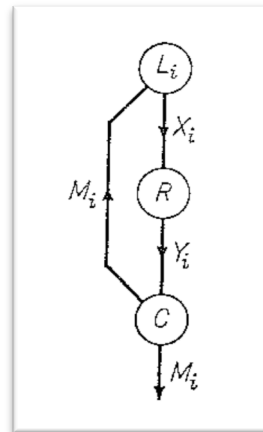

Figure 2. The circuit diagram underlying the Goodwin model (1963, 6).

The main structure of the model is a negative feedback loop. It consists of a genetic locus L_i, synthesizing $mRNA$ in quantities represented by the variable X_i. The $mRNA$ leaves the nucleus and enters the ribosome, which reads out the information from the $mRNA$ and synthesizes proteins in quantities denoted by Y_i. The proteins are connected to metabolic processes. At the cellular locus C the proteins influence a metabolic state, for example by enzyme action, which results in the production of metabolic species in quantity M_i. A fraction

of the metabolic species is traveling back to the genetic locus L_i where it represses the expression of the gene.

This mechanism leads to oscillations in the protein level Y_i regulating temporal processes in the cell, such as the circadian rhythm. Goodwin described the mechanism by a set of coupled differential equations, which were due to the feedback mechanism of a non-linear character. Non-linear systems display complex behavior and in general no analytical solutions exist for them. The differential equations are of the following form:

$$\frac{dX_i}{dt} = a_i Y_i - b_i$$

$$\frac{dY_i}{dt} = c_i X_i - d_i.$$

Where $a_i Y_i$ describes the rate of *mRNA* synthesis and b_i its degradation. In the same way $c_i X_i$ describes the synthesis of the protein and d_i its degradation. The set of kinetic equations describes a deterministic dynamic.

In formulating his model Goodwin had to make simplifying assumptions through which he attempted to deal with two important constraints: the complexity of the system consisting of a variety of biochemical components and processes, and the complexity due to the non-linear dynamics of the assumed mechanism. Firstly, he had to leave aside many known biochemical features of the circadian clock mechanism, and, secondly, he had to make assumptions that would allow him to simplify the mathematical model in such a way that he

could use numerical methods to explore its complex dynamic behavior without having to solve the non-linear coupled differential equations.

Goodwin was able to show by performing very basic computer simulations on an analogue computer that the change in the concentration of proteins Y_i and concentration of $mRNA, X_i$ form a closed trajectory. This means that the model system is able to perform regular oscillations, like those exhibited by circadian rhythms—but for the wrong reasons. Goodwin wrote: "The oscillations which have been demonstrated to occur in the dynamic system […] persist only because of the absence of damping terms. This is characteristic of the behavior of conservative (integrable) systems, and it is associated with what has been called weak stability" (Goodwin 1963, 53). He went on to explain that a limit-cycle dynamic would have been the desirable dynamic behavior with respect to biological organisms. In this case, after small disturbances, the system moves back to its original trajectory—a characteristic of non-conservative systems. But conceiving biological systems as open systems would have required the use of non-equilibrium statistical mechanics that would have meant giving up some mathematical advantages of treating them as closed systems. Goodwin wrote: "This approximation allows us to use the powerful tools of statistical mechanics to study the macroscopic properties of a control network which involves a large number of coupled non-linear equations" (Goodwin 1963, 53). Consequently, in order to simultaneously fulfill the aim of modeling a mechanism that produces oscillatory behavior *and* the aim to formulate a

statistical mechanics for biological systems, Goodwin ended up presenting a model, which he believed could only approximate the behavior he suspected to actually be taking place.

Even though Goodwin's model was not a complete success, it nevertheless provided a basic template of circadian rhythm upon which subsequent modeling endeavors were built. In those modeling efforts the aim of formulating a statistical mechanics for biological systems was left behind. Instead, the notion of a feedback mechanism was made the centerpiece, the main constraints of the modeling approach now being the mathematical difficulties related to the non-linear, coupled differential equations and the related problem of tractability. This meant that only some components and biochemical processes in natural systems could be taken into account. However, the subsequent models became more detailed as the first experiments exploring the molecular basis of the circadian rhythms became possible in the mid-1970s. These experiments, as we will show below, came with their very own constraints. While modelers were able to partly bracket the complexity of biological systems by simply ignoring most of it, this was not possible in the experimental work.

3.2. Experimentation

The first circadian clock gene was discovered in experiments performed by Ron Konopka and Seymour Benzer (Konopka and Benzer 1971) in the beginning of the 1970s. They named the gene *period* (*per*). The experimental research on circadian rhythms in molecular biology and

genetics progressed slowly after Konopka and Benzer published their results. In the mid-1980s and -1990s the situation started to change: as a result of further advances in molecular biology and genetics, more genes, proteins, and possible mechanisms were discovered in experiments on model organisms such as *Drosophila, Neurospora* and *Arabidopsis.* However, some basic constraints of the experimental approach remained and had to be handled by the use of specific strategies.

Two constraints of the experimental approach in circadian clock research deserve special mention:

- The experiments do not allow direct but only indirect observation of the network architecture and dynamic.

- It is difficult to discover whether one has captured the complete gene regulatory network underlying the circadian rhythm.

Before setting up and performing experiments, the first and probably most important decision to be made regards the choice of a model organism. What makes an organism a good organism to study circadian clock mechanisms? One strategy is to look for a model organism, which has a low degree of complexity and which, in addition, can be easily manipulated by using methods from molecular biology and genetics. The least complex model organisms allowing for the study of circadian clock mechanism are prokaryotes[3] like cyanobacteria, also known as

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[3] Prokaryotes are organisms lacking a cell nucleus.

blue-green algae. These bacteria obtain their energy through photosynthesis, which is regulated by a circadian clock. Kondo and colleagues spell out the advantages of using prokaryotes in the following way:

> The realization that prokaryotes express circadian behavior is significant from the perspective of designing an optimal strategy to discover the hitherto elusive secret of the circadian mechanism. That is, if prokaryotes display the phenomenon, then progress in elucidating its basis will probably be most rapid while using an appropriately chosen prokaryotic model; in prokaryotes, the mechanism itself may be simpler, and the average size of prokaryotic genomes, which is smaller than that of eukaryotic genomes; facilitates the goal of saturation mutagenesis[4] for clock-related genes. (Kondo et al. 1993, 5672)

Looking at the tree of life, prokaryotes occupy a very early stage in evolution, from which more complex organisms have evolved. This points to two further interesting observations: first, some components of the clock mechanism may be conserved in more complex organisms. But, second, given the fact that "[…] reflecting their close interface with the environment, clock genes are among the most rapidly evolving genes in an organism" (Dunlap 1999, 273), one may also find different realizations of circadian mechanisms among the organisms at

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[4] Saturation mutagenesis is a form of site-directed mutagenesis, in which one tries to generate all possible mutations.

different stages of evolution. Thus studying circadian clocks in prokaryotes and comparing them with clock mechanisms in more complex organisms offers researchers both the possibility of finding elements, which are conserved, but also the possibility of exploring different clock mechanisms.

But these conserved structures and clock mechanisms are not directly retrievable from experiments, even though scientists have a large variety of experimental methods and background knowledge at their disposal. Theoretical concepts, like negative and positive feedback and mathematical modeling, help researchers in the piecemeal experimental work of identifying possible gene regulatory mechanisms. In the next section we will discuss in more detail the complex interplay of experimental results and modeling efforts by looking at a concrete example: the discovery of the second interlocked feedback loop in the circadian clock of *Drosophila* (Glossop et al. 1999).[5]

3.2.1. An Episode in the Experimental Exploration of the Circadian Clock in Drosophila

At the time when Glossop and his colleagues started their experimental research on the circadian clock in *Drosophila* five genes of the clock had been identified: *Period* (*per*),

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[5] For an insightful and comprehensive account of the interplay between modeling and experimentation in the circadian clock research, see the work of Bechtel and Abrahamsen (e.g., Bechtel 2011; Bechtel and Abrahamsen 2011).

timeless (*tim*), *Drosophila Clock* (*dClk*), *Cycle* (*Cyc*) and *double time (dbt).* Three of these genes are rhythmically expressed: *per* mRNA and *tim* mRNA levels peak early in the evening ZT 13—16. *dclk* mRNA peak late at night to early in the morning, from ZT 23 to ZT 4. ZT, in other words, the zeitgeber time provides the environmental cues that each day reset the rhythms. In this case light has been used to entrain the rhythm of the circadian clock of *Drosophila*. At ZT 0 lights were turned on and at ZT 12 turned off.

It was known that dCLK played an important role in the activation of the transcription of *per* and *tim*. Little was known about the regulation of the *dClk* cycle. To find out about the regulation of *dClk,* Glossop et al. performed mutation experiments. It was known that the levels of *dClk* mRNA are low in mutants per^{01} and tim^{01}, lacking functioning PER and TIM proteins. This observation led to the assumption that PER and TIM activate *dClk* transcription in addition to their roles as transcriptional repressors. Three models had been introduced by Isaac Edery et al. (1994 a,b), which aimed to explain the PER-TIM dependent activation of *dClk*. In the first two models (figures 3a and 3b) PER and TIM promote the *dClk* transcription by bringing in transcriptional activators into the nucleus or by co-activating a transcriptional complex. In the third model (figure 3c) PER, or TIM, or both inhibit the activation of a transcriptional repressor. The question thus became how to distinguish between the three different mechanisms and to discover how the three genes interacted. At this point, all three models suggested equally possible explanations for the observed *dClk* regulation via PER and TIM proteins.

Glossop et al. (1999) systematically explored the three models in order to distinguish

between them. Based on the idea that dCLK together with CYC is necessary for the activation

of *per* and *tim*, they created a clock gene mutant dCk^{JrK}, which led to a non-functional dCLK

protein, and measured the level of *dClk* mRNA. They expected low levels of *dClk* mRNA

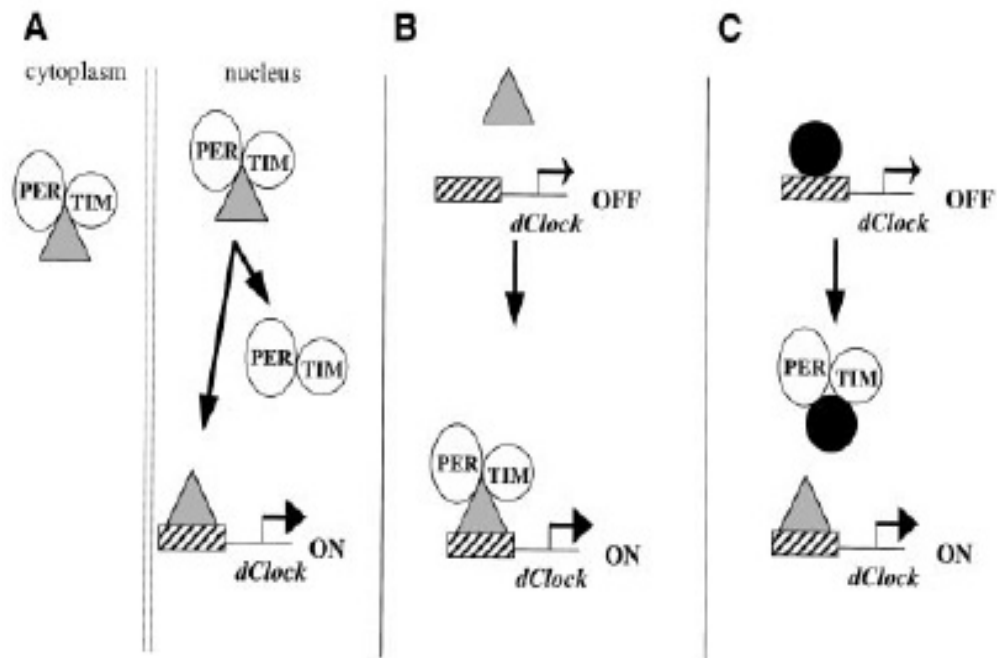because the concentration of PER and TIM proteins would be low.

Figure 3. The three different models of *dClk* activation. A. PER and TIM bring in a transcriptional activator

for the activation of *dClk*. B. PER and TIM co-activate a transcriptional complex. C. PER or TIM inhibit the

activation of a transcriptional repressor.

Instead they found the surprising result that the level of *dClk* mRNA is indistinguishable from the wild type. The only difference to the wild type was that the level of *dClk* mRNA at both times ZT1 and ZT13 was almost the same, which means a non-functional circadian clock. These results ruled out that *dClk* activation is PER-TIM dependent. In further experiments, Glossop et al. (1999) tested the following double mutations $per^{01}dC^{JrK}$ and $per^{01}Cyc^{0}$. In both cases the level of *dClk* mRNA was comparable with the level of *dClk* mRNA wild type. Only in the case of the single mutant per^{01} was the level of *dClk* mRNA low. This observation, which in the first place led to the assumption of a PER-TIM dependent activation of *dClk*—but which had been ruled out by a series of subsequent mutation experiments—had now to be explained by a different model. In this model (fig. 4), the binding of PER-TIM dimers to the dCLK-CYC dimer releases dCLK-CYC dependent repression of dCLK, thus enabling *dClk* transcription. The eventual result of the investigation by Glossop et al. was a description of the mechanism underlying the circadian rhythm *as two interlocked feedback loops* (Glossop et al. 1999). The first loop, the *per*/*tim* loop, is activated by dCLK-CYC and repressed by PER-TIM. The second loop, the *dclk* loop, is repressed by dCLK-CYC and de-repressed by PER-TIM
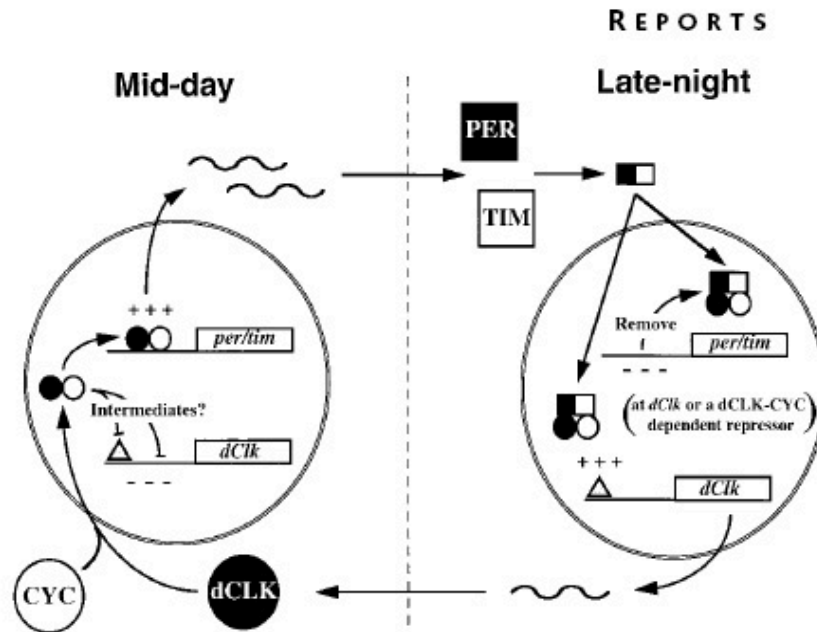
Figure 4. The interlocked feedback loops (Glossop et. al. 1999, 766).

This example of how the second feedback mechanism of the circadian clock of

Drosophila was established gives some insights into the challenges of experimentation. The

first and foremost constraint on experiments is due to the complexity and opacity of biological

systems. In mutation experiments different possible mechanisms are tried out by varying the

experimental parameters in systematic ways, and the results are related to such feedback

mechanisms that could account for the observed behavior.[6] These feedback mechanisms do not result from experimentation alone; insight into them is gained also by computational modeling. This particular strategy has many constraints. Firstly, there are technical constraints related to the available methods from molecular biology and genetics—such as creating the mutations and measuring the effects of the mutations. Due to the relaxation of some of these constraints the experimental approach started to flourish from the mid-1980s onwards. But even though, as in the case of Glossop et al. (1999), it was possible to find a mechanism or parts of a mechanism, due to the complexity of biological systems there always remains the haunting question of whether all the components and their interactions have been found, i.e. whether the network is complete, that is a typical problem of the bottom-up experimental approach in molecular biology. Another question is whether the hypothetical mechanism discovered is the only one that could explain the observations—which amounts to the traditional problem of underdetermination. A further important question to be considered concerns whether, and how, gene regulatory networks like the circadian clock interact with the rest of the cell and how (stochastic) fluctuations in the number of proteins within the cell might influence the behavior of the clock. As we will see, to answer these questions one needs

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[6] This complexity barrier draws simulations nearer to experimentation than what is the case with traditional mathematical modeling. Whereas in the latter one manipulates highly idealized model systems, in simulation one gains understanding through experimenting with a more epistemically opaque model (see Lenhard 2006). Synthetic modeling, as we will argue below, provides one strategy for dealing with complex systems.

a new kind of modeling approach—synthetic modeling.

3.3. Synthetic Modeling

Although mathematical modeling (and simulation) and experimentation informed each other in circadian clock research—mathematical modeling suggesting possible mechanism templates and experimentation in turn probing them and providing more biochemical detail— there remained a gap between them. The modeling effort was based on rather schematic templates and related concepts often originating from fields of inquiry other than biology. It was unclear whether biological organisms really functioned in the way suggested by them, that is, whether the general mechanisms suggested by mathematical models could be realized by biological organisms. This points to the modal nature of mathematical modeling: it typically sets forth only *possible* mechanisms. The phenomena mathematical models are designed to account for could have been produced by other kinds of mechanisms. Synthetic modeling suggests a way to deal with this problem due to the specific construction of synthetic models. They are mixed, double-faced entities: On the one hand, they are of the same materiality as model organisms since they are made of biological material, such as genes and proteins. On the other hand, they differ from model organisms in that they are not the results of any evolutionary process, being instead designed on the basis of mathematical models. In the construction of a synthetic model a mathematical model is used as a blueprint: it specifies the

structures and dynamic giving rise to particular behaviors or functions. Thus the synthetic

model has its origin in the mathematical model, but it is not bound by the same constraints: the

model is constructed from the "same stuff" (Morgan 2003) as the biological genetic networks

and it even works in the cell environment. Consequently, even if the synthetic model is not

understood in all of its details, it provides a kind of simulation device of the same natural kind

as its real world counterparts.

3.3.1. The Repressilator

The *Repressilator* is one of the first and most famous synthetic models. It is an oscillatory

genetic network, which was introduced in 2000 by Michael Elowitz and Stanislas Leibler

(2000). The first step in constructing the *Repressilator* consisted in designing a mathematical

model, which was used to explore the known basic biochemical parameters and their

interactions. Next, having constructed a mathematical model of a gene regulatory network

Elowitz and Leibler performed computer simulations on the basis of the model. They showed

that there were two possible types of solutions: "The system may converge toward a stable

steady state, or the steady state may become unstable, leading to sustained limit-cycle

oscillations" (Elowitz and Leibler 2000, 336). Furthermore, the numerical analysis of the

model gave insights into the experimental parameters relevant for constructing the synthetic

model in showing that "[...] oscillations are favoured by strong promoters coupled to efficient

ribosome binding sites, tight transcriptional repression (low 'leakiness'), cooperative

repression characteristics, and comparable protein and mRNA decay rates" (ibid, 336). The

latter point helped in choosing the three genes used in the design of the network. Elowitz and

Leibler also explored the continuous as well as the stochastic dynamics of the model in order

to analyze the role of internal noise in the mechanism. Internal noise in biological systems is

caused by the low number of molecules in the cell.

The mathematical model functioned as a blueprint for the engineering of the biological

system. The mathematical model is of the following form:

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1 + p_j{}^n)} + \alpha_0,$$

$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

$$\text{with} \begin{pmatrix} i = lacI, tetR, cl \\ j = cl, lacl, tetR \end{pmatrix}.$$

In this set of equations p_i is the concentration of the proteins suppressing the function of the

neighbor genes and m_i (where i is *lacI, tetR*, or *cl*) the corresponding concentration of mRNA.

All in all one has six molecule species (3 proteins functioning as repressors and 3 genes) all

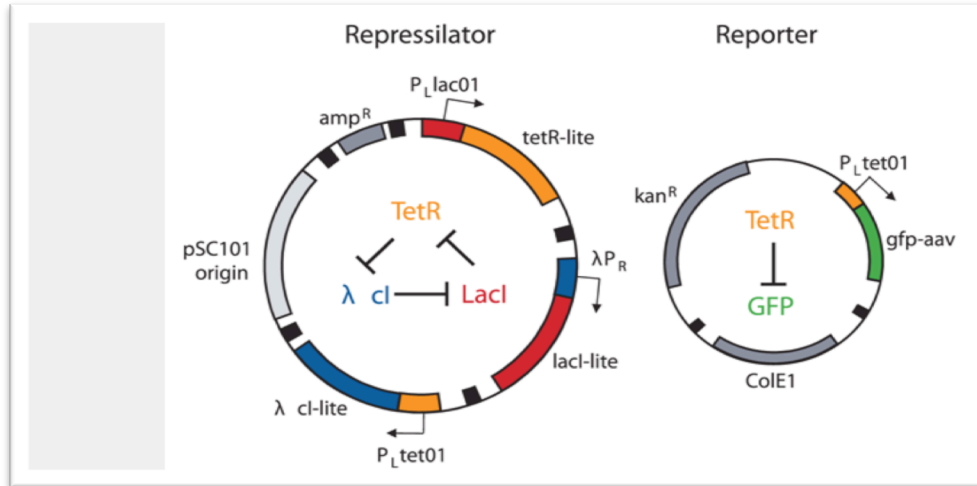taking part in transcription, translation, and degradation reactions.

Figure 5. The main components of the Repressilator (left-hand side) and the Reporter (right-hand side) (Elowitz and Leibler 2000, 336).

The construction of the novel biological system, the *Repressilator*, was enabled by the development of new methods and technologies, such as the construction of plasmids and Polymerase Chain Reactions (PCR). In figure 5 the synthetic genetic regulatory network, the *Repressilator,* is shown on the left-hand side and it consists of two parts. The outer part is an illustration of the plasmid constructed by Elowitz and Leibler. The plasmid is an extra-chromosomal DNA molecule integrating the three genes of the *Repressilator*. Plasmids occur naturally in bacteria. In the state of competence, bacteria are able to take up extra chromosomal DNA from the environment. In the case of the *Repressilator,* this property

allowed the integration of the specific designed plasmid into *E.coli* bacteria. The inner part of the illustration represents the dynamics between the three genes, *TetR*, *LacI* and *λcl*. The three genes are connected by a negative feedback loop. The left-hand side of the diagram shows the *Reporter* consisting of a gene expressing a green fluorescent protein (GFP), which is fused to one of the three genes of the *Repressilator*. The GFP oscillations in the protein level made visible the behavior of transformed cells, allowing researchers to study them over time by using fluorescence microscopy.

Thus with the formation of synthetic biology a novel tool was introduced into the research on the organizational principles in biological organisms: the possibility of constructing novel engineered genetic networks, synthetic models, specially designed for answering certain kinds of theoretical questions. Their construction has so far been limited to simple networks such as the *Repressilator* whose construction components (and their number) had to be chosen in view of what would be optimal for the behavior under study.[7] This means that such networks need not be part of any naturally occurring system. For example the genes used in the *Repressilator* do not occur in such a combination in any biological system but are chosen and tuned on the basis of the simulations of the underlying mathematical model and other background knowledge in such a way that the resulting mechanism would allow for (stable) oscillations. These technical constraints imply a constraint on what can be explored by such synthetic

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[7] In the case of the *Repressilator* the order in which the genes are connected to each other turned out to be crucial, too.

models: possible design principles in biological systems. Indeed, the search for the possible design principles has replaced in systems and synthetic biology the aim of formulating a fundamental theory for biological systems such as a statistical mechanics. Finally, it needs to be noted that the *Repressilator* is also constrained by the engineering notions that guided its construction such as feedback mechanism, robustness and modularity. The *Repressilator* provides a nice example of how these constraints in fact functioned as affordances for theoretical reasoning: the important motivation behind the construction of the *Repressilator* was precisely to test the suitability of those notions for modeling biological circuits.

4. Synthetic Models: Models or Experiments?

Coming back to the philosophical discussion on the similarities and differences of modeling and experimenting, let us first discuss the argument from isolation. The first thing to be noted is that the mathematical models of the circadian clock are difficult to consider as experiments in the sense of being abstractions of real world systems that permit the study of the effects of an isolated causal factor. First, instead of targeting the influence of isolated causal factors they focus on their interaction. Second, in the study of the circadian clock, the modeling endeavor started well before experimentation and the availability of more specific knowledge on the biochemical details. Third, and perhaps most importantly: the circadian clock phenomena were probed with the model templates and concepts borrowed from other disciplines and

subjects. This serves to show that it is often not possible to decompose a model into its assumptions as the isolation account of modeling suggests. The idealizations, omissions and approximations follow frequently from the model template used and are also a result of balancing the different kinds of constraints with one another, as the example of the Goodwin model shows. The philosophical discussion tends to neglect that model assumptions are typically linked to certain mathematical abstractions, and there are limited ways in which they can be relaxed or corrected, constrained by the available mathematical methods. In the case of circadian clock oscillations, the oscillatory phenomena had already been studied by physicists for a long time and there are many well-established ways of mathematically creating them. There exists a whole body of literature on this topic such as Steven Strogatz's book *Nonlinear Dynamics and Chaos* (Strogatz 1994). Therefore, the modelers were not that uncertain of how their models were able to create the oscillations sought for.[8] The problem was rather that the alternative models were too general and underdetermined by available data.

Consequently, although modelers were able to produce the kind of phenomena sought for, that is, robust oscillations, the problem was whether the possible network designs proposed by the mathematical models were really the ones that work in biological organisms. This problem was aggravated by the fact that the model templates, methods and concepts used were not originally devised with biological organisms in mind. In fact, a big part of the modelers in the

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[8] Elowitz was inspired for the design of the *Repressilator* from Thomson and D'Ari's book *Biological Feedback* (1990), which presents a formal methodology for analyzing the dynamic behavior of complex systems.

field of systems and synthetic biology have a physics background. Moreover, the problem of the generality and foreignness of theoretical concepts and mathematical methods used could not be conclusively settled by experimentation since the work with model organisms had to deal with the immense complexity of even such simple organisms as *E.coli*. Thus even though the empirical research was able to find genes and proteins involved in the circadian clock phenomena, the results were still inconclusive as regards the basic mechanisms.

Synthetic models, as we have shown, partly fill the gap between mathematical modeling and experimentation on model organisms by offering a tool for identifying possible network design principles, and showing whether they might be implemented in biological organisms. Their epistemic functioning is due to their hybrid nature: On the one hand they share with mathematical models their tightly constrained nature, which makes it possible to study certain theoretical questions in a regimented manner. This shows that constraints do not just limit reasoning, but instead also afford it, and that modeling can be seen as a specific theoretical strategy precisely making use of purposefully designed artefacts – models.

On the other hand, synthetic models are like experiments in that they are constructed of the same kinds of components as the natural systems being implemented, moreover, in the natural cell environment. In the case of the *Repressilator* the natural kind was replaced by the engineered "natural kind," which gave more control to the researchers on the system under study than in the case of domesticated natural kinds (such as model organisms). Being of a lower degree of complexity compared to the like natural systems *yet* being simultaneously

exposed to the same kind of *biological* constraints as natural biological systems is regarded as an important advantage of synthetic models. By reducing the degree of complexity the synthetic model becomes more tractable and easier to manipulate which draws it closer to modeling. Moreover, the hypothetical nature of the *Repressilator* is highlighted by the fact that it was not supposed to imitate any natural circuit but rather to identify the *sufficient components* and *interactions* of a mechanism able to produce a specific behavior, such as oscillations in protein levels. The components of the network were chosen to get the most optimal behavior and not to get as close as possible to a naturally evolved genetic network. This explains why the genetic network of the *Repressilator* is comprised of a different constellation of components than any known naturally occurring network.

On the other hand, by implementing the synthetic genetic network into a cell it is exposed to some further constraints of natural biological systems. Those are in general not known in all their details but knowing those details need not always be necessary as long as they are there.[9] The cell provides the simulation environment, which is of the same "natural kind" such as the model under investigation. This is a feature commonly associated with experiments. They are considered particularly useful in the contexts in which one has an imperfect understanding of the causal mechanism at work. The control over the "same," often domesticated or

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[9] In so far unpublished work Waters (2012) has considered material *experimental models* by pointing out that this strategy "avoids having to understand the details of the complexity, not by assuming that complexity is irrelevant but by incorporating the complexity in the models." For experimental models, see also Weber (2012).

technologically altered, natural kind is expected to give some handle on the causal mechanism (see Guala 2002).

Still another feature of experiments shared by the *Repressilator* was the way in which the researchers received the undesired results. In the case of experimentation anomalous or unexpected results are commonly taken seriously, whereas if the model does not produce what is expected, modelers usually try to devise a better model. The *Repressilator* in turn sparked a new line of research precisely due to its limited success. In contrast to the mathematical model underlying it, the *Repressilator* did not show the expected behavior: regular oscillations. Instead the oscillations turned out to be noisy. Computer simulations taking into account stochastic fluctuations did show that such fluctuations could be the cause of the noisy behavior that provides another nice example of how the research progressed by combining the different epistemic activities, according to their characteristic affordances. Despite the results of the simulation, there remained the possibility that the noisy behavior could have also been caused by external noise coming from the cell environment. A new line of research emerged exploring the different sources of noise and their effects on biological systems (Swain et. al. 2002). In the context of this research, and in line with the results gained by studying complex systems in physics and in neuroscience, noise based on stochastic fluctuations gained a functional status: molecular mechanisms in biological systems seem to make use of internal noise, for example in decision processes such as cell division.

5. Conclusions

Above we have argued that although from a general philosophical perspective modeling and experimentation share some common features, from the perspective of scientific practice they nevertheless can be considered as separate practices with different constraints and affordances. This in turn explains why they are typically triangulated with each other—yet often a gap remains between the two in actual scientific practices. We have discussed one strategy of closing this gap—synthetic modeling. Interestingly, the hybrid nature of synthetic models serves to show what is distinctive about modeling and experimentation. Synthetic models share with mathematical models their highly constrained construction, which makes it possible to study various theoretical possibilities in a relatively transparent manner. Moreover, mathematical models and synthetic models are both used to study the *dynamics* of systems under study (see Bechtel 2011). Synthetic models have made the dynamics of intracellular processes visible, leading to new kinds of research questions such as noise. The specific affordance of mathematical models is in turn due to the way they are fairly easily reconfigured, which can be seen in the way they are used to study the results of synthetic modeling as well as the experimental results and setups more generally. On the other hand, synthetic models like experiments are expected to have the same material and causal makeup as the systems under study—which simultaneously makes them more opaque, complex, and difficult to control. In actual practice these differences of modeling and experimenting often play a crucial

role, which makes modeling and experimenting complementary activities rather than possible substitutes for each other.

As to the question of materiality, we think that the philosophical discussion has remained partly too crude to appreciate its epistemic dimensions. For instance, in downplaying the importance of materiality, Parker (2009) appears to take Guala and Morgan too literally. In fact, their conception of the "same materiality" includes, apart from the "same stuff," strictly speaking, also same forms and causal make-up (e.g., Morgan 2005, 32; Guala 2002). The example of the *Repressilator* shows that the "same stuff" (e.g., genes and proteins) is not enough: the notion of the "same materiality" should also include the mechanism of their interaction (e.g., the feedback loops), which, taken together, produce a biological function. In this respect, we suggest, further study on the place and role of materiality in modeling and experimentation could do well to adapt some resources from the discussion on mechanisms and natural kinds.

References

Barberousse, Anouk, Sara Franceschelli, and Cyrille Imbert. 2009. "Computer Simulations as
Experiments." *Synthese* 169: 557–574.

Bechtel, William. 2011. " Mechanism and Biological Explanation." *Philosophy of Science* 78:
533–557.

Bechtel, William, and Adele Abrahamsen. 2011. "Complex Biological Mechanisms: Cyclic,
Oscillatory, and Autonomous." In *Philosophy of Complex Systems. Handbook of the
Philosophy of Science*, vol. 10, ed. C. A. Hooker, 257–285. Oxford: Elsevier.

Cartwright, Nancy. 1999. "The Vanity of Rigour in Economics: Theoretical Models and
Galilean Experiments." Discussion Paper Series 43/99, Centre for Philosophy of
Natural and Social Sciences, London School of Economics.

Dunlap, Jay C. 1999. "Molecular Bases for Circadian Clocks." *Cell* 96: 271–290.

Edery, Isaac, Laurence J. Zwiebel, Marie E. Dembinska, and Michael Rosbash. 1994a.
"Temporal Phosphorylation of the Drosophila Period Protein." *Proc. Natl. Acad. Sci.
USA* 91(6): 2260–2264.

Edery, Isaac, Joan E. Rutila, and Michael Rosbash. 1994b. "Phase Shifting of the Circadian
Clock by Induction of the Drosophila Period Protein." *Science* 263: 237–240.

Elowitz, Michael B., and Stanislas Leibler. 2000. "A Synthetic Oscillatory Network of
Transcriptional Regulators." *Nature* 403(6767): 335–338.

Giere, Ronald N. 2009. "Is Computer Simulation Changing the Face of Experimentation?" *Philosophical Studies* 143: 59–62.

Gilbert, Nigel, and Klaus Troitzsch. 1999. *Simulation for the Social Scientists.* Philadelphia: Open University Press.

Glossop, Nick R., Lisa C. Lyons, and Paul E. Hardin. 1999. "Interlocked Feedback Loops Within the *Drosophila* Circadian Oscillator." *Science* 286: 766–768.

Godfrey-Smith, Peter. 2006. "The Strategy of Model-Based Science." *Biology and Philosophy* 21: 725-740.

Goodwin, Brian C. 1963. *Temporal Organization in Cells: a Dynamic Theory of Cellular Control Processes*. London and New York: Academic Press.

Guala, Francesco. 2002. "Models, Simulations, and Experiments." In *Model-Based Reasoning: Science, Technology, Values*, ed. L. Magnani, and N. Nersessian, 59–74. New York: Kluwer.

Humphreys, Paul. 1994. "Numerical Experimentation." In *Patrick Suppes: Scientific philosopher*, Vol. 2, ed. P. Humphreys, 103–121. Boston: Kluwer.

——— 2004. *Extending Ourselves: Computational Science, Empiricism, and Scientific Method*. Oxford: Oxford University Press.

Jacob, François, and Jacques Monod. 1961. "Genetic Regulatory Mechanisms in the Synthesis of Proteins." *J. Mol. Biol.* 3: 318–356.

Kerner, Edward H. 1957. "A Statistical Mechanics of Interacting Biological Species." *Bulletin of Mathematical Biophysics* 19: 121–146.

Knuuttila, Tarja, and Andrea Loettgers. 2012. "The Productive Tension: Mechanisms vs. Templates in Modeling the Phenomena." In *Representations, Models, and Simulations*, ed. P. Humphreys, and C. Imbert, 3–24. New York: Routledge.

Kondo, Takao, Carl A. Strayer, Resham D. Kulkarni, Walter Taylor, Masahiro Ishiura, Susan Golden, and Carl Hirschie Johnson. 1993. "Circadian Rhythms in Prokaryotes: Luciferase as a Reporter of Circadian Gene Expression in Cynobacteria." *Proc. Natl. Acad. Sci*. 90: 5672–5676.

Konopka, Ronald J., and Seymor Benzer. 1971. "Clock Mutants of Drosophila Melanogaster." *Proc. Nat. Acad. Sci.* 68(9): 2112–2116.

Lenhard, Johannes. 2006. "Surprised by Nanowire: Simulation, Control, and Understanding." *Philosophy of Science* 73: 605–616.

——— 2007. "Computer Simulation: The Cooperation Between Experimenting and Modeling." *Philosophy of Science* 74: 176–194.

Mäki, Uskali. 1992. "On the Method of Isolation in Economics." *Poznań Studies in the Philosophy of Science and Humanities* 26: 316–351.

——— 2005. "Models are Experiments, Experiments are Models." *Journal of Economic Methodology* 12: 303–315.

Morgan, Mary S. 2003. "Experiments Without Material Intervention: Model Experiments, Virtual Experiments and Virtually Experiments." In *The Philosophy of Scientific Experimentation*, ed. H. Radder, 216–235. Pittsburgh: University of Pittsburgh Press.

——— 2005. "Experiments Versus Models: New Phenomena, Inference and Surprise." *Journal of Economic Methodology* 12: 317–329.

Morrison, Margaret. 2008. "Fictions, Representations, and Reality." In *Fictions in Science: Philosophical Essays on Modeling and Idealization*, ed. M. Suárez, 110–135. New York & London: Routledge.

——— 2009. "Models, Measurement and Computer Simulations: the Changing Face of Experimentation." *Philosophical Studies* 143: 33–57.

Norton, Stephen D., and Frederick Suppe. 2001. "Why Atmospheric Modeling is Good Science." In *Changing the Atmosphere: Expert Knowledge and Environmental Governance*, ed. Clark Miller, and Paul N. Edwards, 67–105. Cambridge: MIT Press.

Parker, Wendy. 2009. "Does Matter Really Matter? Computer Simulations, Experiments and Materiality." *Synthese* 169: 483–496.

Peschard, Isabelle. 2012. "Is Simulation an Epistemic Substitute for Experimentation?" In *Simulations and* Networks, ed. S. Vaienti. Paris: Herman.

Prigogine, Ilya. 1955. *Introduction to Thermodynamics of Irreversible Processes*. Springfield: Charles C. Thomas Publishers.

Sprinzak, David, and Elowitz, Michael B. 2005. "Reconstruction of genetic circuits." *Nature* 438(7067): 443-438.

Strogatz, Steven H. 1994. *Nonlinear Dynamics and Chaos: With Applications to Physics, Biology, Chemistry, and Engineering*. Reading: Perseus Books Publishing, LLC.

Swain, Peter S., Michael B. Elowitz, and Eric D. Siggia. 2002. "Intrinsic and Extrinsic Contributions to Stochasticity in Gene Expression." *Proc. Natl. Acad. Sci*. 99(20): 12795–12800.

Swoyer, Chris. 1991. "Structural Representation and Surrogative Reasoning." *Synthese* 87(3): 449-508.

Thomas, René, and Richard D'Ari. 1990. *Biological Feedback.* Boca Raton, FL: CRC Press.

Waters, C. Kenneth. 2012. "Experimental Modeling as a Form of Theoretical Modeling." Presentation in Philosophy of Science Association 23rd Meeting (San Diego, CA).

Weber, Marcel. 2012. "Experimental Modeling: Exemplification and Representation as Theorizing Strategies." Presentation in Philosophy of Science Association 23rd Meeting (San Diego, CA).

Weisberg, Michael. 2007. "Who is a modeler?" *British Journal for the Philosophy of Science* 58: 207–233.

Winsberg, Eric. 2003. "Simulated Experiments: Methodology for a Virtual World." *Philosophy of Science* 70: 105–125.

——— 2009. "A Tale of Two Methods." *Synthese* 169: 575–592.