The Importance of Repressor-Activator Balance in Various Models of Repression for Circadian Clocks

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Abstract

To understand protein networks, scientists use mathematical models to simulate the various molecules and their interactions. These mathematical models are differential equations for the concentrations of molecules over time. When constructing a model, it is important to choose a level of abstraction that makes models as simple as possible, without losing their key robustness properties. For oscillating systems, the ability to maintain oscillations is key. In a recent publication Kim and Forger (2012) discuss the relationship between the ratio of repressor and activator proteins and the robustness of a systems oscillations and conclude that a 1:1 ratio is best. Here, we examine three additional models of circadian processes (Leloup and Goldbeter, 2003; Mirsky et al.*, 2009; and Ueda* et al. *2001) and seek to determine which model structures lead to the situation in which a 1:1 repressor to activator ratio gives rise to a more robust system. For each of the models, we modify the mode of repression using three different levels of abstraction: explicit sequestration, implicit sequestration, and direct Hill repression.*

1. Introduction

Organisms across the kingdoms all follow a daily cycle of about 24 hours. Their daily functions are controlled by an internal clock that keeps track of the time of day. This internal clock can be interpreted by the concentrations of key proteins that oscillate over an approximately 24 hour period. Systems biologists can model these proteins by limit cycle ordinary differential equations (ODEs). Using these equations, we can study the function of this cycle called the circadian clock.

Circadian clock proteins oscillate by the existence of feedback loops in the gene regulatory network as shown in figure 1. Sequestration is a specific mechanism used in gene regulation in which a protein indirectly represses the transcription of a gene instead of directly repressing the gene itself. This is done by binding to an activator to form an inactive complex that prevents the activator from promoting the transcription of the gene as shown in figure 2.

Systems with sequestration often exhibit a 1:1 ratio, or stoichiometry, of their repressors to activators. Only when the stoichiometry is 1:1 will the system with sequestration oscillate between having significant free activator concentrations (causing a high transcription rate) and having excess repressor (causing low transcription because all activator is bound).

Circadian models often do not simulate sequestration explicitly. If these models correctly approximate systems involving sequestration, we would expect to see a 1:1 stoichiometry emerge when they oscillate.

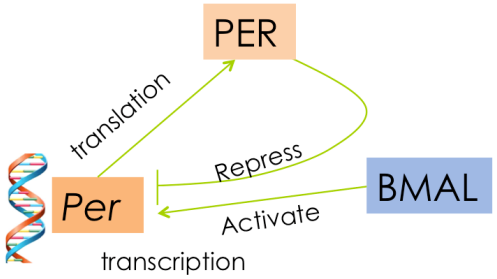

Figure 1. Basic mechanism for a feedback loop. BMAL is the activator and PER is the repressor. Per is the gene encoding for the PER protein.

In section 2 we describe previous work that inspired our project. Section 3 details the process we used to modify existing models. In Section 4 we consider various methods of measuring stoichiometry. Our main experiment and results are in section 5, and concluding remarks are in section 6.

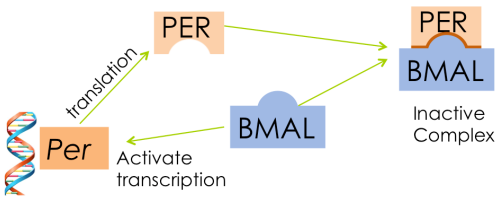

Figure 2. Mechamism for a simple sequestration feedback loop. Again, BMAL is the activator and PER is the repressor that forms the inactive complex with the activator BMAL. PER does not directly interact with Per.

2. Background

Kim and Forger recently modified the Goodwin oscillator (Goodwin, 1965) and proved that it only oscillates when the repressor/activator ratio is near 1 when evaluated at the system's fixed point. They went on to show that this is reflected in the limit cycle by a relationship between the average repressor/activator ratio and the oscillation amplitude. Furthermore, their more complex mechanistic model also displays higher amplitude oscillations when the ratio is near 1.

They modified the Goodwin oscillator to use a transcription expression that approximates true sequestration, given by

$$\frac{(A-R-k)+\sqrt{(A-R-k)^2+4Ak}}{2A} \tag{1}$$

where A and R are the concentrations of activator and repressor, respectively, and K is a dissociation constant indicating how tightly the proteins bind. We call this approach "implicit sequestration."

Kim and Forger's highly detailed mechanistic model explicitly models sequestration of the activator by the repressor. In addition, it separates the processes of transcription and translation, using far more complex expressions than commonly found.

We sought to determine under what conditions the relationship between amplitude and stoichiometry appears in other models. We chose the models of Leloup and Goldbeter (2003), Mirsky, *et al*. (2009) and Ueda *et al*. (2001) (Table 1) because they had clear activating and repressing transcription factors. In particular, we wanted to see if the relationship emerges only in certain models of repression. We considered three different forms.

The first is implicit sequestration, as presented in equation 1. The second is explicit sequestration. When using this model, we treat the activator as the only transcription factor. We use hill kinetics, so the rate is represented as

$$\frac{(A/K)^n}{1+(A/K)^n} \tag{2}$$

The third repression model we consider is competitive binding with Hill kinetics. The models by Ueda and Mirsky both use Hill repression, but gate it with activation in different ways. The repression used by Mirsky is given by

$$\frac{(A/K)^n}{1+(A/K)^n}\prod_i\left(\frac{1}{1+(R_i/K)^n}\right) \tag{3}$$

whereas Ueda used

$$\frac{(A/K_1)^{n_1}}{1+(R/K_2)^{n_2}+(A/K_1)^{n_1}} \tag{4}$$

Note that Mirsky modeled many forms of the repressor protein, which yields many transcription factors.

3. Selecting and Modifying Models

To test the three repression models, we modified each clock model to use the other two types of repression. For all modifications, we used a genetic algorithm to find a new base parameter set that has strong oscillations and a 24 hour period.

3.1. From Hill to Implicit Sequestration

The models of Mirsky *et al*. and Ueda *et al*. use Hill kinetics to describe the affects of the repressor. To modify them to use implicit sequestration, we simply replaced their gated transcription terms with expression 1.

3.2. From Hill to Explicit Sequestration

Changing to explicit sequestration requires the addition of a new state to the model to represent the inactive complex. Its concentration increases using mass-action kinetics on the product of its components, which themselves have a new decreasing term to account for binding. We allow the inactive complex to degrade following the kinetics of the original model. The final change is to remove the repressor's role from the transcription term.

3.3. From Explicit Sequestration to Hill

This is simply the reverse process of "from Hill to Explicit Sequestration." It is only necessary when modifying the model by Leloup. We use expression 4 since there is only one repressor protein.

4. Measuring Stoichiometry

The theoretical expectation of balanced stoichiometry is based on the state at the fixed point of the system. It seems reasonable to expect a balance at the fixed point to occur with balance on the limit cycle, but we needed to verify this assumption.

To do so, we calculated both stoichiometry from concentrations over time and the ratio at the fixed point for various

	Modeled After	Repression Model	Number of States
Leloup and Goldbeter 2003	Mammalian	Explicit Sequestration	16
Mirsky 2009	Mouse	Direct Hill	21
Ueda 2001	Drosophila	Direct Hill	10

Table 1. Circadian Models. We selected three existing models of circadian clocks. We then modify each to use the other two models of repression.

models using different parameter sets. We primarily analyzed the circadian model of Ueda. Based on the published parameters, we found a strong correlation between the two measures of stoichiometry. We then found a new parameter set, optimizing for high amplitude, nonzero concentrations, 24-hour period, and a balance between activator and repressor concentrations. Sampling around these parameters produced very different results, where the limit cycle stoichiometry did not predict the fixed-point ratio. One difference to note is that there is greater variation in the parameters when sampling around the published set.

5. Experimental Results

For each clock and repression model pair, we sampled around our generated parameter sets to find 500 distinct sets. For each of these, we numerically solved the system of ODEs to find the amplitude of Per mRNA (repressor) and the stoichiometry of repressors to activators. Surprisingly, Figure 4 shows that very few models have the highest amplitude at the one-to-one ratio.

The original model of Leloup and Goldbeter (which has explicit sequestration) demonstrates the relationship most clearly. The other two original models do not show the expected trend, even though Leloup's model does when modified to use a direct Hill repression.

Since the implicit sequestration model was shown to theoretically require a one-to-one ratio at the fixed point, it is reasonable to expect it to follow the predicted relationship. It is roughly visible in the models developed by Mirsky and Ueda, but this is the one repression model for which Leloup's model does not exhibit any correlation.

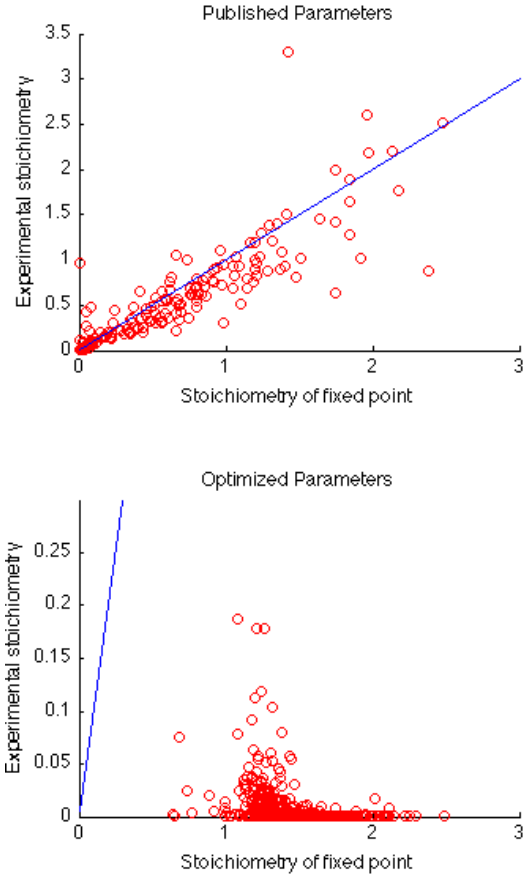

Figure 3. Comparison of stoichiometry evaluated at the fixed point and over the limit cycle. When sampling around the published parameters, there is overall agreement, but sampling a different region exhibits no correlation.

6. Conclusions and Future Direction

There is not a clear relationship between the importance of activator and repressor balance and the repression model used in the system. The various mechanistic models show stronger correlation between balanced stoichiometry and amplitude for different repression models. Since no one repression model stands out as better for all circadian models, there must be other aspects playing a role. Further analysis of modified models, such as omitting feedback loops, could enlighten the situation.

Our result that limit-cycle stoichiometry does not always match fixed-point stoichiometry should be considered when performing theoretical analysis. It is important to figure out when discrepancies arise so that such cases, if appropriate, may be avoided.

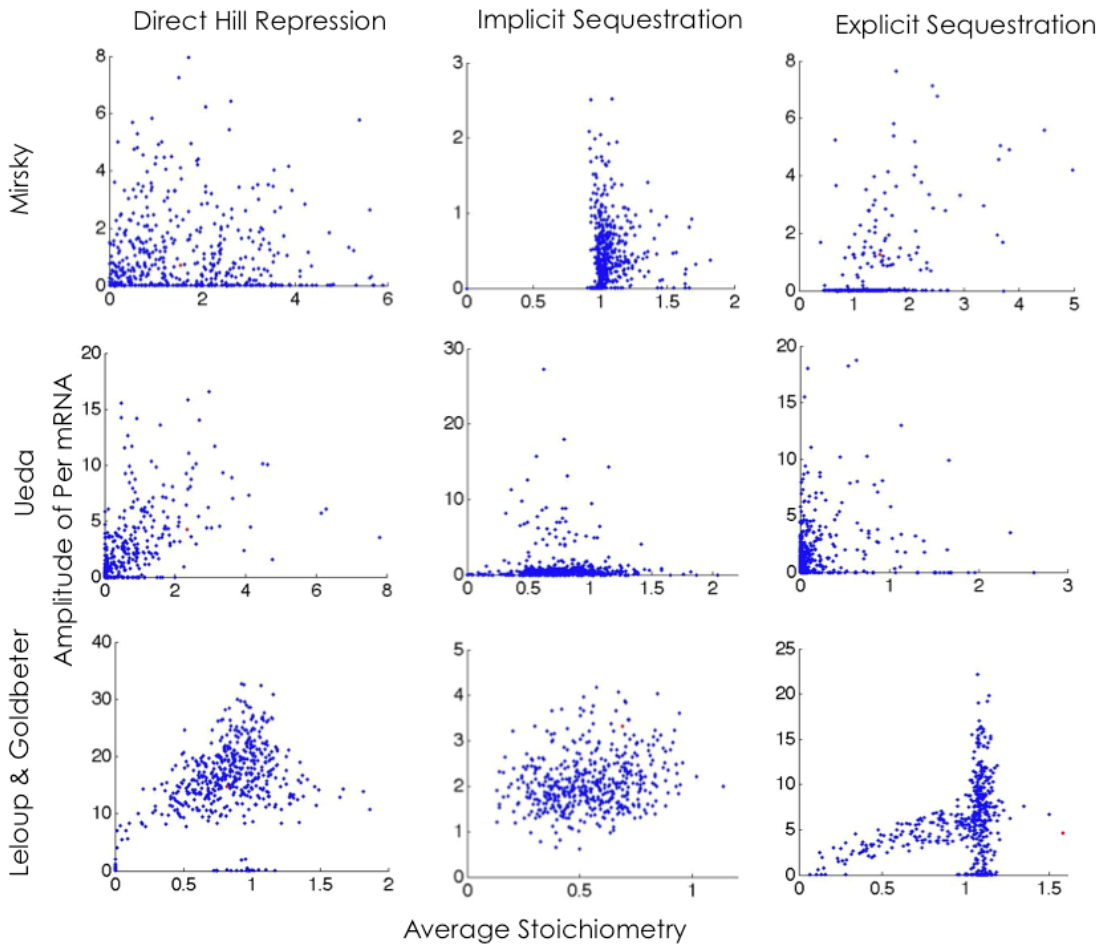

Figure 4. High amplitudes are not always associated with balanced stoichiometry. For each circadian and repression model pair, we simulate the system for 500 parameter sets. A peak around an average stoichiometry of 1 indicates that a balance is important for oscillation.

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