

The Effects of Light and Temperature on the Circadian Rhythms of *Neurospora crassa*

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ABSTRACT

In this study, research was conducted to collect information pertaining to circadian rhythmicity in *Neurospora crassa*. After compiling concepts from several independent research groups, a working biological circuit that considers the effects of light and temperature was proposed. This circuit was used to model certain emergent properties of circadian rhythm. A study of these emergent properties led to the consideration of social issues that would be created by alteration of the human biological clock. With this information, the circuitry was programmed into the computer to simulate the effects of several perturbations.

INTRODUCTION

Neurospora crassa, a common bread mold, has the most completely documented circadian pathway of any organism (McClung et al. 1989, Bell-Pedersen 2000). In this project, our goal was to build a circuit in the hopes of not only gaining a better understanding of rhythmicity, but also developing applications of that knowledge.

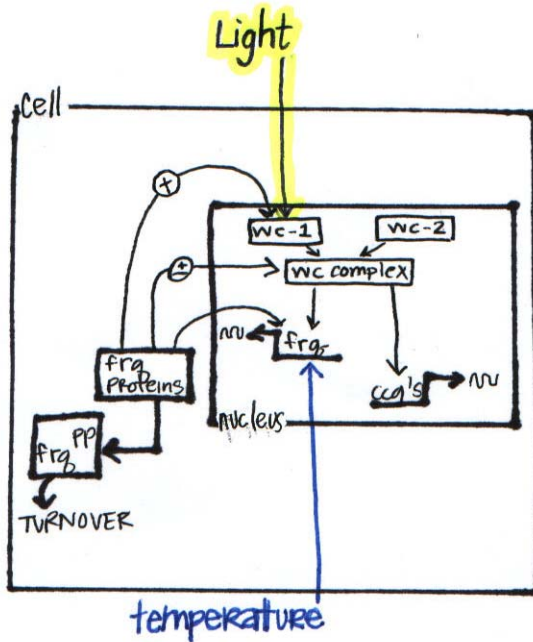
An effective circadian oscillator contains a feedback mechanism to ensure its continuity (Garceau 1997). The feedback loop of *Neurospora crassa* allows clock proteins to serve more than one purpose throughout the day. A feedback loop can be composed of positive and/or negative feedback elements. Discovery of the feedback elements in the *Neurospora crassa* circadian circuit is necessary to complete the rhythmic pathway. Our hypothesis for the central feedback element acting as both a positive and negative regulator is the FRQ protein.

When studying circadian rhythms, an important issue is testing the limits of the circadian system. For example, Thomas Edison's anti-sleep revolution, the light bulb, has put the human circadian rhythm under tremendous stress (Coren 1997). For nearly all of human existence, people have gone to sleep when it got dark and up when it was light. This sleep pattern averaged at around fourteen hours of sleep every night (Coren 1997). The human circadian rhythm was used to this pattern. Then, in the past two centuries, Thomas Edison came along and introduced artificial light, enabling people to sleep less. Now, people get on average about seven and a half hours of sleep a night, whereas their bodies need eight to ten hours of sleep per night (Coren 1997). This immediate stress put on the body's sleep cycle affects the rest of the body's activity cycles. Obtaining a greater understanding of circadian rhythms in general, such as from *Neurospora crassa*, we can relate the learned information to the human body's activity cycles.

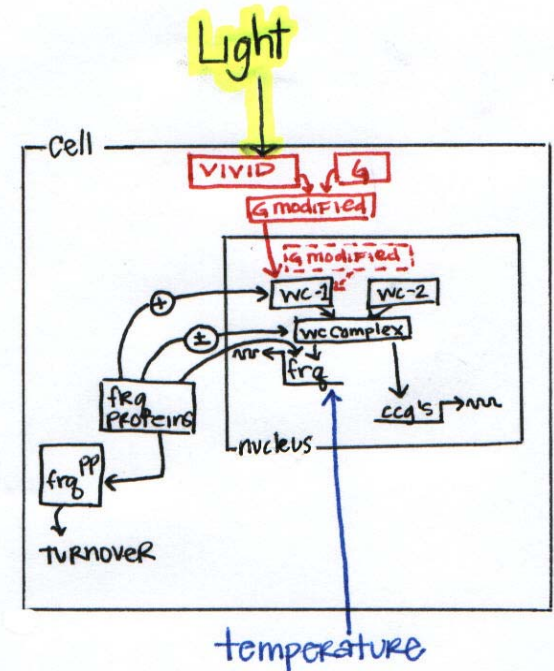
MATERIALS AND METHODS

Using scientific literature and correspondence with Dr. Deborah Bell-Pederson of Texas A&M University, we constructed a biological circuit to model the effects of light and temperature on the circadian system of *Neurospora crassa*. After building the circuit, we explored the in depth effects of light and temperature manipulation. Several key articles provided an abundance of information about these factors. These articles were used to infer the effects of perturbations on the circuit. Once a circuit was created, it was programmed into the simulator by Berndt Schuttler, doctor of Physics and Astronomy at the University of Georgia. The system was perturbed environmentally by removing sunlight, genetically by eliminating the *wc-1* gene, and the G receptor protein was poisoned. Following, we explored the ethical implications and philosophical drawbacks of meddling in the system using the book *Sleep Thieves* by Stanley Coren.

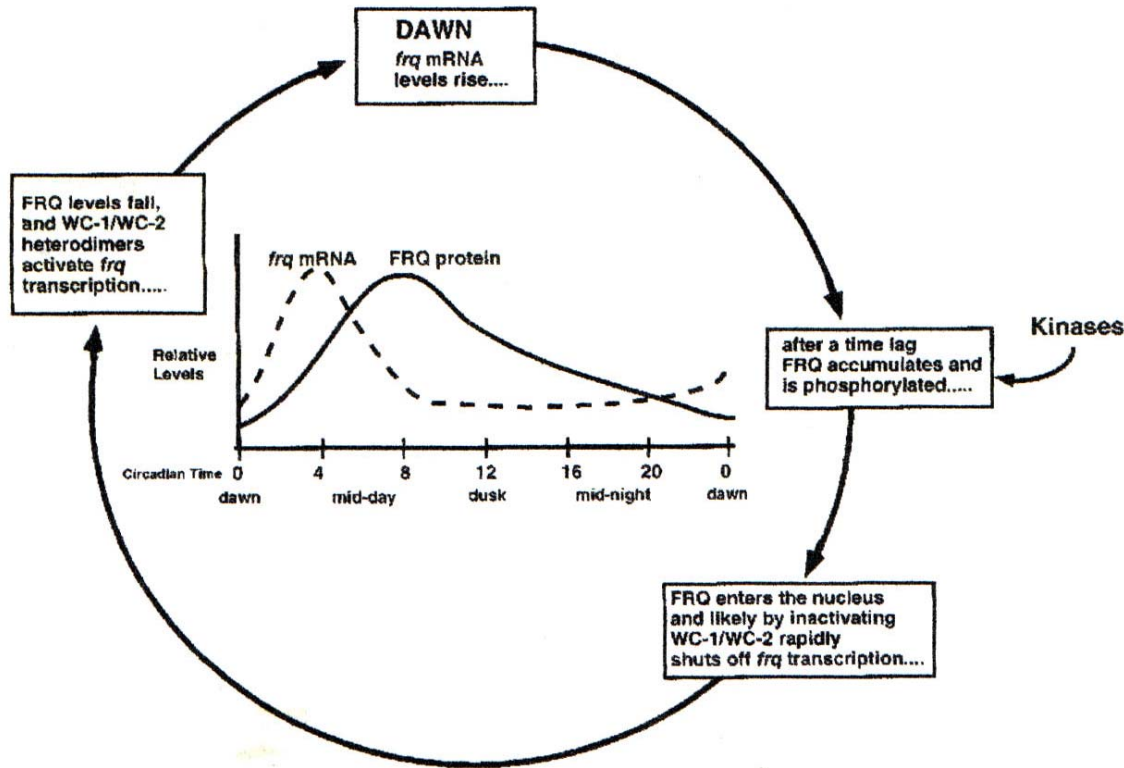
RESULTS



This figure shows a model of the circuit as taken from the articles of Dunlap¹ and Lee². The most important element in the figure is the presence of the feedback loop, which is controlled by the frq proteins. The WC complex activates the translation of frq proteins. The frq proteins activate the transcription wc-1 and can both activate or inhibit the role of the WC complex in creating more frq protein. The figure also shows where light and temperature are introduced into the system.

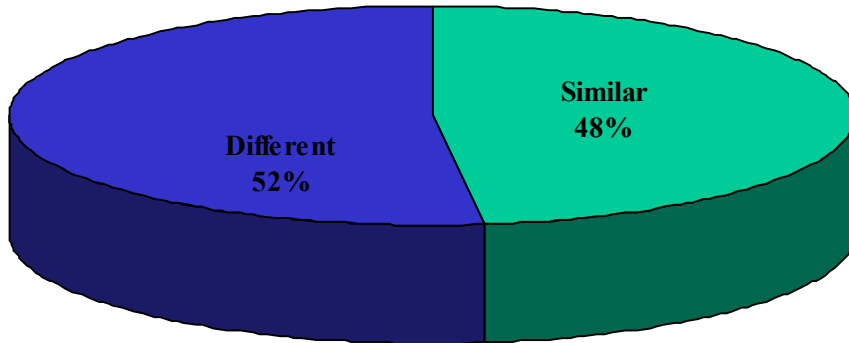


This figure shows the model of the circuit, including the hypothesized vivid and G proteins. The light signal goes into the cell through the vivid protein. Then the vivid protein modifies the G protein so G can take the signal into the nucleus and activate wc-1. It is unknown at this point whether the G modified protein is created inside or outside of the nucleus, yet it is most probable that the complex is formed outside of the nucleus. Therefore, there is a dotted box indicating the possibility that the G modified protein is formed inside of the cell.



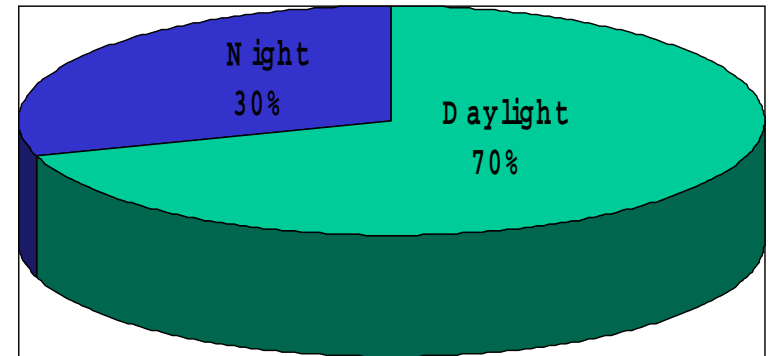
This figure, taken from Bell-Pedersen 2000, shows the feedback loop of *frq* in *N. crassa*. The central graph shows the relative levels of *frq* mRNA and FRQ protein throughout the day. The graph shows a 4-6 hour lag between the peak of *frq* mRNA levels and the peak in FRQ protein levels. Then the *frq* mRNA levels remain low while FRQ protein levels slowly drop. This shows that soon after *frq* mRNA is created, it is translated. However, once FRQ protein is made, it quickly enters the nucleus to keep the *frq* mRNA levels low, by interacting with the *wc* proteins as indicated in the previous figures. The increase in *frq* mRNA between circadian time 20 and 0 shows that once FRQ protein levels drop to a certain amount, it can no longer inhibit the production of *frq* mRNA. These mechanisms create rhythmicity in the cell.

Percentage of Similar Sequences Within the FRQ and BMAL Genes



A BLAST protein search indicated that 48% of the FRQ and BMAL genes contain identical or very similar sequences. BMAL is a mammalian gene. No other Vertebrate protein has shown comparable similarities. These percentages show the close relation of fungal and mammalian biological clocks.

Percent of Human Activity Throughout the Day



This chart shows the percentage human activity during daylight hours and nighttime hours. A large majority of a human's daily activity is during daylight hours; a person does less activity when it is dark outside. This is an emergent property of the fact that light can induce *wc-1* to transcriptionally activate the *frq* gene. This sets the clock to earlier hours and keeps the person awake and energized longer.

Normal ccg output

Environmental:

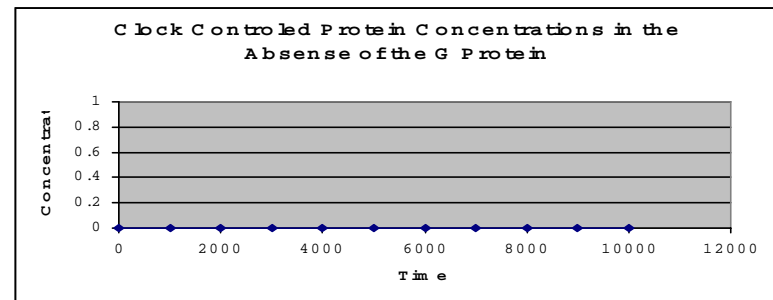
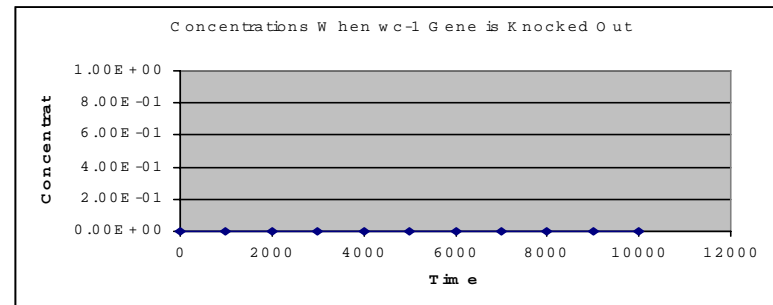
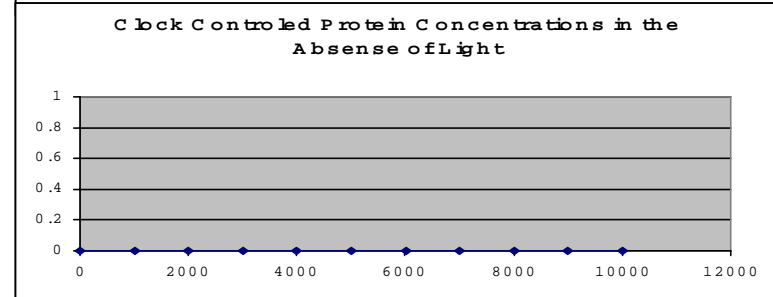
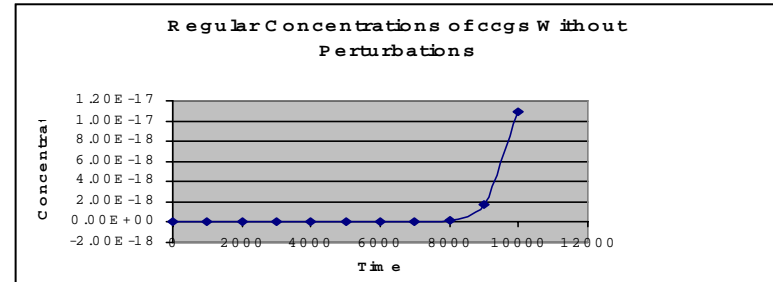
Taking out sunlight eliminated ccg output from the circuit.

Genetic:

When wc-1 gene was knocked out, there was no ccg production.

Poison:

When the G protein was poisoned, there was no ccg output.



CONCLUSION

A better understanding of factors that influence rhythm can be gained through creating the model of the biological pathway. The feedback loop is controlled by the FRQ protein, which positively activates the activation of the WC-1 gene and both positively and negatively regulates the WC protein complex. In the case of *N. crassa*, light and temperature conclusively have an effect on rhythmicity (Garceau et al. 1997). In order to complete this biological circuit, the function of certain genes had to be hypothesized. In our circuit, Vivid protein brings the light signal into the cell while a protein named G carries this message into the nucleus. Light has an indirect effect by triggering the receptor protein, G, through the transfer protein, vivid, but temperature directly effects the translation of FRQ. Temperature's direct influence into the biological clock has a greater influence on rhythmicity than the indirect influence of light.

The WCC complex actually turns on the clock-controlled genes (ccg), and interfering in that circuitry automatically turns off ccg production. Thus, the elimination of sunlight eliminates ccg production because sunlight initiated the circuit through the G modified complex with vivid. The G modified complex initiated transcription of both the wc-1 and wc-2 genes. Knocking out the wc-1 gene blocks the production of the wc-1 protein that forms half of the WCC complex. This also explains the absence of ccg proteins in the cell. Poisoning the G protein eliminated the formation of the G modified complex, which initiates transcription of wc-1 and wc-2 genes. Once again, this interrupted the formation of the WCC complex, and ccg output was eliminated. These perturbations illustrate that all players in the circuit are needed for the emergent clock properties.

There is a definite mammalian connection through the genome of the mouse. The mammalian oscillator contains many similarities to the fungal circadian loop. The CLOCK and BMAL proteins form a complex much like the WC complex. This complex regulates clock controlled genes, and, in return is regulated by a phosphorylated group such as the PER proteins (Dunlap et al. 1997). With this in mind, the mammalian connection validates the study of fungal circadian rhythms.

After discovering the capability to alter biological rhythm, the question becomes whether or not manipulation of a genuine biological system serves an applicable purpose in society.

FUTURE WORK

In the future, we would like to develop a computer simulator capable of simulating oscillation in circuitry. The results today actually represent manipulations in rate constants to allow for data. However, they do not actually fully represent the oscillation of the system.

Also, with an improved computer simulated circuit, additional environmental perturbations such as the addition of drugs like caffeine into the cell could provide a more accurate portrayal of the circadian system in *Neurospora crassa*.

LITERATURE CITED

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