The Effect of Circadian Alteration and Temperature on Reproductive Fitness in Cyanobacteria

Lowell A. Safren, Patrick Donahue, Tara Shrout, Aynalem Ameha, Hollie Black, Stephen Derryberry, Hayley Pionk, and Abby Pribish
*All authors contributed equally to the design and execution of this project.

College of Arts and Science, Vanderbilt University

The reproductive fitness of cyanobacteria is altered by variation of temperature conditions and differential mutation of Kai genes that contribute to circadian rhythms. The degree and significance of these effects were tested in three distinct strains of *Synechococcus elongatus* cyanobacteria: a wild-type strain (AMC149) and two mutated strains with either an optimized or a disrupted circadian rhythm. These strains were allowed to compete in mixed cultures to determine the adaptive value of circadian clocks in relation to the reproductive fitness of cyanobacteria. Pure cultures served as controls, ensuring that growth rates were the same. Results illustrate that under constant light conditions at high temperatures, the strains with mutant circadian rhythms were both more reproductively fit than the wild-type strain. In conclusion, we found that circadian clocks do not confer a reproductive advantage for cyanobacteria when the cycle of the circadian clock does not match the light/dark cycle of the environment.

Introduction

The circadian rhythm is a cyclical period of biological activity that adapts living cells to daily cycles of light and temperature in the environment. The machinery that controls this rhythm is known as the circadian clock. Cyanobacteria, also called blue-green algae, are photosynthetic prokaryotes that closely resemble eukaryotic algae. Although circadian rhythms are present in almost all plants and animals, cyanobacteria are the simplest organisms to study because only three genes primarily regulate the cycle (Karatsoreos et al. 2011).

Mutation of one of these genes, *KaiC*, causes variation in the functionality of the circadian clock. In the cyanobacteria *Synechococcus elongatus* wild-type strain (AMC149) (WT), this gene is unaltered and contributes to the maintenance of a biological free-running period of twenty-four hours, but only at temperatures no less than 30°C. A free-running period is the amount of time it takes to complete one circadian cycle without any environmental feedback. One specific mutation of the *KaiC* gene generates higher *KaiC* expression, and allows this strain to maintain a free-running period (FRP) of twenty-four hours at any temperature. This population served as the Optimized (Opt) strain because the maintained FRP conferred a reproductive advantage at environments below 30°C. The Disrupted strain was also generated by mutation of the *KaiC* gene; however, this specific mutation results in no observable or consistent circadian rhythm of any free-running period at any temperature (Mackey et al. 2011).

The function of the circadian clock is important for continuation of the species for any organism: "Circadian clocks enhance the innate ability of organisms to survive under ever-changing environments by enabling them to efficiently anticipate periodic events such as availability of food, light and mates" (Paranjpe and Sharma 2005). The degree to which an intact or mutated circadian clock affects the fitness of cyanobacteria has not yet been adequately studied.

Previous studies have addressed the importance of an intact circadian clock by measurement of reproductive fitness in other organisms with altered circadian clocks. Studies have shown that *Drosophila melanogaster* and *Neurospora crassa*, for example, do not illustrate decreased reproductive fitness when their circadian clock is disrupted (Kyriacou 1990) (Lakin-Thomas 2000). Another study found that mutated circadian clocks do not negatively impact reproductive fitness in mammals (DeCoursey 1997). Thus, there is little evidence that a mutated circadian clock would significantly alter the reproductive fitness of cyanobacteria under the conditions used in previous studies. One study, however, illustrates how cyanobacteria strains with a circadian rhythm that most closely matches the experimental light/dark cycle were reproductively favored under competitive conditions over strains that did not maintain matching circadian rthythms (Ouyang 1998). Given such evidence, this study aimed to determine if an intact circadian clock enhances the reproductive fitness of cyanobacteria when exposed to an unnatural light/dark

cycle condition of constant light.

We hypothesized that the cyanobacteria strains with a circadian rhythm that matches the exposed light/dark condition would achieve a higher survival rate. To test the hypothesis, the three cyanobacteria strains were allowed to compete in mixed cultures at varying temperatures under constant light. If the fitness of cyanobacteria in competition is enhanced by a regular circadian rhythm independent of light/dark cycle, then the Opt strain survival rate should surpass that of the WT cyanobacteria at low temperatures but not at high, and the WT cyanobacteria survival rate should surpass that of Dis strain at high temperatures but not at low.

Methods

Cyanobacteria

Three variants of *Synechococcus elongatus* strain PCC 7942, a species of photosynthetic cyanobacteria, were used. The strains differed in the functionality of their circadian clock and antibiotic resistance genes. The wild-type (WT) transgenic *S. elongatus* (AMC149) had a precise circadian rhythm at temperatures at or above 30°C and was resistant to Spectinomycin (Kondo 1994). The optimized strain (Opt) had a circadian rhythm that would oscillate regularly despite temperature conditions and was resistant to Kanamycin. The disrupted strain (Dis) had no observable circadian rhythm at any temperature and was resistant to Gentamycin.

Growth

The cultures were initially diluted to a cell density of 0.500 AU. Cultures were grown in liquid BG-11 media in constant light with a supply of air for one week. Cell density was measured via absorbance periodically throughout the growth period. Each competition or pure culture (described below) was held at 20°C and 30°C separately to determine the effect of temperature on reproductive fitness , with two replicates each.

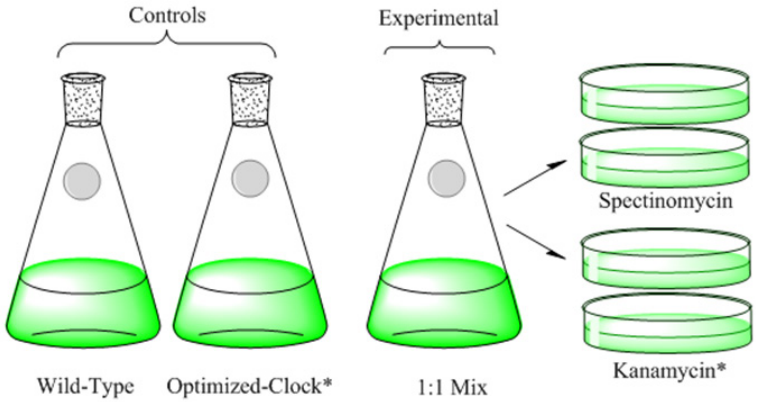

Figure 1. The WT v Opt experiment and control setup. Cell density in controls was measured concurrently to ensure growth rate equality. Spectinomycin plates were used to determine number of wild-type colonies and Kanamycin plates were used to determine the number of optimized-clock colonies (*Gentamycin was used to determine Dis strain colonies in the WT v Dis competition). Each competition was repeated twice at each temperature (20°C and at 30°C).

Competitions

Reproductive fitness was evaluated through competition of different strains of cyanobacteria in mixed cultures and determining the final proportion of each strain. Two competitions were tested: WT v Opt and WT v Dis. For each competition, the two competing strains were initially mixed at a 1:1 ratio. After one week of growth, the percentage of the strains in each culture were determined by plating a sample of the culture on agar plates with antibiotics and then allowing colonies to grow. The WT v Opt competition was plated with Spectinomycin (25 µg/L) to determine the number of WT colonies and on separate plates with Kanamycin (25 µg/L) to determine the number of Opt colonies. The WT v Dis competition was also plated with Spectinomycin (25 µg/L) to determine the number of WT colonies and on separate plates with Gentamycin (5 µg/L) to determine the number of Dis colonies. Each plate was replicated twice per culture. The overall setup of the experiment is briefly illustrated in Figure 1.

Controls

For each competition, liquid cultures of the pure strains involved in the competition were grown separately. Cell density was measured to ensure growth rates were similar and thus the competitions fair.

Results

There were no significant differences found between the growth rates of pure cultures for each competition (df = 2; WT v. Opt, 20: t= 0.5187, p = 0.5187; WT v. Opt, 30: t= 0.4370, p = 0.7377; WT v. Dis, 20: t = 0.0822, p = 0.9478; WT v. Dis, t = 1.5784, p = 0.2252) (Figure 2).

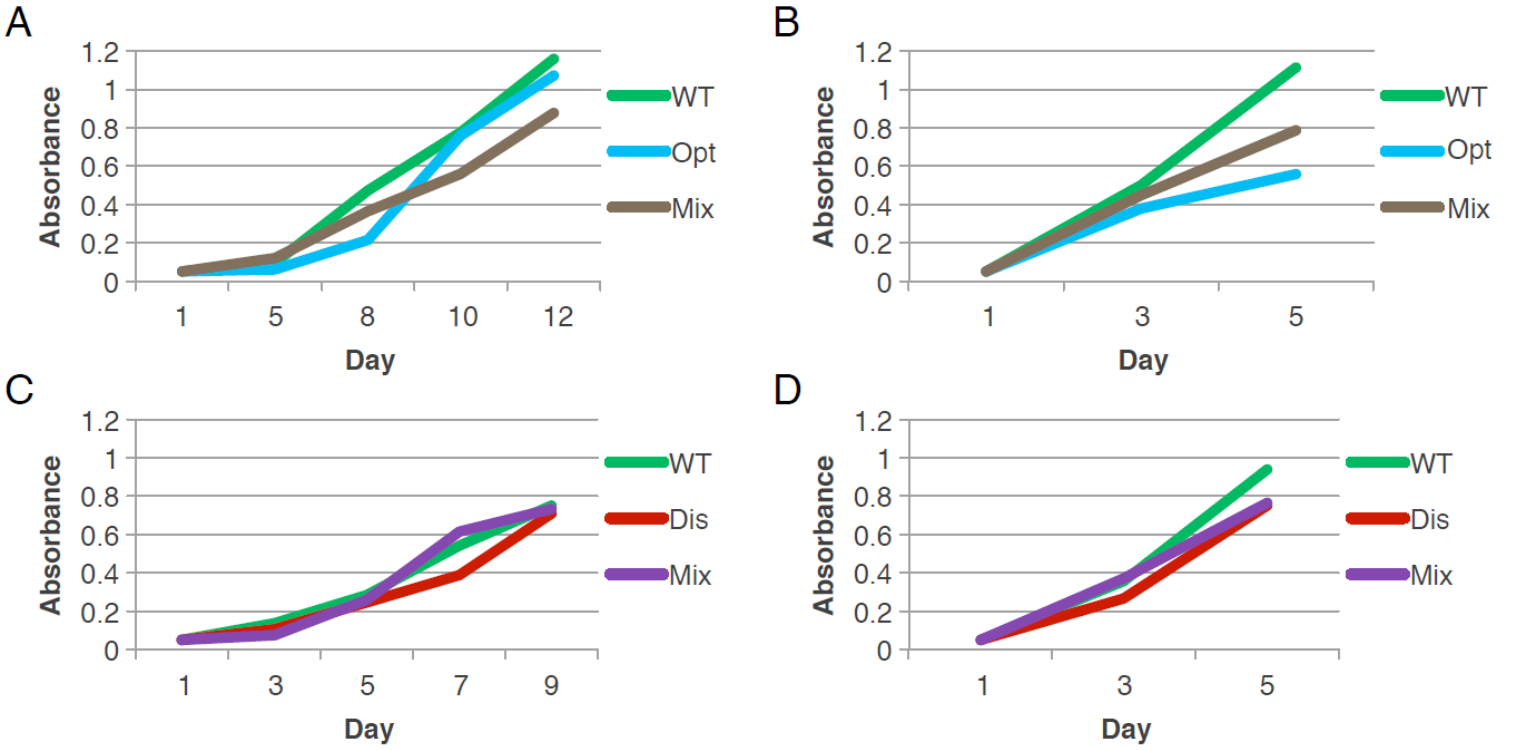

Figure 2. Comparisons of growth rates of pure cultures within the competitions yielded no significant differences in either competition (A/B - WT v Opt, C/D -WT v Dis) or at either temperature (A/C - 20°C, B/D - 30°C), p > 0.2, see text for details.

An ANOVA concluded that there was a significant effect of differential strain on reproductive fitness in both the WT v. Opt and WT v. Dis competitions (df = 2, F= 7.086, $p < 0.001$ and $p = 0.033$, respectively). In the 20°C competition, the Opt strain accounted for 66.1% of the bacteria, while at 30°C, it accounted for 96.2% of the culture. The Dis strain accounted for 73.3% of the culture in its competition after the 20°C trial and 57.6% of the culture after the 30°C. We were unable to determine a mean effect of temperature on reproductive fitness.

Although the data in both competitions trended against the WT, the differences were not significant at 20°C (Figure 3). At 30°C, the fraction of Opt, 0.962, was higher than that of WT ($p < 0.01$), and the fraction of Dis, 0.576, was higher than that of WT ($p = 0.016$) (Figure 3).The ANOVA mentioned above also showed an interaction of temperature and strain for the WT v Opt competition ($p = 0.01$), meaning that the temperature affected the fitness of each strain differently.

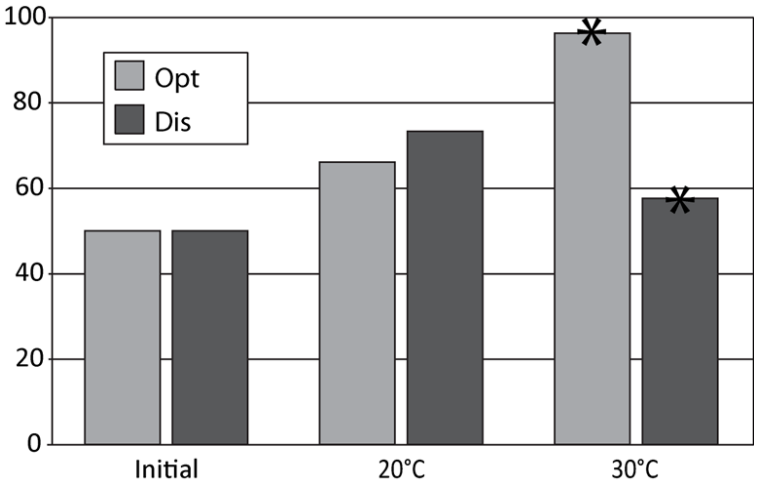

Figure 3. Percentage of culture composed of non-WT strains after one week of growth. Initial compositions were 50%:50% WT:non-WT. *Percentage is significantly different from that of the WT.

Discussion

The Opt strain was significantly more reproductively successful in constant light than the WT strain when in competition at 30°C, but not at 20°C . The natural advantage of the Opt strain at 20°C was lost because its circadian rhythm did not match the light/dark cycle, so the WT bacteria grew comparatively, despite its circadian clock that was also rendered nonfunctional by the low temperature.

In the 30°C constant light cycle competition, the Dis strain also demonstrated significantly greater fitness

than the WT strain. Although 30°C is the optimal temperature for the WT to maximize its reproductive fitness , the Dis strain outcompeted the WT strain because the WT strain's advantage was lost due to the exposed constant light conditions. Ouyang et al. (1998) demonstrated the same results: the wild-type strain used in our experiment reached a certain cell density , then stopped growing, due to loss of reproductive advantage under constant light conditions. Since the Dis strain's growth did not stop, it makes sense that the Dis strain outperformed the WT strain. At 20°C under constant light, neither the Dis nor the WT strain was more reproductively fit, because at this temperature, the circadian clock of the WT is also non-functional.

In conclusion, circadian clocks only confer an advantage in competition when the cycle of the clock matches the light/dark cycle of the environment. To further test this postulate and increase statistical power, future experiments could use more replicates over several weeks. In addition, expanding the range of light/dark cycles used in the experiment to include the natural 12:12 LD , constant dark cycles, and other previously tested cycles such as16:8 and 8:16, would provide more data to determine the effect of varying circadian rhythms on the reproductive fitness in cyanobacteria.

Acknowledgements

The authors would like to acknowledge Peijun Ma for her guidance and assistance throughout the project.

References

DeCoursey, P.J., Krulas, J. R., J. R., Mele, G. & Holley, D.C. (1997). Physiol. Behav. 62, 1099-1108

Karatsoreos, IN, S. Bhagat, EB Bloss, JH Morrison, and BS McEwen. "Disruption of Circadian Clocks Has Ramifications for Metabolism, Brain, and Behavior." Proceedings of the National Academy of Sciences of the United States of America 108.4 (2011): 1657-662. Jean and Alexander Heard Library---Log-in. Web. 20 Feb. 2011. <http://www.ncbi.nlm.nih.gov.proxy.library.vanderbilt.edu/pubmed/21220317>.

Kondo, T, Tsinotremas, N, F, Golden, S. S., Johnson, C. H., Kutsuna, S., & Ishiura, M. (1994). Science 266, 5188, p1233

Kyriacu, C.P, Oldroyd, M., Wood, J., Sharp, M. & Hill, M. (1990). Heredity 64, 395-401

Lakin-Thomas PL. Circadian rhythms: new functions for old clock genes. Trends Genet. 2000 Mar; 16(3):135-42. Review. PubMed PMID: 10689355.

Mackey SR, Golden SS, Ditty JL. The itty-bitty time machine genetics of the cyanobacterial circadian clock. Adv Genet. 2011; 74:13-53. doi: 10.1016/B978-0-12-387690-4.00002-7. Review. PubMed PMID: 21924974; PubMed Central PMCID: PMC3319097.

Paranjpe, Dhanashree A., and Vijay K. Sharma. "Evolution of Temporal Order in Living Organisms." Journal of Circadian Rhythms 3.7 (2005). BioMed Central. Web. 20 Feb. 2011. <http://www.biomedcentral.com/content/pdf/1740-3391-3-7.pdf>.

Ouyang, Y, Anderson, C. R., Kondo, T., Golden, S. S, & Johnson, C. (1998). Proc. Natl. Acad. Sci. USA 95, 8660-8664