Chaos, Solitons & Fractals 50 (2013) 65–74

Contents lists available at SciVerse ScienceDirect

Chaos, Solitons & Fractals

Nonlinear Science, and Nonequilibrium and Complex Phenomena

journal homepage: www.elsevier.com/locate/chaos

An evolutionary fitness enhancement conferred by the circadian system in cyanobacteria

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ARTICLE INFO

Article history:
Available online 21 December 2012

ABSTRACT

Circadian clocks are found in a wide variety of organisms from cyanobacteria to mammals. Many believe that the circadian clock system evolved as an adaption to the daily cycles in light and temperature driven by the rotation of the earth. Studies on the cyanobacterium, *Synechococcus elongatus* PCC 7942, have confirmed that the circadian clock in resonance with environmental cycles confers an adaptive advantage to cyanobacterial strains with different clock properties when grown in competition under light–dark cycles. The results thus far suggest that in a cyclic environment, the cyanobacterial strains whose free running periods are closest to the environmental period are the most fit and the strains lacking a functional circadian clock are at a competitive disadvantage relative to strains with a functional clock. In contrast, the circadian system provides little or no advantage to cyanobacteria grown in competition in constant light.

To explain the potential mechanism of this clock-mediated enhancement in fitness in cyanobacteria, several models have been proposed; these include the limiting resource model, the diffusible inhibitor model and the cell-to-cell communication model. None of these models have been excluded by the currently available experimental data and the mechanistic basis of clock-mediated fitness enhancement remains elusive.

1. Background

Circadian clocks are endogenous timing mechanisms that function to regulate a variety of cellular, metabolic and behavioral activities over the course of the day–night cycle. Circadian systems allow organisms to anticipate daily changes in environmental signals such as light and temperature. Regulated by circadian clocks, organisms sustain roughly 24-h rhythms even in the absence of environmental timing cues, and these clock-driven rhythms sustain stable free-running periods (FRPs) within the physiologically tolerable temperature range [1,2].

Circadian clocks have been found in a broad range of organisms from cyanobacteria to mammals. Given their ubiquity, circadian clocks are considered to be an evolutionary adaptation that enhances the fitness of organisms possessing them [3]. For instance, chipmunks with disrupted circadian clocks were more susceptible to predation in the wild than those with intact circadian systems. Ecological observations suggested that the nighttime restlessness of the arhythmic chipmunks resulted in elevated detection rates by predators [4]. Studies of *Drosophila melanogaster* showed that the life span of flies with altered circadian periods was significantly reduced by up to 15% [5], and that the disruption of the circadian clock also reduced sperm production in males [6]. Furthermore, *Arabidopsis* strains lacking a circadian clock showed lower viability, less carbon fixation and slower photosynthesis rates than wild-type strains [7,8]. Moreover, *Arabidopsis* is more resistant to herbivory when plants were entrained in the same phase as the herbivores, indicating that the circadian system in *Arabidopsis* assists in defending against herbivory [9].

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http://dx.doi.org/10.1016/j.chaos.2012.11.006

Although these studies demonstrate that circadian regulation of cellular, metabolic and behavioral events is beneficial, few studies have rigorously tested the adaptive value of circadian clocks in terms of their contribution to fitness and adaptation. Fitness primarily describes reproductive success [10], whereas longevity, growth and development are secondary factors affecting the fitness of an organism. An adaptation is an acquired feature as a result of natural selection that enhances the fitness of an organism under certain selective pressures [10]. An adaptation can only be presumed to be adaptive when it first emerges [3]. In the process of evolution, the adaptation may retain an "extrinsic" value only if the selective pressure remains. Alternatively, the adaptation may acquire an "intrinsic" value by becoming integrated with other processes. In this case, even if the original adaptation persists in the absence of the selective pressures, it is no longer considered to be an adaptation [10]. In order to fully test the adaptive value of circadian clocks, two questions must be addressed. Does the presence of a circadian clock (i) enhance the fitness and (ii) if so, is the adaptive value conferred by the circadian clock intrinsic or extrinsic? [3] To date, most studies have only partially or indirectly addressed these questions. Furthermore, little if any research has addressed the potential mechanisms by which circadian clocks mediate fitness enhancement.

The cyanobacterium, *Synechococcus elongatus* PCC 7942 (*S. elongatus*) is an ideal model system to address these questions for several reasons [3,11,12]. First, the central clock mechanism is relatively simple; the core circadian clock in *S. elongatus* is composed of three proteins (KaiA, KaiB, and KaiC) that are encoded by the genes, *kaiA*, *kaiB* and *kaiC* [13] and a number of clock mutants have been generated [14]. Among these mutants are those with short and long periods as well as arhythmic mutants. These mutant strains allow us to test the adaptive value of the cyanobacterial circadian clock by directly comparing them to the wild-type strain under different growth conditions. Second, *S. elongatus* reproduces asexually by binary fission and therefore growth rates are a direct measurement of fitness [15]. Third, the growth conditions of *S. elongatus* are relatively simple and therefore laboratory conditions can approximate the relevant features of natural conditions. Fourth, *S. elongatus* can grow in either constant light or in light/dark cycles, thus the extrinsic versus intrinsic adaptive value can be determined by artificially introducing or removing selective pressures [3]. Finally, *S. elongatus* represents one of the most evolutionarily ancient organisms possessing a circadian system; therefore, elucidating the mechanisms of clock-mediated adaptation in this species could help us understand the selective pressures that may have led to the evolution of circadian clocks.

In this chapter, the previous work that has been done to test the adaptive value of the circadian clock in *S. elongatus* will be described and the possible mechanisms that might explain how the cyanobacterial circadian system exerts its influence on overall fitness will be discussed. The models that have been considered to explain these mechanisms are "the limiting resource model", the "diffusible inhibitor model" and the "cell-to-cell communication model" [3].

2. Testing the adaptive value of the circadian clock in cyanobacteria

The adaptive value of circadian clocks in cyanobacteria was tested by using growth in competition between the wild-type *S. elongatus* and several different clock mutant strains [3,11,12]. These clock mutants are due to point mutations in the *kaiA*, *kaiB* or *kaiC* genes respectively, resulting in altered clock properties such as arhythmicity or rhythmicity that exhibits FRPs that are longer or shorter than 24 h [14]. In pure cultures, neither these mutant strains nor the wild-type strain have growth rates that are significantly different from each other in constant light (LL) or in light/dark (LD) cycles [11,12]. This observation does not exclude the possibility that the circadian clock system enhances fitness in cyanobacteria; however the adaptive value may only be detectable under some selective circumstances such as competition. For this reason, competition experiments were designed to assess the reproductive fitness of the wild-type strain (WT) and the clock mutant strains under controlled environmental conditions (Fig. 1) [3,11,12]. In these experiments, two cyanobacterial strains with different clock phenotypes were mixed and grown together in either constant light or in light/dark cycles and the composition of these mixed cultures was assayed over time as a test of reproductive fitness.

For example, to test whether the circadian clock enhances reproductive fitness in cyanobacteria, competition experiments were conducted between the WT strain with a FRP of approximately 24–25 h and an arhythmic mutant (CLAb) whose circadian clock was disrupted by a point mutation (G460E) in the *kaiC* gene (Fig. 2A) [11,13,14]. In LD 12:12 (12 h of light followed by 12 h of darkness) cycles, the WT strain quickly (within 20 generations) became the predominant strain in mixed cultures (Fig. 2B). As a control, the point mutation in CLAb was rescued by introducing a wild-type copy of the *kaiC* gene into the genome. When the rescued CLAb strain was grown in competition with the WT strain, the proportions of the WT and mutant strain remained approximately equal in the mixed cultures over many generations indicating that the reduction in fitness of CLAb was due to altered clock properties rather than some other difference in the genetic background of the two strains in competition [11]. This experiment confirmed that the circadian clock in cyanobacteria confers adaptive value in light/dark cycles, but it does not reveal whether this adaptive value is an intrinsic or extrinsic property of the clock. If the value is an intrinsic property of the clock, one would expect that the WT strain would also defeat CLAb when grown in mixed cultures in constant conditions as well as when grown together in light/dark cycles. To address this question, the WT and arhythmic strains were co-cultured and grown in constant light condition removing the presumed selective pressure of the day–night cycles. Surprisingly (at least, to some chronobiologists who favor the intrinsic adaptiveness of circadian timekeeping!), the CLAb strain was not only able to successfully maintain itself in mixed cultures with WT, but the proportion of CLAb significantly increased in these