Coupled oscillators and circadian gating in the cyanobacterial circadian clock

Qiong Yang[1], Guogang Dong[2], Susan Golden[2], Alexander van Oudenaarden[1]

***Short Abstract* — Cyanobacteria, the simplest organisms known to have circadian behavior, comprise only three core clock proteins: KaiA, KaiB and KaiC, and nevertheless show precise self-sustained rhythm that is fundamentally the same as circadian rhythms of higher multi-cellular organisms. To understand how such simple unicellular organisms resist perturbations from frequent cell divisions and high intracellular noise in the absence of intercellular interactions, we hypothesize that the interlocking between two oscillators and the circadian gating are important for the resilient rhythms in cyanobacteria. We test these hypotheses with quantitative methods based on our long term time-lapse single cell fluorescent microscopy.**

I. Introduction

Circadian clocks are important to improve fitness of almost all living beings on Earth, from algae to humans, by timing their internal activities to match the patterns of day and night, the day warm and bright and the night cold and dark. As the daylight is essential for cyanobacteria to obtain energy, these photosynthetic organisms have developed an amazingly precise circadian clock system to control their daily activities. Current studies [1,2] for the cyanobacteria clock have mostly focused on its biochemical and structural properties outside cells. A milestone experiment [1] reconstituted the biochemical oscillator *in vitro* and claimed it as the core pacemaker in cyanobacteria. However, such studies failed to demonstrate the relevance of this biochemical oscillator *in vivo*. In fact, our data showed that cells still oscillate even with the biochemical oscillator disrupted. We hence aim to fill the gap between *in vitro* and *in vivo* experiments by tracking dynamic circadian behaviors in live cells. Specifically, we aim to understand why cells keep precise and synchronized rhythms over many generations by testing two hypotheses on coupled oscillators and circadian gating.

II. Methodology and Results

Our broad objective is to develop a quantitative understanding of the precise circadian behaviors in cyanobacteria and eventually develop mathematical models for this simple system that might lead to better understanding of more complex clock systems in higher organisms.

A. Methodology

We developed a long term time-lapse single cell fluorescent microscopy with automated light and temperature control based on our previous work [3]. The method suits for tracking circadian behaviors in individual cyanobacteria.

B. Coupled oscillators

We showed that **cells still oscillate even with the biochemical oscillator disrupted, but with less synchrony.** We hypothesize that at least two important oscillators – the biochemical oscillator and the auto-regulatory transcription translation oscillator – interlock together and are the basis for the precise rhythms.

C. Circadian gating

Cyanobacteria have to simultaneously deal with two different time circuits: the cell cycle circuit with period ranging from 5 hrs to a day and the circadian circuit with a constant about 24 hr period. The 'circadian gating' study [4] suggests that the two circuits are not independent. We test whether the interaction between two time circuits may be a strategy for cyanobacteria to keep their temporal stability by limiting the activity of one circuit during the sensitive phase of the other circuit and whether KaiC plays an important role in the regulation.

III. Conclusion

The results will improve our understanding of the coupling of multiple oscillators, a phenomenon that has been found in many higher organisms and help us to build predictive models. They will also help us understand the role of circadian gating at the single cell level. Both of them require the single cell study, which makes our single cell method the most appreciate for addressing these questions.

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[1]Department of Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. E-mail: qiongy@mit.edu

[2]Department of Biology, Texas A&M University, College Station, Texas 77843-3258, USA

[†]Correspondence and requests for materials should be addressed to A.v.O.

E-mail: avano@mit.edu

[1] T.Kondo, *Science*, **308**, 414-415 (2005)

[2] M.J. Rust, J.S. Markson, W.S. Lane, D.S. Fisher & E.K. O'Shea, *Science*, **318**, 809-812 (2007).

[3] Kaufmann, B.B. Yang, Q. Mettetal, J.T. & van Oudenaarden, A., PLoS Biology **5**, e239 (2007).

[4] Mori, T., Binder, B., & Johnson, C.H, Proc. Natl. Acad. Sci. USA. **93**, 10183 (1996).