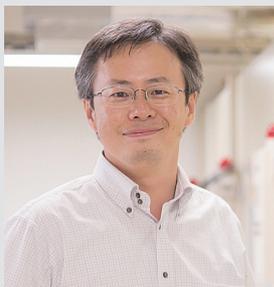


Biological Rhythm and Dynamics through Chemistry

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Education

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Professional Employment

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2005 JST-PRESTO Researcher
2008 Junior Associate Professor, Nagoya University
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2012 Professor, Institute for Molecular Science
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Awards

2022 NAGASE Research Promotion Award
2016 The 13th (FY2016) JSPS PRIZE
2008 The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
The Young Scientists' Prize
2007 Young Scientist Prize, The Biophysical Society of Japan
2006 SAS Young Scientist Prize, IUCr Commission on Small-angle Scattering
2002 The Protein Society Annual Poster Board Award

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Living organisms on Earth evolved over time to adapt to daily environmental alterations, and eventually acquired endogenous time-measuring (biological clock) systems. Various daily activities that we perform subconsciously are controlled by the biological clock systems sharing three characteristics. First, the autonomic rhythm repeats with an approximately 24-hour (circadian) cycle (self-sustainment). Second, the period is unaffected by temperature (temperature compensation). Third, the phase of the clock is synchronized with that of the outer world in response to external stimuli (synchronization). We seek to explain these three characteristics, and consider the biological clock system of cyanobacteria to be an ideal experimental model.

The major reason that cyanobacteria are considered to be the ideal experimental model is that the core oscillator that possesses the three characteristics of the clock can be easily reconstructed within a test tube. When mixing the three clock proteins KaiA, KaiB, and KaiC with ATP, the structure and enzyme activity of KaiC change rhythmically during a circadian cycle. Taking advantage of this test tube experiment, we used an approach combining biology, chemistry, and physics

to elucidate the means by which the clock system extends from the cellular to atomic levels.

Among the three Kai proteins, KaiC is the core protein of the oscillator. In the presence of KaiA and KaiB, KaiC reveals the rhythm of autophosphorylation and dephosphorylation; however, the cycle of this rhythm depends on the ATPase activity of KaiC independent of KaiA or KaiB. For example, when the ATPase activity of KaiC doubles as a result of amino acid mutations, the frequencies of both the *in vitro* oscillator and the intracellular rhythm also double (the cycle period is reduced to half). This mysterious characteristic is called a transmural hierarchy, in which the cycle (frequency) and even the temperature compensation both *in vitro* and *in vivo* are greatly affected (controlled) by the function and structure of KaiC.

How are the circadian activities and temperature compensation features encoded in KaiC and then decoded from it to propagate rhythms at the cellular level? We are committed to better understanding biological clocks and other dynamic systems through the chemistry of circadian **rhythm, structure**, and evolutionary **diversity**.

Selected Publications

- A. Mukaiyama, Y. Furuike, K. Ito-Miwa, Y. Onoue, K. Horiuchi, K. Kondo, E. Yamashita and S. Akiyama, *Nat. Commun.* **16**, 4541 (2025).
- Y. Furuike, Y. Onoue, S. Saito, T. Mori and S. Akiyama, *PNAS Nexus* **4**, pgaf136 (2025).
- Y. Furuike, A. Mukaiyama, S. Koda, D. Simon, D. Ouyang, K. Ito-Miwa, S. Saito, E. Yamashita, T. Nishiwaki, K. Terauchi, T. Kondo and S. Akiyama, *Proc. Natl. Acad. Sci. U. S. A.* **119**, e2119627119 (2022).
- Y. Furuike, A. Mukaiyama, D. Ouyang, K. Ito-Miwa, D. Simon, E. Yamashita, T. Kondo and S. Akiyama, *Sci. Adv.* **8**, eabm8990 (2022).
- J. Abe, T. B. Hiyama, A. Mukaiyama, S. Son, T. Mori, S. Saito, M. Osako, J. Wolanin, E. Yamashita, T. Kondo and S. Akiyama, *Science* **349**, 312–316 (2015).
- Y. Murayama, A. Mukaiyama, K. Imai, Y. Onoue, A. Tsunoda, A. Nohara, T. Ishida, Y. Maeda, T. Kondo and S. Akiyama, *EMBO J.* **30**, 68–78 (2011).

1. Structure: Reasons for Seeking Structure and Dynamics of Circadian Clock Components in Cyanobacteria¹⁻⁴⁾

A great deal of effort has been devoted to characterizing structural changes in the clock proteins along the circadian reaction coordinate. However, little is known about the mechanism driving the circadian cycle, even for the simple cyanobacterial protein KaiC that has ATPase and dual phosphorylation sites in its N-terminal C1 and C-terminal C2 domains, respectively. Nearly all KaiC structures reported to date share a nearly identical structure, and they do not appear to be suggestive enough to explain the determinants of circadian period length and its temperature compensation. We are studying the structural and dynamical origins in KaiC using high-resolution x-ray crystallography,¹⁻⁴⁾ real-time fluorescence detection,⁵⁾ and quasielastic neutron scattering.⁶⁾

2. Rhythm: Cross-Scale Analysis of Cyanobacterial Circadian Clock System⁶⁻⁸⁾

KaiC ATPase is of particular interest here, as it finely correlates to the frequencies of *in vivo* as well as *in vitro* oscillations and also it is temperature compensated. This unique property has inspired us to develop an ATPase-based screening⁷⁾ for KaiC clock mutants giving short, long, and/or temperature-dependent periods.⁸⁾ A developed HPLC system with a 4-channel temperature controller has reduced approximately 80% of time costs for the overall screening process (Figure 1). Using the developed device, we are screening a number of temperature-dependent mutants of KaiC.^{6,7)}

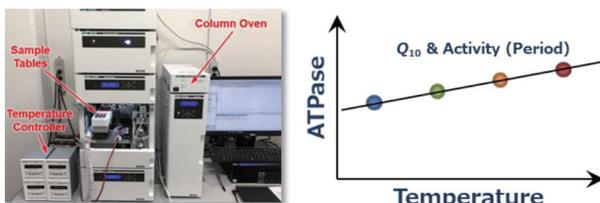


Figure 1. Development of a quick ATPase assay system.

3. Beyond Evolutionary Diversity⁹⁾

In the presence of KaiA and KaiB, the ATPase activity of KaiC oscillates on a 24-hour cycle. KaiC is not capable of maintaining a stable rhythm on its own, but its activity was observed to fluctuate with reduced amplitude over time (Figure 2A). We have identified a signal component that is similar to damped oscillation, and propose that it encodes the specific frequency, equivalent to a 24-hour cycle.

The habitats of cyanobacteria are diverse, so the space of their sequence is immense. Furthermore, some KaiA and KaiB genes are missing in several strains of cyanobacteria. This is understandable to some extent if KaiC possesses the specific frequency. Given this assumption, *what specific frequencies*

are possessed by KaiC homologues in other species and ancestral cyanobacteria? (Figure 2B)

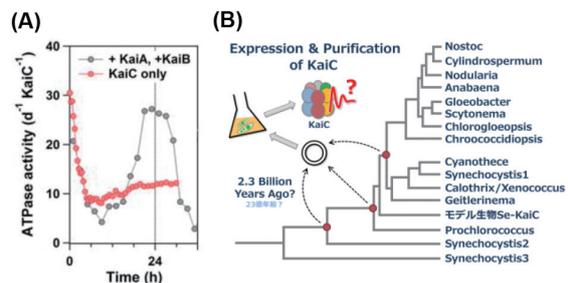


Figure 2. Damped oscillation of KaiC ATPase activity (A) and evolutionary diversity of cyanobacteria (B).

To address these questions, we restored the amino acid sequences of ancestral Kai proteins (Figure 2B) and studied their function (oscillation) and structures to determine the evolutionary origin of the self-sustained Kai-protein oscillators.⁹⁾ Our results clearly demonstrate that the oldest Kai-protein oscillator emerges in the most recent common ancestor (MRCA) of cyanobacteria at approximately 2.2 Ga ago and is able to synchronize with temperature cycles of 18 h; this is shorter than the current rotation period of the Earth.

4. Bio-SAXS Activity in IMS¹⁰⁾

We have supported SAXS users so that they can complete experiments smoothly and publish their results.¹⁰⁾

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