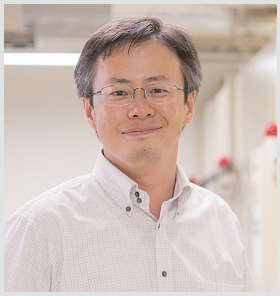
Biological Rhythm and Dynamics through Chemistry

Research Center of Integrative Molecular Systems
Division of Trans-Hierarchical Molecular Systems



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Professional Employment
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Awards
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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 revelas 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

- D. Ouyang, Y. Furuike, A. Mukaiyama, K. Ito-Miwa, T. Kondo and S. Akiyama, *Int. J. Mol. Sci.* **20**, 2789–2800 (2019).
- A. Mukaiyama, D. Ouyang, Y. Furuike and S. Akiyama, *Int. J. Biol. Macromol.* **131**, 67–73 (2019).
- A. Mukaiyama, Y. Furuike, J. Abe, E. Yamashita, T. Kondo and S. Akiyama, *Sci. Rep.* **8**, 8803 (2018).
- 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. Maéda, T. Kondo and S. Akiyama, *EMBO J.* **30**, 68–78 (2011).
- S. Akiyama, A. Nohara, K. Ito and Y. Maéda, *Mol. Cell* **29**, 703–716 (2008).

1. *Structure*: Reasons for Seeking Structure and Dynamics of Circadian Clock Components in Cyanobacteria[1–3)]

A great deal of effort has been devoted to characterizing structural changes in the clock proteins along the circadian reaction coordinate.[1)] 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[2)] and quasielastic neutron scattering,[3)] respectively.

2. *Rhythm*: Cross-Scale Analysis of Cyanobacterial Circadian Clock System[3–5)]

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 screening for KaiC clock mutants[4)] giving short, long, and/or temperature-dependent periods. 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.[3)]

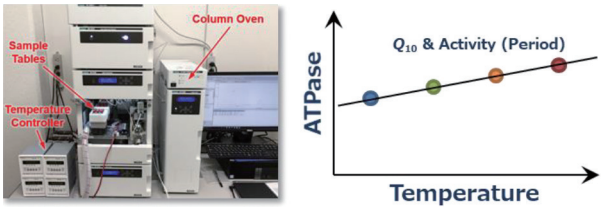

Figure 1. Development of a quick ATPase assay system.

We also collaborated with Drs. Ito-Miwa and Kondo (Nagoya University) to identify a series of KaiC mutations altering circadian periods dramatically, from 0.6 to 6.6 d.[5)]

3. beyond Evolutionary *Diversity*[6)]

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.[1)]

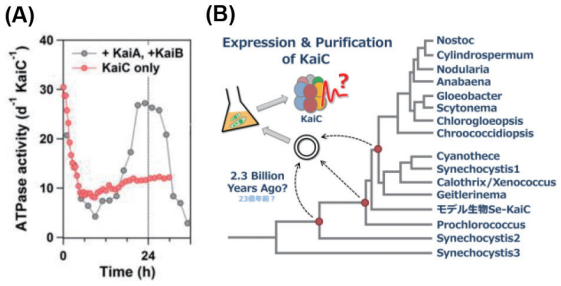

Figure 2. Damped oscillation of KaiC ATPase activity (**A**) and evolutionary diversity of cyanobacteria (**B**).

The habitats of cyanobacteria are diverse, so the space of their sequence is immense.[6)] 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 our current understanding of this phenomenon, *what specific frequencies are possessed by KaiC homologues in other species and ancestral cyanobacteria?* (Figure 2B) If you strain your ears, the rhythms of the ancient Earth may be heard from beyond evolutionary diversity.

4. Bio-SAXS Activity in IMS[7)]

We have supported SAXS users so that they can complete experiments smoothly and publish their results.[7)]

References

1)S. Akiyama, *Circadian Rhythms in Bacteria and Microbiomes*, 138–145 (2021).
2) Y. Furuike, A. Mukaiyama, D. Ouyang, K. Ito-Miwa, D. Simon, E. Yamashita, T. Kondo and S. Akiyama, *bioRxiv* doi: 10.1101/2021.08.30.457330 (2021).
3) Y. Furuike, D. Ouyang, T. Tominaga, T. Matsuo, A. Mukaiyama, Y. Kawakita, S. Fujiwara and S. Akiyama, *bioRxiv* doi: 10.1101/2021.08.20.457041 (2021).
4) D. Ouyang, Y. Furuike, A. Mukaiyama, K. Ito-Miwa, T. Kondo and S. Akiyama, *Int. J. Mol. Sci.* **20**, 2789–2800 (2019).
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6) A. Mukaiyama, D. Ouyang, Y. Furuike and S. Akiyama, *Int. J. Biol. Macromol.* **131**, 67–73 (2019).
7) M. Okumura, S. Kanemura, M. Matsusaki, Y. H. Lee, S. Akiyama and K. Inaba, *Structure* **29**, 1–14 (2021).