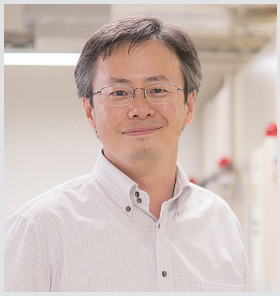
Biological Rhythm and Dynamics through Chemistry

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



AKIYAMA, Shuji
Professor
[akiyamas@ims.ac.jp]

Education
1997 B.E. Kyoto University
1999 M.E. Kyoto University
2002 Ph.D. Kyoto University

Professional Employment
2001 JSPS Research Fellow
2002 JSPS Postdoctoral Fellow
2003 RIKEN Special Postdoctoral Researcher
2005 JST-PRESTO Researcher
2008 Junior Associate Professor, Nagoya University
2011 Associate Professor, Nagoya University
2012 Professor, Institute for Molecular Science
Professor, The Graduate University for Advanced Studies

Awards
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

Member
Assistant Professor
MUKAIYAMA, Atsushi
FURUIKE, Yoshihiko
Post-Doctoral Fellow
OUYANG, Dongyan
Graduate Student
SIMON, Damien
Technical Fellow
WASHIO, Midori
TAKAYA, Mari
SUGISAKA, Kanae
WADA, Kotoe
Secretary
SUZUKI, Hiroko

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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*: Atomic-Scale Origins of Clock Slowness in Cyanobacterial Circadian Clock System[1,2)]

To identify the structural origins of slowness encoded in KaiC, its N-terminal ATPase domain was analyzed using high-resolution x-ray crystallography.[1)] Water molecules are prevented from attacking into the ideal position (Figure 1) for the ATP hydrolysis by a steric hindrance near ATP phosphoryl groups. In addition, this hindrance is surely anchored to a spring-like structure derived from polypeptide isomerization. The ATP hydrolysis, which involves access of a water molecule to the bound ATP and reverse isomerization of the polypeptide, requires a much larger amount of free energy than for typical ATP hydrolysis. The atomic structure explains why the ATPase activity of KaiC is so much lower (by 100- to 1,000,000-fold) than that of typical ATPases.[2)]

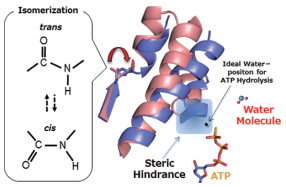

Figure 1. Structural basis for steady slowness. The steric barrier prevents access of a water molecule to the catalytic site (indicated by a black dot).

2. *Rhythm*: Transmural Hierarchy in 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. This unique property has inspired us to develop an ATPase-based screening for KaiC clock mutants giving short, long, and/or temperature-dependent periods.[3)] A developed HPLC system with a 4-channel temperature controller has reduced approximately 80% of time costs for the overall screening process (Figure 2).

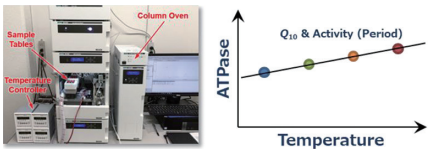

Figure 2. Development of a quick ATPase assay system.

How is the intra-molecular slowness encoded in KaiC (Figure 1) transmitted to the inter-molecular interactions with other Kai proteins? To address this question, a tryptophan residue was introduced in the N-terminal ring of KaiC as the fluorescent probe for KaiBC complex formation.[4)] Our kinetic data indicated that KaiB exclusively selects the post-ATP-hydrolysis state of KaiC to form the KaiBC complex. This process follows a mechanism called conformational selection (CS), in which proteins (KaiC) first undergoes a structural change to form a specific intermediate. Ligands (KaiB) are then recognized specifically through the intermediate state to form a tight ligand-protein complex. The CS mechanism is elegantly designed in KaiC so that the slow intra-molecular ATPase reaction in KaiC can be the rate-liming step of the overall KaiBC complex formation.

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*[1,6)]

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 3A). 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)]

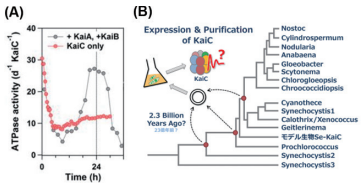

Figure 3. Damped oscillation of KaiC ATPase activity (**A**) and evolutionary diversity of cyanobacteria (**B**).

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 3B) 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) 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).
2) S. Akiyama, *Circadian Rhythms in Bacteria and Microbiomes*, in press (2020).
3) D. Ouyang, Y. Furuike, A. Mukaiyama, K. Ito-Miwa, T. Kondo and S. Akiyama, *Int. J. Mol. Sci.* **20**, 2789–2800 (2019).
4) A. Mukaiyama, Y. Furuike, J. Abe, E. Yamashita, T. Kondo and S. Akiyama, *Sci. Rep.* **8**, 8803 (2018).
5) K. Ito-Miwa, Y. Furuike, S. Akiyama and T. Kondo, *Proc. Natl. Acad. Sci. U. S. A.* **117**, 20926–20931 (2020). doi.org/10.1073/pnas.2005496117
6) A. Mukaiyama, D. Ouyang, Y. Furuike and S. Akiyama, *Int. J. Biol. Macromol.* **131**, 67–73 (2019).
7) I. Anzai, E. Tokuda, S. Handa, H. Misawa, S. Akiyama and Y. Furukawa, *Free Radical Biol. Med.* **147**, 187–199 (2020).