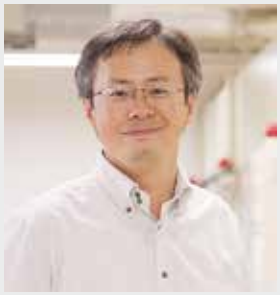
The Origin of 24 Hour Period in Cyanobacterial Clock System

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



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Keywords Circadian Clock, Clock Proteins, Cyanobacteria

Circadian (approximately 24 h) clocks are endogenous time-keeping systems encapsulated in living cells, enabling organisms to adapt to daily fluctuation of exogenous environments on the Earth. These time-keeping systems, found ubiquitously from prokaryotes to eukaryotes, share the three characteristics. First, the circadian rhythmicity of the clocks persists even without any external cues (self-sustainability). Second, the period is little dependent on ambient temperature (temperature compensation). Third, the phase of the clock can be reset by external stimuli such as lightning, humidity, or temperature so as to be synchronized to the external phase (synchronization).

KaiC, a core protein of the circadian clock in cyanobacteria, undergoes rhythmic structural changes over approximately 24 h in the presence of KaiA and KaiB (Kai oscillator). This slow dynamics spanning a wide range of both temporal and spatial scales is not well understood, and is central to a fundamental question: What determines the temperature-compensated 24 h period? The Kai oscillator reconstitutable *in vitro* is advantageous for studying its dynamic structure through a complementary usage of both X-ray crystallography and solution scattering, its transient response by using physicochemical techniques, and its molecular motion through a collaborative work with computational groups (Abe *et al. Science* 2015). Our mission is to explore the frontier in molecular science of the circadian clock system from many perspectives.

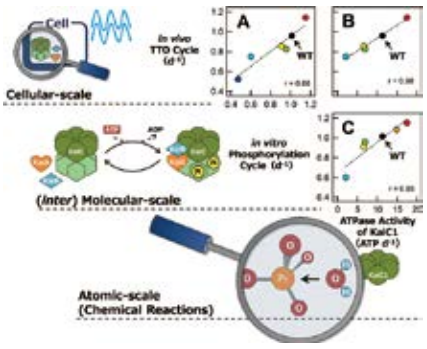

Figure 1. *Trans*-hierarchic nature of the circadian clock system in cyanobacteria. Cross-correlational plots (A–C) among frequency of *in vivo* transcription and translation oscillation (TTO) cycle, frequency of *in vitro* phosphorylation cycle, and ATPase activity of KaiC for cyanobacteria carrying period-modulating KaiC mutants (circles). Fine correlations in three panels indicate regulatory mechanisms of KaiC ATPase as the core basis for *trans*-hierarchic nature of cyanobacterial circadian clock system.

Selected Publications

- Y. Furuike, J. Abe, A. Mukaiyama and S. Akiyama, *Biophys. Physicobiol.* **13**, 235–241 (2016).
- 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, *Cell. Mol. Life Sci.* **69**, 2147–2160 (2012).
- S. Akiyama, A. Nohara, K. Ito and Y. Maéda, *Mol. Cell* **29**, 703–716 (2008).

1. Atomic-Scale Origins of 24 Hour Period in Cyanobacterial Clock System[1,2)]

The cyanobacterial circadian clock can be reconstructed *in vitro* by mixing three clock proteins (KaiA, KaiB, and KaiC) and ATP. As shown in Figure 2, KaiC ATPase activity exhibits a robust circadian oscillation in the presence of KaiA and KaiB. Astonishingly, the temporal profile of KaiC ATPase activity exhibited an attenuating and oscillating component even in the absence of KaiA and KaiB. A detailed analysis revealed that this signal had a frequency of 0.91 d^{-1}, which approximately coincided with the 24 h period. KaiC is thus the source of a steady cycle that is in tune with the Earth's daily rotation.

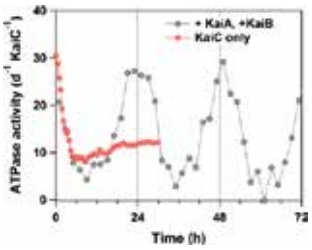

Figure 2. Time-course of KaiC ATPase activity.

To identify the structural origins of slowness encoded in KaiC (Figures 1B & 1C), its N-terminal ATPase domain was analyzed using high-resolution x-ray crystallography. A water molecule is prevented from attacking into the ideal position (a black dot in Figure 3) 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, is expected to require a significantly larger amount of free energy than for typical ATP hydrolysis. The atomic structure discovered by us explains why the ATPase activity of KaiC is so much lower (by 100- to 1,000,000-fold) than that of typical ATPase molecules.

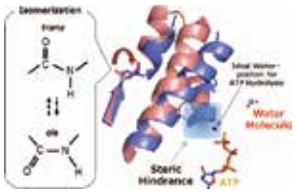

Figure 3. Structural basis for steady slowness. The steric barrier prevents access of a water molecule to the catalytic site (indicated by a black dot).

2. *Trans*-Hierarchic Nature of Cyanobacterial Circadian Clock System[3)]

How is the intra-molecular slowness encoded in KaiC (Figures 2 and 3) transmitted to the inter-molecular interactions with other Kai proteins? Protein-ligand interactions are often discussed whether a structural change of the protein comes before or after the ligand binding (Figure 4). The conformational selection (CS) scheme predicts that the protein first undergoes a structural change to form a specific intermediate. The ligand is then recognized specifically through the intermediate state to form a tight ligand-protein complex. On the other hand, in the induced-fit (IF) scheme, the ligand and protein form an encountered complex without meaningful structural changes, and then both the ligand and protein undergo structural changes to form the tight protein-ligand complex. Under the ligand-saturating conditions, the rate of forming the protein-ligand complex differs between CS (k_f) and IF ($k_f + k_b$) schemes (Figure 4).

A tryptophan residue was introduced in the N-terminal ring of KaiC as the fluorescent probe for KaiBC complex formation.[3)] Our detailed analysis of the kinetic data indicated that KaiB exclusively selects the post-ATP-hydrolysis state of KaiC to form the KaiBC complex. The CS mechanism is elegantly designed in KaiC so that the slow intra-molecular reaction (k_f: The slow rate of ATPase) in KaiC can be the rate-liming step of the overall KaiBC complex formation.

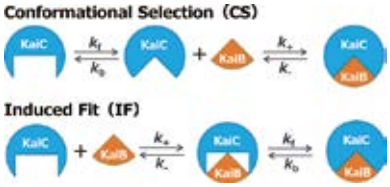

Figure 4. Conformational selection and induced-fit schemes.

3. Instrumentation for Studying Biological Clock Systems[4)]

We have improved stability over time, signal-to-noise ratio, time resolution, temperature control, automated high-throughput measurements each for fluorescence tracking system,[3)] auto-sampling device,[4)] HPLC,[1)] FTIR, and small-angle x-ray scattering (SAXS). The developed devices were utilized in identifying the core process of generating circadian periodicity in cyanobacterial circadian clock.[2,3)]

4. Bio-SAXS Activity in IMS[5)]

We have supported SAXS users so that they can complete experiments smoothly and publish their results.[5)]

References

1) S. Akiyama, A. Mukaiyama, J. Abe and Y. Furuike, *Biological Clocks: With Reference to Suprachiasmatic Nucleus*, 73–77 (2017).
2) 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).
3) A. Mukaiyama, Y. Furuike, J. Abe, E. Yamashita, T. Kondo and S. Akiyama, *Sci. Rep.* **8**, 8803 (2018).
4) Y. Furuike, J. Abe, A. Mukaiyama and S. Akiyama, *Biophys. Physicobiol.* **13**, 235–241 (2016).
5) Submitted.

* IMS International Internship Program