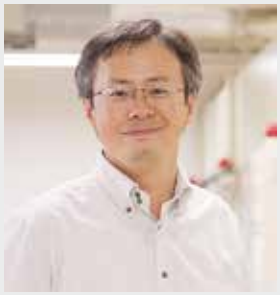
The Origin of 24 Hour Period in Cyanobacterial Clock System

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



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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.

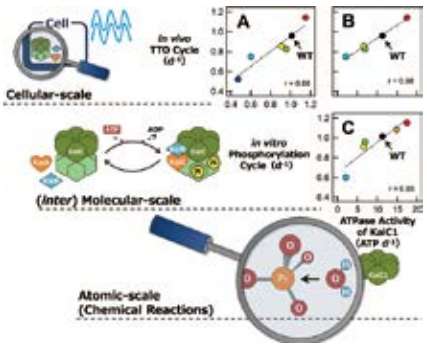

Figure 1. *Trans*-hierarchic nature of the circadian clock system in cyanobacteria. Cross-correlational plots (A–C) among frequency of *in vivo* transcription and translation oscillation (TTO) cycle, frequency of *in vitro* phosphorylation cycle, and ATPase activity of KaiC for cyanobacteria carrying 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)]

In accordance with diurnal changes in the environment resulting from the Earth's daily rotation around its axis, many organisms regulate their biological activities to ensure optimal fitness and efficiency. The biological clock refers to the mechanism whereby organisms adjust the timing of their biological activities. The period of this clock is set to approximately 24 h. A wide range of studies have investigated the biological clock in organisms ranging from bacteria to mammals. Consequently, the relationship between the biological clock and multiple diseases has been clarified. However, it remains unclear how circadian rhythms are implemented.

Our group have addressed this question using cyanobacteria. The cyanobacterial circadian clock can be reconstructed 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.

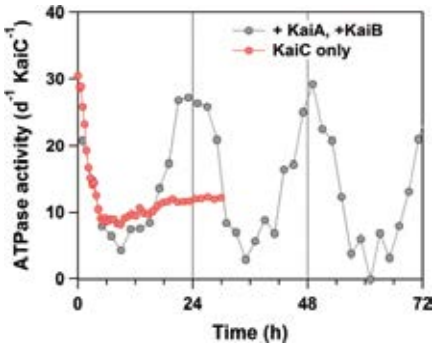

Figure 2. Time-course of KaiC ATPase activity.

To identify the structural origins, the N-terminal domain of KaiC was analyzed using high-resolution x-ray crystallography. The resultant atomic structures revealed the underlying cause of KaiC's slowness relative to other ATPases (Figure 3). 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. Thus, the three-dimensional 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.

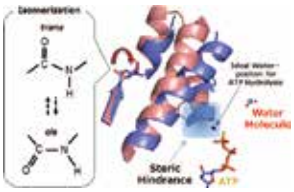

Figure 3. Structural basis for steady slowness. The steric barrier prevents access of a water molecule to the catalytic site (indicated by a black dot).

The fact that a water molecule, ATP, the polypeptide chain, and other universal biological components are involved in this regulation suggests that humans and other complex organisms may also share a similar molecular machinery.

2. Instrumentation for Studying Biological Clock Systems[2)]

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

3. Bio-SAXS Activity in IMS[3–5)]

We have supported SAXS users so that they can complete experiments smoothly and publish their results.[3–5)]

4. Other Activities

We have conducted joint research projects in collaboration with other universities and research facilities.

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) Y. Furuike, J. Abe, A. Mukaiyama and S. Akiyama, *Biophys. Physicobiol.* **13**, 235–241 (2016).
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