

The Origin of 24 Hour Period in Cyanobacterial Clock System

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Professional Employment

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Awards

2008 The Young Scientists' Prize, The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, Japan
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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 physico-chemical techniques, and its molecular motion through a

collaborative work with computational groups (Figure 1).

Our mission is to explore the frontier in molecular science of the cyanobacterial circadian clock from many perspectives. This Annual Report summarizes our recent activities.

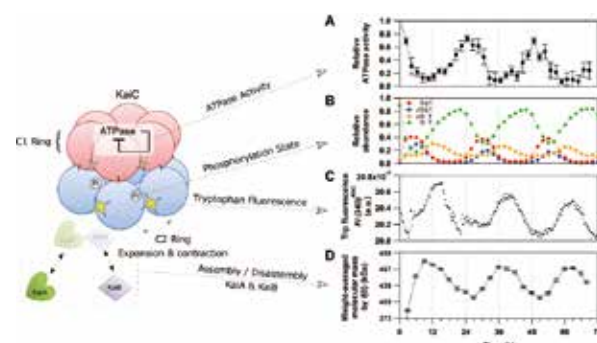


Figure 1. Circadian dynamics of cyanobacterial clock protein KaiC. The C1 and C2 domains in each protomer of KaiC are drawn as red and blue spheres, respectively. Expansion and contraction motions of the C2 ring (B, C) in solution serves as a timing cue for assembly/disassembly of KaiA and KaiB (D), and is interlocked with its C1 ATPase under the control of negative-feedback regulation (A).

Selected Publications

- S. Akiyama, A. Nohara, K. Ito and Y. Maéda, *Mol. Cell* **29**, 703–716 (2008).
- 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).
- A. Mukaiyama, M. Osako, T. Hikima, T. Kondo and S. Akiyama, *Biophysics* **11**, 79–84 (2015).
- 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).

1. Atomic-Scale Origins of 24 Hour Period in Cyanobacterial Clock System^{1,2)}

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.

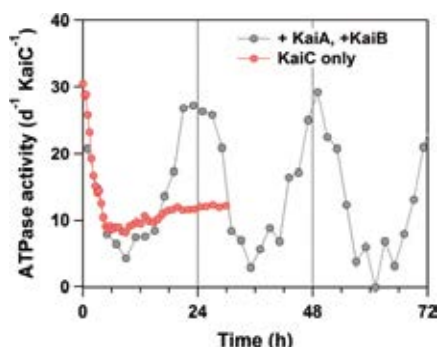


Figure 2. Time-course of KaiC ATPase activity.

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.

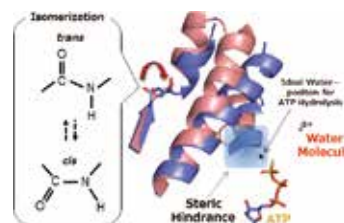


Figure 3. Structural basis for steady slowness. The steric barrier prevents access of a water molecule to the catalytic site (indicated by a black dot).

The circadian clock's period is independent of ambient temperature, a phenomenon known as temperature compensation. One KaiC molecule is composed of six identical subunits, each containing duplicated domains with a series of ATPase motifs. The asymmetric atomic-scale regulation by the aforementioned mechanism suggests a feedback mechanism that maintains the ATPase activity at a constant low level. Our results indicate that the circadian period is implemented as the time constant of the feedback mechanism mediated in this protein structure.

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 Systems³⁾

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,³⁾ HPLC,²⁾ 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^{1,4)}

We are supporting motivated SAXS users so that they can complete experiments smoothly and publish their results.

References

- 1) A. Mukaiyama, M. Osako, T. Hikima, T. Kondo and S. Akiyama, *Biophysics* **11**, 79–84 (2015).
- 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) Y. Furuie *et al.*, to be submitted.
- 4) R. Kojima, M. Okumura, S. Masui, S. Kanemura, M. Inoue, M. Saiki, H. Yamaguchi, T. Hikima T, M. Suzuki, S. Akiyama and K. Inaba, *Structure* **22**, 431–443 (2014).