Crystal Structure of C-terminal Clock-oscillator Domain of KaiA from *Thermosynechococcus elongatus* BP-1

Circadian rhythms, the daily activity cycles exhibited by most organisms, are sustained even in the absence of outside cues. Organisms acquired the rhythms on the genome to match the rotation of the earth by the process of revolution. The biological clock, which is the clock including the body of an organisms, oscillates the circadian rhythms. For example, the oscillation of waking up and sleeping of humans or other organisms is regulated by the biological clock. However, our biological knowledge obtained to date has not yet explained the properties of the biological clock, which are a time constant of ~24 hours and temperature compensation [1].

Cyanobacteria are the most primitive organisms known to exhibit circadian rhythms. The *kaiABC* circadian clock gene cluster in *Synechococcus* sp. strain PCC7942 consists of two operons, *kaiA*, and *kaiBC*. The KaiA protein, which is the *kaiA* product, enhances *kaiBC* promoter activity, and *kaiBC* is repressed by the KaiC protein [2] (Fig. 1(a)). KaiA, KaiB and KaiC proteins, which are necessary to oscillate the rhythms, can directly interact with one another. This interaction has been proposed to be involved in the mechanism underlying circadian rhythms as feedback loop models. Many amino acid substitutions in KaiA, KaiB and KaiC that affect the period length and amplitude of circadian rhythms have been isolated, but the roles of the altered residues have not been elucidated. Therefore, we have started to study the properties and the three-dimensional structure of the KaiA protein.

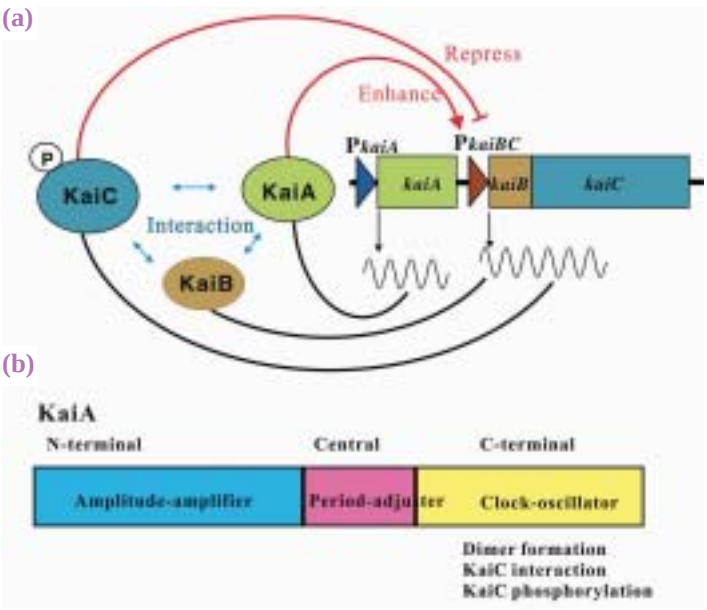

Fig.1. **(a)** Schematic drawing of circadian rhythms of Cyanobacteria. **(b)** Function of KaiA domain.

The limited proteolysis of KaiA from *Themosynechococcus elongatus* BP-1 with trypsin, V8 protease and chymotrypsin showed that KaiA is composed of three regions, which are the N-teminal domain (residue 1-138), central domain (139-173) and C-terminal domain (174-283). In 11 cyanobacteria strains, the C-terminal domain of KaiA shows sequence similarity, whereas the N-terminal and central domains show little similarity, suggesting that the N-terminal and central domains of KaiA may not be fundamental to the clock function. In particular, Anabaena KaiA (110 amino acids) retains only the portion corresponding to the C-terminal domain. Cyanobacteria usually oscillate the circadian rhythms, whereas a *kaiA*-null mutant strain of *Synechococcus kaiA* gene exhibits an arrhythmic phenotype. In order to understand the function of each domain of KaiA, we examined the circadian rhythms when various regions of the *kaiA* gene were transferred into the truncated *Synechococcus kaiA* gene. The C-terminal domain of KaiA showed rhythms although the amplitude of the rhythms was reduced and the period lengthened to about 40 hours. The central and C-terminal domains of KaiA modulated the 24 hours rhythms, suggesting that the central domain has a period-adjuster function. Thus, the C-terminal domain of KaiA has the fundamental clock-oscillator function. The amplitude of the rhythms of full-length KaiA was larger than those of the central and C-terminal domains of KaiA, suggesting that the N-terminal domain has an amplitude-amplifier function (Fig. 1(b)). In addition, the C-terminal domain of KaiA contributes to the dimerization of KaiA, KaiA - KaiC binding, and the enhancement of KaiC phosphorylation [3].

We attempted to crystallize the full-length KaiA and the C-terminal domain of KaiA from *T. elongates*, and obtained only the C-terminal domain crystals. X-ray diffraction data were collected at the RIKEN Structural Genomics Beamlines **BL26B1** and **BL26B2**. The crystal

structure of the C-terminal clock oscillator domain of KaiA was determined at 1.8 Å resolution by the multiwavelength anomalous dispersion method (MAD) [3]. As shown in Fig. 2(a), the subunit structure forms a right-handed superhelix comprising six helices. The four-helix bundle consists of two sets of antiparallel helices. The KaiA C-terminal domain forms a dimer whose interface mainly consists of the h6 helix. The concave surface formed by the dimer is composed of three helices (h3, h6 and the adjacent h4 of the second subunit). In the 11 strains examined, the 23 conserved residues locate almost in the interior of a subunit or on the dimer interface and are involved in maintaining the stability of the structure.

As shown in Fig. 2, the conserved residue His270 is at the center of the concave dimer surface, extending the side chains outside the concavity. The KaiA (174-283) H270A mutant showed KaiC-binding activity and KaiC phosphorylation enhancement of about 30% of the wild type, and the correct folding of H270A was identified by CD spectrum analysis. In addition, we analyzed the *in vivo* rhythm functions of the residue using a mutated *Synechococcus kaiA* gene and *Synechococcus* host cells. His270 of *T. elongates* KaiA corresponds to His271 of *Synecchococcus* KaiA. As shown in Fig. 3, the H271A KaiA mutant of *Synechococcus* could not oscillate the circadian rhythms. Thus, His271 of *Synechococcus* KaiA (His270 of *T. elongates* KaiA) is crucial for the generation of circadian oscillations. In the present work we identified the residue directly involved in the circadian rhythms for the first time.

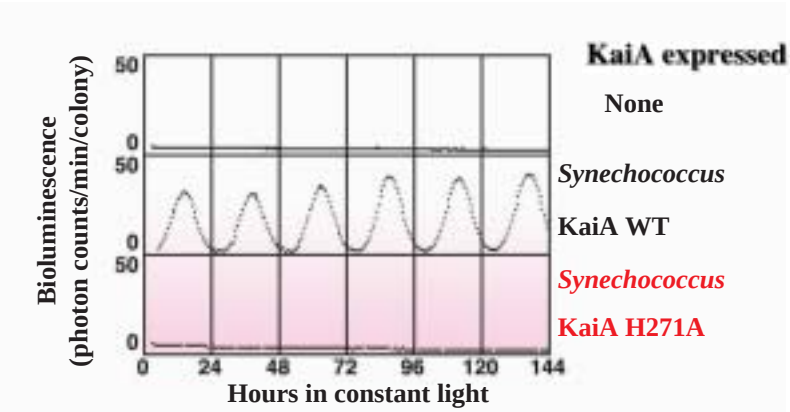

Fig. 3. Bioluminescence rhythms of *Synechococcus* host cells that expressed no KaiA (top), wild-type KaiA (middle) and H271A KaiA mutant (bottom) [3].

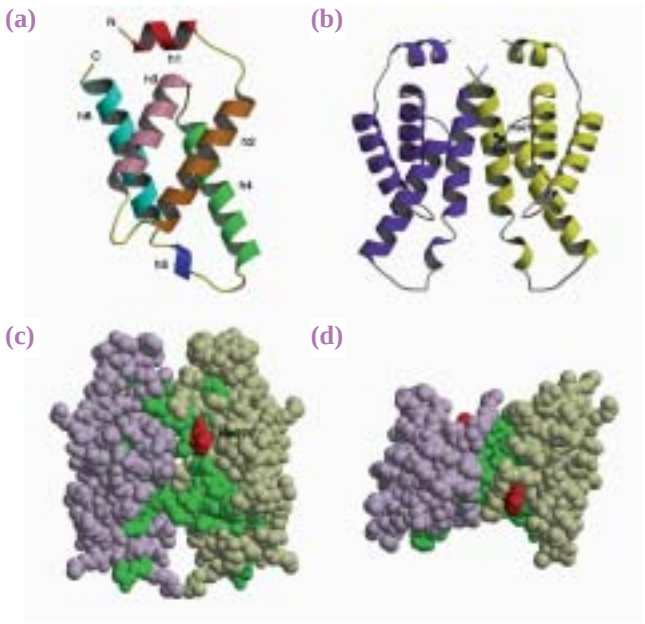

Fig. 2. Crystal structure of KaiA C-terminal domain. Ribbon representation of subunit **(a)** and dimer structure **(b)**. Space filling model of dimer **(c)** and view of **(c)** from different angle **(d)**. The 23 residues conserved in the 11 strains are green, except for His270, which is red [3].

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