Absolute slowness encoded in the circadian clock protein KaiC

Intracellular events are rhythmically regulated by an oscillator with a 24 h period (circadian clock) to adapt to daily changes in the environment on Earth. The slowness of the clock oscillator is one of the characteristics distinguishing it from well-known faster chemical oscillators (with periods of seconds or minutes) [1], raising a fundamental question about the origins of slow but ordered rhythms. We thus searched for determinants of the circadian period using a protein-clock system: the cyanobacterial circadian clock can be reconstituted in a test tube by mixing three clock proteins, KaiA, KaiB and KaiC, with adenosine triphosphate (ATP) [2].

We found that the activity of ATP hydrolysis (ATPase) in KaiC is a determinant of the oscillatory frequency (reciprocal of the period). As the ATPase activity of KaiC itself is increased by a series of single amino acid replacements in KaiC (Fig. 1), the frequency of the *in vitro* protein-clock system (Fig. 1(a)) as well as that of the *in vivo* system (Fig. 1(b)) increased in a well-correlated manner. We thus conducted X-ray crystallographic studies of KaiC [3] at beamline **BL44XU** to search for the structural origins of the slow ATPase orchestrating the frequency of the intracellular events in cyanobacteria.

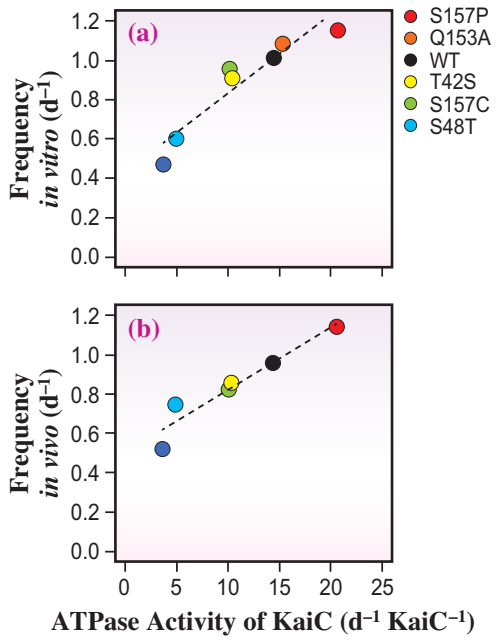

Fig. 1. ATPase activity of KaiC governing the frequencies of both protein-clock rhythms in a test tube **(a)** and intracellular rhythms **(b)**. These figures are reproduced from Ref. 3.

We identified two structural factors slowing the ATP hydrolysis reaction to the circadian time scale (Fig. 2(a)). The first is an unusual positioning of a lytic water molecule surrounding a γ-phosphate of ATP in the crystal structure with the $P2_12_12_1$ space group (right inset in Fig. 2(a)). The lytic water molecule is sequestered from an ideal position to attack a phosphorus atom of the γ-phosphate by hydrogen bonding to the carbonyl oxygen atom of F199 and to the nitrogen atom (N_η) of R226 (Fig. 2(b)). In another crystal structure with the $P3_121$ space group, the lytic water molecule is positioned farther from the γ-phosphate of ATP than that in the $P2_12_12_1$ crystal structure (right inset in Fig. 2(a)). The impact of these two different states on ATPase activity was studied by introducing KaiC mutations perturbing their relative stability (S157P, Q153A, and S157C in Fig. 1). On the basis of the experimental evidence, we conclude that the water positions in both states are basically unfavorable to the ATP hydrolysis (right inset in Fig. 2(a)) as they deviate considerably from the ideal position, similarly to the behavior of other hyperactive enzymes (right inset in Fig. 2(a)), and that the position in the $P3_121$ crystal structure is much more unfavorable than that in the $P2_12_12_1$ crystal structure.

The second is peptide isomerization, one of the slowest reactions in protein chemistry. The coupling of ATP hydrolysis to a *cis-trans* isomerization is clearly reflected in the crystal structure of a KaiC hexamer harboring ATP-bound and adenosine diphosphate (ADP)-bound subunits asymmetrically. In the pre-hydrolysis state, the peptide bond between D145 and S146 mainly adopted the *cis*-conformation, whereas, in the post-hydrolysis state, the peptide completely adopted the *trans*-conformation (left inset in Fig. 2(a)). Our molecular dynamics simulations indicate that the pre-hydrolysis state should overcome a barrier of 14 to 16 kcal·mol^{-1} for *cis-trans* isomerization itself and a barrier of 11 to 17 kcal·mol^{-1} for ATP hydrolysis [3].

The two structural origins uncovered in the present study indicate an unexpectedly high energy barrier upon ATP hydrolysis concomitant with peptide isomerization (Fig. 3). Assuming a frequency factor of 10^{12} s^{-1}, the barrier can be estimated to be approximately 22 kcal·mol^{-1} using the rate constant of 0.5 h^{-1} (12 ATP d^{-1}) at 30°C. Our results show that the circadian time scale is designed in the atomic structure of KaiC to regulate the efficiency of ATP hydrolysis.

An analogous mechanism may be found in other organisms, because all of the key factors such as

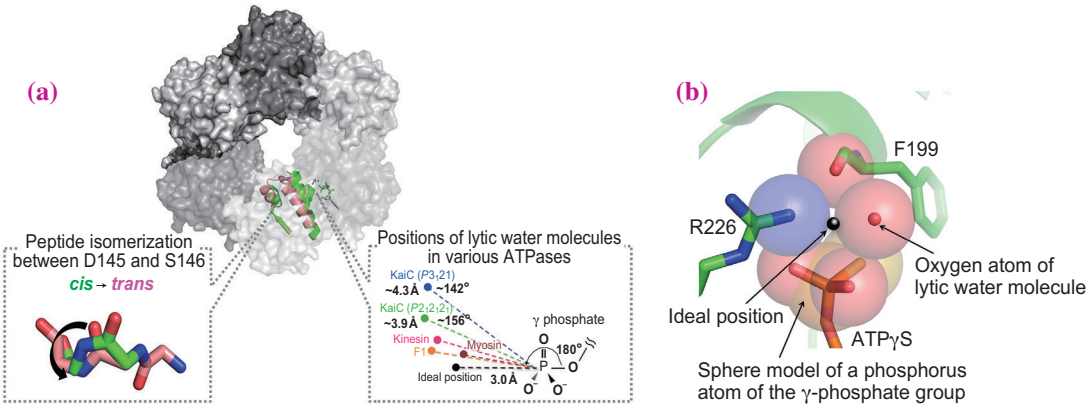

Fig. 2. Structural origins of slowness in a KaiC hexamer. **(a)** Unusual positioning of a lytic water molecule relative to ATP (right inset) and coupling of the peptide isomerization with the ATP hydrolysis reaction (left inset). **(b)** Steric hindrance surrounding a γ-phosphate of ATP. The black filled circle represents the ideal position for a lytic water molecule to attack a phosphorus atom of the γ-phosphate of ATP. Van der Waals radii of the oxygen atom of the lytic water molecule, a carbonyl oxygen atom of F199, N_η of R226, and a phosphorus atom of the γ-phosphate group of ATP γS are drawn using sphere models.

the water molecule, ATP and peptide isomerization are ubiquitously shared and available for achieving absolute slowness. In mammals, the accepted model of the circadian clock is the transcriptional and translational oscillation (TTO); the clock proteins negatively regulate their own expression, thereby producing rhythmic transcription and translation. However, the slowness of the TTO model is often questioned because the transcriptional and translational events can proceed within minutes [4]. Recently, a circadian clock independent of the TTO in mammals was reported [5]. In the future, a structural analysis focusing on the slowness and the correlation with the period of a chemical reaction (the ATP hydrolysis reaction in this study) might reveal the design principle of the circadian clock.

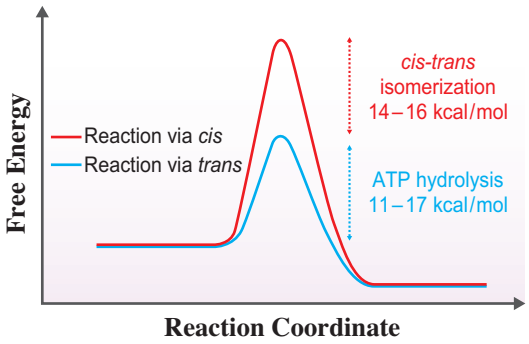

Fig. 3. Circadian time scale encoded in the atomic structure of KaiC. The pre-hydrolysis state with the *cis*-conformation (red line) overcomes a higher energy barrier (exceeding 22 kcal·mol^{-1}) during the reaction than that with the *trans*-conformation (blue line).

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References

[1] K.I. Agladze *et al.*: Nature **308**, (1984) 834.
[2] M. Nakajima *et al.*: Science **308** (2005) 414.
[3] *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*** *(2015) 312.*
[4] B. Schwanhäusser *et al.*: Nature **473** (2011) 337.
[5] J.S. O'Neill and A.B. Reddy: Nature **469** (2011) 498.