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Commentary and Perspective

Beyond multi-disciplinary and cross-scale analyses of the cyanobacterial circadian clock system

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Near the Earth's surface, many environmental factors, such as sunlight, temperature, and humidity, fluctuate with a 24-hour cycle as the Earth rotates. Most of the organisms have a time-measuring system called circadian clocks, and adapt to the day-night environmental cycle using approximately 24-hour (circadian) rhythm emitted by the circadian oscillator as a guide. We would like to understand the shape and mechanism of the circadian systems that incorporate the Earth's rotation period [1].

Studies in a wide variety of species, from bacteria to mammals, have shown that three physiological features of the circadian clock system are conserved across species [2]. The first is the self-sustained 24-hour oscillation even under constant conditions without external stimuli. The second is the temperature insensitivity of the period length, so called temperature compensation. While ordinary chemical reactions are accelerated in a temperature-dependent manner, the frequency (the reciprocal of the period) of the circadian clock systems remains constant over the physiological range of temperature. The third is the ability to update own phase in response to signals from the outside world (temperature, light, etc.) to synchronize with environmental cycles. Researchers in this field seek to build a reasonable model that explains these three characteristics without exception.

The cyanobacterium *Synechococcus elongatus* PCC7942 is one of the ideal experimental organisms for this purpose, as its core oscillator satisfying the three characteristics can be easily reconstructed within a test tube [3]. When mixing the three clock proteins KaiA, KaiB, and KaiC with ATP, the structure and enzyme activity of KaiC change rhythmically with the 24-hour period [4]. Taking advantage of this *in vitro* system, we have studied the cyanobacterial circadian clock system extensively using a multidisciplinary approach, including biophysics, structural biology, chronobiology, molecular biology, and protein engineering, under the financial support from Grant-in-Aid for Scientific Research (S) (No. 17H06165).

The recent research using the latest biophysical techniques are remarkable. Nowadays, the period length of the cyanobacterial circadian clock system can be freely designed from half day to one week, while maintaining its temperature-compensatory ability intact [5]. Novel ideas are emerging, such as a mechanical model that describes a wide-range but temperature-compensated period-tunability [6], and a stochastic model in which an ensemble of many KaiC molecules exhibits coherent oscillations through multiple feedbacks [7,8]. Emerging experimental techniques enable to detect and characterize key KaiC complexes within the ensemble in solution. The recent neutron study revealed that KaiC actively uses fluctuations as an autonomous means to achieve temperature-compensatory responses [9]. A recent crystallographic study has visualized the entire phosphor-transfer cycle of KaiC [10].

In order to summarize these achievements and discuss the prospects for the next stage, we invited five distinguished speakers to a symposium held at the 59th annual meeting of the Biophysical Society of Japan on Friday, November 26,

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2021. Dr. Kumiko Ito-Miwa (Nagoya University) will give us a talk about the mechanical clock model that covers wide-range but temperature-compensated period-tunability. Dr. Masaki Sasai (Nagoya University) is supposed to share with us recent update on single-molecule and ensemble-level models in terms of temperature compensation. Dr Hironari Kamikubo (Nara Institute of Science and Technology) will describe the first application of continuous titration small-angle X-ray scattering technique to elucidate unique binding behaviors of KaiA for phosphorylated/dephosphorylated KaiC ensemble. Dr. Atsushi Mukaiyama (Institute for Molecular Science) will discuss his recent challenges in analyzing the evolutional origin of the time-measuring ability of KaiC and its homologues. Dr. Yoshihiko Furuike (Institute for Molecular Science) will report a structural basis for the minimal set of KaiC allostery that is crucial for the oscillation of the cyanobacterial circadian clock system.

Cyanobacterial circadian clock research has progressed along the line of "simplification of complexities and diversities," in which redundancies are reduced from complex and diverse oscillatory phenomena to the extent that biological functions are maintained intact. At the present stage, however, where the research has reached the molecular to atomic scale, we are faced with several barriers that prevent further simplification. In this symposium, we envision discussing the next stage of simplification-oriented approaches to elucidate the functional-structural relationships underlying the physiological properties of the circadian clock.

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