【Grant-in-Aid for Scientific Research (S)】

Simplifying complexity and diversity of circadian clocks

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Purpose and Background of the Research

●Outline of the Research

Circadian clocks (also called biological clocks) are systems that regulate the time information of life [**Figure 1A**]. The core of the circadian clock consists of a 24-hour oscillator that receives input from the environment upstream, adjusts its phase, and passes time information downstream to express a 24-hour rhythm in gene expression and behavior.

Three features are commonly observed in the circadian clock [**Figure 1A**]. The first is that it exhibits autonomous 24-hour rhythm even in the absence of environmental inputs. The second is the temperature compensability of the period length. The third is the ability to synchronize to external rhythms via input signals. Understanding the circadian clock means explaining all three physiological properties, but a unified understanding has yet to be achieved.

Circadian clock research in cyanobacteria [**Figure 1B**] has progressed under the strategy of trimming components from complex and diverse rhythmic phenomena

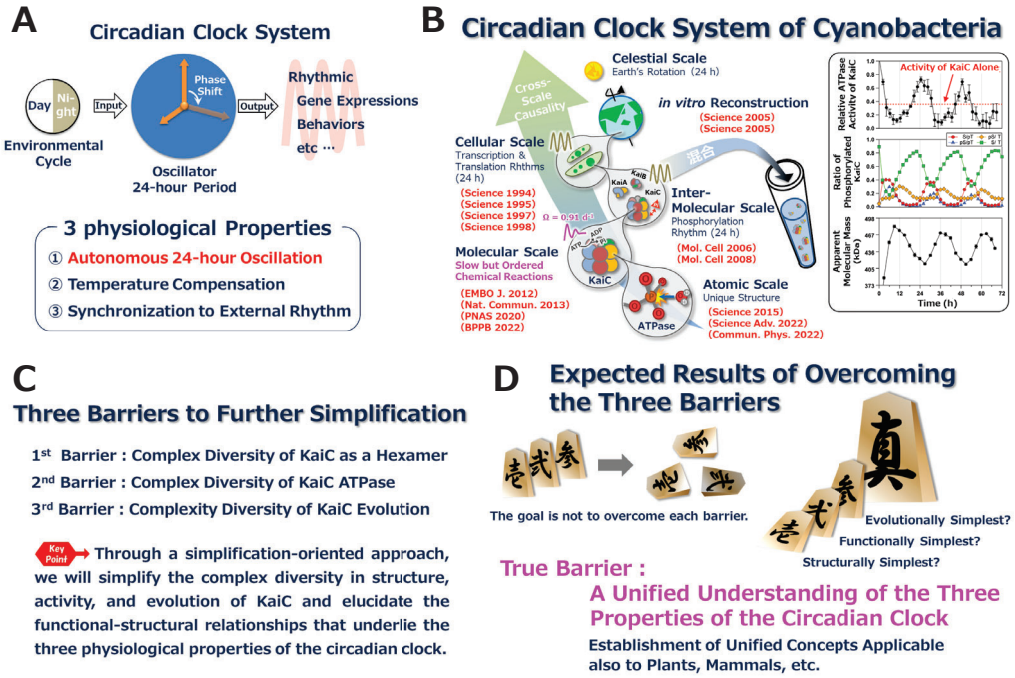

Figure 1. A Simplification-oriented Approach to Understanding the Three Properties of the Circadian Clock.

and analyzing core properties more directly. However, at the current stage, when the research stage has reached the molecular to atomic scale, we are faced with three barriers to further simplification (**Figure 1C**). In this project, through a simplification-oriented approach, we will simplify the complex diversity in structure, activity, and evolution of the clock protein KaiC and elucidate the functional-structural relationships that underlie the three physiological properties of the circadian clock [**Figure 1D**].

●1st Barrier: Complex Diversity of KaiC as a Hexamer

KaiC is composed of a C1 domain, which is responsible for the hydrolysis (ATPase) of adenosine triphosphate (ATP), and a C2 domain, which contains the phosphorylation site, forming a hexamer after binding ATP at each domain interface [**Figure 2**]. The key point is how much of the complex diversity as the hexamers remains after simplification, but it is difficult to verify this experimentally.

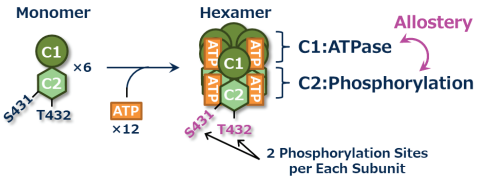

Figure 2. Hexamer Formation and ATP Binding of KaiC Consisting of C1 and C2 Domains

●2nd Barrier: Complex Diversity of KaiC ATPase

The rate of ATP consumption of KaiC is an important factor that defines the three physiological properties of the circadian clock. However, since KaiC has 12 active sites per hexamer [**Figure 2**], and the details of the reaction mechanism consuming ATP cannot be analyzed by conventional methods.

●3rd Barrier: Complex Diversity of KaiC Evolution

Whether cyanobacteria acquired a circadian clock before or after photosynthesis became active is an important question that addresses the physiological and evolutionary origins of the circadian clock.

Expected Research Achievements

●1st Barrier: Complex Diversity of KaiC as a Hexamer

Since there are two phosphorylation sites in KaiC [**Figure 2**], at which the phosphorylation and dephosphorylation occur separately, four states are defined for each subunit of KaiC. Because this four-state cycle occurs in each of the subunits that make up the hexamer, 700 different hexamers are defined even in terms of the phosphorylation state [**Figure 3**]. We will use biochemical and single-particle analyses in a complementary manner to elucidate whether all or only a small fraction of these complex diversities are essential.

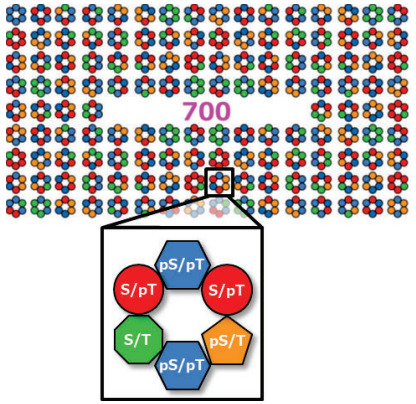

Figure 3. Diversity of KaiC Hexamers

●2nd Barrier: Complex Diversity of KaiC ATPase

We will explore the interplay of C1 and C2 active sites (Abe et al. Science 2015) by establishing a simplified method for quantifying ATP consumption by KaiC.

●3rd Barrier: Complex Diversity of KaiC Evolution

We will elucidate the physiological and evolutionary origins of the circadian clock by analyzing functional and structural aspects of ancestral KaiCs.

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