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

Title of Project : Understanding the design principle of circadian oscillator in mammals through reconstitution

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Research Area : Biological Sciences
Keyword : Synthetic biology

【Purpose and Background of the Research】

Our group has been investigating transcriptional and protein-level networks which drive mammalian circadian oscillation, and provided experimental evidences showing that the design principle of circadian-transcriptional network is negative feedback loop working with time delay [ref.1].

This mechanism, however, is difficult to explain temperature compensation -- a critical feature of circadian clock that allows the period of circadian oscillation is 24h regardless of the environmental temperature. This is because the rate of transcription, translation and protein degradation processes are generally sensitive to the reaction temperature. Interestingly, we have revealed that phosphorylation processes of CKIε/δ are rate-limiting and also temperature-insensitive step in mammalian circadian clock [ref.2]. Theoretical investigation further demonstrates that temperature-insensitive oscillator can be created only by reversible phosphorylation [ref.3].

The aim of this project is designing an autonomous and temperature-compensated oscillator based on the molecular mechanism of temperature insensitive kinase action of CKIε/δ.

【Research Methods】

To understand the temperature-compensation mechanism of CKIε/δ activity, we will firstly measure the rate of elemental process in enzymatic reaction at different temperatures: i) binding of enzyme and substrate, ii) transfer of phosphatase group and iii) release of phosphorylated products. Because most enzymatic reactions are theoretically reversible, we will also focus on the reverse reaction.

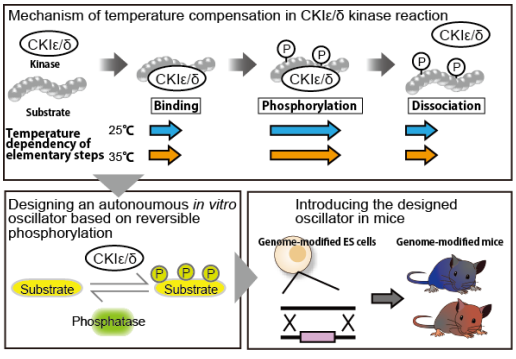

Fig1. Designing a robust oscillator.

Based on these measurements, critical steps for the temperature compensated circadian period will be revealed. CKIε/δ are then used for designing oscillator based on reversible phosphorylation *in vitro*. The substrates and phosphatases playing important roles in circadian oscillator will be investigated and included in the designed oscillator.

Finally, the temperature-compensated periodic phosphorylation scheme will be implemented into mice *in vivo* using a high-throughput method to produce genome-modified mice.

【Expected Research Achievements and Scientific Significance】

Molecular mechanism of temperature compensation in CKIε/δ reaction will provide a novel insight into the robustness of enzymatic reaction as well as specific solution to genetically/pharmacologically control the period of circadian clock.

Designing an autonomous and robust enzymatic dynamics *in vitro* and *in vivo* based on precise measurement of reaction steps will be a model case to understand a complex and dynamical system in biology through constructing one.

【Publications Relevant to the Project】

1. Ukai-Tadenuma et al, Delay in feedback repression by Cryptochrome 1 is required for circadian clock function. *Cell*, 144, 268-281 (2011)
2. Isojima et al, CKI ε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proc. Natl. Acad. Sci. USA*, 106, 15744-15749 (2009)
3. Jolly et al, A design principle for a post-translational biochemical oscillator. *Cell Reports*, 2, 938-950 (2012)

【Term of Project】

FY2013-2017

【Budget Allocation】

159,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://sys-pharm.m.u-tokyo.ac.jp/