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

Broad Section G

Title of Project：Designing the mammalian biological oscillators

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Keyword：Synthetic biology

【Purpose and Background of the Research】

We have demonstrated that the activity of Casein kinase I (CKI) δ/ε plays a critical role in the determination of the period length of mammalian circadian clocks, of which the transcription and translation loop was believed to be the core design principle of the oscillator. We revealed that CKIδ/ε's phosphorylation activity is almost constant over the physiological range of temperature and is partly responsible for the temperature compensation of mammalian circadian clocks, that is, the period length of the circadian clock does not depend on the environmental temperature (Refs 1-2). Therefore, part of the design principle of mammalian circadian oscillators lies in the phosphorylation reaction.

In vivo phosphorylation dynamics is reversibly controlled by the presence of dephosphorylation activity. The primary purpose of this study is to clarify the mechanisms responsible for the dephosphorylation reaction antagonizing the phosphorylation of CKIδ/ε in the control of mammalian circadian clocks.

【Research Methods】

We have established an in vitro system to reconstitute the CKIδ/ε phosphorylation reaction corresponding to the control of the mammalian circadian clocks. With this system, the dephosphorylation enzyme activity antagonizing phosphorylation by CKIδ/ε will be searched. Furthermore, how this dephosphorylation activity is controlled by the phase of the circadian clock will be investigated (Fig. 1). The significance of identified dephosphorylation mechanism in vivo will be rigorously tested by the circadian-functional complementary system in mice (Ref. 3).

We will then reconstitute the dephosphorylation mechanism in the in vitro CKIδ/ε assay system to ask whether the reconstituted reversible phosphorylation reaction shows the property as the oscillator of the mammalian circadian clocks. Given the fact that temperature-independent phosphorylation property is conserved in CKI homolog of yeast, which apparently shows no circadian clock function, we will also design the reversible phosphorylation system to ask whether the idea of phosphorylation-driven oscillators is applicable for the non-circadian biological oscillators.

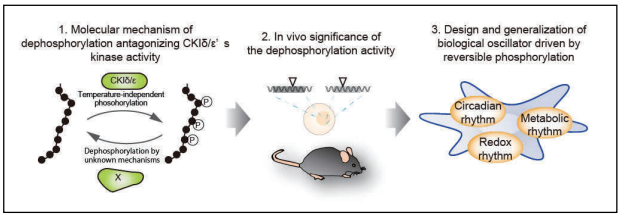

Figure 1　Oscillators driven by phosphorylation

【Expected Research Achievements and Scientific Significance】

This research will propose a new paradigm of the design principle of a mammalian oscillator by reversible phosphorylation. Also, by examining the possibility that reversible phosphorylation can drive biological oscillation involving CKI other than circadian clocks, the impact will be beyond the field of the circadian clock.

【Publications Relevant to the Project】

1. 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)
2. Shinohara *et al.*, Temperature-Sensitive Substrate and Product Binding Underlie Temperature-Compensated Phosphorylation in the Clock. *Mol. Cell*, 67, 783-798 (2017)
3. Ode *et al.*, Knockout-rescue embryonic stem cell-derived mouse reveals circadian-period control by quality and quantity of CRY1. *Mol. Cell*, 65, 176-190 (2017)

【Term of Project】 FY2018-2022

【Budget Allocation】 154,100 Thousand Yen

【Homepage Address and Other Contact Information】

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