Genetic, Genomic and Reconstructive Approaches to Understand Protein-Network-Based Prokaryotic Circadian Rhythms

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Circadian rhythms are endogenous oscillations with a period of ~24 h and observed from bacteria to higher plants and mammals. A dogmatic model has been believed in any model organisms that circadian oscillations are driven by an autoregulatory transcription/translation feedback loops. However, we recently broke this scheme in cyanobacteria (1).

Cyabacteria are the simplest organisms known to show circadian rhythms. In the cyanobacterium *Synechococcus elongatus*, almost all gene promoter activities show circadian rhythms. Such transcriptional rhythms require three clock genes, *kaiA*, *kaiB* are *kaiC*. KaiC shows circadian change in its phosphorylation state (2). We found in continuous dark conditions that the KaiC phosphorylation cycle sustained even after all clock gene transcripts disappeared and *de novo* transcription and translation were abolished in the presence of excess transcription/translation inhibitors. KaiC has both autophosphorylation and autodephosphorylation activities that are modified by KaiA and KaiB. KaiA, KaiB and C proteins form transient complexes during a circadian cycle. Thus, we proposed that a protein dynamics among the three Kai proteins is the core of circadian timing mechanism in cyanobacteria. Indeed, we succeeded in reconstitution of circadian oscillation of KaiC phosphorylation *in vitro* by incubating the three Kai proteins with ATP (3).

How does the Kai-based chemical oscillator drive genome-wide circadian transcription rhythms in *Synechococcus*? I will report DNA microarray analyses on *Synechococcus* genome-wide circadian expression profiles, and identification of the first cyanobacterial DNA-binding protein that is essential for circadian transcription. The protein is a response regulator protein in the bacterial two-component (His-to-Asp) signaling system, and is a *bona fide* cognate partner of the KaiC-interacting histidine kinase, SasA (4).

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