Robust Circadian Timing from a Three-Component Clock

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***Short Abstract* — Circadian clocks are ubiquitous biological oscillators that coordinate behavior with the daily cycling of the environment. To ensure synchronization with the environment, the phase and amplitude of the clock must be tunable by external signals while still maintaining a robust period close to 24 hours. The KaiABC cyanobacterial clock presents a unique opportunity for experiments and modeling because the core oscillator and input mechanisms can be reconstituted in a test tube using three purified proteins. Using quantitative measurements of multisite phosphorylation and enzymatic activity, we present an experimentally constrained mathematical model in which stable oscillations arise from a relaxation oscillator-like mechanism whose characteristic timescale is set by intramolecular enzymatic steps, a potentially general design for building robust timers from biochemical reaction networks.**

***Keywords* — Robustness, circadian rhythms, biochemistry, phosphorylation, oscillators, mathematical modeling.**

I. Introduction

CIRCADIAN clocks are endogenous oscillatory systems that allow organisms to anticipate daily rhythmic variations in the external environment. The functional properties of circadian clocks are remarkably conserved across most organisms that have been studied. Even when deprived of rhythmic input, circadian clocks continue to generate self-sustaining oscillations that have a robust period close to 24 hours [1]. In contrast, the phase and amplitude of the circadian rhythm are generally plastic, and input signals can reset the phase to rapidly bring the clock into synchrony with a rhythmic environment.

Remarkably, a core circadian oscillator from the cyanobacterium *Synechococcus elongatus* can be biochemically reconstituted using three purified proteins: KaiA, KaiB and KaiC [2]. This network of purified proteins can accept input signals encoded in the ATP/ADP ratio and generates stable ~24 hour oscillations in KaiC multisite phosphorylation [3,4]. This biochemical oscillator displays robustness properties that are similar to the clock of an intact organism: altering protein concentration or introducing small molecule input signals gives rise to phase shifts and altered amplitude, but oscillations persist with a period that is robustly close to 24 hours [4].

II. Results

Here we report a combined biochemical and mathematical modeling-based study of the robustness of the circadian rhythm. KaiC consists of two homologous catalytic domains: one domain is an autokinase goes through a well-studied sequence of autophosphorylation, and the other has ATPase activity but does not become phosphorylated. We show that the autokinase domain is inhibited by input signals (ATP/ADP ratio), but the ATPase domain is insensitive and maintains an invariant, slow activity. By selectively mutating conserved residues, we show that both phosphorylation and progression through a slow ATPase cycle are required to form inhibitory complexes and close the negative feedback loop that drives oscillations.

We propose a compensatory scheme where the ATPase domain acts as a slow invariant delay, and the autokinase integrates input signaling by accumulating phosphorylation while waiting for ATPase catalysis to occur. In this architecture, the amplitude decreases when kinase rates are inhibited, but the system retains ~24-hour timing. We show that a mathematical model with enzymatic rate constants estimated from biochemical assays is able to reproduce the robustness effects seen in the full oscillator [5].

III. Conclusion

We propose a model with sequential slow enzymatic reactions that have differential input sensitivity as a means for generating tunable oscillations with a robust period. We show that the design principles of this model are implemented by the biochemistry of the KaiABC circadian oscillator. We suggest that a generalized architecture of this type may find wide use throughout biology where there is a requirement for robust timing.

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References

[1] Winfree AT (2000) *The Geometry of Biological* Time. Springer-Verlag, New York.
[2] Nakajima M, *et al*. (2005) *Science* **308,** 414.
[3] Rust MJ, *et al.* (2007) *Science* **318**, 809.
[4] Rust MJ, Golden SS, O'Shea EK. (2011) *Science* **331**, 220.
[5] Phong C, *et al.* (2013) *PNAS* **110**, 1124.