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主　論　文　の　要　旨

論文題目　**ATPase activity of KaiC is a key determinant of the circadian oscillation of cyanobacterial KaiABC clock (シアノバクテリア由来の KaiABC 概日振動子において KaiC による ATP 加水分解が果たす重要な役割について)**

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論　文　内　容　の　要　旨

Circadian clock is a biological machinery inducing a rhythm of approximately 24 hours, which pertains to all kingdoms of life and helps to organize the behavior of organisms with the day-night cycle. In the test tube, the circadian rhythm of cyanobacterial Kai proteins can be reconstituted when three Kai proteins, KaiA, KaiB and KaiC, are incubated in the presence of ATP; the phosphorylation level of KaiC exhibits a robust circadian oscillation. KaiC forms a hexamer complex and shows ATPase activity; each subunit of KaiC hexamer hydrolyzes about 15 ATP molecules/day. This ATPase activity is very low compared to the other ATPase enzymes and plays an important role in defining the circadian period. In particular, the frequency of the phosphorylation cycle of KaiC is closely correlated to the ATPase activity of KaiC. Furthermore, the ATPase activity of the truncated CI domain of KaiC shows correlation to the circadian frequency in the CII domain. All these observations suggest that ATPase activity in the CI domain plays a central role to generate the circadian period of the Kai oscillator. However, how the ATP hydrolysis in the CI domain drives the oscillation remains elusive. Here, we quantitatively investigate the role of ATP hydrolysis of KaiABC clockwork using mathematical modeling. We thereby propose two coarse-grained theoretical models, the many-molecule model (MM) and the single-molecule

model (SM) to understand the macroscopic synchronization of a large number of KaiC molecules and the microscopic reactions and structural transition in individual KaiC molecules; these two models were developed to unify macroscopic and microscopic viewpoints of KaiC system. Our models are based on the assumptions that conformational transition of KaiC hexamer takes place between two structural states and that the binding interactions among Kai proteins, phosphorylation/dephosphorylation reactions, and ATP hydrolysis depend on this transition. We also assume that the structural transition is induced by these reactions; therefore, multifold feedback relations are constituted among reactions and structural transition in the proposed models. Results of these two theoretical models give insights to elucidate the role of ATP hydrolysis. In the simulation results with these models, ATP hydrolysis in the CI domain of KaiC hexamer is a driving mechanism of the oscillation of individual KaiC hexamers, which brings oscillation in the ensemble-level molecules and the ATP hydrolysis is necessary for synchronizing oscillations of a large number of KaiC hexamers. Sensitive temperature dependence of the lifetime of the ADP bound state in the CI domain makes the oscillation period temperature insensitive. ATPase activity is correlated to the frequency of phosphorylation oscillation in a single molecule of KaiC hexamer, which should be the origin of the observed ensemble-level correlation between the ATPase activity and the frequency of phosphorylation oscillation. Thus, the simulation results with the MM and SM models suggest that ATP hydrolysis stochastically occurring in each CI domain of individual KaiC hexamers is a key process for oscillatory behaviors of the ensemble of many KaiC hexamers.