Endogenous clock-mediated regulation of intracellular oxygen dynamics is essential for diazotrophic growth of unicellular cyanobacteria

Anindita Bandyopadhyay, Annesha Sengupta, Thanura Elvitigala, Michelle Liberton, Alexandra Siemer and Himadri B. Pakrasi

Washington University, 1 Brookings Drive, St. Louis, MO 63130
anindita@wustl.edu

The ability of unicellular diazotrophic cyanobacteria to perform nitrogen fixation and photosynthesis in the same cellular platform is an enigma that continues to intrigue biologists. The observation made decades ago, that unicellular cyanobacteria can perform nitrogen fixation under continuous light, provided the first clues to the existence of a circadian clock in prokaryotes. However, owed to their recalcitrance to any genetic manipulation, the clock-mediated segregation of processes remained largely unexplored in this group of prokaryotes. To investigate its function in these diazotrophs, we disrupted the circadian clock defined by the *kaiABC* genes in the now well-established model strain *Cyanothece* ATCC 51142. Unlike non-diazotrophic cyanobacteria, *Cyanothece* 51142 exhibits conspicuous self-sustained rhythms in various discernable phenotypes, offering a platform to directly study the effects of the clock on the physiology of an organism. Disrupting the clock by deleting *kaiA* led to impairment in nitrogen fixation and growth under continuous light or long day length conditions. Under such conditions, the conspicuous endogenous rhythms in oxygen cycling observed in the WT was disrupted in the mutant, suggesting that a loss in the regulation of oxygen cycling is detrimental to nitrogenase function and growth. This work provides the first molecular evidence of the involvement of the circadian clock in segregating essential yet incompatible processes in unicellular diazotrophic cyanobacteria. Our findings suggest that the addition of KaiA to the KaiBC clock was likely an adaptation that ensured optimal nitrogen fixation as microbes evolved from an anaerobic to an aerobic atmosphere under nitrogen constraints.

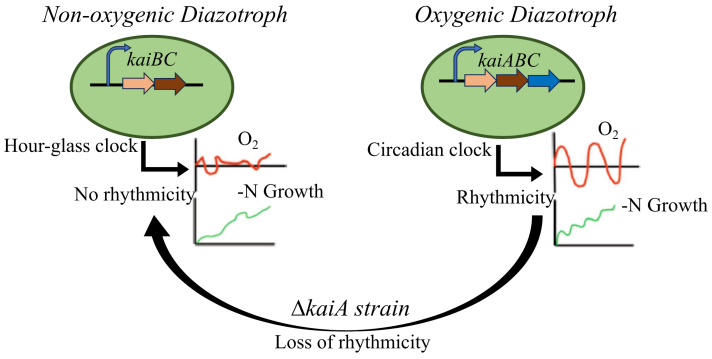

Figure 1. Schematic showing the importance of KaiA in regulating cellular oxygen dynamics in unicellular diazotrophic cyanobacteria.

This work was supported by the National Science Foundation (MCB 1933660) and by the U.S. Department of Energy (DOE), Office of Science Energy Earthshot Initiative, as part of the CyaNofertilizer project (DE-SC0024702).