BACTERIAL PHYSIOLOGY

All around the clock

The coexistence of photosynthesis and nitrogen fixation in marine unicellular cyanobacteria presents a challenge because of their high iron demands and also because the molecular oxygen generated by the first pathway inhibits the second. This conflict is apparently solved by an internal circadian clock which determines that nitrogen fixation is carried out only during the night, when photosynthesis is turned off. However, the mechanisms for transduction of environmental information into the clock and for the ordered alternation of photosynthesis and nitrogen fixation have remained unclear. Two papers now report that the circadian clock is directly entrained by changes in energy metabolism and that key enzymes involved in photosynthesis and nitrogen fixation share a common pool of iron atoms.

> "the circadian clock is directly entrained by changes in energy metabolism"

In the marine cyanobacterium *Synechococcus elongatus*, the core circadian clock consists of three proteins, KaiA, KaiB and KaiC, that mediate daily changes in gene expression. KaiA and KaiB modulate the autophosphorylation of KaiC, resulting in stable oscillations in the amount of phosphorylated KaiC. To study how changes in illumination affected the circadian clock, Rust and co-workers used synchronized cultures of a strain of *S. elongatus* that carried a clock-driven bioluminescent reporter. When placed under constant illumination, the cultures retained a subjective 'day–night' cycle of reporter activity. Pulses of darkness produced a decrease in the cellular ATP/ADP ratio; however, only those pulses applied during the subjective 'day' induced phase shifts in the circadian clock and the rhythm of KaiC phosphorylation. Remarkably, manipulation of the ATP/ADP ratio in an *in vitro* system containing purified KaiA, KaiB and KaiC resulted in similar phase shifts in the rhythm of KaiC phosphorylation. Further *in vitro* experiments indicated that increased ADP concentrations alter the oscillation phase through inhibition of the kinase activity of KaiC. Thus, KaiC seems to integrate clock information (through the kinase-stimulating activity of KaiA) with metabolic information (through the adenosine pool).

Saito and colleagues used proteomic analyses of cultures of *Crocosphaera watsonii* (another marine cyanobacterium that fixes nitrogen and photosynthesizes) to study possible adaptations to the low-iron environment. Large-scale daily changes were observed in the bacterial proteome; in particular, certain iron-containing components of the photosynthesis machinery were partially degraded during the night, whereas key nitrogen fixation metalloenzymes showed near complete degradation during the day. This proteomic cycling probably prevents oxygen disruption of the nitrogenase complex and results in a daily recycling of iron atoms to provide a ~40% reduction in the metabolic iron demand. Incorporating these findings into a global ocean and ecosystem model highlighted the competitive advantage of this strategy in iron-poor sea water.

Together, these reports shed new light on the physiology of marine cyanobacteria. Remarkably, the internal clock is directly entrained by the availability of biochemical energy in the cell (which ultimately depends on light as an energy source) and, thus, no dedicated signalling pathway seems to be required. Also, an energetically expensive strategy — the daily synthesis and degradation of abundant iron-containing enzymes — seems advantageous in the sea, where iron is scarce. These findings are significant, as cyanobacteria are major contributors to oxygen production and nitrogen fixation in the oceans.

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ORIGINAL RESEARCH PAPERS Rust, M. J., Golden, S. S. and O'Shea, E. K. Light-driven changes in energy metabolism directly entrain the cyanobacterial circadian oscillator. *Science* **331**, 220–223 (2011) | Saito, M. A. *et al.* Iron conservation by reduction of metalloenzyme inventories in the marine diazotroph *Crocosphaera watsonii*. *Proc. Natl Acad. Sci. USA* 19 January 2011 (doi: 10.1073/pnas.1006943108)

FURTHER READING Golden, S. S. & Canales, S. R. Cyanobacterial circadian clocks — timing is everything. *Nature Rev. Microbiol.* **1**, 191–199 (2003)