

This study analysis the transcriptome of *Microcystis aeruginosa* PCC7806 cultured under a L/D cycle and describes a clear diel rhythm for the gene expression of *ccyA*, a gene documented in the literature for a putative role in intracellular CaCO<sub>3</sub> precipitation in cyanobacteria, i.e. intracellular amorphous calcium carbonates (iACC). Hence a roughly 2.5 variation in abundance has been observed with highest values of *ccyA* expression at the end of the dark period. Nevertheless, the exact role of *ccyA* remains unknown and the documentation of its diel rhythm assumes that it can be used as a marker gene to learn about the function of this intracellular CaCO<sub>3</sub> precipitation (iACC) in cyanobacteria. As the authors write it could be linked to CCM, intracellular pH buffering, and creating “ballast” for regulating buoyancy and floatability. In their Introduction and Discussion the authors neglect that such functions for iAAC would imply a necessity of mechanisms for the dissolution of iAAC in concert with its precipitation; fine-tuning of both resulting in homeostasis or cyclic temporal patterns of increasing decreasing iAAC.

To obtain more indications about the role of iACC precipitation the authors have studied the transcriptomes very largely, to detect gene-expressions with temporal patterns that positively or negatively (anticorrelate) with *ccyA*. A particular interest was put on neighboring genes (both upstream and downstream) to detect a possible operon comprising *ccyA* with other genes. Very interestingly they discovered that some genes coding for Ca<sup>2+</sup>/H<sup>+</sup> antiporter systems, which occur as neighbors showed transcripts with abundances that correlate with the *ccyA*. Hence, the existence of an operon with *ccyA* and these Ca<sup>2+</sup>/H<sup>+</sup> antiporter systems seems plausible. It can be considered as the other main finding of this study.

Strikingly, most CCM transcripts are higher during daytime, while some others are higher during night time. This may appear confusing (in the Discussion the authors explain it by CCM being coupled to photosynthesis and therefore more related to daytime), but is perhaps more interesting than suggested. We realize that CCM comprises a multitude of inorganic carbon uptake systems differing by use of substrate (CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup>) and different affinities and maximum uptake rates, that can operate in parallel and can be complementary. It could thus be envisioned that some of them are better suited to accumulate inorganic carbon during night time, and why not we can hypothesize that such nighttime CCM operates in concert with the iAAC precipitation to create a temporal stock of sequestered inorganic carbon that could be liberated during daytime.

In the Introduction the authors mention that diel patterns are controlled by Circadian rhythms, but this is not necessarily the case as the control of gene expression may also be based on sensing physiological conditions as e.g. pH, amount of storage compounds etc. A first indication whether a Circadian clock is involved can be obtained by transferring the culture to continuous light conditions (the cycle should then be maintained for several roughly 24 h periods). Has it been studied whether this strain PCC7806 has a Circadian clock? For another strain of *Microcystis aeruginosa* PCC7820 this has been documented by Huang et al (Huang, J., Wang, J. & Xu, H. The circadian rhythms of photosynthesis, ATP content and cell division in *Microcystis aeruginosa* PCC7820. *Acta Physiol Plant* **36**, 3315–3323 (2014). <https://doi.org/10.1007/s11738-014-1699-1>) and the authors may consider citing this paper and any others that could support the occurrence and role of a Circadian clock in PCC7806. The authors could perhaps even check if genes for KaiA3 and KaiB3-KaiC3 or analogues are expressed in this strain PCC7806.

Please could you consider these aforementioned points and adapt the Introduction and Discussion section, accordingly. In addition I have spotted a number of editorial points that need correction:

**A most general important point: At least, in the bioRxiv preprint,** the quality of the Figures is generally low, particularly of Figs. 4 and 6, and also 3. Please can you provide better quality Figures with more attractive lay-out and easier to read. This will be very important to improve the understanding for the readers and thus the impact of your paper.

Line 113 : Methods: In the Methods, first point to measure is the species and strain used (with its reference allowing to obtain the strain), before describing the culture conditions.

Line 115 : (50 mmol photons.m<sup>-2</sup>.s<sup>-1</sup> appears excessively high (20 to 25 times Zenith intensities outside in the Tropics), I think it should be (50 μmol photons.m<sup>-2</sup>.s<sup>-1</sup> (micromolar), which is low but not surprising when using artificial light.

Line 163 : rephrase “Raw RNA-seq reads (available online, see reference Raw transcriptomics data)” by “Raw RNA-seq reads (available online, see section Data, scripts, code, and supplementary information availability).

**Upon publication you should make these data available (relieve the private constraint).**

Line 210-211 : Each replicate (i.e. three independent cultures) at a single time showed only minor variations between them, at least along axis 1 (accounting for almost 50% of the variance), and were distinctly separated from the replicates at other time points.” – should be reformulated as follows: “Among the triplicates (i.e. three independent cultures for each time-point) only minor variations were observed, at least along axis 1, and their values were clearly separated from the samplings at other time points.”

Line 240 : replace “Figure 2 - Abundance profile of ccyA transcripts during a day/night cycle” by “Figure 2 – Time course of the abundance of ccyA transcripts during a day/night cycle”

Please use terminology consistently

Note that diurnal = during daytime (i.e. L period) as opposed to nocturnal (during night or D period).

Diel = variation during the entire 24 h cycle comprising both L (day) and D (night) periods.

To prevent confusion, please consistently use either the term “log base 2” or the term “binary logarithm” but not both (personally I prefer “log base 2”, which is more commonly used and clear). You may also use the mathematical formulation “log<sub>2</sub> (x)”

End of Review