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The Response Regulator Npun_F1278 is Essential for Scytonemin Biosynthesis in the Cyanobacterium Nostoc Punctiforme ATCC 29133

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Abstract
Following exposure to long-wavelength ultraviolet radiation (UVA), some cyanobacteria produce the indole-alkaloid sunscreen scytonemin. The genomic region associated with scytonemin biosynthesis in the cyanobacterium Nostoc punctiforme includes 18 adjacent co-transcribed genes. A two-component regulatory system (Npun_F1277 and Npun_F1278) directly upstream of the biosynthetic gene cluster was identified through comparative genomic analysis and is likely involved in scytonemin regulation. In this study, the response regulator Npun_F1278 was evaluated for its ability to regulate scytonemin biosynthesis using a mutant strain of N. punctiforme deficient in this gene, hereafter strain Δ1278. Following UVA radiation, the typical stimulus to initiate scytonemin biosynthesis, Δ1278 was incapable of producing scytonemin. A phenotypic characterization of Δ1278 suggests that, aside from the ability to produce scytonemin, the deletion of Npun_F1278 does not affect the cell morphology, cellular differentiation capability, or lipid-soluble pigment complement of Δ1278 compared to the wild type. The mutant, however, had a slower specific growth rate and the amount of phycocyanin yielded by Δ1278 differed from the wild type. Given the inability of Δ1278 to produce scytonemin, this study demonstrates that the response regulator Npun_F1278 in N. punctiforme is essential for scytonemin biosynthesis. While most of the evaluated effects of this gene appear to be specific for scytonemin, this regulator may also influence the overall health of the cell and specifically, phycobiliprotein biosynthesis. This is the first study to identify a regulatory gene involved in the biosynthesis of the sunscreen scytonemin and understanding the transcriptional regulation of scytonemin biosynthesis may allow for mass production in future applications.

Methods
Scytonemin Biosynthesis: Scytonemin was induced following exposure to UVA for one week and the pigments were measured by absorbance at 384 nm. A comparative genomic analysis of scytonemin biosynthesis in N. punctiforme using strain Δ1278 suggested that, aside from the ability to produce scytonemin, the deletion of Npun_F1278 does not affect the cell morphology, cellular differentiation capability, or lipid-soluble pigment complement of Δ1278 compared to the wild type. The mutant, however, had a slower specific growth rate and the amount of phycocyanin yielded by Δ1278 differed from the wild type. Given the inability of Δ1278 to produce scytonemin, this study demonstrates that the response regulator Npun_F1278 in N. punctiforme is essential for scytonemin biosynthesis. While most of the evaluated effects of this gene appear to be specific for scytonemin, this regulator may also influence the overall health of the cell and specifically, phycobiliprotein biosynthesis. This is the first study to identify a regulatory gene involved in the biosynthesis of the sunscreen scytonemin and understanding the transcriptional regulation of scytonemin biosynthesis may allow for mass production in future applications.

Results
Table 2. Transcript abundance of scyA in the wild type and Δ1278 mutant strain following 48 hrs of UVA irradiation.

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Strain Δ1278 was unable to produce scytonemin following UVA exposure compared to the wild type under both conditions (Table 2). During logarithmic growth under white light, the wild type was 0.335 ± 0.10 (doublings per day, g) while the mutant strain Δ1278 was lower at 0.139 ± 0.08 (n = 3, p ≤ 0.05). Despite these differences, deletion of Npun_F1278 did not appear to affect the production of chlorophyll a, the carotenoids (Figs. 4-5), or the phycobilin pigment phycocerythrin (Table 1) under white light or UVA. There was, however, a significant increase in the amount of phycocyanin produced by the mutant strain compared to the wild type under both conditions. Furthermore, since scyA gene expression was minimal, the absence of the Δ1278 gene in strain Δ1278 may not only affect the ability of the cell to respond to UVA, but also impair any scytonemin production due to basal expression of Δ1278. With an impaired regulatory gene, it is unclear as to what extent the UVA/light-associated stress response is affected in strain Δ1278.

Conclusions
In this study we sought to determine the relationship between the putative response regulator gene Npun_F1278 and scytonemin biosynthesis in the cyanobacterium Nostoc punctiforme. This was demonstrated visually through microscopy, spectroscopically through pigment extraction and analysis, and molecularly through scyA transcript quantification. Future studies will continue to evaluate how Δ1278 responds to a variety of light-associated conditions at both the physiological and molecular levels.

References

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