Protein abundance dynamics in the unicellular cyanobacterium *Cyanothece* ATCC 51142 during light-dark cycle

Uma K. Aryal[1], Jon M. Jacobs[1], Jana Stöckel[2], Carrie D. Nicora[1], Himadri B. Pakrasi[2], David W. Koppenaal[1], Louis A. Sherman[3] and Richard D. Smith[1]

[1]Biological Science Division, Pacific Northwest National Laboratory, WA, [2]Department of Biology, Washington University St. Louis, MO, [3]Department of Biological Sciences, Purdue University, West Lafayette, IN

Pacific Northwest NATIONAL LABORATORY

Overview

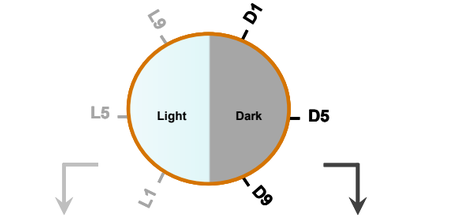
- *Cyanothece* 51142 is a unicellular marine cyanobacterium capable of temporally separating oxygenic photosynthesis and N_2-fixation in a single cell.
- Goal: To characterize the cellular strategy utilized to achieve temporal separation of these processes.
- Time-course pulse metabolic labeling combined with mass spectrometry (MS) to determine dynamics of protein abundance changes in response to diurnal growth. Enzymatic data confirmed that N_2-fixation is limited to the dark cycle.
- Results suggest that *Cyanothece* can utilize various metabolic processes for acquisition and recycling of ammonia under nitrogen limitation.

Introduction

- Cyanobacteria are able to perform oxygenic photosynthesis, a key metabolic process that converts light to chemical energy. Many strains have also evolved strategies for reducing atmospheric nitrogen to ammonia, referred as biological nitrogen fixation.
- To accommodate the two incompatible processes, the intracellular environment oscillates between aerobic and anaerobic conditions during a day-night cycle; photosynthesis occurring during the day and N_2-fixation at night.
- Previous studies have examined changes at the transcript level during diurnal cycle[1], but protein level regulation of the two biological processes is unclear. To better understand how they synchronize these two incompatible biological processes, we *in-vivo* metabolically labeled cells in *Cyanothece* sp. ATCC 51142 and determined the dynamic changes in protein abundances over time during growth.

Methods

- *Cyanothece* cells were first grown in normal ASP2 medium without $NaNO_3$ under 12 h light-dark conditions at 30°C with ambient air bubbling for 7 days. Then, heavy isotope of leucine ($^{13}C^{15}N$-Leu) was added to the same growing culture at 95 μg/mL of the culture.
- Following addition of labeled isotope, cells were grown for 12 h before sampling. Time point samples were collected every 4 h for two consecutive diurnal cycles.



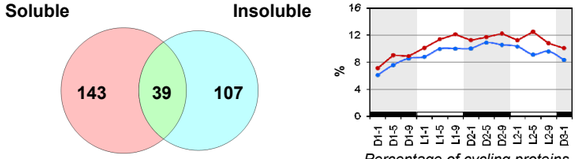
Overview of sample collection and preparation

- Soluble and insoluble proteins from each point were tryptically digested, and subjected to capillary liquid chromatography (LC) combined with tandem MS (MS/MS) to identify peptides and create an accurate mass and time (AMT) tag database[2].
- Time-course pulse metabolic labeling strategy utilizes incorporation of stable isotope labeled amino acid to identify new protein synthesis. Dynamic quantitative information for this new protein was obtained by measuring peak intensities of labeled versus natural version of the same peptides.

CONTACT: Uma K Aryal, Ph.D.
Biological Sciences Division, K8-98
Pacific Northwest National Laboratory
P.O. Box 999, Richland, WA 99352
E-mail: Uma.Aryal@pnl.gov

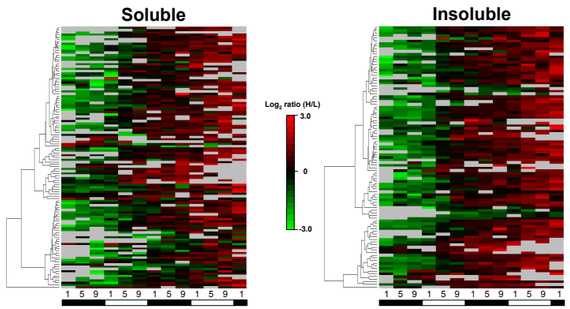
Results

Overview of proteins with dynamic expression



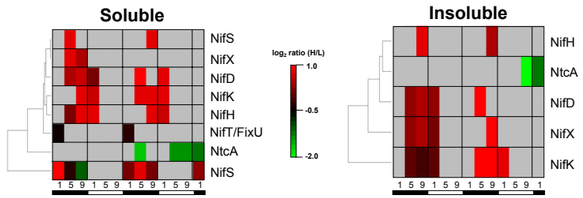
Cycling proteins with differential abundances

Percentage of cycling proteins over total identification



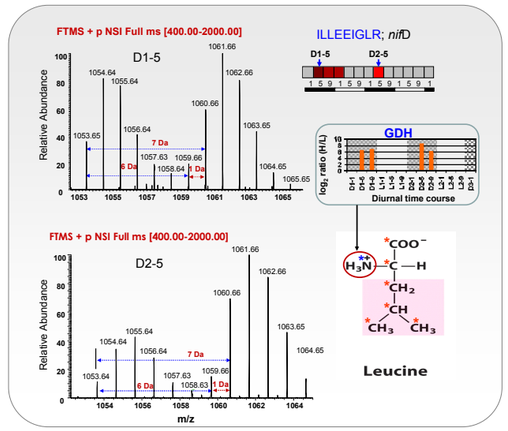
Changes in protein abundances at different time points. Proteins were hierarchical clustered based on similarities of heavy to light peptide ratios. Color patterns indicate higher rate of heavy isotope incorporation for new protein synthesis as cells continue to grow.

Expression of various nitrogenase



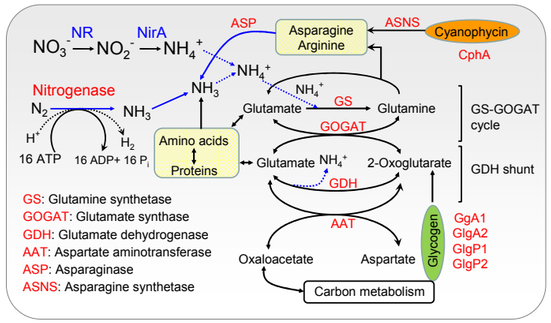
Nitrogenase showed strong cycling and synchronous expression in both fractions and patterns were mostly similar in two consecutive diurnal cycles. ***Interestingly, NtcA, a global nitrogen regulator was only transiently expressed and expression was not synchronized with nitrogenase.***

Incorporation of heavy isotope into protein



Spectra showing heavy isotope incorporation (7 Da mass shift) into protein. D1-5, and D2-5 represent samples collected 5 h into dark cycle 1 and 2, respectively. Spectra show higher incorporation at later time point. Interestingly, a loss of 1 Da mass was also evident which might be due to selective scavenging of ammonia from the heavy isotope.

Pathway for nitrogen metabolism



Results provide evidence that Cyanothece 51142 utilizes various metabolic pathways for nitrogen assimilation and internal re-mobilization.

Conclusions

- Enzymes involved in N_2-fixation displayed strong cycling behavior with maximum expression in the dark, and near complete degradation during the light period, showing that N_2-fixation is limited to the dark cycle.
- Proteins involved in photosynthesis, respiration, and phycobilisome proteins also showed diurnal oscillation, but their changes in abundances were not as tightly regulated as nitrogenase.
- *Cyanothece* appears to utilize multiple metabolic pathways, including GS-GOGAT pathway and GDH shunt, for nitrogen assimilation. Expression of GDH only in the dark suggests its possible role in ammonia detoxification and internal mobilization.
- MS data also indicated nitrogen scavenging from heavy labeled amino acid.
- Nitrate reductase (NR) and nitrite reductase (NiR) were not differentially expressed. Interestingly, NtcA, a global nitrogen regulator was only transiently expressed at the late time points, which suggests that NtcA may not be involved in regulating nitrogen fixation. Expression of NR, NiR and NtcA seems to be nitrate dependent.
- This study provides novel information concerning the impact of temporal separation of photosynthesis and N_2-fixation processes on global proteome regulation, and provides an important resource for future studies involving in-depth analysis of unique metabolic pathways in cyanobacteria.

Acknowledgements

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References

1. Stöckel, J. et al. "Global transcriptomic analysis of *Cyanothece* 51142 reveals robust diurnal oscillation of central metabolic processes. *Proc Natl Acad Sci USA* **105**, 6156-6161 (2008).
2. Jacobs, J.M. et al. "Ultra-sensitive, high throughput, and quantitative proteomic measurements". *Int J Mass Spectrom* **240**, 195-212 (2005).