

SCIENTIFIC REPORTS

OPEN

Multi-Omic Dynamics Associate Oxygenic Photosynthesis with Nitrogenase-Mediated H₂ Production in *Cyanothece* sp. ATCC 51142

Received: 01 July 2015
Accepted: 21 September 2015
Published: 03 November 2015

Hans C. Bernstein^{1,2}, Moiz A. Charania², Ryan S. McClure², Natalie C. Sadler², Matthew R. Melnicki², Eric A. Hill², Lye Meng Markillie², Carrie D. Nicora², Aaron T. Wright², Margaret F. Romine² & Alexander S. Beliaev²

To date, the proposed mechanisms of nitrogenase-driven photosynthetic H₂ production by the diazotrophic unicellular cyanobacterium *Cyanothece* sp. ATCC 51142 have assumed that reductant and ATP requirements are derived solely from glycogen oxidation and cyclic-electron flow around photosystem I. Through genome-scale transcript and protein profiling, this study presents and tests a new hypothesis on the metabolic relationship between oxygenic photosynthesis and nitrogenase-mediated H₂ production in *Cyanothece* 51142. Our results show that net-positive rates of oxygenic photosynthesis and increased expression of photosystem II reaction centers correspond and are synchronized with nitrogenase expression and H₂ production. These findings provide a new and more complete view on the metabolic processes contributing to the energy budget of photosynthetic H₂ production and highlight the role of concurrent photocatalytic H₂O oxidation as a participating process.

Photobiological H₂ production is still a nascent technology with long-term potential for sustainable energy production with a low environmental impact. Although direct biophotolytic H₂ production has been documented and studied for decades¹, significant challenges remain for the development of microbial strains and conditions that can directly and efficiently use sunlight and water to produce H₂. Chief among them is the low production rate, which is largely due to feedback inhibition of the H₂ producing enzymes by O₂, an obligate byproduct of oxygenic photosynthesis. Limitations imposed by O₂ sensitivity of the native hydrogenase and nitrogenase enzymes have motivated significant efforts to identify and even engineer O₂ tolerant variants² and multi-stage processes that temporally separate O₂ and H₂ evolution³. However, to date, the kinetic rates and sustainability of hydrogenase-mediated H₂ production are low in comparison to those reported for some diazotrophic organisms that produce H₂ in oxic-environments as a byproduct of nitrogenase catalyzed N₂ fixation⁴⁻⁶.

Nitrogen-fixing cyanobacteria have been recognized as one of the most promising photolytic platforms for sustainable H₂ production⁷⁻¹¹. A unicellular marine strain *Cyanothece* sp. ATCC 51142 (hereafter *Cyanothece* 51142) has emerged as a model system because of its ability to produce H₂ at rates > 100 μmol-H₂ hr⁻¹ mg-Chl⁻¹ under photosynthetic conditions associated with continuous

¹Chemical and Biological Signature Science, Pacific Northwest National Laboratory, Richland, WA 99352.

²Biological Sciences Division, Pacific Northwest National Laboratory, Richland WA, 99352. Correspondence and requests for materials should be addressed to H.C.B. (email: hans.bernstein@pnnl.gov) or A.S.B. (email: alex.beliaev@pnnl.gov)

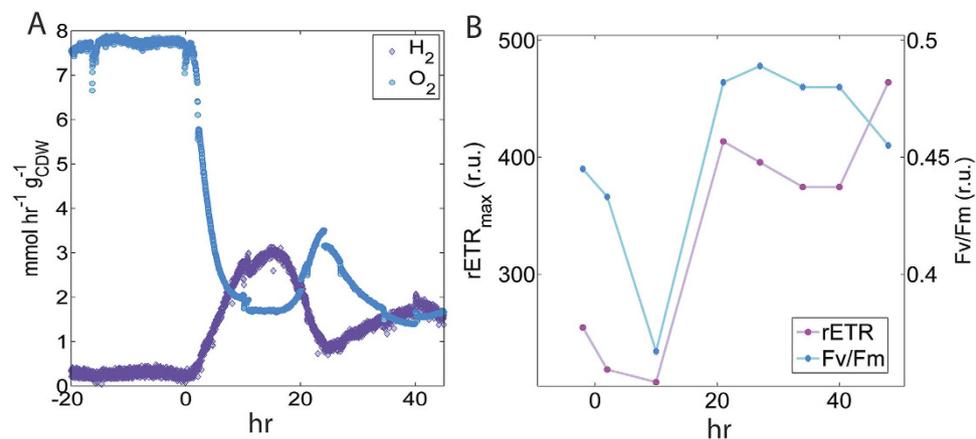


Figure 1. (A) Specific rates of net H₂ and O₂ production. Time zero indicates onset of nitrogen-depletion and absence of media addition (dilution rate = 0). (B) Variable chlorophyll fluorescence originating from PS II: the maximum relative electron transfer rate (rETR_{max}) and the optimal quantum yield (Fv/Fm). Data plotted at times prior to t = 0 represent measurements taken during the ammonia limited chemostat (i.e., steady-state precondition).

illumination^{7,12}. Although recent genome-enabled studies have provided systems-level insights into the mechanisms governing diurnal growth and metabolism of *Cyanotheca* 51142^{7,13–17}, the processes that support H₂ production in this organism have yet to be fully resolved. The current and prevailing view assumes that H₂ production mediated by energetically expensive nitrogenase activity in *Cyanotheca* 51142, and other closely related strains, is exclusively supported by ATP and reductant derived from oxidation of intracellular glycogen and/or cyclic-electron flow around photosystem (PS) I^{7,13,18}. Here we present evidence to support a new model whereby energy derived directly from oxygenic photosynthesis (i.e., linear electron flow through PS II) is an important process in funding the energy budget required for nitrogenase activity under illuminated, nitrogen-deplete conditions. These conclusions are supported by a combined analysis of detailed process and integrated transcriptome and proteome profiles across a photosynthetically driven H₂ production process.

Results

H₂ production kinetics. High levels of H₂ production were achieved by interrupting medium flow to an ammonium-limited chemostat of *Cyanotheca* 51142, grown in an Ar/CO₂ atmosphere under continuous illumination (Fig. 1). The resulting N-depletion yielded metabolically active but non-growing cells, as confirmed by constant cell dry weight (CDW) concentrations (0.086–0.005 g_{CDW} L⁻¹) (Fig. S1). The maximum specific rate of H₂ production (q_{H₂}) by *Cyanotheca* 51142 was reached after 14.5 hours of N-depletion and measured to be 3.12 mmol-H₂ hr⁻¹ g⁻¹_{CDW} (279 μmol-H₂ mg-Chl a⁻¹ hr⁻¹) which, to our knowledge, is the highest reported rate of H₂ production per unit biomass under photoautotrophic conditions⁷. H₂ production persisted throughout the experiment and is known to be sustainable through this method for more than 100 hours¹². The average specific rates were 2.24 and 1.70 mmol-H₂ hr⁻¹ g⁻¹_{CDW} over 20 and 40 hours, respectively. Notably, the *Cyanotheca* 51142 cells were maintained under continuous irradiance under all culturing steps; hence, H₂ production was not dependent on diel cycling as reported in previous studies^{7,14}.

Concurrent oxygenic photosynthesis. In this study, H₂ production was only measured under aerobic conditions maintained by active oxygenic photosynthesis (i.e., PS II activity) in the bioreactor environment. Dissolved O₂ concentrations ranged from steady-state values of 10 μM to 2.4 μM, observed during H₂ production. As this specific rate of O₂ production (q_{O₂}) is non-zero and represents the net rate of photosynthesis, whereby the gross rate of O₂ evolution is greater than the rate of respiration¹⁹; concurrent observations of O₂ and H₂ production provide direct evidence of energy acquisition from photocatalytic H₂O oxidation via linear electron flow through PS II. Electrons originating from H₂O are available for photosynthetic electron transport, which can result in proton reduction and generation of the proton motive force. The q_{O₂} values ranged from 7.5 ± 0.2 mmol-O₂ hr⁻¹ g⁻¹_{CDW} to 1.4 mmol-O₂ hr⁻¹ g⁻¹_{CDW} for steady-state and N-deplete conditions, respectively (Fig. 1). Net photosynthesis rates decreased immediately upon initiation of N-depletion but then increased inversely with q_{H₂}. The capacity for linear electron flow through PS II was also confirmed via measurements of variable Chl *a* fluorescence (Fig. 1B). The maximum relative rates for electron transport (rETR_{max}) increased throughout the H₂ production profile and at their lowest levels were near the values measured from the steady-state precondition. The optimal photochemical quantum yield of PS II (Fv/Fm) decreased initially with the onset of N-depletion but then increased beyond the steady-state value for the remainder of the H₂ production

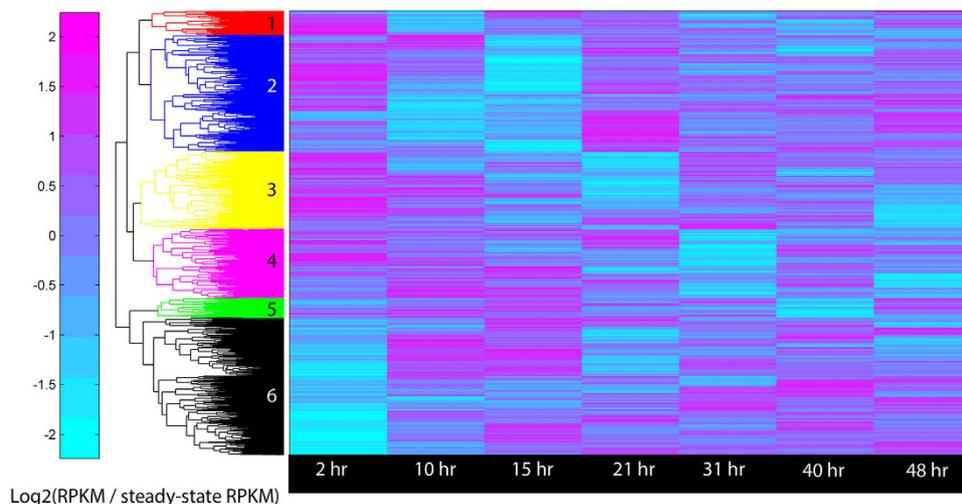


Figure 2. Hierarchical clustering of the relative mRNA abundances given in reads per kilobase per million reads (RPKM) normalized by steady-state. 3319 unfiltered genes (of 5303) categorized into six main cluster groups: 1-red (181 genes), 2-blue (872 genes; enriched for PS I and electron transport, $p < 0.05$), 3-yellow (580 genes), 4-magenta (516 genes), 5-green (146 genes) and 6-black (1024 genes; enriched for PS II and N-fixation, $p < 0.05$).

period. Evidence for cyclic electron flow, as inferred from rises in post-illumination Chl *a* fluorescence (Fig. S2) was only observed during the ammonium-limited chemostat precondition.

Global transcriptional and translational dynamics. Absolute and relative expression of key metabolic processes varied temporally across eight time-resolved sample points within the H_2 production profile. Dynamic expression patterns of energy (catabolic and photosynthetic) and nitrogen metabolism genes were observed within the global transcriptome (Fig. 2). The transcriptome was directly compared to and showed general agreement with the global proteome patterns (Fig. S3), which was not entirely unexpected in pre-synchronized, non-growing cells. Enrichment of key functional roles identified correlations between photosynthesis and nitrogen fixation processes (Table S1 and S2): transcripts specific for the main subunits for PS I were enriched ($p < 0.004$) in a separate group (Fig. 2; blue) and distinct from the main subunits of PS II, which co-clustered ($p < 0.05$) with the nitrogen fixation machinery (Fig. 2; black).

Nitrogenase and hydrogenase expression. Transcriptional and translational expression patterns of *nif* genes, as compared to those displayed by the bi-directional hydrogenase (Hox), implicated nitrogenase as the key catalytic process for H_2 production in this study. This result is consistent with other studies reporting the physiology of *Cyanothece* 51142 functioning within N-limited (or deplete) environments^{7,20}. The relative transcript and protein abundance profiles containing all of the *nif* transcripts, were positively correlated ($R^2 > 0.71$) with the multi-modal (damped oscillatory) pattern of H_2 production (Fig. 3C and S4). In general, Nif protein expression corresponded with the transcriptional dynamics and was significantly increased during H_2 production (Fig. 4C). The abundances (RPKM) for the encoding transcripts and NifHDK proteins were very high (compared within the global expressome) during chemostat growth as well as throughout H_2 production (see supplementary data file). At the same time, the relative abundances of transcripts for the uptake hydrogenase (*hupLS*) varied during H_2 production (Fig. S5), displaying no distinctive patterning with respect to H_2 or O_2 production. The bidirectional hydrogenase (*hox*) genes exhibited significantly lower RPKM values than *nif* (e.g., *hoxH* was only 0.15% of *nifH* at max q_{H_2}) and the relative abundances did not cluster uniformly or share common profile patterning with H_2 production or photosynthesis. Unique peptides were not detected for either the Hup or Hox proteins; hence they were not represented within the global proteome.

Photosynthetic units. The relative abundances of the PS II reaction center transcripts and proteins (PsbA and PsbD) increased with H_2 production (Figs 3B and 4B), while relative abundances of PS I reaction centers (PsaA and PsaB) were generally decreased (Figs 3A and 4A). Accordingly, the relative transcript abundances of genes encoding PS II and PS I components displayed positive and negative correlation ($R^2 > 0.6$) with nitrogenase expression, respectively (Fig. S6). Transcript and protein levels of PS II genes correlated with each other and increased with H_2 production. In contrast, relative abundance profiles for all PS I transcripts showed multi-modal (oscillatory) patterning (Fig. 3A). The dynamics of

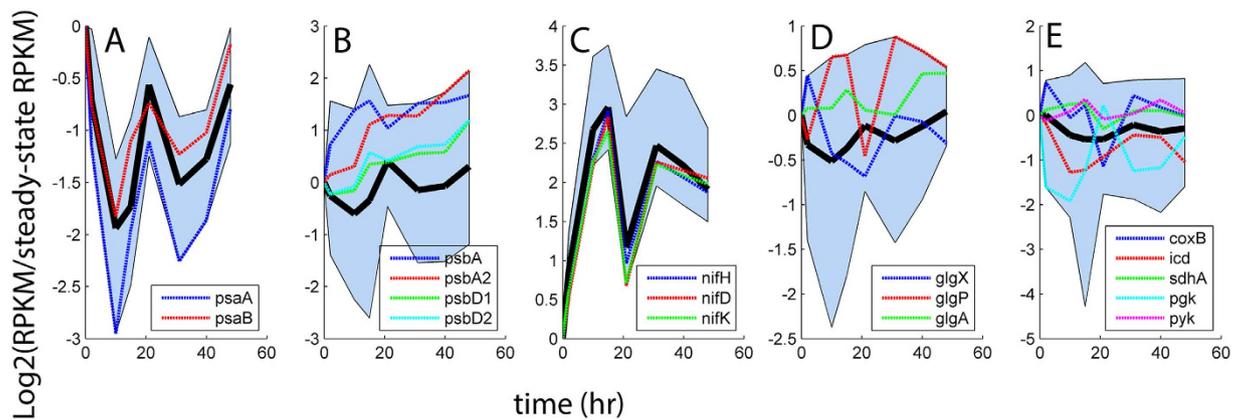


Figure 3. Relative abundance profiles of functionally categorized gene transcripts. Solid black lines indicate the mean profile taken from each collection of genes. Edges of each shaded region represent the respective maximum and minimum relative abundances of transcripts measured at each sampling point. (A) PS I (11 *psa* genes); (B) PS II (31 *psb* genes); (C) Nitrogenase (20 *nif* genes); (D) Glycogen metabolism (8 *glg* genes); (E) Catabolic energy metabolism (18 genes involved in glycolysis, TCA and oxidative phosphorylation).

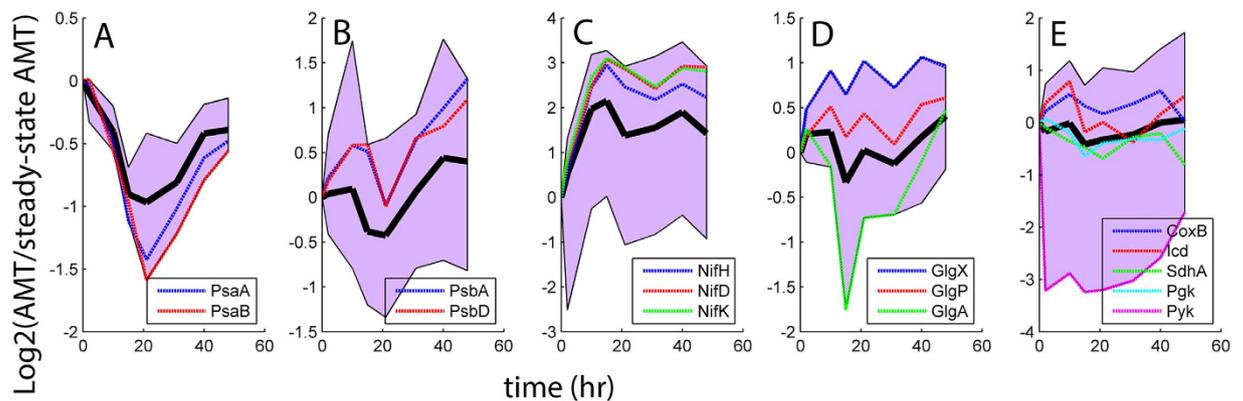


Figure 4. Relative abundance profiles of functionally categorized proteins. Solid black lines indicate the mean profile taken from each collection of proteins. Edges of each shaded region represent the respective maximum and minimum relative abundances of proteins measured at each sampling point. (A) PS I (6 *Psa* proteins); (B) PS II (15 *Psb* proteins); (C) Nitrogenase (12 *Nif* proteins); (D) Glycogen metabolism (7 *Glg* proteins); (E) Catabolic energy metabolism (13 proteins involved in glycolysis, TCA and oxidative phosphorylation).

PS I proteins differed from the transcripts and displayed mono-modal behavior which was inversely correlated with the net O_2 production at ~ 21 hr (Figs 1 and 4A). Taken together with the net photosynthesis rates and Chl *a* fluorescence kinetics (Fig. S2), expression dynamics of PS II and PS I machinery provides key evidence linking the H_2 production in *Cyanothece* 51142 with increase in linear electron flow through and concurs with decreased capacity for cyclic electron flow as the dominant ATP generating process.

Energy and glycogen metabolism. The dynamic patterns of key glycogen metabolism pathways were variable and did not correlate with the nitrogenase expression at either the transcriptional or translational levels (Fig. 3D and S6). Evidence for glycogen degradation was observed in the expression profiles of the glycogen debranching enzyme (*glgX*; *cce_3465*) and glycogen phosphorylase (*glgP*, *cce_1629*), which showed increased abundances relative to the ammonium limited precondition and positive correlation with nitrogenase expression at the translational level (Fig. 4D and S6). However, relative abundance patterns of these glycogen degradation genes were decreased at the transcriptional level. Glycogen synthesis genes revealed opposite expression dynamics as compared to degradation and the relative protein abundance of glycogen synthase (*GlgA*; *cce_3396*) was significantly decreased during peak H_2 production (Fig. 4D).

The principal processes involved in catabolic energy metabolism, downstream of glycogen degradation, generally showed decreased relative abundances during H₂ production and negative correlation with nitrogenase expression (Fig. S6). The relative protein abundance profiles of the two ATP generating steps of glycolysis (phosphoglycerate kinase and pyruvate kinase; *cce_4219* and *cce_3420*, respectively) were significantly decreased ($p < 0.05$; Fig. 4E). Expression at the transcriptional level (*pgk* and *pyk*) was more varied but generally demonstrated a decrease across the H₂ production profile (Figs 3E and 4E). The relative transcript and protein abundance profiles for the key reductant generating reactions of the tricarboxylic acid cycle (TCA), such as isocitrate dehydrogenase (*Icd*; *cce_3202*) and succinate dehydrogenase (*SdhA*; *cce_0663*), were generally decreased (Figs 3E and 4E); although some variability was observed in the *Icd* protein profile during the early time points of the H₂ production profile. A principal gene in respiration and oxidative phosphorylation for *Cyanothece* 51142 is cytochrome *c* oxidase (*coxB*; *cce_1977*). The relative abundance profiles of *CoxB* proteins generally increased with H₂ production (Fig. 4E) but showed very weak correlation with nitrogenase expression (Fig. S6). The transcriptional profiles for *coxB* did not correlate with nitrogenase expression and showed relative decreases during the local net-photosynthesis minima corresponding to peak H₂ production (Figs 1 and 3E).

Discussion

The results of this study yield kinetic and genome-scale evidence that supports a new level of insight into the metabolic processes that supply the energy requirements for nitrogenase-mediated H₂ production in *Cyanothece* 51142. These results frame the concept of active reductant and ATP generation originating from a combination of energy metabolism processes including linear electron flow through PS II. The H₂ production profiles measured under these experimental conditions correlate positively with nitrogenase and PS II expression but negatively with these of PS I and some of the key catabolic processes required to harvest energy from intercellular glycogen stores. In addition, expression of the principal downstream ATP and reductant generating steps of glycolysis and the TCA cycle were generally decreased relative to the nitrogen limited steady-state precondition. Taken together, these results suggest that the high levels of ATP and reductant required to continuously support nitrogenase catalyzed H₂ production do not originate solely from glycogen catabolism but also from linear electron flow through PS II. This is an exciting development, since it has been previously reported and generally assumed that H₂ production, via nitrogenase activity in *Cyanothece* 51142 and highly related strains, is exclusively supported by ATP and reductant derived from glycogen degradation and/or cyclic-electron flow^{7,13,18,21}.

The importance of linear electron flow through PS II, as it relates to supporting nitrogenase driven H₂ production in *Cyanothece* 51142, has been a topic of scientific ambiguity. Multiple studies have investigated the effect of PS II inhibition with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) during N-depleted H₂ production. It has been reported that the addition of DCMU to Ar-flushed *Cyanothece* 51142 suspensions had no effect on H₂ production⁷. However, another study (that supports the current hypothesis) reported that the addition of DCMU to cells had delayed but dramatic inhibitory effect of H₂ production and a similar effect was observed after addition of the electron transport inhibitor p-benzoquinone (BQ), a plastoquinone analog¹². The inference from these studies was that cyclic electron flow around PS I was the key mechanism supplying ATP for nitrogenase activity. However, the comprehensive gene and protein profiles presented here show that expression of critical subunits for energy acquisition in PS I (*PsaA* and *PsaB*) are decreased compared to the reaction centers of PS II (*PsbA* and *PsbD*). Additionally, peak PS II performance, as inferred from variable Chl *a* fluorescence parameters $rETR_{max}$ and Fv/Fm , was observed when the relative abundance PS I reaction center proteins were lowest (~20 hr). Hence, reduced expression of PS I did not restrict linear electron flow. This strongly suggests the involvement of PS II in nitrogenase-mediated H₂ production. In contrast to prior belief, it is likely that ATP generation is primarily facilitated by linear electron flow through each photosystem accounting for the full potential for proton translocation, ATPase activity and generation of reducing equivalents.

Although changes in expression patterns do not directly represent enzymatic activity, the H₂ production and photosynthesis kinetics are in strong agreement with the global transcript and protein measurements. The current results are in agreement with previously reported Chl *a* fluorescence kinetics, across similar H₂ production profiles, which also revealed that the maximum electron transfer rates (ETR_{max}) increased substantially along the H₂ production profiles under different carbon availability regimes¹². This evidence complements the *in situ* reaction measurements that show the gross rate of oxygenic photosynthesis exceeding the rate of respiration which confirms that linear electron flow through PS II is active during nitrogenase mediated H₂ production. This result is in contrast to observations made in cyanobacterium *Trichodesmium* which has been observed to facilitate nitrogen fixation concurrently with oxygenic photosynthesis by scavenging O₂ via the PS I mediated Mehler reaction, resulting in negative net-O₂ production rates²². We note that results from the current study only represent the physiology and metabolic potential of *Cyanothece* 51142 under a specific photobioreactor-facilitated H₂ production process, which is not directly comparable to any natural cyanobacterial ecosystem.

Nitrogenase-mediated H₂ production under aerobic conditions has been observed for *Cyanothece* 51142 in a number of reported studies^{7,12}. However, previous reports assumed that glycogen acts as the electron donor for oxidative phosphorylation and cyclic-electron flow⁷. The expression patterns for enzymes required for catabolic energy acquisition within glycolysis (phosphoglycerate kinase and

pyruvate kinase) and the TCA cycle (isocitrate dehydrogenase and succinate dehydrogenase) do not support this previously proposed scheme. It is unlikely that glycogen serves as the sole electron donor to nitrogenase while there is a net-positive oxygenic photosynthesis rate; as observed under the conditions reported in this study. Supporting evidence for this realization has previously been presented in a similar study reporting that intracellular carbohydrates levels (glucose equivalents) and glycogen levels are dynamic during H₂ production. In fact, the previous report showed evidence for both glycogen synthesis and degradation during the H₂ production and demonstrated that the maximum theoretical reductant derived from glycogen oxidation alone could not account for the rate of H₂ production measured¹².

The current study did not directly investigate nitrogen fixation or diel-regulated cell cycling. Hence, the presented results are not assumed to be ubiquitous and there is no precedent to infer that these extend toward nitrogen fixation in natural ecosystems or N₂-replete conditions. Also, it has been proposed that *Cyanothece* 51142 possesses temporal gene regulation processes which operate as circadian clocks^{15–17,23,24}. This study does not refute that concept; in fact, the experiments here avoided external temporal cues by incubating *Cyanothece* 51142 cells exclusively under constant light through a period of weeks.

In summary, photolytic hydrogen production by autotrophic cyanobacteria represents a promising source of clean, renewable energy. This has been the focus of many fundamental and applied research investments made by both private enterprises and government funding agencies. However, significant developments still need to be made on the basic physiological understanding of these processes before bioprocess optimization may take full effect. This study enables an advance in the field through high resolution multi-omics analyses coupled with detailed kinetics which present a direct relationship between photosynthetic energy acquisition and nitrogenase enzyme activity. These insights bring us closer to resolving the mechanistic underpinnings of energy metabolism used to drive the complex nitrogenase machine for biotechnology that may ultimately enable robust and efficient biophotolytic conversion processes for the production of fuel from H₂O, atmospheric CO₂ and sunlight.

Methods

Media and culturing conditions. *Cyanothece* 51142 was cultured in modified ASP-2 medium²⁷, supplemented with 0.75 mM NH₄Cl, 0.03 mM FeCl₃ and 0.75 mM K₂HPO₄. Photobioreactor (PBR) cultures were operated by a previously described method¹². Briefly, di-chromatic (680 and 630 nm LEDs) PBRs were operated as nitrogen limited chemostats with a 5.5 L working volume diluted at a 0.05 hr⁻¹, 30 °C, pH 7.5 and controlled for constant incident and transmitted irradiance (250 and 10 μmol photons m⁻² sec⁻¹, respectively). Cells were never exposed to dark conditions during these experiments. The PBR cultures were sparged at 4.08 L min⁻¹ with 1.3% CO₂ in argon. H₂ production profiles were initiated by arresting flow to the PBR while maintaining sparging with 1.3% CO₂ in argon. Steady-state biomass concentrations were measured directly as ash-free cell dry weight (g_{CDW} L⁻¹) as previously reported²⁵.

Transcriptomics. Global RNA sequencing was performed by a previously reported method utilizing SOLiDTM sequencing technology²⁶. RNA was extracted from *Cyanothece* 51142 cells collected at different time points from the bioreactor using TRIzol[®] Reagent (Invitrogen), followed by genomic DNA removal and cleaning using RNase-Free DNase kit and Mini RNeasyTM kit (Qiagen). The 50-base short read sequences produced by the 5500XL SOLiDTM sequencer were mapped in color space using SOLiDTM LifeScopeTM software version 2.5 using the default parameters against the genome of *Cyanothece* sp. ATCC 51142 (<http://www.ncbi.nlm.nih.gov/genome>). Resulting BAM files were then analyzed with the Rockhopper program as described previously²⁷ to determine gene expression levels (RPKM).

Proteomics. Global samples were measured via previously reported LC-MS methods²⁸. Briefly, an accurate mass and time (AMT) tag approach was used that utilized tandem mass spectrometry to generate a reference peptide database of observed peptides. Samples were analyzed using an LTQ-Orbitrap VelosTM (Thermo Fisher Scientific) MS interfaced with a reverse phase HPLC system for peptide separation (LC-MS). Only peptides unique in identifying a single protein were used; however, the uniqueness criteria was dropped for analysis of the subset of PS II (PsbA and PsbD) proteins to accommodate for the high degree of similarity between isoforms. Only proteins represented by >2 unique peptides were considered. All AMT values are represented as the mean from duplicate samples.

Clustering and expression profiling. All filtering, clustering and profile analyses were performed with custom scripts (available upon request) written in Matlab and the Bioinformatics Tool Box (Mathworks). The steps used to filter expression profiles are detailed in a supplementary file. Hierarchical clustering was performed on the filtered mRNA data and grouped into six distinguished clusters. Matching protein and mRNA expression profiles synchronized by using K-means clustering to identify six distinct clusters (both protein and mRNA abundances) containing identical genes. The detailed algorithm for synchronized K-means clustering is described in the supplementary file. Eigen-gene and protein profiles are represented as the centroid of all profiles contained in a cluster.

Statistics. Statistical significance ($p < 0.05$) in the proteomic data sets was determined by the Dunnett Test used to compare expression to the steady-state preconditions. Enrichment, within the transcriptomic and proteomic data sets, is defined as the percentage of genes within the profile for which a

function has been assigned being significantly higher than the percentage of genes of the same function in the entire genome with a p-value of <0.05 according to Fisher's exact test.

References

- Gaffron, H. & Rubin, J. Fermentative and photochemical production of hydrogen in algae. *J. Gen. Physiol.* **26**, 219–240 (1942).
- Ghirardi, M. L., Togasaki, R. K. & Seibert, M. in *Biotechnology for Fuels and Chemicals* 141–151 (Springer, 1997).
- Melis, A., Zhang, L., Forestier, M., Ghirardi, M. L. & Seibert, M. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. *Plant. Physiol.* **122**, 127–136 (2000).
- Levin, D. B., Pitt, L. & Love, M. Biohydrogen production: prospects and limitations to practical application. *Int. J. Hydrogen. Energ.* **29**, 173–185 (2004).
- Reddy, K., Haskell, J. B., Sherman, D. & Sherman, L. Unicellular, aerobic nitrogen-fixing cyanobacteria of the genus *Cyanothece*. *J. Bacteriol.* **175**, 1284–1292 (1993).
- Tsygankov, A., Fedorov, A., Kosourov, S. & Rao, K. Hydrogen production by cyanobacteria in an automated outdoor photobioreactor under aerobic conditions. *Biotechnol. Bioeng.* **80**, 777–783 (2002).
- Bandyopadhyay, A., Stöckel, J., Min, H., Sherman, L. A. & Pakrasi, H. B. High rates of photobiological H₂ production by a cyanobacterium under aerobic conditions. *Nat. Comm.* **1**, 139 (2010).
- Bothe, H. & Newton, W. E. in *Microbial BioEnergy: Hydrogen Production* 137–153 (Springer, 2014).
- Kosourov, S. *et al.* Hydrogen photoproduction by immobilized N₂-fixing cyanobacteria: understanding the role of the uptake hydrogenase in the long-term process. *Appl. Environ. Microbiol.* **80**, 5807–5817 (2014).
- Masukawa, H., Sakurai, H., Hausinger, R. P. & Inoue, K. Sustained photobiological hydrogen production in the presence of N₂ by nitrogenase mutants of the heterocyst-forming cyanobacterium *Anabaena*. *Int. J. Hydrogen. Energ.* **39**, 19444–19451 (2014).
- Park, J.-W., Nam, S. W., Kim, H. S., Youn, S.-H. & Yih, W. Enhanced photobiological H₂ production by the addition of carbon monoxide and hydrogen cyanide in two unicellular N₂-fixing cyanobacterial strains isolated from Korean coasts. *Ocean. Sci. J.* **49**, 11–18 (2014).
- Melnicki, M. R. *et al.* Sustained H₂ production driven by photosynthetic water splitting in a unicellular cyanobacterium. *MBio* **3**, e00197–00112 (2012).
- Aryal, U. K. *et al.* Proteome analyses of strains ATCC 51142 and PCC 7822 of the diazotrophic cyanobacterium *Cyanothece* sp. under culture conditions resulting in enhanced H₂ production. *Appl. Environ. Microbiol.* **79**, 1070–1077 (2013).
- Bandyopadhyay, A., Elvitigala, T., Liberton, M. & Pakrasi, H. B. Variations in the rhythms of respiration and nitrogen fixation in members of the unicellular diazotrophic cyanobacterial genus *Cyanothece*. *Plant. Physiol.* **161**, 1334–1346 (2013).
- Colon-Lopez, M. S., Sherman, D. M. & Sherman, L. A. Transcriptional and translational regulation of nitrogenase in light-dark and continuous-light-grown cultures of the unicellular cyanobacterium *Cyanothece* sp. strain ATCC 51142. *J. Bacteriol.* **179**, 4319–4327 (1997).
- Colón-López, M. S. & Sherman, L. A. Transcriptional and translational regulation of photosystem I and II genes in light-dark and continuous-light-grown cultures of the unicellular cyanobacterium *Cyanothece* sp. strain ATCC 51142. *J. Bacteriol.* **180**, 519–526 (1998).
- Welsh, E. A. *et al.* The genome of *Cyanothece* 51142, a unicellular diazotrophic cyanobacterium important in the marine nitrogen cycle. *Proc. Natl. Acad. Sci.* **105**, 15094–15099 (2008).
- Skizim, N. J., Ananyev, G. M., Krishnan, A. & Dismukes, G. C. Metabolic pathways for photobiological hydrogen production by nitrogenase-and hydrogenase-containing unicellular cyanobacteria *Cyanothece*. *J. Biol. Chem.* **287**, 2777–2786 (2012).
- Bernstein, H. C. *et al.* Direct measurement and characterization of active photosynthesis zones inside wastewater remediation and biofuel producing microalgal biofilms. *Bioresour. Technol.* **156**, 206–215 (2014).
- Vu, T. T. *et al.* Genome-scale modeling of light-driven reductant partitioning and carbon fluxes in diazotrophic unicellular cyanobacterium *Cyanothece* sp. ATCC 51142. *PLoS Comput. Biol.* (2012).
- Min, H. & Sherman, L. A. Hydrogen production by the unicellular, diazotrophic cyanobacterium *Cyanothece* sp. strain ATCC 51142 under conditions of continuous light. *Appl. Environ. Microbiol.* **76**, 4293–4301 (2010).
- Berman-Frank, I. *et al.* Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*. *Science* **294**, 1534–1537 (2001).
- Červený, J. & Nedbal, L. Metabolic rhythms of the cyanobacterium *Cyanothece* sp. ATCC 51142 correlate with modeled dynamics of circadian clock. *J. Biol. Rhythm.* **24**, 295–303 (2009).
- Schneegurt, M. A., Sherman, D. M., Nayar, S. & Sherman, L. A. Oscillating behavior of carbohydrate granule formation and dinitrogen fixation in the cyanobacterium *Cyanothece* sp. strain ATCC 51142. *J. Bacteriol.* **176**, 1586–1597 (1994).
- Pinchuk, G. E. *et al.* Constraint-based model of *Shewanella oneidensis* MR-1 metabolism: a tool for data analysis and hypothesis generation. *PLoS Comput. Biol.* **6**, e1000822 (2010).
- Beliaev, A. S. *et al.* Inference of interactions in cyanobacterial–heterotrophic co-cultures via transcriptome sequencing. *ISME J.* (2014).
- McClure, R. *et al.* Computational analysis of bacterial RNA-Seq data. *Nucleic. Acids Res.* **41**, e140–e140 (2013).
- Sadler, N. C. *et al.* Live cell chemical profiling of temporal redox dynamics in a photoautotrophic cyanobacterium. *ACS Chem. Biol.* **9**, 291–300 (2013).

Acknowledgements

The authors would like to thank Allan Konopka, Jim Fredrickson, Lindsey Anderson and Bobbi-Jo Webb-Robertson for helpful discussions and technical assistance. Author HCB is grateful for support given by the Linus Pauling Distinguished Postdoctoral Fellowship, a Laboratory Directed Research and Development Program of PNNL. This research was supported by the Genomic Science Program (GSP), Office of Biological and Environmental Research (BER), U.S. Department of Energy (DOE), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Biofuels Scientific Focus Area (BSFA). The genome annotation efforts used customized processes and resources developed and supported by the Foundational Scientific Focus Area at PNNL. MS-based proteomic measurements used capabilities developed partially under the GSP Panomics project. A significant portion of the research was performed using the Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility sponsored by DOE BER and located at PNNL. PNNL is operated for the DOE by Battelle Memorial Institute under Contract DE-AC05-76RLO 1830.

Author Contributions

ASB designed the experiment. M.A.C., E.A.H., N.C.S., M.R.M., L.M.M., C.D.N. and H.C.B. performed the experiment. M.F.B., R.S.M., N.C.S., A.T.W. and H.C.B. analyzed the data. H.C.B., A.S.B. and R.S.M. interpreted the data and wrote the paper.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Bernstein, H. C. *et al.* Multi-Omic Dynamics Associate Oxygenic Photosynthesis with Nitrogenase-Mediated H₂ Production in *Cyanothece* sp. ATCC 51142. *Sci. Rep.* **5**, 16004; doi: 10.1038/srep16004 (2015).



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>