

# Global Proteomics Reveal an Atypical Strategy for Carbon/Nitrogen Assimilation by a Cyanobacterium Under Diverse Environmental Perturbations\*

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Cyanobacteria, the only prokaryotes capable of oxygenic photosynthesis, are present in diverse ecological niches and play crucial roles in global carbon and nitrogen cycles. To proliferate in nature, cyanobacteria utilize a host of stress responses to accommodate periodic changes in environmental conditions. A detailed knowledge of the composition of, as well as the dynamic changes in, the proteome is necessary to gain fundamental insights into such stress responses. Toward this goal, we have performed a large-scale proteomic analysis of the widely studied model cyanobacterium *Synechocystis* sp. PCC 6803 under 33 different environmental conditions. The resulting high-quality dataset consists of 22,318 unique peptides corresponding to 1955 proteins, a coverage of 53% of the predicted proteome. Quantitative determination of protein abundances has led to the identification of 1198 differentially regulated proteins. Notably, our analysis revealed that a common stress response under various environmental perturbations, irrespective of amplitude and duration, is the activation of atypical pathways for the acquisition of carbon and nitrogen from urea and arginine. In particular, arginine is catabolized via putrescine to produce succinate and glutamate, sources of carbon and nitrogen, respectively. This study provides the most comprehensive functional and quantitative analysis of the *Synechocystis* proteome to date, and shows that a significant stress response of cyanobacteria involves an uncommon mode of acquisition of carbon and nitrogen. *Molecular & Cellular Proteomics* 9: 2678–2689, 2010.

Cyanobacteria are a morphologically diverse group of Gram-negative bacteria capable of oxygenic photosynthesis.

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Received, April 29, 2010 and in revised form, September 1, 2010  
Published, MCP Papers in Press, September 21, 2010 DOI 10.1074/mcp.M110.000109

These organisms occur in almost every habitat and are credited with the transition of the Earth's atmosphere from an anaerobic state to the aerobic condition, the evolution of planetary primary production, and as being the progenitor of chloroplasts in higher plants (1–3). Cyanobacteria contribute significantly to global photosynthetic productivity. It is estimated that more than half the total primary production essential for sustaining life on Earth is produced by cyanobacteria (4). Additionally, cyanobacteria have recently attracted significant interest because of their crucial role in the biogeochemical cycle (5), and their ability to produce renewable carbon-neutral biofuels. Despite these important evolutionary, ecological, environmental, and biotechnological factors, many aspects of cyanobacterial physiology remain poorly understood.

The physiological activities of cyanobacteria are intricately linked with the daily changes in environmental conditions. This is because the energy requirements for cyanobacterial cellular metabolism including fixation of carbon dioxide and assimilation of nutrients are derived from sunlight. Thus cyanobacteria must constantly modify the cellular machinery for efficient capture of light energy under continually changing natural conditions. Typically, they tightly coordinate growth with cellular energy levels to survive unfavorable conditions. Integration of nutrient specific pathways with photosynthetic processes is a key survival mechanism employed by cyanobacteria under changing environmental conditions (6–8). Such adaptation strategies allow cyanobacteria to balance the supply of electrons from photosynthetic processes with the demands of cellular metabolism, and prevent the generation of damaging reactive oxygen species by excess reducing power.

Assimilation of carbon and nitrogen in photosynthetic organisms is one of the main sinks for the reducing power produced by the light reactions of photosynthesis. Accordingly, cyanobacteria have developed intricate mechanisms to control and coordinate several pathways involved in the acquisition of carbon and nitrogen. For example, PII, a regulatory protein, has been suggested to balance the acquisition of

the two nutrients by sensing the carbon and nitrogen ratio (9). Thioredoxins have also been shown to link the activity of the photosynthetic electron transport chain with carbon and nitrogen assimilation (6). Despite the active participation of these proteins, cyanobacteria can assimilate carbon and nitrogen at disparate levels exceeding cellular demands. Excess carbon and nitrogen are stored in the forms of glycogen and cyanophycin granules, respectively, and are subsequently utilized under limiting conditions. *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*), a model cyanobacterium, utilizes the oxidative pentose phosphate and glycolytic pathways to obtain carbon from glycogen granules (10). However, the pathway for utilization of cyanophycin is not well understood. Cyanophycin is a polymer of aspartic acid and arginine, which must be further catabolized to meet the nitrogen requirement for cellular metabolism. There are at least five known pathways for the catabolism of arginine in prokaryotes. Among them, a pathway utilizing arginase and the urea cycle has been shown to be active in *Synechocystis* (11).

Our understanding of gene regulation linked to the assimilation of carbon and nitrogen, as well as broader cellular adaptation mechanisms under different environmental conditions, have significantly benefited from global transcriptional analysis of *Synechocystis* (12). Generally, it has been observed that such perturbations lead to down-regulation of genes involved in light absorption and the photosystems, as well as in carbon fixation and nitrogen assimilation. However, many of the studies have reported a complex regulation of genes involved in carbon and nitrogen assimilation. For example, upon preferential illumination of photosystem II, genes involved in nitrogen assimilation using nitrate as a substrate respond negatively, whereas those involved in utilization of either ammonia, urea, or arginine as substrate respond positively (13). Despite these transcriptomic studies, the impact of transcript regulation on actual protein levels remains poorly understood, in part because of several previous studies showing poor correlation between transcriptomic and proteomic datasets (14, 15). Because proteins are directly responsible for cellular functions, measurements of protein abundance are expected to provide significant clues to the modulation of cellular functions during different environmental perturbations. Several proteomic studies under diverse environmental conditions have been undertaken in *Synechocystis* (16–34). However, such studies have not yielded a comprehensive understanding of cellular adaptations, either because of low proteome coverage or because of the limited information on the changes in protein abundance.

Recent advances in high-throughput proteomic technology and informatics tools have allowed high-confidence quantitative and qualitative proteome determination of several model organisms such as *Escherichia coli*, yeast, *Drosophila*, and *Caenorhabditis elegans* (35–38). These results have enabled a systems-level analysis of cellular functions. In the current

study, we have used liquid chromatography-tandem MS<sup>1</sup> (LC-MS/MS) to analyze the proteome of *Synechocystis* across 33 different environmental conditions. Our efforts have led to a 53% proteome coverage, resulting in the most complete functional and quantitative description of the proteome of *Synechocystis*, to date. Our analyses of differentially regulated proteins show that *Synechocystis* activates alternate pathways for the acquisition of carbon and nitrogen under diverse environmental conditions.

#### EXPERIMENTAL PROCEDURES

**Culture Conditions**—*Synechocystis* cultures were grown to a density of  $2 \times 10^8$  cells/ml as described (39). Cells were harvested by centrifugation at  $6000 \times g$  for 5 min at 22 °C and washed twice with 100 mM 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]ethanesulfonic acid (TES) pH 8.0. The washed cells were inoculated into complete BG11 medium and sampled following 0, 4, 6, 8, and 16 days. For growth under nutrient deplete conditions, washed cells were grown in BG11 depleted of either nitrate (nitrogen depletion), sulfate (sulfur depletion), or phosphate (phosphorus depletion), and sampled following 6 days. For iron depletion, cells were incubated twice in 20 mM 4-morpholineethanesulfonic acid (MES), 10 mM EDTA, pH 5.0 for 10 min, then inoculated in BG11 depleted of iron, and sampled following 6 days. Following 6 days, starved cultures were supplemented with 17.65 mM nitrate, 32  $\mu$ M ammonium, 301  $\mu$ M sulfate, 175  $\mu$ M phosphate, or 30  $\mu$ M iron as appropriate, and sampled following 4 and 24 h. For salt stress, 0.5 M NaCl was added to cells grown in complete BG11 and sampled following 0.5, 6, and 24 h. Cells were also collected following 3 and 6 days growth in the presence of 5 mM glucose and 10  $\mu$ M 3-(3',4'-dichlorophenyl)-1,1-dimethylurea. For high CO<sub>2</sub> treatment, cells grown in 3% CO<sub>2</sub> were sampled following 1 and 25 h, transferred back to air level (0.3%) CO<sub>2</sub>, and sampled following 2 h. Last, cells were subjected to heat (38 °C) or cold (22 °C) shock and sampled following 1, 4, and 24 h.

**Sample Preparation**—Cells were harvested by centrifugation at  $6000 \times g$  for 5 min at 4 °C. Membrane and soluble fractions from total cell extracts were prepared as described (35) with minor modifications. The lysis buffer lacked any detergent and cells were broken by using 6 cycles of 1 min break, 1 min rest on ice. The cell lysates were fractionated by centrifugation at  $150,000 \times g$  at 4 °C for 20 min. The supernatant comprising soluble fractions were transferred to separate tubes. The pellet comprising membrane fractions was washed with 100 mM ammonium bicarbonate buffer (pH 8.0) and centrifuged again at  $150,000 \times g$  at 4 °C for 20 min. The protein concentrations of soluble and membrane fractions were determined by bicinchoninic acid assay (Pierce, Rockford, IL). Soluble fractions were denatured and reduced using 8 M urea and 5 mM dithiothreitol (Sigma-Aldrich) at 37 °C for 60 min. Membrane fractions were treated identically, except for the addition of 1% 3-[[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid (CHAPS) for 45 min prior to digestion. All samples were then diluted with five volumes of 25 mM ammonium bicarbonate prior to tryptic digestion using sequencing-grade modified porcine trypsin (Promega, Madison, WI) at a 1:50 (w/w) trypsin-to-protein ratio for 5 h at 37 °C. The digestions were stopped by boiling for 5 min followed by cooling on ice. Samples were separated using strong cation exchange chromatography with a PolySulfoethyl A, 200 mm  $\times$  2.1 mm, 5  $\mu$ M, 300-Å column and a 10 mm  $\times$  2.1 mm guard column (PolyLC, Inc., Columbia, MD) at a flow rate of 0.2 ml/min. The strong cation exchange chromatography peptide fractionation was

<sup>1</sup> The abbreviations used are: LC-MS/MS, liquid chromatography-tandem MS.

carried out as previously described (39). The peptides were resuspended in 900  $\mu$ l of mobile phase A, and separated on an Agilent 1100 high pressure liquid chromatography system (Agilent, Palo Alto, CA) equipped with a quaternary pump, degasser, diode array detector, Peltier-cooled autosampler, and fraction collector (both set at 4 °C). A total of 25 fractions were collected for each sample.

**Reversed Phase Liquid Chromatography (LC) Separation and Tandem MS (MS/MS) Analysis of Peptides**—The LC separation and MSMS analysis have been extensively reported (40) with the coupling of a constant pressure (5000 psi) high-resolution reversed phase capillary liquid chromatography system (150  $\mu$ m i.d.  $\times$  360  $\mu$ m o.d.  $\times$  65 cm capillary; Polymicro Technologies Inc., Phoenix, AZ). Analyses performed utilized both Finnigan LCQ and LTQ ion trap mass spectrometers (ThermoFinnigan, San Jose, CA) using an electrospray ionization source manufactured in-house. Each unfractionated and strong cation exchange chromatography fraction was analyzed via capillary LC-MS/MS.

**LC-MS/MS Data Analysis**—ExtractMSn (version 4.0) and SEQUEST (Version v.27, Rev 12, Thermo Fisher Scientific, Waltham MA) (41) analysis software was used to match the MS/MS fragmentation spectra to sequences from the 2004 Cyanobase (<http://genome.kazusa.or.jp/cyanobase>) annotation of *Synechocystis* (3663 total entries, no enzyme search,  $\pm 3$ -Da tolerance for parent MS peak). Search was performed using default parameters with no-enzyme rules within a  $\pm 1.5$ -Da parent mass window,  $\pm 0.5$ -fragment mass window, average parent mass, and monoisotopic fragment mass. The criteria selected for filtering for both LCQ and LTQ data followed methods based upon a reverse database false positive model, which provides a target of 95% confidence in peptide identifications (42). No fixed or variable modifications were considered. Additional metrics of individual peptide confidence included Peptideprophet (43) and discriminate score (44). Specific SEQUEST based filter criteria for LTQ level data includes  $\text{DeICN} \geq 0.1$  coupled with  $\text{Xcorr}$  of  $\geq 1.6$  for full tryptic charge state +1,  $\geq 2.4$  for charge state +2, and  $\geq 3.2$  for charge state +3. For partial tryptic,  $\text{Xcorr} \geq 4.3$  for charge state +2 and  $\geq 4.7$  for charge state +3. An additional eight proteins and 180 peptides were included from wild type spectra MSMS\_01 - MSMS\_06 (NCBI GEO accession GSE9577) (39).

**Data Processing and Analysis**—Peptides matching multiple proteins were assigned to each of the matching proteins. Protein spectral counts were calculated by summing numbers of observed peptides for each protein in all fractions. For sample replicates, all combinations of soluble and membrane replicate pairings were summed, and the average and standard deviation of these combinations were used for the final values. Differentially expressed proteins were identified using three criteria: [(i)  $\text{mean1}/\text{mean2} \geq 1.5$ , (ii)  $\text{mean1} - \text{mean2} > 1$ , (iii)  $(\text{mean1} - 2\text{stddev1}) - (\text{mean2} + 2\text{stddev2}) > 0$ ] where mean1 and stddev1 are the values of largest mean of the treatment or control samples. Proteins were categorized as up- or down-regulated, on the basis of whether peptide abundances were higher or lower in the treated sample compared with the control sample. Transmembrane helices were predicted with a transmembrane hidden Markov model (45). SignalP (46) was used to predict cleavage sites for signal peptides, using the Gram-negative bacteria setting. Transmembrane hidden Markov models predicted helices shorter than 15 amino acids and those overlapping with signal peptides were discarded. Proteins were considered to be membrane proteins if at least one transmembrane helix was predicted. Peptide hydrophobicities were calculated by summing the hydrophobicities of the amino acid sequences using the Kyte and Doolittle scale. Peptide hydrophobicity, length, and mass histograms were generated for the subset of observed fully tryptic peptides of  $\geq 5$  amino acids in length and  $\geq 500$  Da and compared with ideal tryptic digests of the observed proteins, using the same constraints.

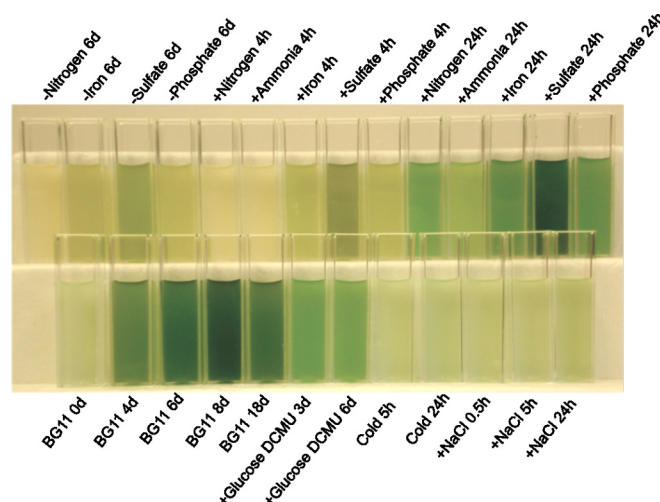


FIG. 1. **Physiological characteristics of *Synechocystis* cultures used for proteomic analysis.** *Synechocystis* cells were grown under various environmental conditions as described in the Experimental Procedures. At specific time points, 3-ml cultures were transferred to cuvettes and photographed.

## RESULTS

**Determination of the Composition of *Synechocystis* Proteome**—To obtain a comprehensive proteomics description of *Synechocystis*, we collected samples from cells grown under 33 different environmental conditions. These included time series studies of *Synechocystis* growth under nutrient-limiting conditions followed by recovery under nutrient-sufficient conditions. The kinetics of pigment loss, a typical observable phenotype associated with nutrient starvation in cyanobacteria, were quite variable between nutritional conditions (Fig. 1), possibly because of the ability of cyanobacteria to store some, but not all, nutrients in the form of inclusion bodies that can be utilized during starvation. Therefore, we used a strategy that involved prolonged starvation for nitrogen, phosphorus, sulfur, or iron for 6 days followed by recovery with the addition of the limiting nutrient. This “starve and recovery strategy” resulted in a uniform recovery response. We also exposed cells to excess sodium chloride (2 M), CO<sub>2</sub> (3%) with a recovery under ambient air, glucose (5 mM), as well as low (20 °C) and high (38 °C) temperatures.

We utilized a sensitive, high-resolution LC-MS/MS peptide-based “bottom-up” approach to maximize the proteome coverage of *Synechocystis* (47). Fig. 2 describes various steps involved in the identification of proteins under multiple environmental conditions. Total cell extracts were prepared from *Synechocystis* cells grown under the 33 environmental conditions. To increase the coverage of membrane proteins, total cellular extracts were separated into membrane and soluble fractions by centrifugation prior to tryptic digestion. The resulting peptide mixtures were then subjected to LC-MS/MS analysis to generate datasets of fragmentation spectra for each sample followed by SEQUEST analysis to generate identified peptide sequences (37). These spectra identifications

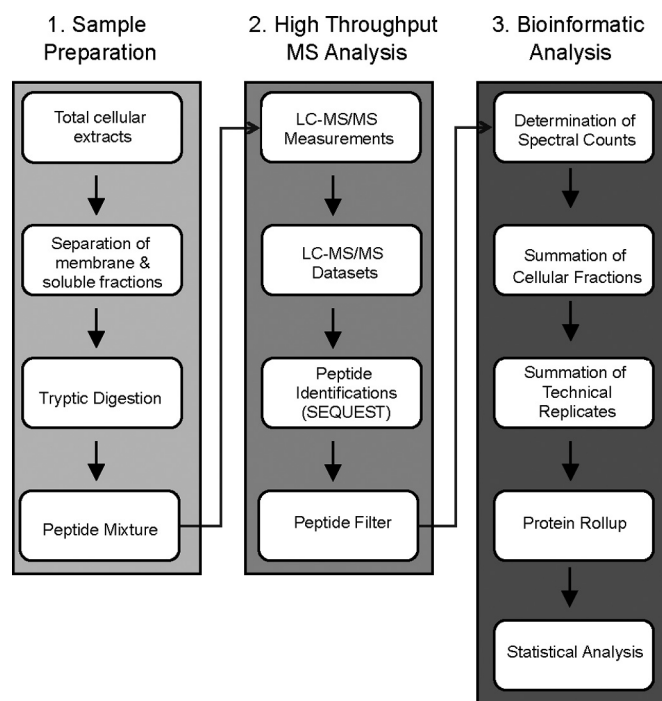


FIG. 2. **Experimental Design.** A flow chart describing various steps involved in the identification of *Synechocystis* proteome. Each step has been described in detail in the Experimental Procedures.

were then used to determine spectral counts for each peptide and protein. The counts from the membrane and soluble datasets were then combined for each condition and the technical replicates were averaged. Last, the peptides were mapped back to their corresponding proteins and the final compiled dataset was then analyzed statistically.

We identified a total of 22,318 unique tryptic peptides with a confidence criterion of 95% (supplemental Tables 1 and 2). These observed peptides correspond to 2369 proteins of the predicted 3663 for the *Synechocystis* genome (supplemental Table 1). Further analysis show that 414 proteins were identified on the basis of a single peptide. These proteins were removed from further analysis. Thus the final analysis was based on 1955 proteins, a coverage of 53% of the total predicted genome, which were identified by multiple distinct peptide identifications (see supplemental Table 3).

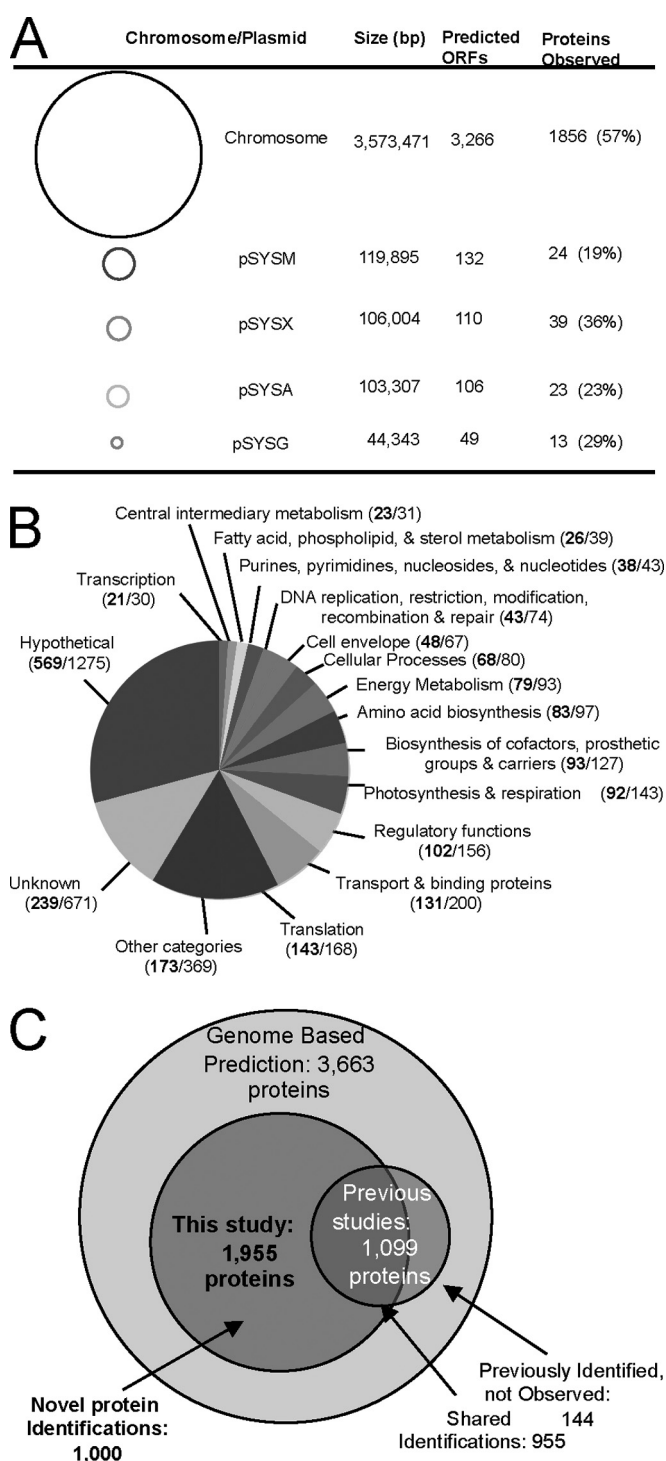
*Synechocystis* contains seven endogenous plasmids in addition to one circular chromosome (48). The plasmids pCC5.2, pCA2.4, and pCB2.4 were not annotated at the time of our initial analysis and thus the 11 predicted proteins contained on these three plasmids were not included in this study. To date, the largest category of predicted gene annotation belongs to unknown or hypothetical gene classification. The distribution of gene functional categories is skewed between chromosomal and endogenous plasmids. For example, hypothetical or unknown genes comprise ~45% of the predicted chromosomal genes whereas they are ~87, 79, 73, and 62% for the four largest plasmids, pSYSA, pSYSX, pSYSM, and

pSYSG, respectively. Overall, the highest observed coverage was for the circular chromosome (57%) (Fig. 3A). Identification of the large number of hypothetical or unknown proteins located on both the plasmids and the chromosome (Fig. 3B) showcases the unbiased discovery based approach of proteomic analysis in the present work. Importantly, this finding represents the magnitude of proteins actively engaged in the physiology of *Synechocystis* with little information known concerning their function.

Of the 53% protein coverage for the proteome, the majority of detected proteins belonged to 14 known functional categories (Fig. 3B). Importantly, the observed proteins were uniformly distributed among different functional categories (Fig. 3B). We have identified more than 85% of proteins involved in amino acid biosynthesis; cellular processes; energy metabolism; purines, pyrimidines, nucleosides, and nucleotides; and translation processes. Similarly, more than 64% of proteins involved in cell envelope, central intermediary metabolism, photosynthesis, transcription, and transport have been identified. As expected, the lowest proteins coverage was obtained for unknown (36%), hypothetical (45%), and other (47%) categories.

Several proteomics studies have been previously undertaken in *Synechocystis* (16–34). These studies have resulted in a combined observation of 1099 proteins (supplemental Table 4). A comparative analysis of these previously identified proteins with those identified in the present study shows that 955 proteins were commonly identified (Fig. 3C). One hundred and forty four previously identified proteins were not observed in our study whereas 1000 proteins were uniquely observed in the present study. A large number of these proteins (498) are currently annotated as either hypothetical or unknown in Cyanobase (49). Thus, our results have provided direct proof of a functional role of over one third of hypothetical and unknown proteins in *Synechocystis*.

Cyanobacteria contain a greater number of membrane proteins compared with heterotrophic bacteria. This is because of the presence of an internal thylakoid membrane system, where the light reactions of photosynthesis occur. Therefore, a special emphasis in the current study was given toward identification of the *Synechocystis* proteome that is not biased toward any of the known factors. Analysis of the proteome data observed in this study shows that it consisted of 57 and 40% of predicted soluble and membrane proteins, respectively (inset, Fig. 4A). Importantly, increasing number of transmembrane helices had little impact on the identification of membrane proteins (Fig. 4A). Identification of a large number of membrane proteins in our study is because of the initial separation of membrane fractions from soluble fractions as well as the use of optimized solubilization buffers. An examination of the hydrophobicity of the observed peptides showed a similar distribution to that of the predicted tryptic peptides from the genome (Fig. 4B). However, analysis of a subgroup of membrane proteins showed that most of the



**FIG. 3. Proteome Coverage.** A, Distribution of observed proteins among the different DNA elements. The numbers in parentheses indicate the observed proteins out of the total predicted proteins. B, Distribution of the observed proteins in various functional categories. The numbers in parentheses indicate the observed proteins (bold font) out of the total predicted proteins. C, Comparative analysis of proteins identified in this study (blue circle) with previously published proteomic studies (red circle) as a fraction of the predicted proteome (gray circle).

identified peptides were from the cytosolic loop regions of the proteins. A study of the peptides identified showed that the masses of peptides observed also followed a similar distribution to that predicted, except in the case of the peptides less than 2000 Da, which were under-identified (Fig. 4E). Other bias analysis determined that detection of the observed peptides was somewhat decreased for the shortest category (5–10 amino acids) (Fig. 4D). However, the observation of peptides was independent of pI, as shown by the similarity of the distribution of identified and predicted peptides (Fig. 4C). Taken together, these bias analyses show that there was very limited technical bias in peptide observation in the present work.

**Quantitative Analysis of Protein Response to Various Perturbations**—We re-examined 12 environmental conditions for quantitative determination of protein abundance (Table I). A total of 1198 differentially regulated proteins compared with cells grown in complete BG11 medium under normal conditions were identified (supplemental Tables 5 and 6). The number of differentially regulated proteins in each condition varied from a low of 262 (cold shock) to a high of 548 (nitrogen depletion) proteins (Table I). A majority of differentially regulated proteins (56%–77%) in most conditions, with the exception of cold shock and nitrogen depletion, were up-regulated as compared with control. Under cold stress and nitrogen depletion, ~61% and 86% of the differentially regulated proteins, respectively, were down-regulated as compared with control. However, addition of either nitrate or ammonia to the nitrogen-depleted cells had significant effects on the expression patterns of proteins. Under these conditions, the number of up-regulated proteins increased from 74 to 230 (with nitrate repletion) and 254 (with ammonia repletion). At the same time, the number of down-regulated proteins decreased from 473 to 148 (with nitrate repletion) and 139 (with ammonia repletion). The number of up- and down-regulated proteins during recovery in the presence of either nitrate or ammonia was similar to those of other nutrients. A small number of proteins were differentially expressed in a stress-specific manner (Table I). Most of these proteins have no known functions.

Examination of cellular processes on the basis of differential regulation of their associated proteins showed that the responses varied widely between conditions and, surprisingly, were not correlated with observed physiological responses (Fig. 5). For example, depletion of iron, phosphorus, sulfur, and nitrogen was accompanied by significant chlorosis and slow growth. However, we observed that most processes were down-regulated significantly only under nitrogen depletion and, to some extent, under iron depletion. In fact, in most other conditions, we noticed that majority of proteins in cellular processes were up-regulated. This is even true for ribosomal proteins, whose expression has been typically linked with the growth of an organism. Though the number of differentially regulated ribosomal proteins varied depending on conditions, we found that ribosomal proteins were down-

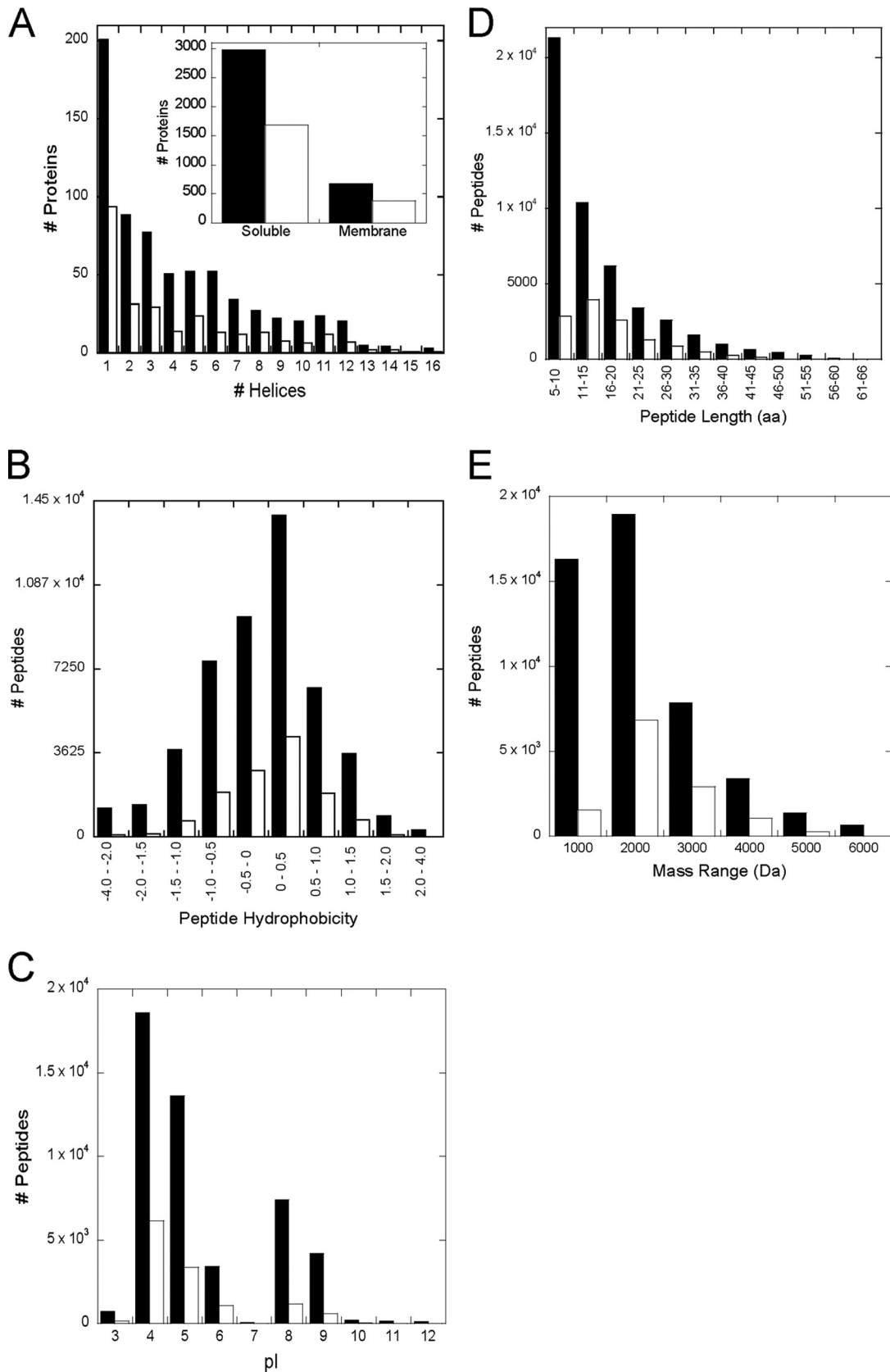


TABLE I  
Numbers of differentially regulated proteins under 12 environmental conditions

| Treatment            | Number of proteins      |              |                |                 |
|----------------------|-------------------------|--------------|----------------|-----------------|
|                      | Differentially abundant | Up-regulated | Down-regulated | Stress specific |
| CO <sub>2</sub>      | 306                     | 189 (62%)    | 117 (38%)      | 9               |
| Cold shock           | 262                     | 101 (39%)    | 161 (61%)      | 5               |
| Heat shock           | 377                     | 211 (56%)    | 166 (44%)      | 6               |
| Iron depletion       | 392                     | 230 (59%)    | 162 (41%)      | 1               |
| Iron repletion       | 369                     | 241 (65%)    | 128 (35%)      | 4               |
| Nitrogen depletion   | 548                     | 74 (14%)     | 473 (86%)      | 4               |
| Nitrogen repletion   | 378                     | 230 (61%)    | 148 (39%)      | 10              |
| Ammonium Repletion   | 393                     | 254 (65%)    | 139 (35%)      | 8               |
| Phosphorus Depletion | 349                     | 263 (75%)    | 86 (25%)       | 5               |
| Phosphorus Repletion | 407                     | 312 (77%)    | 95 (23%)       | 14              |
| Sulfur depletion     | 390                     | 246 (63%)    | 144 (37%)      | 6               |
| Sulfur repletion     | 370                     | 272 (74%)    | 98 (26%)       | 12              |

regulated under nitrogen, phosphorus, and iron depletion, and up-regulated under sulfur depletion and heat shock. As expected, the majority of ribosomal proteins were up-regulated during all recovery stages. A large number of proteins with unknown functions showed significant differential regulation. Several of these proteins showed stress specific regulation, providing evidence of their potential roles in cellular adaptation (Fig. 5).

Proteins involved in amino acid biosynthesis, glucose metabolism, TCA cycle, and cytochrome *b<sub>6</sub>f* complex displayed strong up-regulation in a majority of environmental conditions (supplemental Table 6). In general, enzymes known to catalyze key reactions in any given pathway were differentially regulated. For example, glycogen phosphorylase, which catalyzes the release of glucose from glycogen, was strongly up-regulated in all studied conditions. We also found that fructose-bisphosphate aldolase, which catalyzes the formation of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate from fructose 1,6-bisphosphate, and pyruvate dehydrogenase, which converts pyruvate into acetyl-CoA, were strongly up-regulated in all conditions. Similarly, key proteins involved in the biosynthesis of amino acids belonging to aromatic, aspartic acid, branched chain, serine, and glutamate families were strongly differentially regulated. For example, chorismate synthase was strongly up-regulated in most environmental conditions. Chorismate is a key intermediate involved in the biosynthesis of phenylalanine, tyrosine, and tryptophan. Similarly, diaminopimelate decarboxylase that catalyzes the synthesis of lysine, and acetolactate synthase that catalyzes the first step in the biosynthesis of leucine, isoleucine, and valine were strongly up-regulated under most environmental conditions. In contrast, most photosynthesis related proteins, including phycobiliproteins, did not show

significant changes in their abundance. Similarly, very few proteins involved in pigment biosynthesis showed differential regulation. However, proteins with other critical functions were differentially regulated. For example, heme oxygenase, involved in the multistep monooxygenase reaction to produce biliverdin IX $\alpha$  and CO from protoheme, was down-regulated during nutrient depletion conditions.

**Concordance Between Transcriptomic and Proteomic Datasets**—The global scale of proteome coverage and protein expression profiles obtained in this study enabled the first large-scale comparison of gene expression at the RNA and protein levels in *Synechocystis*. The differentially regulated proteins identified under cold shock, phosphorus, sulfur, nitrogen, and iron depletions were compared with the differentially regulated transcripts identified during the respective DNA microarray studies (50–54). To allow for a uniform comparison, all five transcriptomic datasets were reanalyzed. We used a fold change of 1.5 to identify differentially regulated genes from these datasets (supplemental Table 7). Concordance analysis showed that the expression changes of genes between transcriptomic and proteomic studies were quite low (Table II). However, we note that although the comparisons were done between datasets generated under similar conditions, there are differences in some of the growth and sampling regimes between our proteomic experiments and the previously published microarray studies. Agreement between the two studies was lowest for sulfur depletion and highest for nitrogen depletion (Table II). However, analysis of correlated and anticorrelated genes revealed some interesting results. Stress-specific genes showed similar expression patterns in both transcriptomic and proteomic studies (supplemental Table 7). For example, expression of nutrient specific transporters showed strong concordance. The relatively

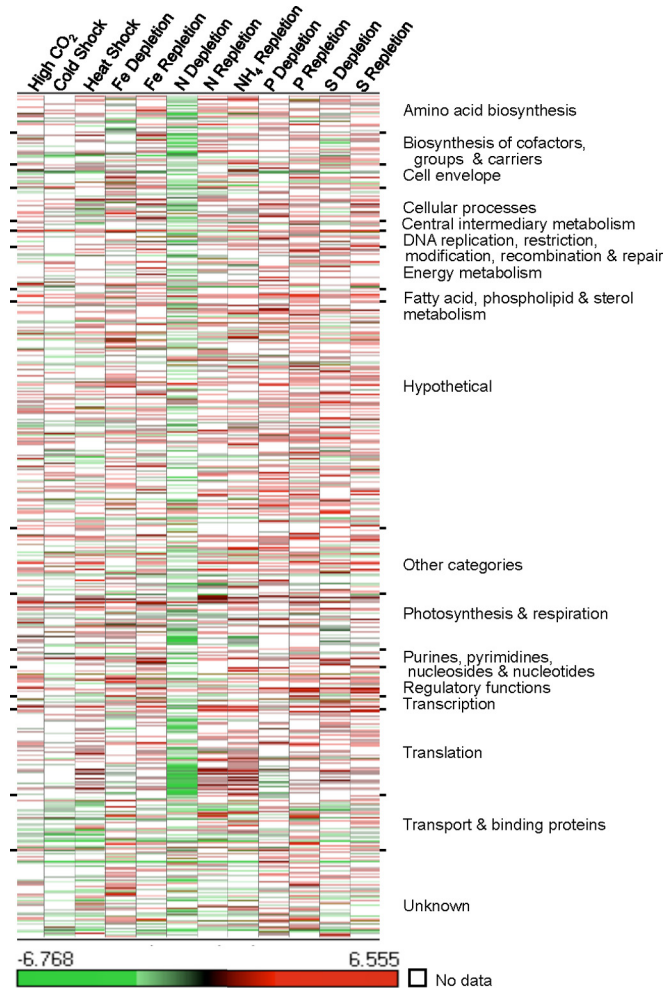
FIG. 4. **Proteome Bias Analysis.** Black and white bars indicate the numbers of predicted and observed proteins, respectively. A, Detection of membrane proteins as a function of transmembrane helices. The inset shows the numbers of soluble and membrane proteins detected in the present study. B, Detection of peptides of varying hydrophobicities versus the predicted peptides. C, Detection of peptides of varying masses versus the predicted peptides. D, Detection of peptides of varying lengths versus the predicted peptides. E, Detection of peptides of varying pIs versus the predicted peptides.

higher concordance seen under nitrogen depletion was because of similar expression patterns of ribosomal and photosynthesis genes. On the other hand, expression patterns of photosynthetic genes were anticorrelated under iron depletion with down-regulation of transcript levels and up-regulation of protein levels.

**Alternate Pathway for Assimilation of Nitrogen and Carbon under Various Perturbations**—Several proteins involved in nitrogen assimilation showed significant differential regulation. Generally, proteins involved in the transport of nitrate were

down-regulated in cells grown under nutrient depletion, cold, and heat shock (supplemental Tables 5 and 6). Repletion of nutrients to starved cells led to the up-regulation of these transporter proteins. As expected, recovery of nitrogen-depleted cells in the presence of ammonia did not lead to the up-regulation of nitrate transport proteins. To compensate for reduced nitrate uptake, cells up-regulated proteins involved in transport and utilization of urea and arginine. UrtE (SII0374) and UrtD (SII0764), which are involved in transport of urea, were strongly up-regulated under most environmental conditions. The data also showed that urease, which converts urea into CO<sub>2</sub> and ammonia, was up-regulated by fourfold under most conditions. Similarly, BgtB (SII1270), and to some extent BgtA (SII1735), the periplasmic and ATP-binding components of an arginine transporter, were up-regulated under most conditions. It was also observed that cyanophycinase, involved in the breakdown of cyanophycin into arginine and aspartic acid, was moderately up-regulated under several conditions. These results suggest that a common response in *Synechocystis* under different perturbations is to reduce the uptake of nitrate and increase the uptake of alternate nitrogen sources.

Arginine and aspartic acid must be further catabolized to acquire nitrogen for cellular metabolism. In *Synechocystis*, an arginine catabolic pathway has been described that combines the arginase pathway and urea cycle (11). All proteins in this pathway were detected in our study. However, none of them showed significant differential levels, suggesting that this pathway is not the preferred route for arginine catabolism under perturbations. Further analysis suggested that arginine is preferentially broken down into agmatine via arginine decarboxylase. In particular, arginine decarboxylase (SII1312 and SII0662), the first committed enzymes in this pathway, were up-regulated 1.5- to threefold as compared with control conditions (Fig. 6, supplemental Table 8). Agmatine can subsequently be catabolized into putrescine via the putative agmatinase (SII1077) or arginase (SII0228). These two proteins were not observed in the control cells; however, peptides corresponding to these proteins were detected under various environmental conditions, providing support for the involvement of this alternative pathway during various perturbations (supplemental Table 3). Putrescine is known to play a critical role in DNA, RNA, and protein synthesis, as well as in cell proliferation and differentiation. Furthermore, putrescine serves as a source for carbon and nitrogen in *E. coli* and *Pseudomonas* (55, 56), where it is converted to succinate.



**FIG. 5. Differential Regulation of Proteins under Different Perturbations.** The 1198 differentially regulated proteins were grouped in functional categories and a heat map was generated using Spotfire 7.0. Color bar indicates protein fold change in experimental conditions as compared with levels in complete BG11.

TABLE II  
Concordance between transcriptomic and proteomic studies in *Synechocystis*

|                                                                               | Cold stress | Iron depletion | Phosphorus depletion | Sulfur depletion | Nitrogen depletion |
|-------------------------------------------------------------------------------|-------------|----------------|----------------------|------------------|--------------------|
| Number of genes correlated                                                    | 8           | 56             | 57                   | 14               | 109                |
| Number of genes anticorrelated                                                | 12          | 52             | 15                   | 5                | 26                 |
| Total genes differentially expressed in proteomic and transcriptomic datasets | 20          | 108            | 72                   | 19               | 135                |

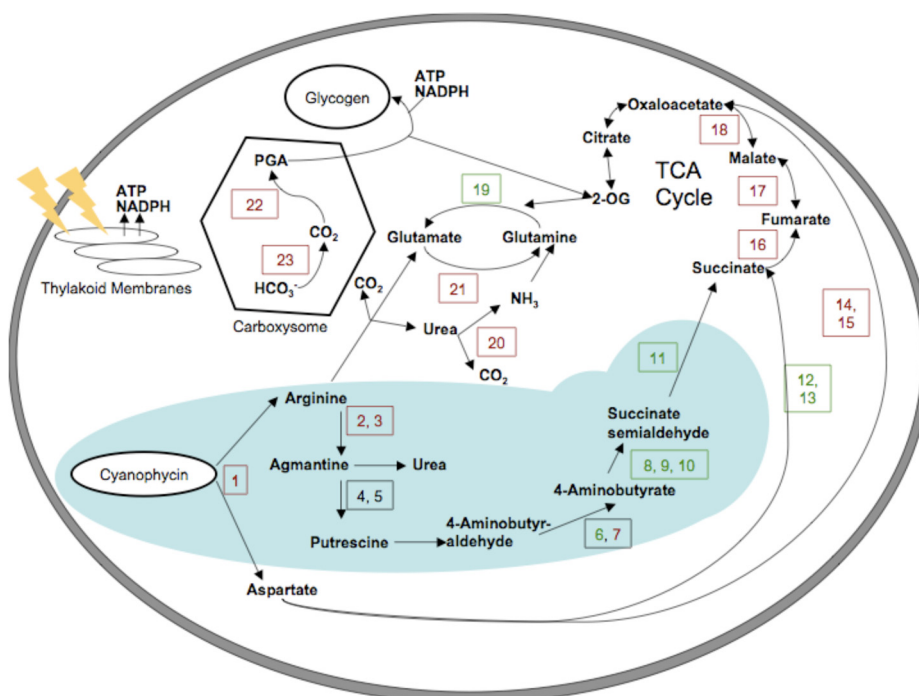


FIG. 6. **Global Stress Response under Different Perturbations.** Numbers denote the enzymes required for various reactions, colors indicate either increased (red) or decreased (green) protein abundances under stress conditions compared with those in the BG11 control sample. Black color indicates that proteins are not observed in BG11 control sample. Numbers correspond to the following enzymes: 1, cyanophycinase; 2 and 3, arginine decarboxylase; 4, agmatinase; 5, arginase; 6 and 7, 4-aminobutyraldehyde dehydrogenase; 8–10, 4-aminobutyrate transaminase; 11, aldehyde dehydrogenase; 12, adenylosuccinate lyase; 13, L-argininosuccinate lyase; 14, L-aspartic acid oxidase; 15, aspartic acid aminotransferase; 16, succinyl-CoA synthetase; 17, succinate dehydrogenase; 18, malate dehydrogenase; 19, Glu synthase; 20, urease; 21, Glu-NH<sub>3</sub> ligase; 22, ribulose 1,5-bisphosphate carboxylase oxygenase; 23, carbon concentrating mechanism proteins. Abundance values for these proteins are provided in [supplemental Table 1S–7S](#).

Examination of proteins involved in putrescine degradation suggested that this pathway is also active in *Synechocystis* (Fig. 6, [supplemental Table 8](#)). Slr1022 shows strong similarity to the proteins (YgjG and SpuC) involved in degradation of putrescine into aminobutyrate in *E. coli* and *Pseudomonas* (55, 56). Further evidence that succinate is produced from arginine came from the strong up-regulation of succinate dehydrogenase and malate dehydrogenase (Fig. 6, [supplemental Table 8](#)). Thus conversion of arginine into succinate not only allows generation of tricarboxylic acid cycle intermediates but in the process releases glutamate, ammonia, and CO<sub>2</sub>. Ammonia is assimilated into glutamate, whereas CO<sub>2</sub> is fixed by ribulose 1,5-bisphosphate carboxylase oxygenase. Indeed, we found that transporters of free inorganic carbon were not differentially regulated. However, several carbon concentrating mechanism proteins, required for concentrating intracellular carbon, showed significant up-regulation.

#### DISCUSSION

We report the most comprehensive functional and quantitative analysis of the *Synechocystis* proteome to date. The resulting proteome consists of 1955 unique proteins (53% of the predicted proteins), 1198 of which have been identified as differentially regulated under 12 different environmental con-

ditions. Several bias analyses show that proteins identified in this study are representative of the entire proteome. Importantly, functional category-based analysis shows that the observed proteins were uniformly distributed. Identification of 239 proteins of unknown function, of which 121 were differentially regulated, provides direct evidence of their roles in *Synechocystis* physiology. Taken together, this study has revealed the global proteomic makeup of *Synechocystis* and has facilitated a systems-level analysis of cellular response under different environmental conditions.

Analysis of 1198 differentially regulated proteins shows that *Synechocystis* utilizes few stress-specific proteins to optimize cellular functions under perturbations (Table I). Many of these proteins have no known function. In contrast, a large number of proteins associated with housekeeping functions were commonly differentially regulated. For example, key proteins involved in the biosynthesis of all amino acid families were strongly up-regulated. These results suggest that despite the prolonged starvation for essential nutrients, cells continue to maintain a metabolically active state by seeking either the limiting nutrients, or alternate nutrients for growth. Typically, transporters involved in the acquisition of iron, sulfur, and phosphorus were up-regulated. Although sulfate transporters were specifically up-regulated under sulfur depletion, expres-

sion of iron transporters was also up-regulated under phosphorus depletion and *vice versa*. In contrast, nitrate transporters were down-regulated under most environmental conditions. Previous studies using DNA microarrays have also shown strong down-regulation of nitrate transporters under various environmental conditions (7, 13). It has also been suggested that changes in environmental conditions lead to reduced transport of nitrate whereas simultaneously activating the pathway involved in the transport of alternate nitrogen substrates including ammonia, urea, and arginine (13). A similar mechanism under changing nutrient conditions is apparent from the analysis of proteomic datasets. Our data shows that the preferred substrates for nitrogen acquisition are urea and arginine.

The most striking cellular strategy revealed from the analysis of differentially regulated proteins is the way in which the cells acquire carbon and nitrogen under different perturbations. Our results suggest that any change in conditions, irrespective of their amplitudes or durations, immediately leads to the activation of alternate pathways toward the acquisition of carbon and nitrogen. Although the majority of perturbations had little impact on levels of proteins involved in photosynthesis, they affected the efficiency of photosynthetic light reactions, resulting in a lower production of energy. It should be mentioned that most photosynthetic proteins were identified as strongly down-regulated under nitrogen depletion and therefore, the lack of differential regulation was not because of our inability in the detection of peptides. The assimilation of carbon and nitrogen is an energy intensive process, requiring significant amounts of ATP, NADPH, and reduced ferredoxin. Therefore, decreased energy production leads to the activation of alternate carbon and nitrogen assimilation pathways. Our data strongly suggest that under the different environmental perturbations, urea and arginine are the preferred substrates for both carbon and nitrogen. Cells also actively seek internal and external carbon sources, as is apparent from the up-regulation of proteins involved in glycolysis and glucose transport. Arginine is preferentially catabolized *via* putrescine using the pathway recently characterized in *E. coli* and *Pseudomonas* (55, 56). Activation of this pathway allows cells to obtain both carbon in the form of succinate, and CO<sub>2</sub> and nitrogen in the form of glutamate and ammonia. Aspartic acid, which can be generated from cyanophycin, can serve two purposes. It can be utilized for the synthesis of methionine, lysine, and threonine. Additionally, aspartic acid can be combined with 2-oxoglutarate to produce glutamine, which can then be combined with ammonia to produce glutamate. This result also indirectly suggests that arginine is not directly converted to glutamate using the pathway identified in (11). The utility of this arrangement is that 2-oxoglutarate generated by glycolysis is channeled toward the production of glutamate.

In conclusion, the proteome analysis presented in this study has provided a unique and comprehensive catalogue of the

proteomic makeup of *Synechocystis* under various environmental conditions. We believe that the knowledge of the functional information of when and how proteins with known as well as unknown functions are expressed is going to be a strong basis for future experimental studies. Analysis of dynamic changes in the proteome has provided insights into cellular adaptations under various environmental perturbations. Our results showed that a key cellular adaptation leads to the activation of alternate pathways for the acquisition of carbon and nitrogen, which are the two major sinks for reducing powers generated by the photosynthetic light reactions in cyanobacteria.

**Acknowledgments**—We thank the members of the Pakrasi and Smith groups for collegial discussions. We thank Dr. N. Murata for providing a transcriptomic dataset for *Synechocystis* under cold stress.

\* This work was supported by the National Science Foundation grants FIBR EF0425749 and MCB 0745611 programs to H. B. P. This work was additionally supported as part of the Membrane Biology Scientific Grand Challenge project at the W. R. Wiley Environmental Molecular Science Laboratory, a DOE/BER national scientific user facility located on the campus of Pacific Northwest National Laboratory (PNNL) in Richland, Washington. The Pacific Northwest National Laboratory is a multiprogram national laboratory operated by Battelle Memorial Institute for the DOE under contract DE-AC05-76RLO-1830.

[S] This article contains [supplemental Tables 1S-7S](#).

§§ Both authors contributed equally to this work.

All proteomics datasets have been deposited with NCBI Peptidome under the accession number PSE117.

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## REFERENCES

1. Kasting, J. F. (2001) The rise of atmospheric oxygen. *Science* **293**, 819–820
2. Cavalier-Smith, T. (2002) Chloroplast evolution: secondary symbiogenesis and multiple losses. *Curr. Biol.* **12**, R62–R64
3. Kasting, J. F., and Siefert, J. L. (2002) Life and the evolution of Earth's atmosphere. *Science* **296**, 1066–1068
4. Johnson, Z. I., Zinser, E. R., Coe, A., McNulty, N. P., Woodward, E. M., and Chisholm, S. W. (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* **311**, 1737–1740
5. Falkowski, P. G., and Godfrey, L. V. (2008) Electrons, life and the evolution of Earth's oxygen cycle. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363**, 2705–2716
6. Lindahl, M., and Florencio, F. J. (2003) Thioredoxin-linked processes in cyanobacteria are as numerous as in chloroplasts, but targets are different. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 16107–16112
7. Singh, A. K., Elvitigala, T., Bhattacharyya-Pakrasi, M., Aurora, R., Ghosh, B., and Pakrasi, H. B. (2008) Integration of carbon and nitrogen metabolism with energy production is crucial to light acclimation in the cyanobacterium *Synechocystis*. *Plant Physiol.* **148**, 467–478
8. Tsinoremas, N. F., Castets, A. M., Harrison, M. A., Allen, J. F., and Tandeau de Marsac, N. (1991) Photosynthetic electron transport controls nitrogen assimilation in cyanobacteria by means of posttranslational modification of the *glnB* gene product. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4565–4569
9. Forchhammer, K. (2004) Global carbon/nitrogen control by PII signal transduction in cyanobacteria: from signals to targets. *FEMS Microbiol. Rev.* **28**, 319–333
10. Yang, C., Hua, Q., and Shimizu, K. (2002) Quantitative analysis of intracel-

- lular metabolic fluxes using GC-MS and two-dimensional NMR spectroscopy. *J. Biosci. Bioeng.* **93**, 78–87
11. Quintero, M. J., Muro-Pastor, A. M., Herrero, A., and Flores, E. (2000) Arginine catabolism in the cyanobacterium *Synechocystis* sp. Strain PCC 6803 involves the urea cycle and arginase pathway. *J. Bacteriol.* **182**, 1008–1015
12. Singh, A. K., Elvitigala, T., Cameron, J. C., Ghosh, B. K., Bhattacharyya-Pakrasi, M., and Pakrasi, H. B. (2010) Integrative analysis of large scale expression profiles reveals core transcriptional response and coordination between multiple cellular processes in a cyanobacterium. *BMC Syst. Biol.* **4**, 105
13. Singh, A. K., Bhattacharyya-Pakrasi, M., Elvitigala, T., Ghosh, B., Aurora, R., and Pakrasi, H. B. (2009) A systems-level analysis of the effects of light quality on the metabolism of a cyanobacterium. *Plant Physiol.* **151**, 1596–1608
14. Corbin, R. W., Paliy, O., Yang, F., Shabanowitz, J., Platt, M., Lyons, C. E., Jr., Root, K., McAuliffe, J., Jordan, M. I., Kustu, S., Soupen, E., and Hunt, D. F. (2003) Toward a protein profile of *Escherichia coli*: comparison to its transcription profile. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 9232–9237
15. Ideker, T., Thorsson, V., Ranish, J. A., Christmas, R., Buhler, J., Eng, J. K., Bumgarner, R., Goodlett, D. R., Aebersold, R., and Hood, L. (2001) Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science* **292**, 929–934
16. Fulda, S., Huang, F., Nilsson, F., Hagemann, M., and Norling, B. (2000) Proteomics of *Synechocystis* sp. strain PCC 6803: Identification of periplasmic proteins in cells grown at low and high salt concentrations. *Eur. J. Biochem.* **267**, 5900–5907
17. Fulda, S., Mikkat, S., Huang, F., Huckauf, J., Marin, K., Norling, B., and Hagemann, M. (2006) Proteome analysis of salt stress response in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Proteomics* **6**, 2733–2745
18. Gan, C. S., Chong, P. K., Pham, T. K., and Wright, P. C. (2007) Technical, experimental, and biological variations in isobaric tags for relative and absolute quantitation (iTRAQ). *J. Proteome Res.* **6**, 821–827
19. Gan, C. S., Reardon, K. F., and Wright, P. C. (2005) Comparison of protein and peptide prefractionation methods for the shotgun proteomic analysis of *Synechocystis* sp. PCC 6803. *Proteomics* **5**, 2468–2478
20. Herranen, M., Battchikova, N., Zhang, P., Graf, A., Sirpiö, S., Paakkari, V., and Aro, E. M. (2004) Towards functional proteomics of membrane protein complexes in *Synechocystis* sp. PCC 6803. *Plant Physiol.* **134**, 470–481
21. Huang, F., Fulda, S., Hagemann, M., and Norling, B. (2006) Proteomic screening of salt-stress-induced changes in plasma membranes of *Synechocystis* sp. strain PCC 6803. *Proteomics* **6**, 910–920
22. Huang, F., Hedman, E., Funk, C., Kieselbach, T., Schröder, W. P., and Norling, B. (2004) Isolation of outer membrane of *Synechocystis* sp. PCC 6803 and its proteomic characterization. *Mol. Cell Proteomics* **3**, 586–595
23. Huang, F., Parmryd, I., Nilsson, F., Persson, A. L., Pakrasi, H. B., Andersson, B., and Norling, B. (2002) Proteomics of *Synechocystis* sp. strain PCC 6803: Identification of plasma membrane proteins. *Mol. Cell Proteomics* **1**, 956–966
24. Kurian, D., Jansen, T., and Mäenpää, P. (2006) Proteomic analysis of heterotrophy in *Synechocystis* sp. PCC 6803. *Proteomics* **6**, 1483–1494
25. Kurian, D., Phadwal, K., and Mäenpää, P. (2006) Proteomic characterization of acid stress response in *Synechocystis* sp. PCC 6803. *Proteomics* **6**, 3614–3624
26. Mata-Cabana, A., Florencio, F. J., and Lindahl, M. (2007) Membrane proteins from the cyanobacterium *Synechocystis* sp. PCC 6803 interacting with thioredoxin. *Proteomics* **7**, 3953–3963
27. Pérez-Pérez, M. E., Florencio, F. J., and Lindahl, M. (2006) Selecting thioredoxins for disulphide proteomics: target proteomes of three thioredoxins from the cyanobacterium *Synechocystis* sp. PCC 6803. *Proteomics* **6**, S186–195
28. Pisareva, T., Shumskaya, M., Maddalo, G., Ilag, L., and Norling, B. (2007) Proteomics of *Synechocystis* sp. PCC 6803: Identification of novel integral plasma membrane proteins. *FEBS J.* **274**, 791–804
29. Sazuka, T., and Ohara, O. (1997) Towards a proteome project of cyanobacterium *Synechocystis* sp. strain PCC 6803: linking 130 protein spots with their respective genes. *Electrophoresis* **18**, 1252–1258
30. Sergeyenko, T. V., and Los, D. A. (2000) Identification of secreted proteins of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *FEMS Microbiol. Lett.* **193**, 213–216
31. Simon, W. J., Hall, J. J., Suzuki, I., Murata, N., and Slabas, A. R. (2002) Proteomic study of the soluble proteins from the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 using automated matrix-assisted laser desorption/ionization-time of flight peptide mass fingerprinting. *Proteomics* **2**, 1735–1742
32. Srivastava, R., Pisareva, T., and Norling, B. (2005) Proteomic studies of the thylakoid membrane of *Synechocystis* sp. PCC 6803. *Proteomics* **5**, 4905–4916
33. Wang, Y., Sun, J., and Chitnis, P. R. (2000) Proteomic study of the peripheral proteins from thylakoid membranes of the cyanobacterium *Synechocystis* sp. PCC 6803. *Electrophoresis* **21**, 1746–1754
34. Sazuka, T., Yamaguchi, M., and Ohara, O. (1999) Cyano2Dbase updated: linkage of 234 protein spots to corresponding genes through N-terminal microsequencing. *Electrophoresis* **20**, 2160–2171
35. Tonella, L., Hoogland, C., Binz, P. A., Appel, R. D., Hochstrasser, D. F., and Sanchez, J. C. (2001) New perspectives in the *Escherichia coli* proteome investigation. *Proteomics* **1**, 409–423
36. Schmidt, M. W., Houseman, A., Ivanov, A. R., and Wolf, D. A. (2007) Comparative proteomic and transcriptomic profiling of the fission yeast *Schizosaccharomyces pombe*. *Mol. Syst. Biol.* **3**, 79
37. Brunner, E., Ahrens, C. H., Mohanty, S., Baetschmann, H., Loevenich, S., Potthast, F., Deutsch, E. W., Panse, C., de Lichtenberg, U., Rinner, O., Lee, H., Pedrioli, P. G. A., Malmstrom, J., Koehler, K., Schimpf, S., Krijgsvel, J., Kregenow, F., Heck, A. J. R., Hafen, E., Schlapbach, R., and Aebersold, R. (2007) A high-quality catalog of the *Drosophila melanogaster* proteome. *Nat. Biotech.* **25**(5), 576–583
38. Mawuenyega, K. G., Kaji, H., Yamuchi, Y., Shinkawa, T., Saito, H., Taoka, M., Takahashi, N., and Isobe, T. (2003) Large-scale identification of *Caenorhabditis elegans* proteins by multidimensional liquid chromatography tandem mass spectrometry. *J. Proteome Res.* **2**, 23–35
39. Wegener, K. M., Welsh, E. A., Thornton, L. E., Keren, N., Jacobs, J. M., Hixson, K. K., Monroe, M. E., Camp, D. G., 2nd, Smith, R. D., and Pakrasi, H. B. (2008) High sensitivity proteomics assisted discovery of a novel operon involved in the assembly of photosystem II, a membrane protein complex. *J. Biol. Chem.* **283**, 27829–27837
40. Shen, Y., Zhao, R., Belov, M. E., Conrads, T. P., Anderson, G. A., Tang, K., Pasa-Tolić, L., and Veenstra, T. D., Lipton, M. S., Udseth, H. R., Smith, R. D. (2001) Packed capillary reversed-phase liquid chromatography with high-performance electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry for proteomics. *Anal. Chem.* **73**, 1766–1775
41. Eng, J. K., McCormack, A. L., and Yates, J. R. (1994) An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J. Am. Soc. Mass Spectrom.* **5**, 976–989
42. Qian, W. J., Liu, T., Monroe, M. E., Strittmatter, E. F., Jacobs, J. M., Kangas, L. J., Petritis, K., Camp, D. G., 2nd, and Smith, R. D. (2005) Probability-based evaluation of peptide and protein identifications from tandem mass spectrometry and SEQUEST analysis: the human proteome. *J. Proteome Res.* **4**, 53–62
43. Nesvizhskii, A. I., Keller, A., Kolker, E., and Aebersold, R. (2003) A statistical model for identifying proteins by tandem mass spectrometry. *Anal. Chem.* **75**, 4646–4658
44. Strittmatter, E. F., Kangas, L. J., Petritis, K., Mottaz, H. M., Anderson, G. A., Shen, Y., Jacobs, J. M., Camp, D. G., 2nd, and Smith, R. D. (2004) Application of peptide LC retention time information in a discriminant function for peptide identification by tandem mass spectrometry. *J. Proteome Res.* **3**, 760–769
45. Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Biol.* **305**, 567–580
46. Emanuelsson, O., Brunak, S., von Heijne, G., and Nielsen, H. (2007) Locating proteins in the cell using TargetP, SignalP and related tools. *Nat. Protoc.* **2**, 953–971
47. Page, J. S., Masselon, C. D., and Smith, R. D. (2004) FTICR mass spectrometry for qualitative and quantitative bioanalyses. *Curr. Opin. Biotechnol.* **15**, 3–11
48. Kaneko, T., Nakamura, Y., Sasamoto, S., Watanabe, A., Kohara, M., Matsumoto, M., Shimpo, S., Yamada, M., and Tabata, S. (2003) Structural

- analysis of four large plasmids harboring in a unicellular cyanobacterium, *Synechocystis* sp. PCC 6803. *DNA Res.* **10**, 221–228
49. Nakamura, Y., Kaneko, T., Hirose, M., Miyajima, N., and Tabata, S. (1998) CyanoBase, a www database containing the complete nucleotide sequence of the genome of *Synechocystis* sp. strain PCC 6803. *Nucleic Acids Res.* **26**, 63–67
50. Osanai, T., Imamura, S., Asayama, M., Shirai, M., Suzuki, I., Murata, N., and Tanaka, K. (2006) Nitrogen induction of sugar catabolic gene expression in *Synechocystis* sp. PCC 6803. *DNA Res.* **13**, 185–195
51. Singh, A. K., McIntyre, L. M., and Sherman, L. A. (2003) Microarray analysis of the genome-wide response to iron deficiency and iron reconstitution in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Physiol.* **132**, 1825–1839
52. Suzuki, I., Kanesaki, Y., Mikami, K., Kanehisa, M., and Murata, N. (2001) Cold-regulated genes under control of the cold sensor Hik33 in *Synechocystis*. *Mol. Microbiol.* **40**, 235–244
53. Suzuki, S., Ferjani, A., Suzuki, I., and Murata, N. (2004) The SphS-SphR two component system is the exclusive sensor for the induction of gene expression in response to phosphate limitation in *Synechocystis*. *J. Biol. Chem.* **279**, 13234–13240
54. Zhang, Z., Pendse, N. D., Phillips, K. N., Cotner, J. B., and Khodursky, A. (2008) Gene expression patterns of sulfur starvation in *Synechocystis* sp. PCC 6803. *BMC Genomics* **9**, 344
55. Chou, H. T., Kwon, D. H., Hegazy, M., and Lu, C. D. (2008) Transcriptome analysis of agmatine and putrescine catabolism in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* **190**, 1966–1975
56. Kurihara, S., Oda, S., Kato, K., Kim, H. G., Koyanagi, T., Kumagai, H., and Suzuki, H. (2005) A novel putrescine utilization pathway involves gamma-glutamylated intermediates of *Escherichia coli* K-12. *J. Biol. Chem.* **280**, 4602–4608