A systems level analysis of the effects of light quality on the metabolism of a cyanobacterium

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Cyanobacteria & light quality

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ABSTRACT

Photosynthetic organisms experience changes in light quantity and light quality in their natural habitat. In response to changes in light quality, these organisms redistribute excitation energy and adjust photosystem stoichiometry to maximize utilization of available light energy. However, response of other cellular processes to changes in light quality is mostly unknown. Here, we report a systematic investigation into the adaptation of cellular processes in *Synechocystis* to light that preferentially excites either photosystem II or photosystem I. We find that preferential excitation of photosystem II and photosystem I induce massive reprogramming of the *Synechocystis* transcriptome. Identification of these responsive genes has revealed the coordinated regulation of cellular processes with the excited photosystem. The rewiring of cellular processes begins as soon as *Synechocystis* senses the imbalance in the excitation of reaction centers. We find that *Synechocystis* utilizes the cyclic photosynthetic electron transport chain for ATP generation, and a major part of the respiratory pathway to generate reducing equivalents for nutrient assimilation during preferential excitation of photosystem I. In contrast, cytochrome c oxidase and photosystem I act as terminal components of the photosynthetic electron transport chain to produce sufficient ATP and limited amounts of NADPH and reduced ferredoxin during preferential excitation of photosystem II. To overcome shortage of NADPH and reduced ferredoxin, *Synechocystis* preferentially activates transporters and acquisition pathways to assimilate ammonia, urea and arginine over nitrate as nitrogen source. These results suggest that state transitions and adjustment of photosystem stoichiometry alone are insufficient to reverse the effects of excitation imbalance in cyanobacteria. This study provides the first systematic analysis of cellular processes in cyanobacteria in response to illumination that preferentially excites either photosystem II or photosystem I and shows that the cyanobacterial cell undergoes significant rewiring of cellular processes, many of which were previously unknown.

INTRODUCTION

Light is one of the most important environmental factors for photosynthetic organisms. It is used to drive photosynthesis and to regulate growth and development. The primary reactions of oxygenic photosynthesis are catalyzed by two large protein complexes, photosystem II (PSII) and photosystem I (PSI) [1]. These two complexes act in series to drive light-dependent electrochemical reactions. PSII catalyzes light-dependent oxidation of water and reduction of plastoquinone (PQ). PSI catalyzes light-dependent oxidation of PQ and reduction of ferredoxin and $NADP^+$. The ability of both PSII and PSI to catalyze electrochemical reactions is dependent on the presence of unique light harvesting antennae structures that are used for the efficient absorption of light. These antennae, a pigment-protein complex, optimize the capture of light by absorption at totally different wavelengths. Cyanobacteria utilize two main antenna pigments, chlorophyll (Chl) and bilin, to absorb light energy [2], [3]. Chls are mostly associated with PSI and absorb light of maximum absorbance wavelengths (λmax) ≈ 435 and 680 nm. Bilins are covalently attached to light-harvesting proteins called phycobiliproteins and are mostly associated with PSII. The specific combination of apo-proteins and bilins present in a phycobilisome (PBS) determines its light absorption profile. Two major phycobiliproteins commonly present in the PBS of cyanobacteria are the red-light absorbing allophycocyanin (AP) with λmax ≈ 650 nm and phycocyanin (PC) with λmax ≈ 620 nm.

The maximal quantum yield of photosynthesis is achieved when the rates of delivery of quanta from the respective antenna to PSII and PSI reaction centers are the same. Any change in spectral composition of light which affects the rate of quanta transfer to one reaction center over other leads to decreased photosynthetic efficiency and damages the photosynthetic apparatus [4], [5]. To counteract such imbalance, cyanobacteria, like plants and algae, have developed short term and long term adaptation mechanisms [4]-[9]. As a short-term mechanism which occurs in the timescale of second to minute, cyanobacteria utilize a process known as state transition; the adaptive, complementary transfer of light energy from the overexcited photosystem to the underexcited photosystem [10]. Under light conditions which predominantly excites Chl associated with PSI (PS1 light), cyanobacteria maintain balance by decreasing energy transfer to PSI and increasing energy transfer to PSII. Similarly, preferential excitation of PSII (PSII light) leads to an increase in energy transfer to PSI and decreases energy transfer to PSII in order to maintain balance. In contrast, long term mechanism requires the adjustment of the photosystem stoichiometry to regulate the balance of electron flow between two reaction

centers [4]-[6]. Thus by modulating the composition, structure and functions of the photosynthetic apparatus, cyanobacteria ensure an effective balance of light-driven electron transfer between the two photosystems under changing light quality.

The physiological and molecular basis of short term and long term adaptations under changing light quality have been well characterized in photosynthetic organisms [5], [7], [8]. However, response of cellular processes other than photosynthesis to changing light quality is largely unexplored. This is particularly striking given the wealth of recent data suggesting that photosynthetic process is tightly coupled to other principal metabolic pathways [11]–[14] . In addition, photosynthetic organisms have evolved complex transcriptional networks that dictate the acclimation process in response to environmental cues [15]. Thus, it can be speculated that the acclimation process of cyanobacteria to changing light quality is not limited to photosynthetic apparatus only but also involves other cellular processes. Indeed, few physiological results have previously shown that change in light quality affects other cellular processes. For example, respiration in higher plants and algae is activated by PSI light [16]. In contrast, cytochrome C oxidase activity, the terminal component of respiration, in *Synechocystis* PCC 6714 is reported to be preferentially activated by PSII light [17]. PII (a small nitrogen regulatory protein) has been shown to be covalently modified by PSII light [18]. It has been suggested that PII links the state of central carbon and energy metabolism to the control of nitrogen (N) assimilation in cyanobacteria [19]. In this work, we have utilized a time series DNA microarrays to investigate the response of *Synechocystis* to PSI and PSII lights. Our results show that preferential excitation of PSII and PSI leads to the massive reprogramming of the *Synechocystis* transcriptome. This reprogramming aligns certain cellular processes to be preferentially utilized under a given light condition. Such rewiring enables *Synechocystis* to overcome the limited production of energy and reducing equivalents and in the process, allows growth and development under changing light quality.

RESULTS

Physiological response of *Synechocystis* to PSI light and PSII light

The physiological response of white light grown *Synechocystis* to PSI and PSII lights was monitored by measuring the growth and 77K emission spectra of Chl fluorescence (Fig. 1). The measurement of fluorescence emission at 77K can be used to determine the rate of energy transfer from Chl and PBS to PSI and PSII reaction centers. This is accomplished by quantification of distinct fluorescence peaks originating from PSII and PSI (Fig. 1A & 1B). The peak at 685 nm arises from PSII and possibly from terminal emitters of PBS whereas the 695 nm and 725 nm peaks arise from PSII and PSI, respectively. The relative intensity of PSI fluorescence gradually decreased under PSI light (Fig. 1A). This gradual decrease was observed until 90 min and remained to the same level thereafter. The decrease of PSI fluorescence under PSI light indicates a decreased energy transfer to PSI and increased energy supply to PSII (the so-called “state 1”). In contrast, the relative intensity of PSI fluorescence gradually increased under PSII light (Fig. 1B). This gradual increase in PSI fluorescence was observed until 90 min and was maintained at this level thereafter. The increase of PSI fluorescence under PSII light indicates a decreased energy transfer to PSII and increased energy supply to PSI (the so-called “state 2”). These results show that preferential illumination of *Synechocystis* with PSI light or PSII light lead to the redistribution of excitation energy between the two reaction centers. We also observed that *Synechocystis* grew at a significantly slower rate under PSI light compared to cells grown under white light (Fig. 1C). In contrast, cells under PSII light grew at a rate that was comparable to growth of cells under white light.

Complementary regulation of PS genes by PSI and PSII lights establishes proof of concept

Illumination of *Synechocystis* with PSI light results in the positive regulation of PSII genes including those required for PSII biogenesis (Fig. 2). Transcriptional regulation of PSII genes by PSI light appears to be immediate, as significant regulation could be observed within 15 min of the onset illumination by PSI light. Similarly, PSI genes show positive regulation under PSII light (Fig. 2). However, regulation of PSI genes by PSII light is observed only after 30 min of illumination. Regulation of the *psbA* gene encoding D1 protein of PSII by PSI light and that of the *psaE* gene encoding PsaE protein of PSI by PSII light has been previously reported [20]-[22]. The same results are observed in the present study, supporting the conclusion that

preferential illumination of *Synechocystis* with PSI light or PSII light leads to the complementary regulation of PS genes.

Cellular processes responsive to PSII light

Our data show that the regulation of a large number of genes encoding proteins for various cellular processes is intimately linked to illumination with PSI and PSII lights (Table S1; Fig. 3). In addition to PSI genes, we find that genes coding for heat shock proteins (HSPs), cytochrome c oxidase, and those required for arginine catabolism are positively regulated by PSII light. Most HSP gene expression could be linked to illumination with PSII light (Table S2). We find that only the *sll1384* gene encoding an HSP, DnaJ, is positively regulated by PSI light. Positive regulation of the *sll1384* gene by PSI light could be seen within 15 min of onset illumination and expression remain high throughout the entire illumination period. Induction of several HSPs gene expression during heat shock in cyanobacteria is known to be modulated by light [23], [24], however, to our knowledge, this is the first time that regulation of HSP gene expression has been linked to illumination with a specific light quality.

Genes encoding aa_3-type cytochrome c oxidase are positively regulated by PSII light. These include all three genes present in the *ctaCDE* operon encoding aa_3-type cytochrome c oxidase subunits C, D and E. Neither of the other known terminal oxidases in *Synechocystis*, cytochrome *bd*-quinol oxidase and the alternative respiratory terminal oxidase [25], responded to specific light. We also find that genes encoding cytochrome c oxidase folding protein and Cyt cM, a small c-type cytochrome, are positively regulated by PSII light. It has been reported that cytochrome oxidase activity increases in *Synechocystis* 6714 under PSII light [17]. Our results suggest that a similar regulation of cytochrome c oxidase under PSII light occurs in *Synechocystis* and that its increased activity is due to regulation of genes encoding aa_3-type cytochrome oxidase.

We find that the *sll1831* gene encoding a subunit of glycolate oxidase is positively regulated by PSII light. Glycolate oxidase is the key enzyme involved in the salvage of 2-p-glycolate to phosphoglycerate and O_2 [26]. 2-p-glycolate is produced by the oxidase activity of Rubisco when O_2: CO_2 ratio increases. Preferential excitation of PSII is expected to increase the O_2: CO_2 ratio and therefore positive regulation of glycolate oxidase may constitute an important adaptation under these conditions. It must be noted that the *sll0404* gene encoding glycolate

dehydrogenase, identified by Eisenhunt et al., [27] and annotated as glycolate oxidase subunit in Cyanobase, is not regulated by either PSI light or PSII light in our study.

Cellular processes responsive to PSI light

In addition to PSII genes, a number of processes including CO_2 fixation, energy metabolism, and cytochrome b6f complex responded positively to PSI light. We find that genes encoding Rubisco subunits, CCM proteins, and phosphoribulokinase are positively regulated by PSI light (Table S2). In contrast, genes encoding transporters for the uptake of CO_2 and HCO_3^- are not preferentially regulated by either PSI light or PSII light. This contrasting regulatory pattern of genes involved in transport, and CO_2 fixation has also been observed during high light treatment [14]. It was found that genes encoding Rubisco and CCM proteins were downregulated whereas those coding for transporters required for active uptake of CO_2 and bicarbonate were upregulated during high light. The apparent inconsistency between the regulation of genes involved in transport, and CO_2 fixation was explored by measuring the effect of PSI and PSII lights on the uptake of ^{14}C-bicarbonate by *Synechocystis*. Uptake of bicarbonate in white light grown *Synechocystis* is at the rate of 130 $\mu mole.mg^{-1}chl.h^{-1}$ under our growth condition (Fig. 4). The high uptake rate of bicarbonate in *Synechocystis* is due to the growth of cells at ambient CO_2 concentration which is known to induce transporters involved in uptake of bicarbonate [28]. Illumination of *Synechocystis* with either PSI light or PSII light led to a significant but similar decrease in the uptake of bicarbonate (Fig. 4). These results show that illumination of *Synechocystis* with either PSI light or PSII light does not preferentially affect uptake of bicarbonate which is in accordance with the transcriptional pattern of bicarbonate transporters observed in the present study.

Genes encoding cytochrome b6/f complex, ATP synthase, and some of the NADH dehydrogenase subunits are positively regulated by PSI light (Table S2). These complexes participate in both photosynthetic and respiratory electron transport chains in cyanobacteria [25]. In addition, a number of genes coding for proteins involved in energy metabolism are positively regulated by PSI light (Table S2). These include genes involved in glycogen degradation, glucose transporter, glucose catabolism via the glycolytic and oxidative pentose phosphate (OPP) pathways, and the TCA cycle. The OPP is the major pathway of glucose catabolism in *Synechocystis* [29]. Glucose 6-phosphate dehydrogenase and phosphogluconate dehydrogenase, the two rate-controlling enzymes of OPP pathway, are positively regulated by PSI light. Taken together, these results suggest that illumination of *Synechocystis* with PSI light

leads to an activation of respiration, a conclusion similar to that observed in plants and algae [16]. However, a significant difference in activation of respiration in *Synechocystis* and plants is in the regulation of cytochrome c oxidase, the terminal component of respiration. Our data show that cytochrome c oxidase in *Synechocystis* is regulated by PSII light.

Cellular processes responsive to PSI and PSII lights

We identified several cellular processes, where for a given process some genes are positively regulated by PSI light while other genes involved in the same process respond to PSII light. Such processes include light harvesting, pigment biosynthesis, chemotaxis, and protein degradation. PBS is the major light harvesting complex in cyanobacteria. It consists of a core complex formed by AP and a rod complex formed by PC. The core and rod complexes are connected to each other via linker proteins. Our results show that genes encoding AP and core linker proteins are positively regulated by PSI light, a response similar to the regulation of PSII genes. In contrast, we find that the *cpc* operon encoding PC and rod linker proteins are positively regulated by PSII light, a response similar to the regulation of PSI genes. In addition, we find that the *cpcG1* gene encoding a rod-core linker protein is positively regulated by PSI light whereas the *cpcG2* gene encoding a second rod-core linker protein is not regulated by either light. It has been suggested that PBS containing the CpcG1 consists of both core and rod complexes and associates with PSII whereas PBS containing the CpcG2 consists of only rod complex and efficiently transfers light energy preferentially to PSI [30].

Close coordination between regulation of genes for pigment biosynthesis and pigment associated apoproteins was observed during preferential illumination of *Synechocystis*. Our data show that genes encoding proteins required for the conversion of glutamate to protoporphyrin IX are mostly non-responsive to preferential excitation. However, genes involved in the conversion of protoporphyrin IX to Chl are positively regulated by PSII light whereas genes involved in the conversion of protoporphyrin IX to bilin are positively regulated by PSI light (Table S2). We also find that *chlB* and *chlL* genes encoding the light-independent protochlorophyllide reductase are positively regulated by PSI light whereas the *por* gene encoding light-dependent protochlorophyllide reductase is not affected by either light. Similarly, the *sll1184* gene is positively regulated by PSI light whereas the *sll1875* gene is positively regulated by PSII light. Both these genes encode heme oxygenase, however, only the *sll1184* gene has been suggested to be involved in the multi-step monooxygenase reaction to produce biliverdin IXα and CO from protoheme [31].

A significant number of genes involved in motility show light quality dependent transcriptional regulation. These include genes coding for proteins involved in the pili formation and signal transduction pathways. Most of the genes including the *sll0038-sll0043* operon (loss of these genes result in negative phototaxis) and the *sll1291-sll1296* operon (loss of these genes has no effect on phototaxis) are positively regulated by PSII light. The *pilA9* operon is positively regulated by PSI light (Table S2). Regulation of these genes by PSI and PSII lights is in accordance with the importance of motility in photosynthetic organisms in response to changes in light quality and/or quantity [32].

Genes encoding proteins with significant function in cellular protection and redox homeostasis also show light quality dependent transcriptional regulation. Genes encoding ClpB, HtrA, HhoA, and two processing proteases are positively regulated by PSII light whereas genes encoding Clp and carboxy terminal proteases are positively regulated by PSI light. We find that *trxM* and *ntr* genes encoding thioredoxin M and NADP-thioredoxin reductase (NTR), respectively, are positively regulated by PSI light. These two genes were also upregulated during high light [14]. *hliB* and *hliD* genes encoding high light inducible proteins (HLIPs) are positively regulated by PSI light. The *sll1621* gene encoding a type II peroxiredoxin (Prx) and the *ssr2061* gene encoding a glutaredoxin (Grx) are positively regulated by PSII light. The PerR repressor which controls the expression of the *sll1621* and *ssr2061* gene is also positively regulated by PSII light. We find that a glutathione synthase is positively regulated coordinately with the *sll1159* gene by PSII light. Sll1159 contains a Cys–X–X–Cys motif and has a typical Grx domain.

Opportunistic utilization of N assimilation pathways under PSII light

A major adaptation in *Synechocystis* under PSII light appears to be the regulation of genes involved in transport and assimilation of N substrates. The *nrt* operon involved in transport and assimilation of nitrate is transiently regulated within 15 min of PSI light. In contrast, genes coding for proteins involved in transport and assimilation of ammonium, urea, glutamate, and arginine are positively regulated by PSII light (Fig. 5). This includes genes encoding ammonium permease, urea permease, and urease. In addition, the *gtrA* gene encoding a sodium-dependent glutamate transporter is positively regulated by PSII light. The imported N substrates are first converted to ammonia and then combined with glutamate to produce glutamine through GS-GOGAT pathways. Glutamine synthase (GS) is the first enzyme of GS-GOGAT pathway and combines glutamate and ammonia to produce glutamine. We find

261 that the *glnA* and *glnN* genes encoding (GS) are positively regulated by PSII light.
262 Commensurate with this finding, genes encoding IF7 and IF17 were positively regulated by PSI
263 light. These two proteins inhibit the activity of GS [33]. *ntcA* and *glnB* genes, involved in the
264 regulation of N assimilation in cyanobacteria [19], are also positively regulated by PSII light.
265 Additional genes involved in N assimilation and positively regulated by PSII light include an
266 asparaginase that converts asparagine to ammonia and subunits of carbamoyl-phosphate
267 synthase involved in conversion of L-glutamine to carbamoyl L-P. The *glsF* gene encoding
268 ferredoxin-dependent glutamate synthase and the *gdhA* gene encoding glutamate
269 dehydrogenase are positively regulated by PSI light. We also find that several genes encoding
270 proteins involved in the arginine catabolism [34] are positively regulated by PSII light (Table S2).

271 The DNA microarray data presented in this work suggest that *Synechocystis* cells
272 preferentially transport and assimilate ammonia, urea and arginine under PSII light over nitrate,
273 a common N substrate present in BG11 medium. To study the physiological effect of this
274 adaptation, we measured the growth of *Synechocystis* in BG11 medium in the presence or
275 absence of 2 mM NH_4NO_3 under illumination with either white light or PSII light. Cells grew to a
276 similar rate under illumination with white light and PSII light in BG11 medium (Fig. 7). Addition of
277 2 mM NH_4NO_3 into BG11 medium had no effect on the growth rate of *Synechocystis* under
278 illumination with white light. However, growth rate of *Synechocystis* increased significantly under
279 PSII light in the presence of 2 mM NH_4NO_3 (Fig. 7). The growth of *Synechocystis* in the
280 presence and absence of NH_4NO_3 confirm results obtained from microarray data and show that
281 assimilation of N substrates other than nitrate is beneficial to growth of *Synechocystis* under
282 PSII light.

DISCUSSION

Light is the only source for generation of energy and reduced carbon for cyanobacteria under photoautotrophic conditions. It is critically important that these organisms balance the excitation of PSII and PSI to maximize the quantum yield of photosynthetic light reactions and to protect the photosynthetic apparatus. Acclimation processes to counter imbalances in excitation energy distribution between the two photosystems are well studied [4]-[9]. However, response of cellular processes other than PSII and PSI to changes in light quality is mostly unknown. Thus, we have initiated a systematic investigation into the adaptation of cellular processes in *Synechocystis* during changes in light quality. Using a custom designed light emitting diode (LED) source, we stimulated energy flow through either PSI or PSII with a narrow bandwidth of lights tailored to preferentially excite either PSI or PSII. We find that approximately 33% of genes in *Synechocystis* are regulated by illumination with light that preferentially excites either Chl or PBS. Analysis of these genes has led for the first time to a systematic identification of cellular processes that are required for the growth and development of *Synechocystis* during adaptation to changes in light quality (Fig. 7). Illumination of *Synechocystis* with PSII light results in significant rewiring of cellular processes including the use of multiple terminal components of photosynthetic electron transport chain, induction of nutrient assimilatory pathways and protection mechanisms against oxidants and reactive oxygen species (ROS). Similarly, preferential excitation of PSI results in the activation of energy metabolism, respiration and protection mechanisms against reductants and ROS. These modifications enable *Synechocystis* to circumvent the limitation of energy and reducing power and protect cells from ROS during preferential illumination. Taken together, these results suggest that state transitions and photosystem stoichiometry alone are insufficient to reverse the effects of excitation imbalance. The rewiring of cellular processes begins as soon as *Synechocystis* senses the imbalance in the excitation of reaction centers.

Illumination of *Synechocystis* with PSII light leads to the accumulation of reduced PQ which must pass electrons to acceptor molecules in order to avoid photo-oxidative damage. Our results indicate that *Synechocystis* utilizes at least two routes to transfer electrons from reduced PQ. In the first route, the electrons are received by PSI. This is accomplished by the transfer of rod complex of PBS to PSI as apparent from the positive regulation of PC genes by PSII light. This modification allows *Synechocystis* to transfer electrons from the reduced PQ to PSI under PSII light. Despite an extensive study of the structure and function of PBS, little is known about the regulation of PBS interaction with PSII and PSI [35]. However, our conclusion is in

agreement with a recent finding that rod complex in association with a rod-core linker protein transfers light energy preferentially to PSI [30]. It was suggested that *Synechocystis* possesses two types of PBSs that differ by the presence of rod-core linker proteins (CpcG1 and CpcG2). CpcG1-PBS contains both core and rod complexes, and transfers light energy preferentially to PSII. Indeed, our data show that the *cpcG1* gene is positively regulated by PSI light. In contrast, CpcG2-PBS contains only rod complex and transfers light energy preferentially to PSI. In the second route, electrons from reduced PQ are received by cytochrome c oxidase, the terminal component of respiration. It should be noted that several components of the electron transport chain including PQ are shared by both respiration and photosynthesis in cyanobacteria [25]. Utilization of cytochrome c oxidase as terminal acceptor of electrons originating from PSII suggests that association of rod complex to PSI is not sufficient to accept all the electrons from reduced PQ. The use of two terminal electron acceptors during preferential excitation of PSII enables similar production of ATP as under normal light conditions. However, it leads to the limited production of reduced ferredoxin and NADPH as the electrons received by cytochrome c oxidase are used to reduce O_2. The limiting NADPH and reduced ferredoxin can significantly affect the assimilation of nutrients, especially C and N. The conversion of 1 mole of nitrate (present in BG11 media) to nitrite and then to NH_4^+ by nitrate reductase and nitrite reductase, respectively, requires 8 moles of reduced ferredoxin [36]. Our data show that cells overcome the shortage of reduced ferredoxin by activation of pathways involved in the transport and assimilation of ammonia, urea, arginine and glutamate. This adjustment not only overcomes the shortage of ferredoxin, but also ensures that the electrons from ferredoxin will be maximally used to produce NADPH that can be used to fix C. Further support of such rewiring of cellular processes comes from the measurement of physiological growth in the presence of different N substrate under PSII light. We find that growth of *Synechocystis* increases significantly in the presence of ammonium under PSII light compared to white light (Fig. 6).

Synechocystis is unable to grow under PSI light in our growth conditions (Fig. 1C). We propose that the inability of *Synechocystis* to grow under PSI light is related to a lack of NADPH production. Photosynthesis provides at least two modes for electron flow. In the first mode, a linear electron flow from PSII to PSI allows the production of ATP and reducing power in the forms of reduced ferredoxin or NADPH. In the second mode, a cyclic electron flow driven solely by PSI allows the production of ATP without the accumulation of reduced species. We suggest that *Synechocystis* can only generate ATP via the cyclic electron flow under PSI light. To

overcome the limited production of NADPH (or lack thereof), our data show that *Synechocystis* activates pathways involved in glucose catabolism and respiration. We find that genes encoding rate-controlling enzymes involved in glycogen degradation (glycogen phosphorylase) and catabolism of glucose (glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) are responsive to PSI light. The activation of respiration by PSI light in *Synechocystis* reinforces similar findings in higher plants and unicellular green algae [16]. However, as reported in the results section, cytochrome oxidase, the terminal component of respiration, was responsive to PSII light in *Synechocystis*. These results together suggest that *Synechocystis* utilizes the energy metabolism pathways to generate NADPH and C skeletons for biosynthesis from reduced C under PSI light. These adaptations will allow *Synechocystis* to grow optimally under PSI light in the presence of reduced C. In accordance with our conclusions, it has been previously shown that growth of *Synechocystis* in the presence of glucose requires blue light [37].

The rewiring of cellular processes allows cells to maintain growth and development during changes in light quality. However, cells must protect cellular functions against an excessive production of oxidants and reductants during preferential excitation. Our data show that *Synechocystis* utilizes the glutathione/GRX system in combination with Prxs for protection under PSII light. The glutathione/GRX system is known to protect cells during oxidative stress by reducing peroxides and Prxs, and by protecting thiol groups of enzymes via glutathionylation/ deglutathionylation mechanisms [38]. Additional cellular protection under PSII light is provided by HSPs. Our data show that most HSPs important for cellular functions including GroELS, HtpG, HspA, and Hsp70 are induced under PSII light. A role for HSPs in cellular protection under PSII light is consistent with earlier results obtained in *Synechocystis* suggesting that the transcription of HSP genes is induced in response to a reduced state of PQ and to oxidative stress [23], [24], [39], [40]. Similarly, the TrxM/NTR system and HLIPs appear to protect cellular functions under PSI light (Fig. 7). Recent studies have also suggested a role for TrxM/NTR in antioxidant network involved in mitigating ROS [14], [41]. HLIPs have been suggested to be involved in the protection of PSI during stress [42].

In summary, our analyses provide novel insights into the adaptations of *Synechocystis* to changing light quality. We show that a significant rewiring of cellular processes in addition to previously well studied adaptation mechanisms viz. state transitions and adjustment of photosystem stoichiometry is a must to reverse the effects of excitation imbalance. The preferential excitation of PSII favors the association of rod complexes of PBS to PSI which

allows a linear electron flow from PSII to PSI under light absorbed only by bilins. However, association of rod complex with PSI is not sufficient to accept all of the electrons from reduced PQ and electrons are also transferred to cytochrome c oxidase (Fig. 7). This adjustment creates the transmembrane proton gradient required for the synthesis of sufficient ATP, but leads to an inherently limited production of NADPH and reduced ferredoxin as electrons received by cytochrome c oxidase are wasted in reducing oxygen to water. In this situation, *Synechocystis* stops assimilation of nitrate for its N requirement and activate pathways involved in assimilation of alternative N sources. This adaptation not only minimizes the use of reduced ferredoxin but also ensures that the electrons from ferredoxin will be maximally used to produce NADPH. These cellular rewiring mechanisms allow *Synechocystis* to grow under PSII light at a rate typically observed under white light. In contrast, *Synechocystis* is unable to produce NADPH under PSI light due to a lack of electron flow from PSII. In this situation, cells depend exclusively on metabolic pathways involved in glucose catabolism and respiration to fulfill the requirements of reducing power for the assimilation of nutrients and C skeletons for biosynthesis (Fig. 7). These rewiring of cellular processes begins immediately following perception of the excitation imbalance by *Synechocystis*. Our results provide the first systematic analysis of cellular processes in cyanobacteria during changes in light quality and highlight the importance of preferential adjustments in cellular processes for the survival of photosynthetic organisms.

METHODS

Custom design of a light-emitting diode panel

A LED panel (26 x 26 cm^2) was custom designed and constructed at Photon Systems Instruments (Czech Republic; www.psi.cz). The panel consists of 14 rows of alternating royal blue and red emitting diodes separated by ~1.8 cm. Each row consists of seven LEDs. The royal blue emitting diodes have a wavelength range from 440 nm to 460 nm with a typical light emission at 455 nm. The red emitting diodes have a wavelength range from 620.5 nm to 645 nm with a typical light emission at 627 nm. The LED panel is connected to a programmable box that allows the control of light output intensity from 0 to 200 µE m^{-2} s^{-1} for both royal blue and red emitting diodes.

Illumination of *Synechocystis* with PSI light and PSII light

Synechocystis cells were grown at 30°C in BG11 medium buffered with 10 mM TES-KOH, pH 8.2 and bubbled with air as described previously [43]. Illumination was at 30 µE m^{-2} s^{-1} provided by fluorescent cool white light. Growth of cyanobacteria was spectrophotometrically monitored by measuring OD_{730} on a DW2000 (SLM-AMINCO, Urbana, IL). For illumination with PSI light or PSII light, cells grown under white light were transferred in a long test tube (3 cm in diameter) to a cell density of OD_{730}= 0.037 (~5 X 10^7 cells/ml). The tubes containing cells were transferred in a thermostat water bath maintained at 30°C and illuminated with either PSI light or PSII light provided by a custom designed LED panel. Light intensity was maintained at 10 µE m^{-2} s^{-1}. Cells were air-bubbled during light illumination. Cells were collected after 15 min, 45 min, 1.5 h, 2 h, 3 h and 6 h following illumination with light. Cells were spun down by centrifugation at 6000 x g, frozen in liquid nitrogen and stored at -80°C.

RNA isolation and DNA Microarray Hybridization

Total RNA from *Synechocystis* was isolated using RNAwiz kit (Ambion) as described (8). The quantity and quality of extracted RNA were determined spectrophotometrically (Nanodrop) at 260 and 280 nm and by Bio-analyzer (Agilent) as described by manufacturers. Total RNA isolated from PSI light or PSII light illuminated cells were fluorescently labeled either with Cy3 or Cy5 using the MICROMAX™ ASAP RNA Labeling Kit (PerkinElmer Life Sciences) as described (8). The specific activity of the labeled RNA was determined in a Nanodrop. Fluorescently labeled probes (700 ng of Cy3 and Cy5 labeled RNA to a final specific activity of 50 pmol/µg of

RNA) were hybridized to *Synechocystis* 11K custom oligo DNA microarrays. Hybridization, scanning and data extraction were performed by MOGENE (St Louis) essentially as described (8).

Statistical analysis

The experimental design used to identify regulated genes in response to PSI light and PSII light is essentially same as described (8). For each time points, we have used two biological replicates and each biological replicate consists of three process replicates including a dye-swap. Microarray data was processed using Matlab (The MathWorks, Inc, Novi, MI). Determination of the coefficient of variation (CV) of individual spots showed that the pixel intensity variation within the spots was quite low. We used LOWESS based data normalization procedure with a window size of 25% for removing the intensity based trends observed in the microarray data. The standard t-test was used to quantify the consistency of measurements across different replicates. A gene was considered as positively regulated by PSI light or PSII light if the absolute value of its log-ratio value exceeded a threshold of 0.3785 (i.e. 1.3 fold change) and p-value was less than 0.01 at any of the time points over the time points. The transcriptome data generated in this work has been submitted to the ArrayExpress database at the European Bioinformatics Institute (accession no. E-TABM-339).

Assay for uptake of bicarbonate

The uptake of bicarbonate in white light grown *Synechocystis* illuminated with PSI light or PSII light was measured using $NaH^{14}CO_3$ (Amersham). One milliliter of *Synechocystis* was mixed with 1 µCi of $NaH^{14}CO_3$ in a clear colorless eppendorf tube. Following illumination with white light (500 $\mu E.m^{-2}.s^{-1}$) for 30 sec, reaction was terminated by rapid filtration of the cells onto a glass filter (GF/B, Whatman) by suction, followed by immediate washing of the filter with 5 ml of BG11. The filter was subjected to the measurement of radioactivity.

Fluorescence Measurements

Fluorescence emission spectra of *Synechocystis* at 77 K were measured on a Fluoromax-2 fluorometer with excitation at 435 nm (Jobin Yvon, Longjumeau, France). Fluorescence induction kinetics at room temperature were performed on a dual modulation kinetic fluorometer (model FL-100, Photon Systems Instruments, Brno, Czech Republic) interfaced with a computer.

Supplementary Information

Table S1: Summary of differentially regulated genes

Table S2: List of genes regulated by PSI light and PSII light

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Author Contributions

Conceived and designed the experiments: AKS, HBP. Performed the experiments: AKS. Analyzed the data: AKS. Contributed reagents/materials/analysis tools: TE, RA, BG. Wrote the paper: AKS, MBP.

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Figure Legends:

Figure 1: **Physiological response of *Synechocystis* to PSI and PSII lights.** Chl fluorescence emission spectra of cells illuminated with (A) PSI light and (B) PSII light was measured following excitation of Chl at 435 nm. Curves were normalized to the fluorescence intensity at 695 nm. (C) Cell growth was monitored by measuring absorption at 730 nm under PSI light or PSII light. Error bars represent SD based on mean values of three independent growth experiments.

Figure 2: **A hierarchical cluster display of PS genes regulated by PSI and PSII lights.** Expression ratios {$\log_2$(PSII light/PSI light} of all PSI and PSII genes were used to generate cluster using Spotfire Decisionsite version 8.0 (Somerville, MA). Euclidean distance was used as measure of similarity between various time points and genes were clustered by using the weighted pair gene method with arithmetic mean. Colors description used to define the regulation pattern of a gene is provided in the lower panel of figure. The fold change of genes is provided in Table S2.

Figure 3: **A schematic representation of major cellular processes regulated by PSI and PSII lights.** Blue and orange circles represent responses associated with PSI light and PSII light, respectively. Circles containing both blue and orange colors represent cellular processes regulated by both PSI and PSII lights. The list of regulated genes belonging to these processes is provided in Table S2. The various abbreviations used are PR = photoreceptor(s); PSI = photosystem I; PSII = photosystem II; COX = cytochrome oxidase; HSP = heat shock proteins; EM-AA = energy metabolism-amino acids; PIGM = pigments; PBS = phycobilisome; CHEM = chemotaxis; PROT = proteases; CO_2 = CO_2 fixation; EM = energy metabolism; Cytbf = cytochrome b6f complex.

Figure 4: **Uptake of ^{14}C-bicarbonate in air-grown *Synechocystis* under PSI and PSII lights.** *Synechocystis* cells grown under white light were illuminated with either PSI light (open bar) or PSII light (solid bar) for 1 and 6 h, and rate of bicarbonate uptake was measured as described in Methods. Error bars represent SD based on mean values of four independent measurements.

Figure 5: **Effect of PSI and PSII lights on N assimilation pathways.** Pathways regulated by PSII light are colored red whereas those regulated by PSI light are colored blue. The *nrt*

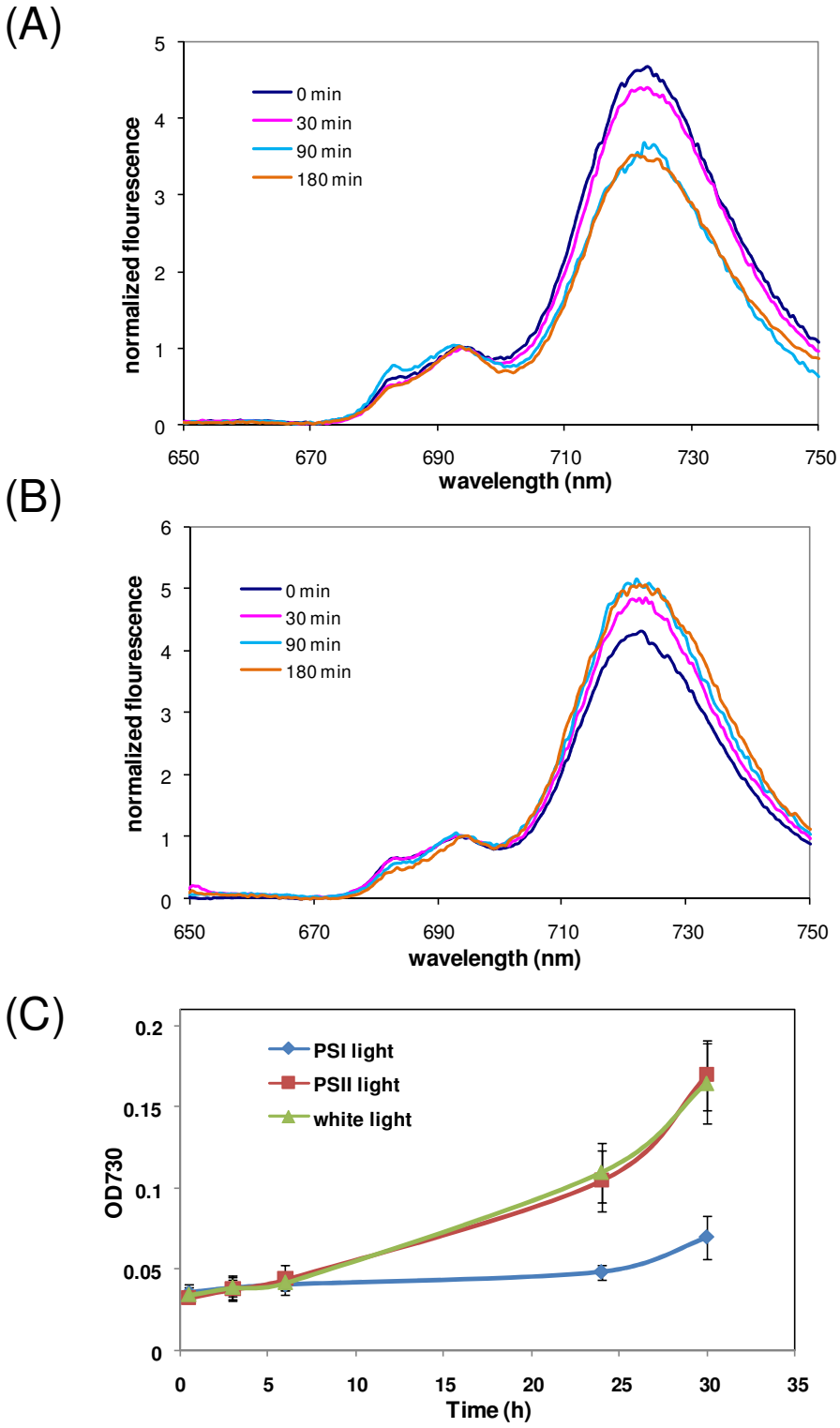
617 transporter is transiently regulated by PSI light and is colored light blue. The list of regulated
618 genes belonging to these processes is provided in Table S2.

619 Figure 6: **Effect of NH_4NO_3 and light quality on the growth of *Synechocystis*.** *Synechocystis*
620 cells were grown under 30 $\mu E.m^{-2}.s^{-1}$ of white light (circle) and 10 $\mu E.m^{-2}.s^{-1}$ of PSII light
621 (square) in BG11 with (solid) or without (open) 2 mM NH_4NO_3. Cell growth was monitored by
622 measuring absorption at 730 nm. Error bars represent SD based on mean values of three
623 independent growth experiments.

624 Figure 7: **A summary of key cellular adaptations during changes in light quality.** Blue and
625 orange colors represent responses associated with PSI light and PSII light, respectively.
626 The photosynthetic linear electron transfer chain is represented by dotted black line. The
627 dotted blue line (from ferredoxin to PQ) denotes the cyclic electron transfer chain under PSI
628 light. The dotted red lines show the electron transfer to cytochrome c oxidase under PSII
629 light. The solid orange line denotes the movement of rod complex to PSI under PSII light.
630 The abbreviations used are 2G = 2-glycolate; 3PG = 3-phosphoglycerate; and 2-OG = 2-
631 oxo-glutarate.

632

633 **Figure 1**



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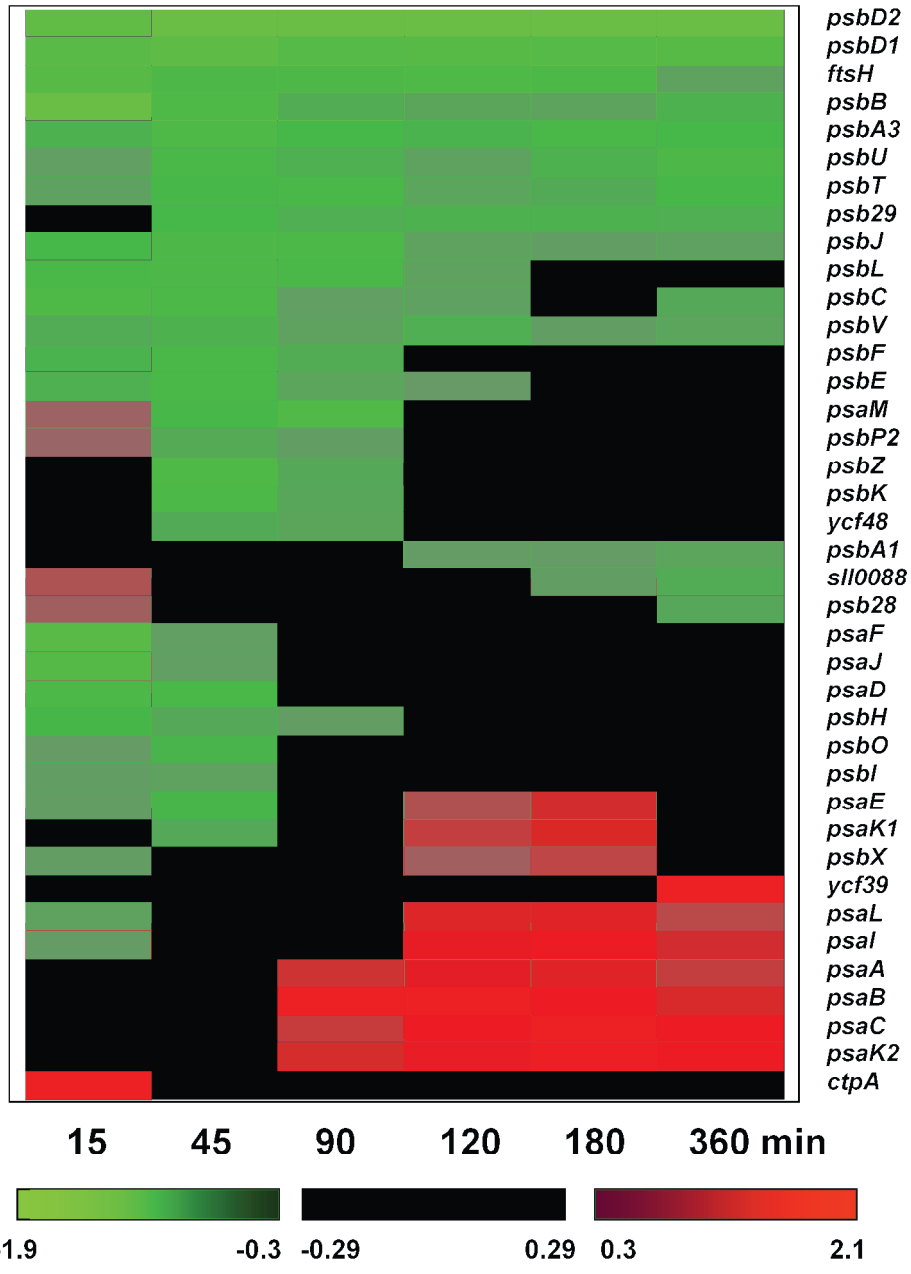
635 **Figure 2**



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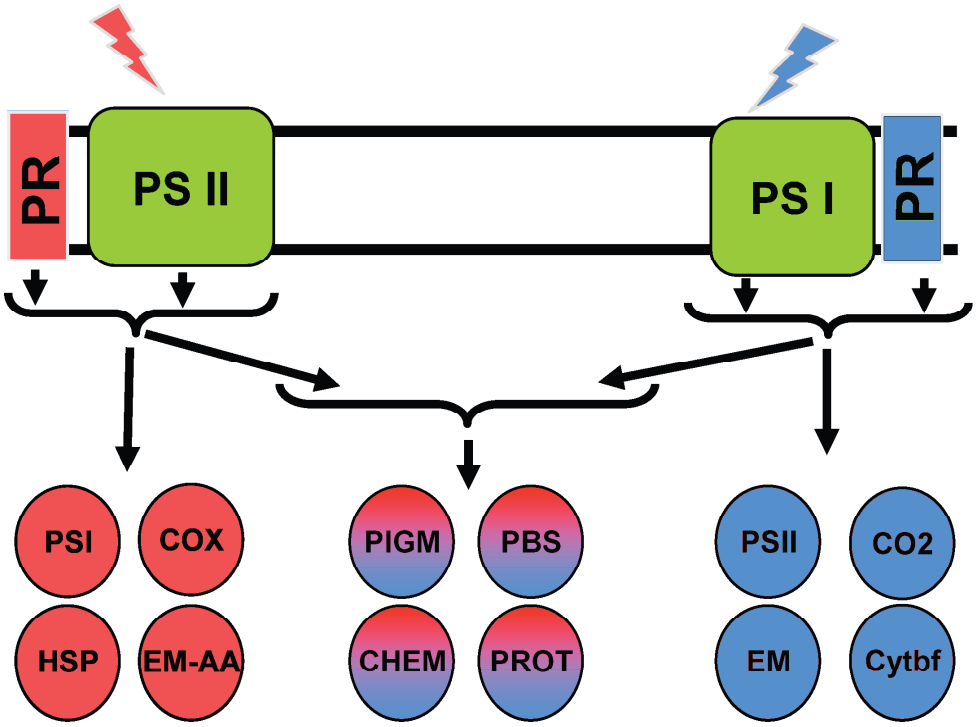
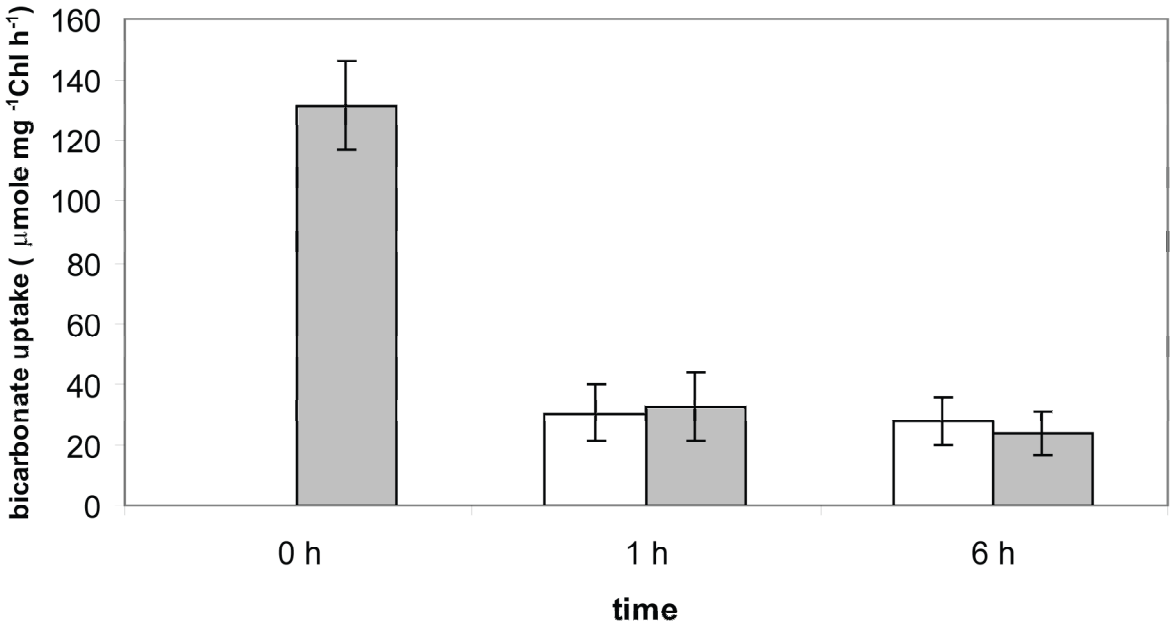
637 **Figure 3**



638

639 **Figure 4**



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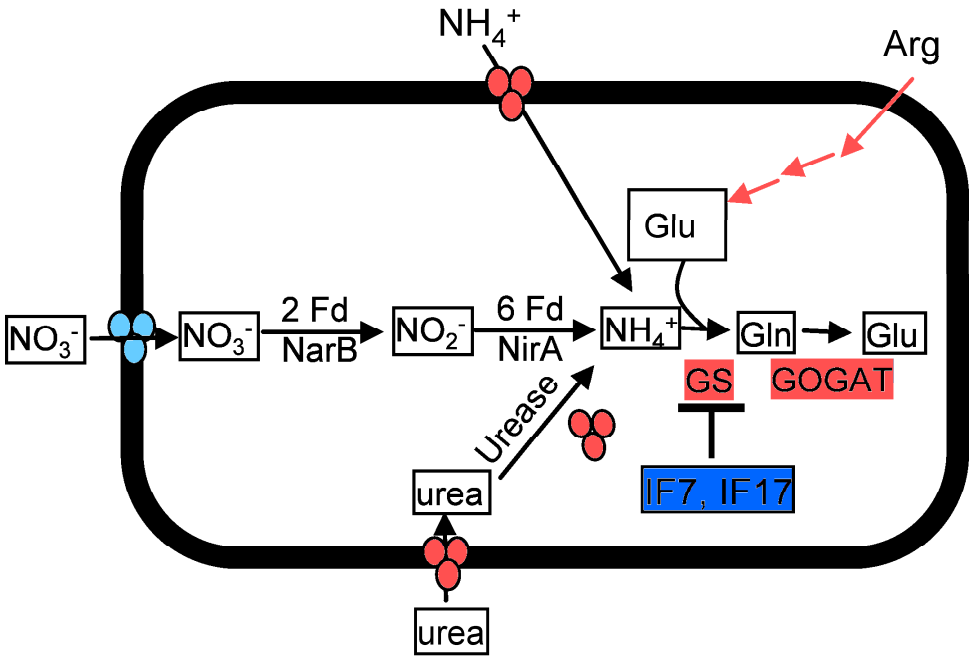
641 **Figure 5**



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643 **Figure 6**

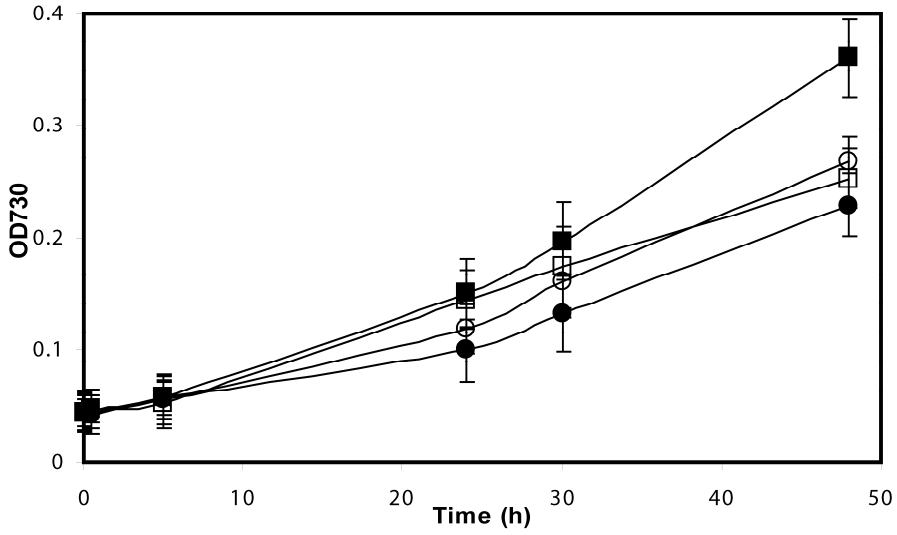

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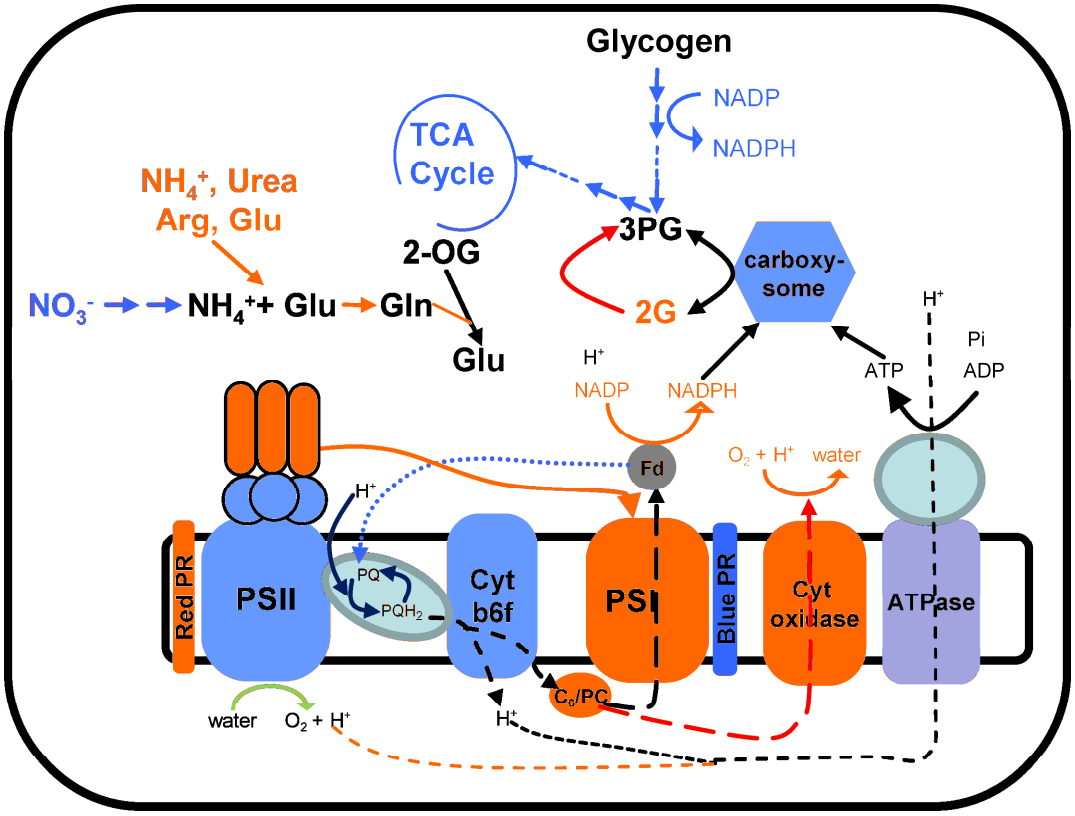
Figure 7


Glycogen
NADP
NADPH
TCA Cycle
NH4+, Urea
Arg, Glu
2-OG
3PG
carboxy-some
NO3-
NH4++ Glu
Gln
Glu
2G
H+
NADP
NADPH
ATP
Pi
ADP
Fd
O2 + H+
water
H+
Red PR
PSII
PQ
PQH2
Cyt b6f
PSI
Blue PR
Cyt oxidase
ATPase
C6/PC
water
O2 + H+
H+