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Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size

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ABSTRACT

A phycocyanin-deletion mutant of *Synechocystis* (cyanobacteria) was generated upon replacement of the *CPC*-operon with a kanamycin resistance cassette. The *Δcpc* transformant strains (*Δcpc*) exhibited a green phenotype, compared to the blue-green of the wild type (WT), lacked the distinct phycocyanin absorbance at 625 nm, and had a lower Chl per cell content and a lower PSI/PSII reaction center ratio compared to the WT. Molecular and genetic analyses showed replacement of all WT copies of the *Synechocystis* DNA with the transgenic version, thereby achieving genomic DNA homoplasmy. Biochemical analyses showed the absence of the phycocyanin α- and β-subunits, and the overexpression of the kanamycin resistance NPTI protein in the *Δcpc*. Physiological analyses revealed a higher, by a factor of about 2, intensity for the saturation of photosynthesis in the *Δcpc* compared to the WT. Under limiting intensities of illumination, growth of the *Δcpc* was slower than that of the WT. This difference in the rate of cell duplication diminished gradually as growth irradiance increased. Identical rates of cell duplication of about 13 h for both WT and *Δcpc* were observed at about 800 μmol photons m^{-2} s^{-1} or greater. Culture productivity analyses under simulated bright sunlight and high cell-density conditions showed that biomass accumulation by the *Δcpc* was 1.57-times greater than that achieved by the WT. Thus, the work provides first-time direct evidence of the applicability of the *T*runcated *L*ight-harvesting *A*ntenna (TLA)-concept in cyanobacteria, entailing substantial improvements in the photosynthetic efficiency and productivity of mass cultures upon minimizing the phycobilisome light-harvesting antenna size.

1. Introduction

Cyanobacteria have evolved an auxiliary light-harvesting antenna, the phycobilisome (PBS) that allows absorption of sunlight, primarily in the 575–675 nm region, and unidirectional excitation energy transfer toward the chlorophyll-pigment bed of PSII reaction centers. Each phycobilisome consists of two main structural parts, the core-cylinders and the peripheral rods. Core cylinders are made of allophycocyanin $(\alpha\beta)_3$ discs stacked next to each other [13]. The core cylinder axis is parallel to the thylakoid membrane surface with at least two of the cylinders resting with their long axes on the stromal side of the thylakoid membrane. These provide a structural and excitation energy transfer link to the chlorophyll-pigment bed of PSII reaction centers [3,10,11,13,14,16]. In *Synechocystis* sp. PCC 6803 (*Synechocystis*), there are three allophycocyanin core cylinders, two of which rest directly onto the thylakoid membrane. A third cylinder is resting on the stromal side of the furrow formed by the other two core cylinders [3,13]. Core cylinders contain the pigment-proteins allophycocyanin-α and allophycocyanin-β, encoded by the *APCA* and *APCB* genes, and a small linker polypeptide L_C, encoded by the *APCC* gene [14,19,36]. These are linked to the thylakoid membrane and the PSII dimer chlorophyll-proteins by a terminal excitation-acceptor allophycocyanin pigment including the linker polypeptide L_{CM}, encoded by the *APCE* gene [2,21]. The latter functions together with the products of the *APCD* and *APCF* genes to facilitate efficient excitation energy transfer from the phycobilisome toward the PSII reaction center [4,6,35,48]. Peripheral to the allophycocyanin core cylinders are phycocyanin-containing rods, also in cylinder form, physically extending outward from the allophycocyanin core cylinders [3,13,14,16]. Similar to the allophycocyanin, the phycocyanin rods are composed of stacked discs, each one made by six hetero-dimers of the pigment-containing CPC-α and CPC-β proteins, encoded by the *CPCA* and *CPCB* genes, respectively [14,19,36]. The CPC-α and CPC-β dimers are connected by linker polypeptides, encoded by *CPCC1*, *CPCC2*, and *CPCD* genes [19,57]. In *Synechocystis*, genes *CPCA*, *CPCB*, *CPCC1*, *CPCC2* and *CPCD* are clustered in a single operon, which is referred to as the *CPC*-operon.

Abbreviations: APC, allophycocyanin; Chl, chlorophyll; Car, carotenoids; dcw, dry cell weight; PBS, phycobilisome; Phc, phycocyanin; TLA, *T*runcated *L*ight-harvesting *A*ntenna

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The phycobilisome substantially increases the sunlight absorption cross-section of PSII [14,16], thereby countering a potential imbalance in excitation energy distribution due to the high PSI/PSII stoichiometric ratio in cyanobacteria [46,50], and the fact that most of the chlorophyll is associated with PSI in these microorganisms [16,37]. Up to 450 phycocyanin (Phc) and allophycocyanin (AP) pigments can be associated with the PBS in *Synechocystis*. This large light-harvesting antenna confers a survival advantage in the wild, where cells grow under light-limiting conditions. Under direct sunlight, however, the rate of photon absorption far exceeds the rate with which photosynthesis can utilize them, and excess light-energy is dissipated by non-photochemical quenching [5,25,26,49]. A soluble carotenoid-binding protein (orange carotenoid protein, OCP) plays essential role in this process in *Synechocystis*. Wasteful dissipation of excess absorbed irradiance prevents unwanted photodamage and photoinhibition [42] but inevitably results in a sub-optimal sunlight energy conversion. As a result, the utmost measured sunlight-to-biomass energy conversion efficiencies of cyanobacterial photosynthesis were reported to be in the range of 1–2%, whereas the theoretical maximum is 8–10% [44]. This pitfall affects all photosynthetic organisms [44]. It was alleviated in green microalgae, upon minimizing the size of the chlorophyll light-harvesting antenna, effectively limiting the capacity of the photosystems to over-absorb sunlight. A smaller photosystem antenna size prevented over-absorption of photons by individual cells, enabling deeper sunlight-penetration into the culture, and affording an opportunity for more cells to be productive, in effect raising photosynthetic productivity of the culture as a whole [27,28,45,54]. This concept of increasing photosynthetic productivity of a mass-culture under direct sunlight upon minimizing the light-harvesting antenna size is known as the *T*runcated *L*ight-harvesting *A*ntenna (TLA) concept [29,44,45]. In this work, we investigated the applicability of the TLA-concept in cyanobacteria by truncating the phycobilisome antenna size and measuring the effect on *Synechocystis* mass-cultures growing under high light conditions. The results showed substantial improvement in the photosynthetic productivity and biomass accumulation of TLA cyanobacterial cultures over that of their wild type counterparts.

2. Materials and methods

2.1. Cell cultivation

Synechocystis sp. PCC 6803 was used as the recipient strain, and is referred to as the wild type. Wild type and transformant strains were maintained on solid BG-11 media supplemented with 10 mM TES–NaOH (pH 8.2), 0.3% sodium thiosulfate at 25 °C and about 50 μmol photons m^{-2} s^{-1}. When indicated, kanamycin was added to a concentration of 50 μg/mL. Liquid cultures were grown in 25 mM phosphate buffered BG11, pH 7.5, at 25.5 °C under constant aeration and were gradually acclimated to the final light intensity. Acclimation times were 3 d at 170 μmol photons m^{-2} s^{-1}, 5 d at 350 μmol photons m^{-2} s^{-1}, 14 d with a step-wise increase in the light intensity to 1500 μmol photons m^{-2} s^{-1}, and 20 d with a step-wise increase in the light intensity to 2000 μmol photons m^{-2} s^{-1}. Cultures grown under 2000 μmol photons m^{-2} s^{-1} were bubbled continuously with 3% CO_2 to ensure that C-availability would not limit the rate of growth. A cylindrical bioreactor was employed for biomass accumulation measurements with internal diameter of 12 cm, simulating conditions for commercial growth. *Synechocystis* inoculum of 0.5 g dcw L^{-1} was initially applied to ensure that >98% of incident irradiance would be absorbed by the culture. Biomass accumulation measurements were conducted in the range of 0.5–1.0 g dcw L^{-1}.

2.2. Nucleic acid extractions

Synechocystis genomic DNA was isolated for PCR analysis using Qiagen's Plant DNA purification kit (Qiagen, USA) according to the manufacturer's protocol.

2.3. Generation of Δcpc-transformants of Synechocystis sp. PCC6803

A 1928 bp DNA construct was synthesized (DNA2.0, USA) containing 550 bp of homologous DNA regions upstream and downstream of the *CPC*-operon, designed to replace the coding region of the *CPC*-operon with a codon-optimized *NPTI* gene conferring kanamycin resistance to transformants.

Transformations of *Synechocystis* were carried out according to the procedures established in this lab [7,34]. Successful replacement of the *CPC*-operon with the *NPTI* construct and complete cyanobacterial DNA copy segregation was verified by genomic DNA PCR analysis, using primers further upstream and downstream of the regions of the *CPC*-operon that were used for homologous recombination, and also by using primers within the *CPC*-operon (primer sequences are reported in Table 1).

2.4. Microscopic imaging analysis

Cells were grown at 350 μmol photons m^{-2} s^{-1} and sampled out of the exponential growth phase when biomass density was 0.3–0.5 g dcw L^{-1}. Imaging analysis of the *Synechocystis* WT and *Δcpc*-transformants was conducted by a Zeiss AxioImager M1, Hamamatsu C8484 equipped with a Sutter Instruments Lambda LS light source and a 100× 1.4 NA objective, and a Zeiss LSM 710 laser scanning confocal microscope quipped with a 100× 1.4 NA objective (UC Berkeley Imaging Facility). Excitation band of 540–580 nm (filter set Chroma #49008) was used to excite predominantly phycocyanin, referred to as F-Phc1 excitation in the Results. Excitation band of 450–490 nm (filter set Chroma #49002) was used to excite predominantly Chl *a*, referred to as F-Chl excitation in the Results. An excitation wavelength of 593 nm was used in the confocal microscope images to excite predominately phycocyanin and was referred to as F-Phc2 excitation in the Results. Imaging of the cells was taken under exactly the same experimental settings for each condition, so as to enable direct comparisons.

2.5. Pigment analysis, biomass quantification and cell size determination

Aliquots were extracted from the exponential growth phase of cultures. Chlorophyll *a* and carotenoid concentrations were determined spectrophotometrically in 100% methanol extracts of the cells according to Lichtenthaler [32]. Culture biomass accumulation was measured gravimetrically as dry cell weight (dcw), whereby 5 or 10 mL aliquots of a culture were filtered through 0.22 μm Millipore filters and the immobilized cells dried at 80 °C for 12 h prior to weighing the dry cell weight. Cell size was determined with a Beckman coulter Multisizer™ 3.

2.6. Measurements of photosynthetic activity

The oxygen evolution activity of the cultures was measured at 25 °C with a Clark-type oxygen electrode (Rank Brothers, Cambridge, England) illuminated with actinic light from a quartz halogen lamp projector. A Corning 3-69 filter (510 nm cut-off filter, Corning, NY) defined the

Table 1
Primers used in the genomic DNA analysis of *Synechocystis* wild type and *Δcpc* transformants. Primer sets refer to Fig. 2.

Primer direction	Used in primer set, see Fig. 2	Sequence, 5′ to 3′
Forward	b, c	GACTTGAATGTCACTAACTACATCCAGTCTTTGC
Forward	d	GCTAAATCCCATGAAGAGAAGGTTTATG
Forward	a, e	CCATTAGCAAGGCAAATCAAAGAC
Reverse	c, d, e	GGTGGAAACGGCTTCAGTTAAAG
Forward	f, g, h	GTTCCCTTTGGTCAAGCAAGTAAG
Reverse	a, f	GGTTGATTCGTTTACATCAGTTCAATAAAG
Reverse	g	CCATTAAACATTGTGCTTACACTCC
Reverse	b, h	GAAGTGCCAGTGACTAACCTTTATCGAG

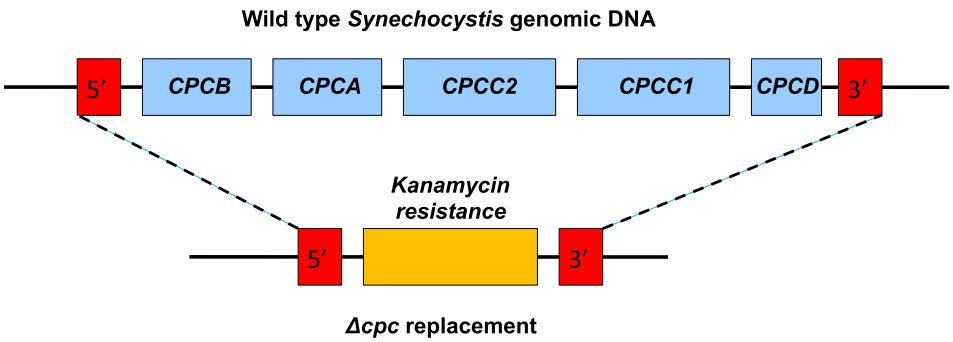


Fig. 1. Genetic map of the *Synechocystis CPC*-operon and replacement of the *CPC*-coding region with the *NPTI* gene conferring a kanamycin resistance. Red: Nucleotide regions of the *Synechocystis* genomic DNA used for homologous recombination; blue: *CPC*-operon genes; orange: *NPTI* gene.

yellow actinic excitation via which photosynthesis measurements were made. Samples of 5 mL cell suspension containing 1.3 μM Chl were loaded onto the oxygen electrode chamber. Sodium bicarbonate (100 μL of 0.5 M solution, pH 7.4) was added to the cell suspension prior to the oxygen evolution measurements to ensure that photosynthesis and oxygen evolution would not be limited by the carbon supply available to the cells. After registration of the rate of dark respiration by the cells, samples were illuminated with gradually increasing light intensities. The rate of oxygen exchange (uptake or evolution) under each of these irradiance conditions was recorded continuously for 2–5 min allowing a linear regression of the slope.

2.7. Cell fractionation

Cells were harvested by centrifugation at 1000 *g* for 3 min at 4 °C. Samples were resuspended with ice-cold lysis buffer containing 50 mM Tricine (pH 7.8), 10 mM NaCl, 5 mM $MgCl_2$, 0.2% polyvinylpyrrolidone-40, 0.2% sodium ascorbate, 1 mM aminocaproic acid, 1 mM aminobenzamidine and 100 μM phenylmethylsulfonyl fluoride (PMSF). Cells were lysed in a French pressure cell operated at 4 °C, upon passing twice under 20,000 psi pressure with 30 s cooling intervals on ice. Unbroken cells were removed by centrifugation at 3000 *g* for 4 min at 4 °C. Membranes were collected by centrifugation of the supernatant at 75,000 *g* for 45 min at 4 °C. The thylakoid membrane pellet was resuspended in a buffer containing 50 mM Tricine (pH 7.8), 10 mM NaCl, and 5 mM $MgCl_2$ for spectrophotometric measurements, or 250 mM Tris–HCl (pH 6.8), 20% glycerol, 7% SDS and 2 M urea for protein analysis.

2.8. SDS-PAGE and Western blot analysis

SDS-PAGE and Western blot analyses were performed with total protein from cell extracts, resolved in precast SDS-PAGE "Any KD™" (BIO-RAD, USA). Loading of samples was based on chlorophyll content and resolved proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon-FL 0.45 μm, Millipore, USA) by a tank

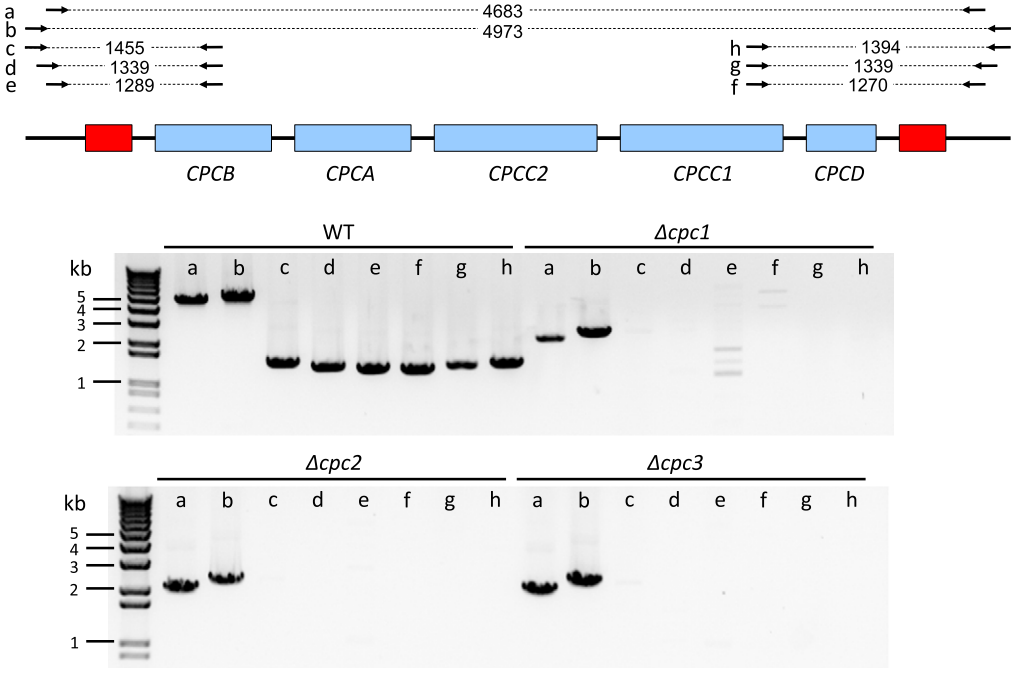
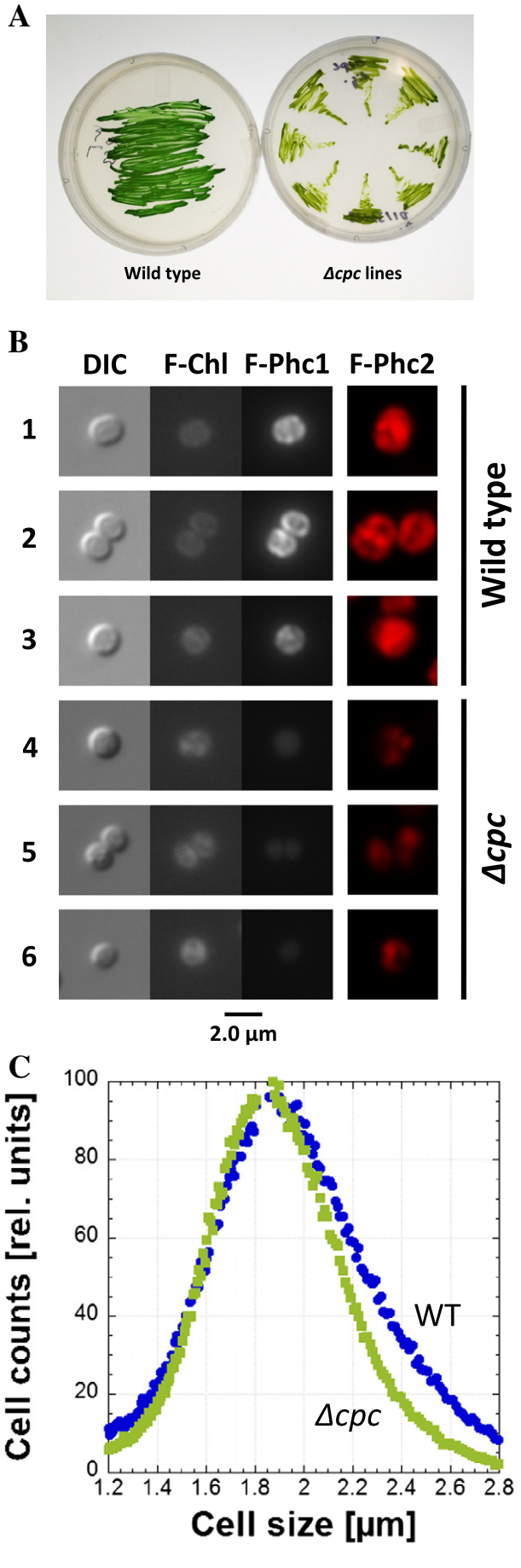


Fig. 2. PCR-analysis of *Synechocystis* wild type and three independent *Δcpc*-transformants to test for insertion locus and DNA copy homoplasmy. Primer-sets a and b tested for the correct replacement of the *CPC*-operon with the *NPTI* gene. Expected product size for the wild type, using primer set a, was 4683 nt. Expected product size for the *Δcpc*-transformants, using primer set a, was 2126 nt. Expected product size for the wild type, using primer set b, was 4973 nt. Expected product size for the *Δcpc*-transformants, using primer set b, was 2416 nt. Primer-sets c through h were designed to test for homoplasmy by specifically amplifying the wild type genomic DNA. No PCR amplification product was obtained using genomic DNA from the *Δcpc*-transformants.



transfer system. Specific polyclonal antibodies were raised against the spinach D1, D1/D2 PSII reaction center proteins (PSII RC), RBCL, and the ATP-β subunit, as well as the orange carotenoid protein (OCP) from *Synechocystis*. Cross-reactions were visualized by Supersignal West Pico Chemiluminescent substrate detection system (Thermo Scientific, USA).

2.9. Spectrophotometric and kinetic analyses

The concentration of the photosystems in thylakoid membrane preparations was measured spectrophotometrically from the amplitude of the light-*minus*-dark absorbance difference signal at 700 nm (P700) for PSI, and 320 nm (Q_A) for PSII [40,46,55]. The functional absorption cross-section of PSII and PSI in wild type and transformant *Synechocystis* and in the respective thylakoid membranes was measured upon weak green actinic excitation (50 µmol photons m^{-2} s^{-1}) of the samples from the kinetics of Q_A photoreduction and P700 photooxidation, respectively [40].

3. Results

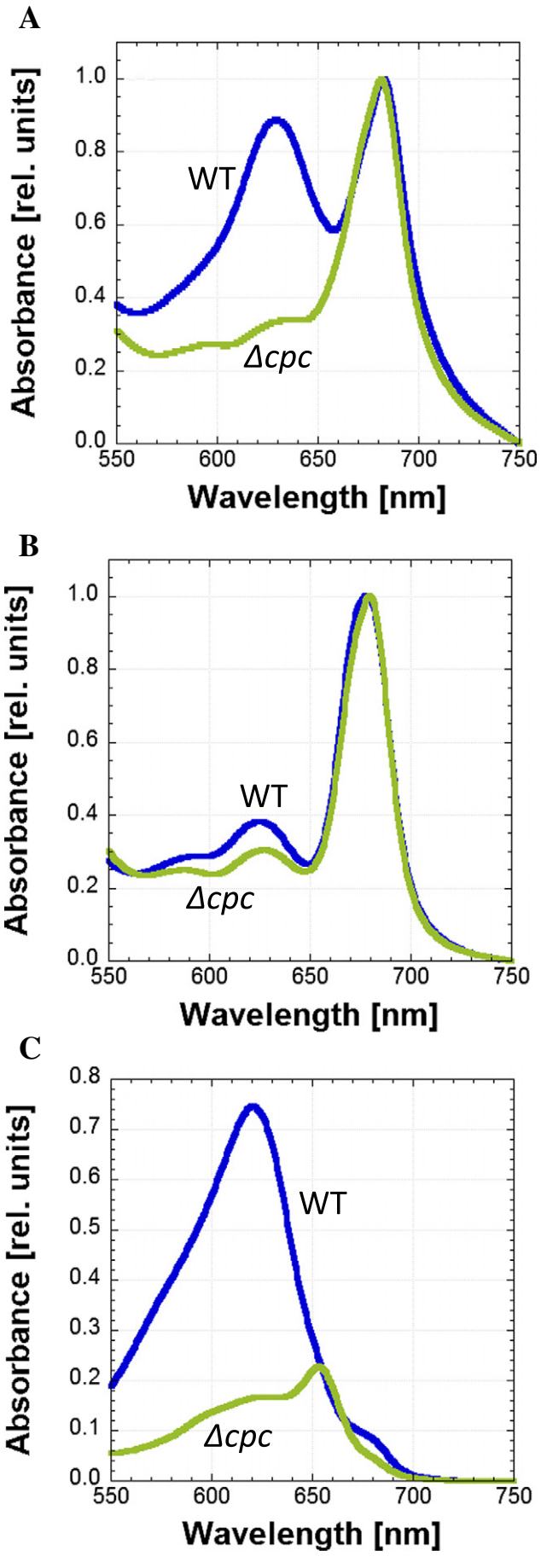
3.1. Construction of a phycocyanin deficient Synechocystis strain

To investigate whether the concept of the *T*runcated *L*ight-harvesting *A*ntenna (TLA) of the photosystems, originally developed in green microalgae [29] can be applied to cyanobacteria, as a way by which to improve culture productivity under bright sunlight and high cell-density conditions, we generated and tested transformants with a severe reduction in the size of the phycobilisome (PBS) light-harvesting antenna. This was achieved upon deletion of the *CPC* operon in *Synechocystis*, encoding for most of the proteins needed for the assembly of the PBS-peripheral phycocyanin rods. The *CPC*-operon, as defined in this work, includes the *CPCA* gene, encoding for the phycocyanin α-subunit, the *CPCB* encoding the Phc β-subunit, the *CPCC1* and *CPCC2* encoding the phycocyanin rod linker polypeptides and the *CPCD* encoding a small linker polypeptide. The *CPC* operon was replaced via double homologous recombination with a neomycin phosphotransferase I (*NPTI*) gene, conferring kanamycin resistance, as a selectable marker, under the control of the endogenous *CPC*-operon promoter (Fig. 1).

3.2. Transformant DNA segregation analysis (homoplasmy)

Genomic DNA PCR reactions were used to map the insertion site and to test for transformant DNA copy homoplasmy, i.e., to ensure that wild type copies of the DNA are deleted and that every copy of the resultant transformant cyanobacterial genome contains the *NPTI* gene and lacks the endogenous *CPC*-operon (Fig. 2). Primer set "a" in Fig. 2 tested for the correct integration of the insert at the *CPC*-operon site by using primers outside of the region of the homologous recombination, designed to amplify the entire *CPC*-operon and yielding different size fragments from the wild type and transformant DNA. In this case, the PCR product size using wild type (WT) genome as a template was a single 4683 bp product (Fig. 2, WT a). Three independent *Δcpc* transformant lines generated a single product of 2126 bp (Fig. 2, *Δcpc1* a, *Δcpc2* a, and *Δcpc3* a) due to the replacement of the *CPC*-operon with the *NPTI* gene.

Fig. 3. Coloration, Chl *a* and Phc fluorescence of *Synechocystis* wild type and *Δcpc*-transformant lines. A: Strains grown on BG-11 agar plates. Note the blue-green coloration of the wild type strain and the green coloration of the *Δcpc*-transformants. B: Microscopic images of wild type and *Δcpc*-transformant cells. DIC: differential interference contrast; F-Chl: Chl *a* fluorescence. F-Phc1: phycocyanin fluorescence; F-Phc2: phycocyanin fluorescence imaged with a confocal microscope. 1–3: Wild type cells; 4–6 *Δcpc*-transformant cells. In each row the set of DIC, F-Chl and F-PhcF1 images were taken from the exact same cells, while the confocal microscope image (F-Phc2) was taken from different cells. C: Cell size analysis. Cells were taken out of the exponential growth phase. The distribution is very slightly shifted toward smaller cells in the *Δcpc*-transformants but overall they have a very similar cell size compared to the wild type.



The smaller product size observed for the transformants in Fig. 2 (*Δcpc1* a, *Δcpc2* a, and *Δcpc3* a) indicated replacement of the *CPC*-operon with *NPTI*. It is also important to note that the *Δcpc1*, *Δcpc2*, and *Δcpc3* transformants did not generate a 4683 bp wild type product, suggesting that the transformants have achieved transgenic DNA copy homoplasmy, meaning that no copy of the wild type genome remains in the transformant cells.

Primer set "b" in Fig. 2 was used to show reproducibility of the above findings by a different set of primers and by amplifying a PCR product that is slightly longer than that in Fig. 2a, with expected sizes of 4973 for the wild type and 2416 bp for the *Δcpc* transformants, respectively. In this case, the PCR product size using WT genome as a template was a single 4973 bp product (Fig. 2, WT b). The three independent *Δcpc* transformant lines generated a single product of 2416 bp (Fig. 2, *Δcpc1* b, *Δcpc2* b, and *Δcpc3* b) due to the replacement of the *CPC*-operon with the *NPTI* gene. Here again it was noted that the *Δcpc1*, *Δcpc2*, and *Δcpc3* transformants did not generate a 4973 bp wild type product, confirming that the transformants have achieved transgenic DNA copy homoplasmy.

Primer sets "c", "d", "e", "f", "g" and "h" were all designed to yield PCR products specific to the wild type genomic DNA because one primer anneals to the native *CPC*-operon and thus, would not generate a product if the *CPC*-operon was quantitatively replaced by *NPTI* in all *Synechocystis* DNA copies. These sets of primers tested specifically for the presence of wild type genomic DNA copies in the transformants and, because of the sensitivity of a PCR reaction, this was designed to identify transformant lines that have not yet reached homoplasmy. The results of Fig. 2 (c, d, e, f, g, h) showed that PCR products were obtained with wild type DNA as the template of the PCR reaction and that none of the three *Δcpc1*, *Δcpc2* and *Δcpc3* independent transformant lines generated products using these primers. This is further evidence showing that *Synechocystis Δcpc1*, *Δcpc2* and *Δcpc3* transformant lines lacked wild type copies of DNA in their respective genomes and have reached the required complete *Δcpc* genomic DNA segregation (homoplasmy).

3.3. Wild type and Δcpc transformant cellular phenotype analyses

A change in the pigmentation of the *Δcpc* transformants was noted by the coloration of the colonies, which were green compared to the blue-green coloration of the wild type (Fig. 3A). This is consistent with observations on other mutants lacking phycocyanin [1]. To investigate the effect of the *Δcpc* mutation at the cellular level, we used differential interference contrast (Fig. 3B, DIC) and fluorescence emission imaging analysis of the cells, specifically exciting either Chl (Fig. 3B, F-Chl) or Phc (Fig. 3B, F-Phc). The DIC imaging of the cells suggested similar size for wild type (Fig. 3B, DIC rows 1–3) and *Δcpc* transformants (Fig. 3B, DIC rows 4–6). The fluorescence difference between the wild type and *Δcpc* transformants was negligible when using blue light, which preferentially excites Chl molecules (Fig. 3B F-Chl), indicating that there is no substantial difference in the cellular Chl content among the two cell types. However, when exiting with green light, which is preferentially absorbed by Phc, the wild type cells showed much higher fluorescence emission than the *Δcpc* transformants (Fig. 3, F-Phc1 and F-Phc2). A more quantitative analysis of size distribution of wild type and *Δcpc* cells is shown in the results of Fig. 3C. Size distribution in the wild type was shifted slightly toward bigger cells compared to the *Δcpc* cells. The mean cell size was measured to be 1.90 ± 0.12 μm for the wild type and 1.86 ± 0.08 μm for the *Δcpc* cells, consistent with the findings of Lea-Smith et al. [31].

Fig. 4. Absorbance spectra of live *Synechocystis* wild type (WT) and *Δcpc*-transformants. A: Spectra of live cells. The *Δcpc*-transformants lacked the phycocyanin absorbance band peaking at 625 nm. B: Absorbance spectra of the membrane fraction of *Synechocystis* wild type (WT) and *Δcpc*-transformant strains. C: Absorbance spectra of the soluble fraction of *Synechocystis* wild type (WT) and *Δcpc*-transformant strains. The absorbance peak for phycocyanin (625 nm) is evident in the WT, whereas allophycocyanin absorbance peaks are most pronounced at 650 and 675 nm in the *Δcpc*-transformant strains.

3.4. Comparative pigment analysis

In order to further characterize pigmentation differences between the two strains, we measured absorbance spectra of cells and phycobilisomes (Fig. 4). The spectrum of wild type cells showed the typical absorbance bands of chlorophyll at 680 nm and phycocyanin at 625 nm [14,15] (Fig. 4A). The *Δcpc* transformants showed the specific Chl absorbance peak at 680 nm, whereas the phycocyanin absorbance peak at around 625 nm was missing. This is attributed to the deletion of the *CPC*-operon and the resulting absence of phycocyanin from the *Δcpc* transformants. Cell lysates were fractionated into crude membrane and soluble fractions. The membrane fraction of wild type and *Δcpc* transformants (Fig. 4B) showed similar absorbance spectra in the red region, dominated by the absorption of Chl at 680 nm. This was expected, because Chl pigments are bound to the transmembrane proteins of the PSI-core and PSII-core complexes, which pelleted with the thylakoid membranes upon centrifugation. The soluble fraction of wild type and *Δcpc* transformants contained the dissociated phycobilisomes (Fig. 4C), and showed substantially different absorbance spectra. The wild type supernatant was blue and dominated by the absorption of phycocyanin at 625 nm, with minor absorbance shoulders at 650 and 675 nm, whereas that of the *Δcpc* transformants showed a featureless low-level absorption in the 625 nm region, a peak at 650 nm, and a minor band at 675 nm. The latter are ascribed to allophycocyanin [14], which remains in the residual phycobilisome as a component of the core-cylinders of the *Δcpc* transformants. Missing from the latter was the dominant 625 nm phycocyanin absorbance.

The chlorophyll content per OD_{750}, dry cell weight (dcw), or per cell basis in the *Δcpc* transformants was 50–70% of that in the wild type, while the carotenoid content did not change significantly (Table 2). Changes in Chl content per dcw may reflect underlining changes in photosystem stoichiometry, as a result of the *Δcpc* transformations. Past work with TLA mutants showed that the PSI/PSII stoichiometry ratio was adjusted and optimized when mutations induced light-harvesting antenna size changes that affected the two photosystems in a dissimilar manner [41]. In this respect, PSI is more abundant than PSII in cyanobacteria and contains 95 Chl *a* and 22 β-carotene molecules, whereas the PSII-core contains only 37 Chl *a* and 11 β-carotene molecules [17,23,58]. The sizable PBS antenna aids absorption of sunlight by PSII, so that a statistically balanced distribution of excitation between the two photosystems is achieved with a PSI/PSII ratio of about 2:1 to 4:1 in the wild type [12,16,46,50]. Deletion of the peripheral phycocyanin rods from the *Δcpc* transformants lowers the capacity and rates of light absorption by PSII and, thus, would tend to tilt the balance of excitation energy distribution in favor of PSI. A decline in the number of the PSI units in the *Δcpc* transformants would then be a compensation response, entailing adjustment and optimization of function [41], thereby explaining the lower Chl per dcw in these transformants (Table 2).

Table 2
Pigment content, photosystem reaction center quantification, and relative absorption cross-section of PSII and PSI in *Synechocystis* wild type and *Δcpc* transformants grown photoautotrophically in the laboratory. Photosystem absorption cross-sections and reaction center concentrations were measured spectrophotometrically [40]. n ≥ 3; means ± SD.

Parameter measured	Wild type	*Δcpc* (Average of *Δcpc1*, *Δcpc2*, *Δcpc3*)
Chl/OD_{750} [μg]	2.6 ± 0.1	1.8 ± 0.1
Chl/dcw [μg·mg^{-1}]	8.5 ± 0.7	5.0 ± 0.5
Chl/cell [fg]	88 ± 31	41 ± 17
Car/OD_{750} [μg]	2.2 ± 0.1	2.1 ± 0.2
Car/dcw [μg·mg^{-1}]	7.2 ± 0.7	6.5 ± 0.1
Chl/Car (w:w)	1.2 ± 0.1	0.7 ± 0.1
Mean cell size [μm]	1.90 ± 0.12	1.86 ± 0.08
P700/Chl × 10^{-3}	5.60 ± 0.34	5.82 ± 0.68
Q_A/Chl × 10^{-3}	2.23 ± 0.11	3.25 ± 0.18
PSI/PSII	2.5 ± 0.08	1.8 ± 0.13
k_{PSII} (Q_A photoreduction kinetics) [s^{-1}]	30.1 ± 6.2	3.7 ± 1.5
k_{PSI} (P700 photooxidation kinetics) [s^{-1}]	2.9 ± 0.2	2.5 ± 0.1

3.5. Photosystem stoichiometry and light-harvesting antenna cross-section

To test the hypothesis of an adjusted and optimized photosystem stoichiometry in the *Δcpc* transformants, and to investigate the functional effect of the truncation of the PBS–PSII antenna size more precisely, we applied light-induced absorbance difference spectrophotometry to quantify PSI and PSII reaction centers and, thus, to estimate the PSI/PSII ratio in wild type and *Δcpc* transformants [40]. For the PSI measurement, the amplitude of the light-induced ΔA_{700} signal measured the amount of P700 in the sample. For the PSII measurement, the amplitude of the light-induced ΔA_{320} signal measured the amount of Q_A [40]. Ratios of P700 per total Chl content and Q_A/Chl in *Synechocystis* thylakoid membranes are shown in Table 2, as is the resulting PSI/PSII stoichiometric ratio. The PSI/PSII ratio declined from about 2.5:1 in the wild type down to 1.8:1 in the *Δcpc* transformants. This adjustment is consistent with the hypothesis of an optimized PSI/PSII ratio in the *Δcpc* transformants in the direction of balancing the excitation energy distribution between the two photosystems [16].

The effective absorption cross-section of PSI and PSII was compared in the wild type and *Δcpc* transformants. This was measured from the light-induced ΔA_{700} oxidation kinetics of P700 for PSI and the Q_A fluorescence induction kinetics for PSII, measured under weak broad-band green actinic excitation defined by CS 3-69 and CS 4-96 Corning filters (half-band width of 40 nm with 50% transmittance at 520 and 560 nm, respectively). It is recognized that such green excitation would sensitize phycocyanin much more than it would sensitize allophycocyanin and chlorophyll. In wild type live cells, rates of light absorption by PSII (k_{PSII}, Table 2) were 30.1 s^{-1}, whereas in the *Δcpc* transformant lines they averaged 3.7 s^{-1}. The substantial, almost 10-fold difference in the effective absorption cross-section of PSII in wild type and *Δcpc* transformant cells is attributed to the presence and absence of phycocyanin between the two respective strains. Rates of light absorption by PSI (k_{PSI}, Table 2) were determined with isolated thylakoid membranes from which the phycobilisome peripheral antenna was disconnected. As such, the results afford a direct comparison of the

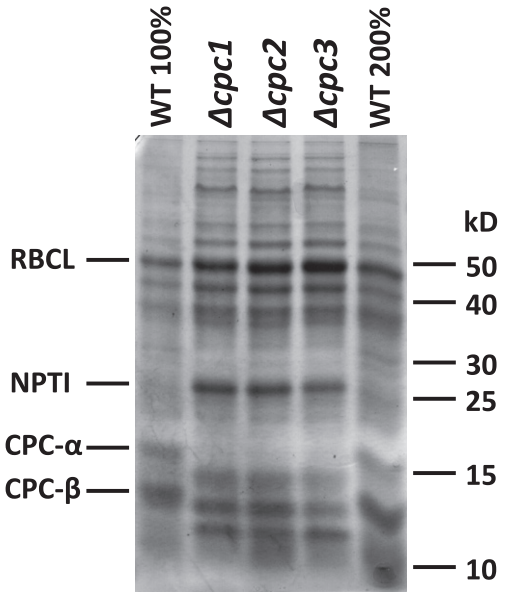


Fig. 5. SDS-PAGE analysis of total protein extracts from *Synechocystis* wild type and *Δcpc* transformants. Phycocyanin α-subunit (CPC-α) and phycocyanin β-subunit (CPC-β) are migrating at around 17 and 13 kDa, respectively, and are clearly absent from the *Δcpc* transformants. The NPTI protein with a molecular weight of 27 kDa is highly abundant in the *Δcpc* transformants.

core Chl antenna size of PSI between wild type and *Δcpc* transformants. Similar rate constants of P700 photo-oxidation with values between 2.9 and 2.5 s^{-1} were measured (k_{PSI}, Table 2), suggesting similar PSI Chl antennae. This finding is consistent with the notion that photosystem stoichiometry adjustments affect the ratio of the two photosystems but not necessarily their core Chl antenna size.

3.6. SDS-PAGE and Western blot analysis

Total protein extracts from wild type and *Δcpc* transformants provided further insight into the phenotype of the latter (Fig. 5). In the wild type, the phycocyanin alpha and beta subunits were visible as abundant low molecular weight proteins (Fig. 5, CPC-α and CPC-β). These protein bands were absent from the *Δcpc* transformants. The latter showed a new pronounced band at around 27 kDa, which was identified by Western blot analysis to be the NPTI protein (not shown), conferring the kanamycin resistance to the *Δcpc* transformants. The absence of the phycocyanin alpha and beta subunits from the *Δcpc* transformants is consistent with the absorbance spectral measurements, and PSII absorption cross section results, and is further consistent with the notion of a severely truncated phycobilisome antenna size in the *Δcpc* transformants, one that contains only the core allophycocyanin component, as the auxiliary antenna of the PSII reaction center.

Western blot analyses of protein extracts from wild type and *Δcpc* transformants, probed with specific polyclonal antibodies raised against the D1 protein, the PSII reaction center D1/D2 heterodimer (PSII RC), the RbcL, the ATP synthase β-subunit (ATP-β), and the orange carotenoid protein (OCP) showed the presence of these reference proteins in wild type and *Δcpc* transformants (Fig. 6). Loaded on a per Chl basis, three lines of the *Δcpc* transformants showed greater relative amount of these proteins, as compared to the wild type. *Δcpc* transformants have only about 60% of the Chl/dcw and a greater PSII/PSI ratio. Loading on a per Chl basis would tend to overload the *Δcpc* transformant lanes for PSII compared to that of the wild type (Fig. 6, D1 and PSII RC). A similar argument can be made for the ATP-β subunit (Fig. 6, ATP-β). On the other hand, non-thylakoid membrane proteins such as the RbcL and OCP occur in about equivalent amounts in wild type and *Δcpc* transformants.

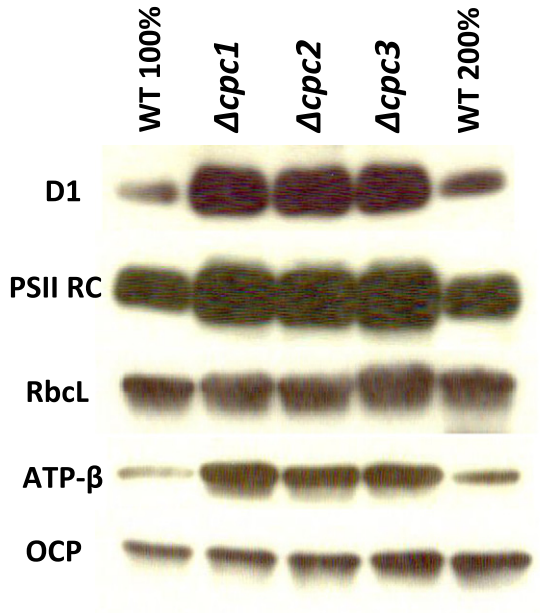


Fig. 6. Western blot analyses of protein extracts from *Synechocystis* wild type and *Δcpc* transformants. Specific polyclonal antibodies were raised against the D1 protein, the PSII reaction center D1/D2 heterodimer (PSII RC), the RbcL, the ATP synthase β-subunit (ATP-β), and the orange carotenoid protein (OCP), and were used in this study.

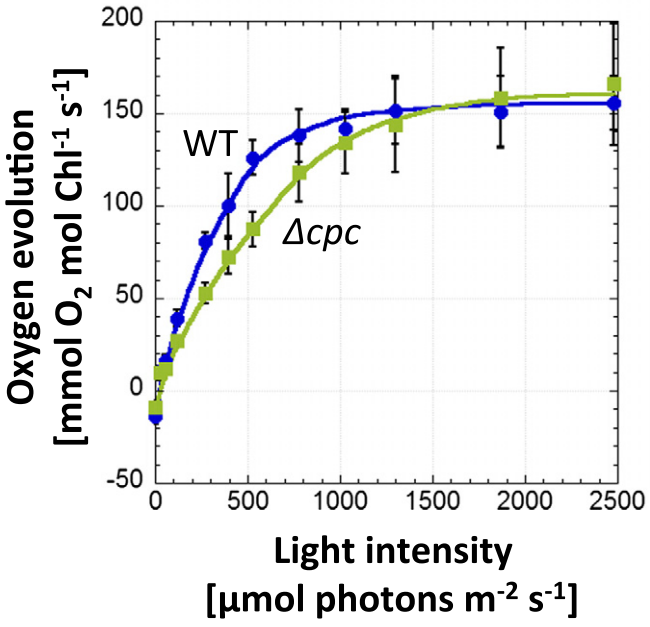


Fig. 7. Light-saturation curves of photosynthesis obtained with the *Synechocystis* wild type and *Δcpc*-transformants. A higher light-intensity was needed to saturate photosynthesis in the *Δcpc*-transformants compared to the wild type. The half-saturation intensity of photosynthesis for the two strains was measured to be 220 μmol photons m^{-2} s^{-1} for the wild type and 500 μmol photons m^{-2} s^{-1} for the *Δcpc* transformants. The experimental points are the average results from three independent cultures, ±SD.

3.7. Functional properties of wild type and Δcpc transformant

The functional consequence of the deletion of phycocyanin from the phycobilisome antenna in the *Δcpc* transformants was assessed upon measurement of the light saturation curves of photosynthesis in the strains. These are shown in Fig. 7 for wild type and *Δcpc* transformants, normalized to the Chl concentration of the samples. At zero light intensity, the oxygen evolution rate was negative, reflecting the respiratory activity of the cells. It was measured to be on the average 13 ± 4 and 9 ± 3 mmol O_2 per mol Chl per s for the wild type and *Δcpc* transformants, respectively. The rate of photosynthesis increased linearly as a function of light-intensity in the range between 0 and 300 μmol photons m^{-2} s^{-1} (Fig. 7). The slope of this line was steeper for the wild type than for the *Δcpc* transformants because of the presence of phycocyanin in the wild type, which affords greater absorption and utilization of actinic light than that in the *Δcpc* transformants. This was also reflected in the half-saturation intensity of photosynthesis for the two strains, which was measured to be 220 μmol photons m^{-2} s^{-1} for the wild type and 500 μmol photons m^{-2} s^{-1} for the *Δcpc* transformants. This difference is consistent with the TLA concept, i.e., the effect of antenna truncation on the light saturation curves of photosynthesis, as previously measured in green microalgae [27,28]. The light-saturated rate of photosynthesis, when measured on a chlorophyll basis (Fig. 7) was slightly greater for the *Δcpc* transformants and the wild type, reflecting changes in cellular chlorophyll content due to the photosystem stoichiometry adjustment.

The TLA concept and the two-fold greater intensity needed to half-saturate the rate of photosynthesis in the *Δcpc* transformants compared to the wild type predict greater productivity for cultures of the transformants under high cell density and saturating illumination (greater than 1000 μmol photons m^{-2} s^{-1}) conditions. This would occur because over-absorption and wasteful dissipation of sunlight by the top layer of cells in the *Δcpc* culture would be diminished, permitting greater transmittance and productivity by cells deeper into the medium [44,45].

The saturation intensity of photosynthesis (I_S) for wild type and *Δcpc* transformants was defined from the intercept between the initial

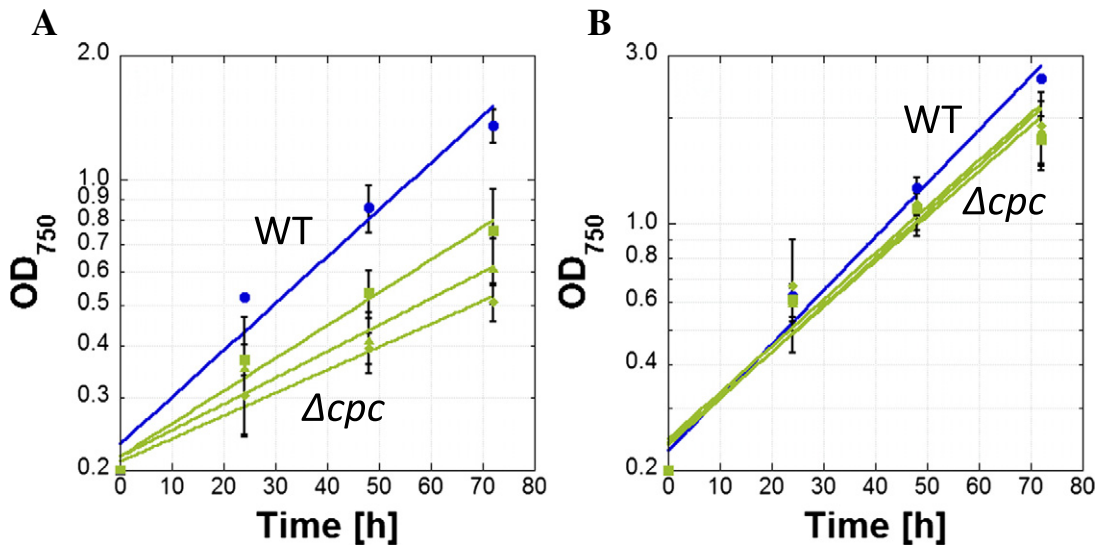


Fig. 8. Early-stage growth of *Synechocystis* wild type and *Δcpc*-transformants under low light conditions. A: Cell growth at 50 μmol photons m^{-2} s^{-1}. B: Cell growth at 170 μmol photons m^{-2} s^{-1}. The *Δcpc*-transformants showed a retarded growth under 50 μmol photons m^{-2} s^{-1}, but they grew with a rate closer to that of the wild type under 170 μmol photons m^{-2} s^{-1}. The experimental points are the average results from three independent cultures, ± SD.

linear increase in the rate of photosynthesis as a function of light intensity and the asymptotic light-saturated rate achieved at maximum intensity, e.g. 2500 μmol photons m^{-2} s^{-1}. This I_S was calculated to be 400 μmol photons m^{-2} s^{-1} for the wild type and 1000 μmol photons m^{-2} s^{-1} for the *Δcpc* transformants. Again, in cultures under high cell density conditions, the two-fold greater intensity for the saturation of photosynthesis in the *Δcpc* transformants compared to the wild type informs that there would be a quantitative productivity difference between the two strains under saturating illumination. This, however, will be manifested at light intensities greater than 1000 μmol photons m^{-2} s^{-1}, i.e., at light intensities greater than what is needed for the saturation of photosynthesis in the *Δcpc* transformants.

To investigate the photosynthetic productivity of wild type and *Δcpc* cultures in greater detail, we measured rates of cell growth and biomass accumulation at different light intensities ranging from sub-saturating to saturating. At the low light intensity of 50 μmol photons m^{-2} s^{-1}, *Δcpc* transformants showed growth slower than that of the wild type (Fig. 8A). The doubling time under these conditions was measured to be 30 h for the wild type and about 49 h for the *Δcpc* transformants. This retarded growth rate under low light intensities is a consequence of the severe light-limitation imposed on the *Δcpc* transformants due to the absence of phycocyanin, whereby the latter do not harvest as much light energy as the wild type and, therefore, photosynthesis and growth are limited in these mutants. This difference in the growth phenotype became less severe, when the cell growth intensity was increased. When grown under 170 μmol photons m^{-2} s^{-1}, the difference in growth rates between wild type and the *Δcpc* transformants was diminished (Fig. 8B) with doubling times of 20.5 h for the wild type and 26.9 h for the *Δcpc* transformants. This finding is consistent with recent studies [33,53], where phycocyanin-less mutants showed a retarded growth under low light intensities between 50 and 150 μmol photons m^{-2} s^{-1}. However, when growth was measured under 350 μmol photons m^{-2} s^{-1} this difference between wild type and *Δcpc* transformants was minimized with doubling times of 16.7 h and 19.5 h for the wild type and *Δcpc* transformants, respectively.

A more extensive presentation of the measured cell duplication time in *Synechocystis* cultures, as a function of growth light intensity, for wild type and the *Δcpc* transformants, is shown in Fig. 9. A substantial difference exists between cell duplication time in wild type and *Δcpc* transformants at low growth intensities. This difference is diminished as the growth intensity increases. Extrapolating the relationship to higher light intensities, we found that wild type and *Δcpc* transformants would reach the same cell duplication time of about 13 h at about 800–1000 μmol photons m^{-2} s^{-1} (dashed line in Fig. 9). It is evident from this analysis that wild type cells would have a competitive advantage when growth is measured under low light-intensities, but when the light-intensity approaches or exceeds the saturation point of photosynthesis, wild type and *Δcpc* transformants can grow with identical rates.

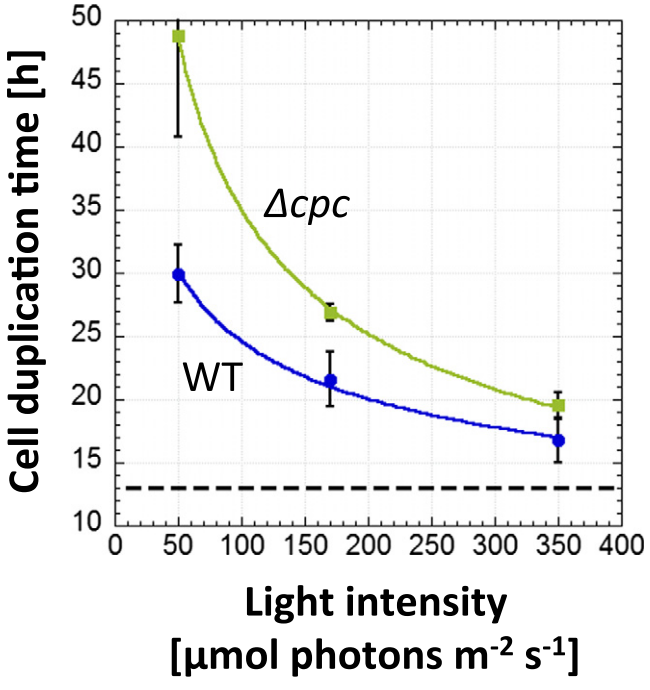


Fig. 9. Cell duplication time as a function of growth intensity for *Synechocystis* wild type and *Δcpc*-transformants. The *Δcpc*-transformants showed a slower than wild type growth under low-light intensities but this difference was diminished as the growth irradiance increased. It was estimated that wild type and *Δcpc* transformants would reach the same cell duplication time of about 13 h at 800–1000 μmol photons m^{-2} s^{-1} (dashed line). The experimental points are the average results from three independent cultures, ± SD.

3.8. Culture productivity under high cell density and saturating irradiance conditions

The TLA technology concept is based on the premise of maximizing sunlight utilization efficiency and photosynthetic productivity in mass microalgal cultures, or high-density plant canopies, upon minimizing the light-harvesting capacity of the photosynthetic apparatus [44]. The rationale for this counterintuitive concept is that, at saturating or greater intensities, a small light-harvesting antenna would alleviate excessive absorption of sunlight by the top layer of cells in a mass culture or canopy and would thus prevent the ensuing wasteful dissipation of the excess absorbed energy, while at the same time permitting light penetration deeper into the culture [29,44,45]. It is worth noting that improved photosynthetic productivity of a culture with TLA cells is solely due to the effect of better sunlight penetration with more cells deeper into a culture having a chance to absorb and perform useful photosynthesis, whereas the photosynthetic productivity of individual TLA cells would not be better than that of the wild type. Thus, it is crucial in such applications that bioreactors are designed to be deep enough with a cell-density sufficient to permit quantitative absorption of all incoming irradiance, and to perform the productivity measurement at light intensities equal to or greater than that needed to saturate photosynthesis.

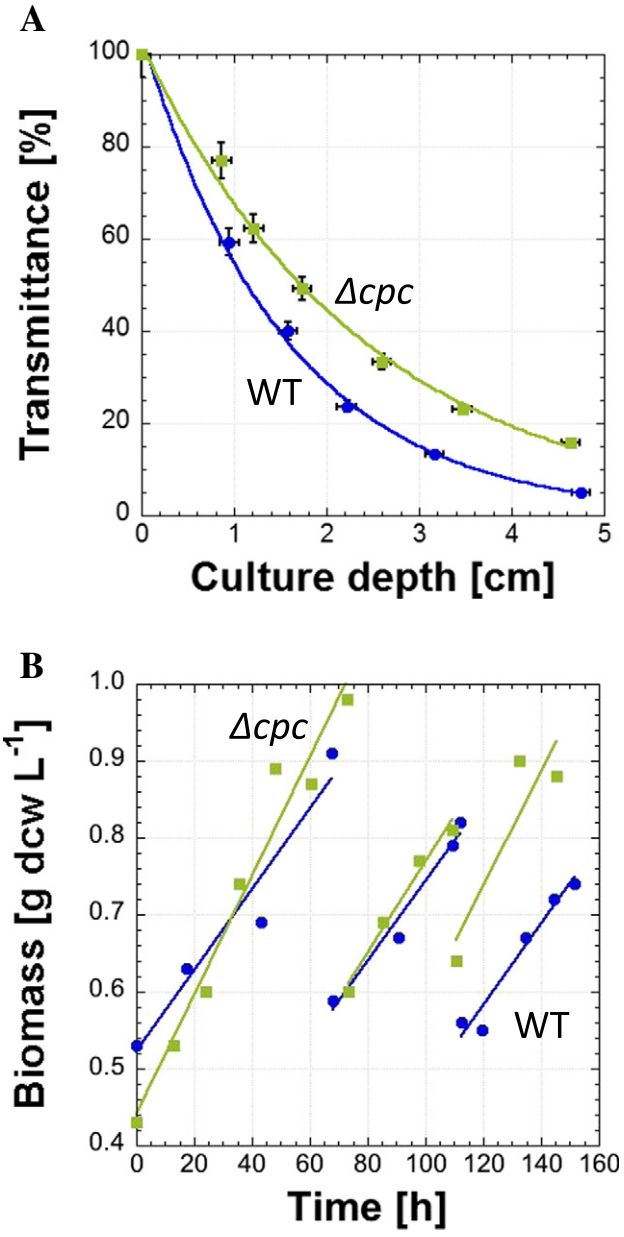


Fig. 10. Batch culture experiments of *Synechocystis* wild type and *Δcpc* transformants, grown under simulated bright sunlight conditions (2000 μmol photons m^{-2} s^{-1}). A: Light-penetration into a wild type and *Δcpc* culture with a biomass content of 0.5 g/L. The exponential decline of transmittance in the wild type culture is steeper compared to the *Δcpc* culture, resulting in a much lower light-intensity deeper in the culture. The experimental points are the average result from three independent measurements ± SD. B: Biomass accumulation. Cultures were diluted with fresh growth media once they approached the end of the linear growth phase, i.e., at about 0.9–1.0 g dcw L^{-1}. The slope of the linear regressions defined the rate of biomass accumulation. This rate was always greater for the *Δcpc* transformants grown under these conditions compared to the wild type.

To investigate the transmittance of light through the wild type and *Δcpc* cultures we measured the light-intensity at different distances from the surface of the culture at a biomass concentration of 0.5 g dcw L^{-1} (Fig. 10A). In both wild type and *Δcpc* cultures, the light intensity declined exponentially as a function of the distance from the surface of the culture. However, the decline was much steeper in the wild type with a 50% transmittance reached at only about 1.1 cm, compared to *Δcpc* where a 50% transmittance was shifted toward about 1.7 cm. At the optical depth of 12 cm (diameter of the cylindrical reactor), intensity of the transmitted light was about zero for both wild type and *Δcpc* transformants. These differential light penetration properties afford credence to the notion that actinic light intensity distribution and photosynthetic productivity would be greater in a *Δcpc* compared to a wild type culture under high light-intensity and mass-culture conditions.

The light-penetration experiments and the light-saturation curves of photosynthesis (Fig. 7) indicated that *Δcpc* transformants are promising in the application of the TLA technology concept. This was tested upon wild type and *Δcpc* growth under simulated bright sunlight conditions (e.g. 2000 μmol photons m^{-2} s^{-1}) in the laboratory, with cultures having optical density sufficient to absorb > 98% of the incident irradiance. These conditions ensured that light-energy input for the wild type and *Δcpc* transformant cultures was about the same. Biomass accumulation results of representative wild type and *Δcpc* transformant cultures are shown in Fig. 10B. In this experiment, cultures were diluted with fresh growth media once they approached the end of the growth phase, i.e., at about 0.9–1.0 g dcw L^{-1}, to ensure that nutrient availability will not adversely affect growth, and to also permit for a continuous production process over a long growth period, during which to assess the effect of the TLA phenotype on the productivity of the culture. The slope of the linear regressions in Fig. 10B showed the rate of biomass accumulation by wild type (Fig. 10B, circles) and *Δcpc* transformant cultures (Fig. 10B, squares), respectively. It is evident from the results that rates of biomass accumulation by the *Δcpc* transformant cultures were always faster than those by the wild type over all cultures and *Δcpc* transformant lines measured. We compiled the results from several such continuous growth experiments for wild type and *Δcpc* transformant cultures (Fig. 11). In this presentation, initial cell density of the cultures was about 0.5 g dcw L^{-1}. The average rate of biomass accumulation in this presentation (Fig. 11) was defined by the slopes of the linear regression of the points and was measured to be about 4.9 mg dcw L^{-1} h^{-1} for the wild type and 7.7 mg dcw L^{-1} h^{-1} for the *Δcpc* transformants. This analysis, therefore, showed that culture productivity of the *Δcpc* transformants exceeded that of the wild type by about 57%.

4. Discussion

Deletion of the key cyanobacterial *CPC*-operon, encoding phycocyanin and associated linker polypeptides of the phycobilisome peripheral rods, caused a highly truncated phycobilisome antenna size, resulting in a substantially smaller absorption cross-section for PSII in the *Synechocystis Δcpc* transformants. A schematic model presentation of the phycobilisome structure and its association with photosystem II is shown in Fig. 12 for the wild type and the *Δcpc Synechocystis*

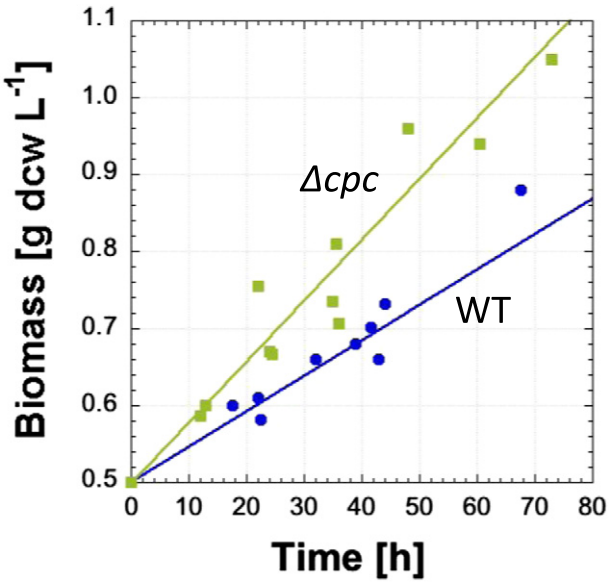


Fig. 11. Summary of biomass accumulation measurements from three batch cultures under simulated bright sunlight conditions (2000 μmol photons m^{-2} s^{-1}). The linear regression of the points represents the average rate of biomass accumulation of wild type (WT) and *Δcpc* transformants. A 57% greater productivity of the *Δcpc* transformants, relative to that of the wild type, was measured.

transformants. Noted is the presence of the allophycocyanin (AP) core cylinders in both strains, and the absence of phycocyanin (Phc) from the *Δcpc* transformants. A compensation reaction of the cells to the smaller PBS–PSII antenna size was a reduction in the number of PSI units, relative to those of PSII (Table 2), which can be viewed as a cellular effort to retain balanced absorption and distribution of excitation energy between the two photosystems [16]. Such adjustment enables the *Δcpc* transformants to operate the linear electron transport process efficiently, compared to an unbalanced *Δcpc* transformant system in which the PSI/PSII ratio would be the same as that in the wild type.

The smaller photon absorption cross-section in the *Δcpc* transformants resulted in a retarded growth under low-light conditions, which is a consequence of a greater than wild type light-limitation in these transformants. This was previously reported by Nakajima and Ueda [51,52], Bernat et al. [8], Kwon et al. [30], Joseph et al. [24], as well as by Page et al. [53]. This result was anticipated because, at light-intensities well below saturation, less light energy is harvested by the TLA strains to operate photosynthesis and, thus, their rate of growth is relatively slower. The TLA property would confer a disadvantage to *Δcpc* transformants in the wild, where growth irradiance is almost always limiting. The TLA strains would be outcompeted by organisms with larger light-harvesting antenna size, because of their slower growth in these light-limiting conditions. However, *Δcpc* transformant cultures as a whole, having a high-density of cells in a photobioreactor under direct sunlight would outperform a corresponding wild type culture as top layers of cells would not over-absorb sunlight and wastefully dissipate the excess excitation energy. It was pointed out before that such TLA property could translate into a greater sunlight-to-biomass energy conversion efficiency of mass culture photosynthesis, helping to elevate the culture performance and productivity beyond what can be achieved with wild type strains [29,44,45]. Results in this work provide evidence of a 57% improvement in the productivity of high cell density *Δcpc* transformant cultures as compared to that of the wild type, consistent with the predictions of the TLA model.

Considering the recent interest in the field of renewable biomass, and fuel and chemical production by photosynthetic microorganisms [8,9,18,20,22,38,39,43,47], improvements in the energy conversion efficiency of photosynthesis can significantly improve the economic outlook of such processes using cyanobacteria or microalgae as a single-celled photocatalyst [7,34,56].

Although a mere +57% improvement in productivity was demonstrated with the *Δcpc* transformant cultures in this work, theoretical improvements in photosynthetic energy conversion efficiency could be as high as 3-fold over that in the wild type [29,44], suggesting that further advances could be made in this work. Reasons for the less-than-optimal improvement in the yield of photosynthesis in the *Δcpc* transformants could be attributed to the over-expression of the NPTI protein. A significant portion of carbon commitment by the cells goes into the synthesis of the NPTI protein in the *Δcpc* transformants as evidenced by the Coomassie-stained gel (Fig. 7). The NPTI protein has no useful function, whenever cells are grown in liquid media in the absence of kanamycin, as the case was in this work. If this carbon was invested into cell constituent proteins that contributed to growth, a faster rate of growth could potentially be achieved. Another consideration is whether heterologous accumulation of such substantial amounts of the NPTI protein may affect fitness of the transformant lines through cellular investment in a non-native protein, or through direct NPTI protein toxic effects. Further fine-tuning of the light-absorption properties of cyanobacteria, e.g. upon the additional deletion of the allophycocyanin phycobilisome subunits, could provide further improvements in the sunlight-to-biomass energy conversion efficiency. The contention of a need for a light harvesting antenna size smaller than that in the *Δcpc* transformants is based on our results of Figs. 7 and 9, where *Δcpc* photosynthesis and growth are saturated at about 1000 μmol photons m^{-2} s^{-1}, suggesting wasteful dissipation of energy at greater light intensities.

Page et al. [53] recently investigated the photosynthetic energy conversion efficiency of a similar phycobilisome-deficient mutant of *Synechocystis* lacking the phycocyanin peripheral antenna. Under low and medium light conditions, the phycocyanin-deletion mutants lagged in growth and productivity when compared to the wild type, as also

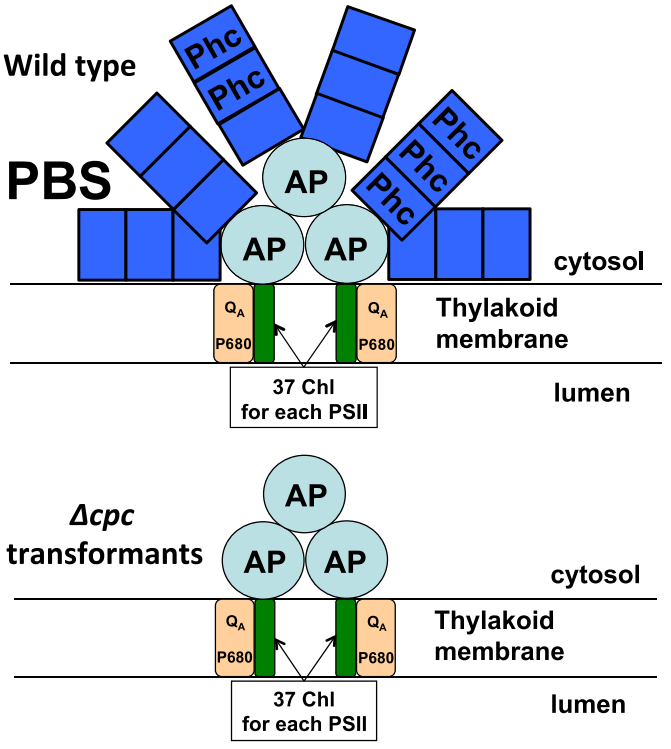


Fig. 12. Phycobilisome–chlorophyll antenna organization in the thylakoid of *Synechocystis* wild type and *Δcpc* transformants. Cyanobacteria may possess up to 850 phycocyanin (Phc), allophycocyanin (AP), and chlorophyll (Chl) molecules per unit photosynthetic apparatus.

reported in this work. These results led Page et al. [53] and Liberton et al. [33] to the generalized conclusion of a lowering in photoautotrophic productivity in the cyanobacterium *Synechocystis* by phycobilisome antenna truncation, opposite to the conclusions drawn by Nakajima and Ueda [51,52], and also opposite to the conclusions drawn in this work. An explanation of the opposite conclusions could be that productivity of the phycocyanin-deletion mutants by Page et al. [53] and Liberton et al. [33] was not properly assessed at high cell-densities and light intensities equal to or above that required for the saturation of photosynthesis, when the TLA property ought to manifest in the form of improved biomass productivity. A high cell density culture, sufficient to absorb all incoming photosynthetically active radiation, and light intensities equal to or above those required for the saturation of photosynthesis are essential requirements for the detection of improvements in the photosynthetic productivity of TLA versus wild type strains. Thus, it cannot be excluded that the Page et al. [53] cultures of *Synechocystis* mutants lacking the peripheral phycobilisome antenna are indeed more productive than the wild type under the proper conditions, and should be further investigated as to their potential to confer a greater photosynthetic energy conversion efficiency in mass cultures under high-light conditions.

While this manuscript was in the peer-review process, we became aware of a new publication by Lea-Smith et al. [31] addressing phycobilisome attenuation in cyanobacteria. Lea-Smith et al. arrived at the conclusion "This study demonstrates that reducing the PBS is not useful for improving industrial production of either cyanobacterial biomass or metabolites, except when carbon is limited, in contrast to the studies performed in *C. reinhardtii*." Our work arrived at the opposite conclusion by demonstrating improved biomass accumulation upon reduction in the phycobilisome size (Figs. 9, 10B, and 11) under conditions of sufficient carbon supply. Moreover, Lea-Smith et al. concluded that "While reduction of the PBS does not improve growth, a possible alternative might involve the development of PBSs containing at least 2 discs with pigments other than PC, such as phycoerythrin, which absorbs light at $\lambda max = 545$ nm, the part of the spectrum where light absorption by *Synechocystis* is lowest." In contrast, we arrived at the opposite conclusion, that further reduction in PBS size is needed, e.g. upon removal of the remaining APC-core, to attain additional biomass accumulation benefits under mass culture and bright sunlight conditions.

There are further substantial differences between our work and that by Lea-Smith et al. [31]. Our *Δcpc* strains, equivalent to the "olive strains" in Lea-Smith et al., showed a half saturation intensity of photosynthesis twice that of the wild type (our Fig. 7). This is in sharp contrast to the results obtained by Lea-Smith et al., where the half-saturation intensity of photosynthesis in the olive strains (their Fig. 2C, inverted solid triangles) was essentially the same as that in the wild type (their Fig. 2C, solid circles).

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