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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