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Loss of CpSRP54 function leads to a truncated light-harvesting antenna size in *Chlamydomonas reinhardtii*

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ABSTRACT

The *Chlamydomonas reinhardtii truncated light-harvesting antenna* 4 (*tla4*) DNA transposon mutant has a pale green phenotype, a lower chlorophyll (Chl) per cell and a higher Chl *a*/*b* ratio in comparison with the wild type. It required a higher light intensity for the saturation of photosynthesis and displayed a greater per chlorophyll light-saturated rate of oxygen evolution than the wild type. The Chl antenna size of the photosystems in the *tla4* mutant was only about 65% of that measured in the wild type. Molecular genetic analysis revealed that a single plasmid DNA insertion disrupted two genes on chromosome 11 of the mutant. A complementation study identified the "chloroplast signal recognition particle 54" gene (*CpSRP54*), as the lesion causing the *tla4* phenotype. Disruption of this gene resulted in partial failure to assemble and, therefore, lower levels of light-harvesting Chl-binding proteins in the *C. reinhardtii* thylakoids. A comparative *in silico* 3-D structure-modeling analysis revealed that the M-domain of the CpSRP54 of *C. reinhardtii* possesses a more extended finger loop structure, due to different amino acid composition, as compared to that of the *Arabidopsis* CpSRP54. The work demonstrated that *CpSRP54* deletion in microalgae can serve to generate *tla* mutants with a markedly smaller photosystem Chl antenna size, improved solar energy conversion efficiency, and photosynthetic productivity in high-density cultures under bright sunlight conditions.

1. Introduction

Photosynthesis depends on the absorption and conversion of sunlight energy by the photosystems, localized in the chloroplast thylakoid membranes. Photosynthetic organisms have developed an extensive light-harvesting system, composed of highly organized photosynthetic pigments within thylakoid membrane-embedded proteins that absorb and channel the light energy towards the photochemical reaction centers. Plants and green algae have developed large arrays of such chlorophyll-carotenoid (Chl-Car) light-harvesting complexes associated with the reaction centers of photosystem-I (PSI) and photosystem-II (PSII). The light-harvesting Chl-Car-binding proteins (LHCPs) comprise up to 50% of the thylakoid membrane proteins [1] and, therefore, are the most abundant membrane proteins on earth [2–5]. The *Lhca* and *Lhcb* gene subfamilies encode LHCA and LHCB proteins for the light-harvesting complex I (LHCI) associated with PSI and light-harvesting complex II (LHCII), associated with PSII, respectively [6].

Nuclear-encoded LHC proteins are synthesized in the cytosol and immediately imported into chloroplasts prior to insertion and assembly in the developing thylakoid membranes. While in transit in the chloroplast stroma, they are guided toward the portion of the thylakoid membrane where assembly of new photosystems takes place by the so-called signal recognition particle (SRP)–dependent pathway [7]. LHCPs and some PSII-core and PSI-core proteins use the chloroplast equivalent of the SRP pathway (CpSRP) for transmembrane assembly [2–5]. In a temporal sequence of events, the chloroplast stromal proteins CpSRP43 and CpSRP54 bind to imported target proteins; the receptor protein CpFtsY recognizes the CpSRP43–CpSRP54–target complex and guides it to the integral thylakoid membrane protein ALBINO3 (ALB3) [8,9]. ALB3 facilitates integration of the target protein into the thylakoid membrane [7]. The function of the CpSRP pathway in green microalgae slightly differs from that in higher plants [10–12]. This is evident from previous work on CpSRP43 and CpFTSY, recently identified and studied in *Chlamydomonas reinhardtii* [10,11]. The respective knockout mutants lacking these proteins exhibited lower total chlorophyll content and LHCP deficiency but normally functional photosystems, which are typical features of a **T**runcated **L**ight-harvesting **A**ntenna (TLA) phenotype.

Abbreviations: CCU, carbon capture and utilization; Chl, chlorophyll; Chl-Car, chlorophyll-carotenoid; CpSRP54, chloroplast signal recognition particle 54; LHCI, light-harvesting complex I; LHCII, light-harvesting complex II; LHCPs, Chl-Car-binding light-harvesting proteins; PSI, photosystem-I; PSII, photosystem-II; TLA, truncated light-harvesting antenna.

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It has been reported that high-density cultures of microalgae with a truncated light-harvesting antenna are photosynthetically more productive under bright sunlight and high-density growth conditions than cultures with fully pigmented cells. This improvement is due to mitigation of over-absorption and wasteful dissipation of excess energy by the outermost layer of cells in the culture, permitting greater transmittance of sunlight and culture productivity [13–17]. Thus, identification of genes that confer a permanent truncated light-harvesting antenna size phenotype in higher plants and green algae is of considerable interest, because such genes could be used to improve solar energy-to-biomass conversion efficiencies [17–19]. Recent results from *in vivo* analysis of *Chlamydomonas reinhardtii* TLA mutants with defects in LHCP transport and integration into thylakoids via the CpSRP pathway have demonstrated a drastic lowering in the cellular content of Chl and light-harvesting complexes in these mutants [10,11]. The mutants with the loss of function of CpFTSY and CpSRP43 were referred to as *tla2* and *tla3*, respectively. However, the role of the CpSRP54 protein in the assembly of the LHC in *Chlamydomonas*, and the role this protein plays as a tool in the generation of TLA-mutants, have not been investigated.

In this work, we isolated a new truncated light-harvesting antenna (TLA)-type strain from a library of *C. reinhardtii* strains generated by DNA insertional mutagenesis. We present a molecular, genetic, and physiological analysis of the newly identified mutant, termed *tla4*, which had a stably truncated light-harvesting Chl antenna. The corresponding *TLA4* gene was cloned and found to encode a homolog of the plant CpSRP54 protein. Functional analyses revealed a smaller light-harvesting Chl antenna size in the *tla4-CpSRP54* mutant in *C. reinhardtii* and a greater per Chl productivity of this strain relative to the wild type.

2. Materials and methods

2.1. Cell growth conditions

Chlamydomonas reinhardtii wild-type strains CC-4349 cw15 mt- (provided by the laboratory of Dr. Jae-Hyeok Lee) and DNA insertional transformants were maintained at 25 °C. Cells were cultivated photoheterotrophically in Tris-acetate phosphate (TAP) medium, or photoautotrophically in high-salt (HS) and Tris-bicarbonate phosphate (TBP) medium [20] under continuous low or medium irradiance (70 or 350 μmol photons m^{-2} s^{-1}, respectively).

2.2. Mutagenesis and mutant selection

Mutants of *C. reinhardtii* CC-4349 were constructed by random DNA insertional mutagenesis by agitating cells with glass beads as described in [21]. A PCR product amplified from the plasmid pPEARL [22], which contained a paromomycin resistance gene, was introduced into the parental strain CC-4349. Specific primers (PAR_vector-F and PAR_vector-R) were used for PCR product amplification (Supplementary Table S1). Transformants were selected on TAP medium containing 1.5% agar with 50 μg/mL paromomycin and screened upon measurement of Chl fluorescence with a Walz Imaging PAM System (M-series) equipped with a CCD camera. Among the initially screened transformants, mutants with altered pigment compositions were further selected on the basis of their Chl *a*/*b* ratios.

2.3. Cell counts and pigment determination

Samples were harvested from the exponential growth phase of cultures grown in HS medium. Cell number in cultures was estimated by counting with a Neubauer Bright Line hemacytometer and an Olympus CH30 microscope. The Chl content of cells was spectrophotometrically determined in 100% (v/v) methanol extracts according to the method of [23]. Total carotenoid was measured as previously described in [24].

2.4. Measurements of photosynthetic activity

Cells were grown in HS medium under 70 μmol photons m^{-2} s^{-1} and aliquots were removed from the culture during the exponential growth phase. Oxygen evolution was measured at 25 °C with a Clark-type oxygen electrode illuminated with red LED light (660 nm). A 1 mL aliquot of cell suspension containing 2 μM Chl was transferred to the oxygen electrode chamber. To ensure that oxygen evolution was not limited by carbon supply available to the cells, 50 μL of 0.5 M $NaHCO_3$ (pH 7.4) was added to the cell suspension before measurements. After registration of dark respiration, the rate of oxygen exchange was measured at increasing light intensities (20, 40, 60, 80, 100, 300, 500, 700, 900, 1200 and 1500 μmol photons m^{-2} s^{-1}). Baseline registration and the rate of oxygen evolution at each light intensity step were recorded for 2 min.

2.5. Isolation of thylakoid membranes

Cells were grown photoautotrophically at 250 μmol photons m^{-2} s^{-1} under continuous bubbling with air in the TBP growth medium. Cells were harvested by centrifugation at 1000*g* for 3 min at 4 °C. Samples were resuspended with ice-cold sonication 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 mM phenylmethylsulfonyl fluoride. Cells were broken by sonication in a Branson 250 Cell Disrupter operated at 4 °C three times for 30 s each time (pulse mode, 50% duty cycle, output power of 5) with 30 s cooling intervals on ice. Cell debris and starch grains were removed by centrifugation at 3000*g* for 4 min at 4 °C. Thylakoid membranes were collected by centrifugation of the first supernatant at 75,000*g* for 30 min at 4 °C. The pellet of thylakoid membrane was resuspended in a buffer containing 50 mM Tricine (pH 7.8), 10 mM NaCl, and 5 mM $MgCl_2$ for spectrophotometric measurements.

2.6. Spectrophotometric and kinetic analyses

Spectrophotometric measurements of the amplitude of the light-minus-dark absorbance difference signal at 700 nm (P700) for PSI and 320 nm (Q_A) for PSII were used to estimate the concentration of the photosystems in thylakoid membranes [25,26]. The kinetics of P700 photooxidation and Q_A photoreduction of DCMU-poisoned thylakoid were measured under weak green actinic excitation and the corresponding rate constants were used to estimate the functional light-harvesting Chl antenna size of PSI and PSII, respectively [26].

2.7. Cell growth analysis

To measure the biomass accumulation properties of the *tla4* culture relative to that of the wild type, we carried out comparative growth analyses with each of the two cultures grown upon bubbling with 6% CO_2, under 450 μmol photons m^{-2} s^{-1}, i.e., a light intensity greater than that required to saturate photosynthesis in the wild type. Cultures were bubbled with airflow velocity of 40 mL/min at 25 °C. The *C. reinhardtii* cells were grown in 200 mL HS media in a bubble column photobioreactor with 4 cm diameter and 50 cm height.

Rate of microalgal growth was measured during the exponential growth phase from the increase in cell number as a function of time. The growth rate (μ) was calculated from the following equation [27]:

$$\mu = \ln(N_2/N_1)/(t_2 - t_1) \quad (1)$$

where N_1 and N_2 are the cell counts at times t_1 and t_2, respectively.