

# Photosynthetic apparatus organization and function in the wild type and a chlorophyll *b*-less mutant of *Chlamydomonas reinhardtii*. Dependence on carbon source

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**Abstract.** The assembly, organization and function of the photosynthetic apparatus was investigated in the wild type and a chlorophyll (Chl) *b*-less mutant of the unicellular green alga *Chlamydomonas reinhardtii*, generated via DNA insertional mutagenesis. Comparative analyses were undertaken with cells grown photoheterotrophically (acetate), photomixotrophically (acetate and  $\text{HCO}_3^-$ ) or photoautotrophically ( $\text{HCO}_3^-$ ). It is shown that lack of Chl *b* diminished the photosystem-II (PSII) functional Chl antenna size from 320 Chl (*a* and *b*) to about 95 Chl *a* molecules. However, the functional Chl antenna size of PSI remained fairly constant at about 290 Chl molecules, independent of the presence of Chl *b*. Western blot and kinetic analyses suggested the presence of inner subunits of the Chl *a-b* light-harvesting complex of PSII (LHCII) and the entire complement of the Chl *a-b* light-harvesting complex of PSI (LHCI) in the mutant. It is concluded that Chl *a* can replace Chl *b* in the inner subunits of the LHCII and in the entire complement of the LHCI. Growth of cells on acetate as the sole carbon source imposes limitations in the photon-use efficiency and capacity of photosynthesis. These are manifested as a lower quantum yield and lower light-saturated rate of photosynthesis, and as lower variable to maximal ( $F_v/F_{\text{max}}$ ) chlorophyll fluorescence yield ratios. This adverse effect probably originates because acetate shifts the oxidation-reduction state of the plastoquinone pool, and also because it causes a decrease in the amount and/or activity of Rubisco in the chloroplast. Such limitations are fully alleviated upon inclusion of an inorganic carbon source

(e.g. bicarbonate) in the cell growth medium. Further, the work provides evidence to show that transformation of green algae can be used as a tool by which to generate mutants exhibiting a permanently truncated Chl antenna size and a higher (per Chl) photosynthetic productivity of the cells.

**Key words:** *Chlamydomonas* (mutant) – Chlorophyll *b*-less mutant – Light-harvesting complex – Photosynthesis – Productivity (photosynthetic)

## Introduction

The process of photosynthesis is initiated upon absorption of light energy by pigments in the light-harvesting chlorophyll (Chl) antenna complexes. Two distinct photosystems in the chloroplast of higher plants and green algae possess, in addition to their Chl *a*-containing core antenna, an auxiliary light-harvesting antenna consisting of specific Chl *a*- and Chl *b*-binding light-harvesting proteins (LHC). The light-harvesting Chl-proteins of photosystem I (PSI) and photosystem II (PSII) are encoded for by the large nuclear *Lhc* gene family and are referred to by the LHCI and LHCII abbreviations, respectively (Jansson 1994).

In higher plants, Chl *b* is specifically required for the assembly and function of several LHC proteins. Studies of Chl *b*-less strains of barley (Thorner and Highkin 1974; Harrison and Melis 1992; Harrison et al. 1993; Krol et al. 1995), rice (Terao and Katoh 1989) and *Arabidopsis thaliana* (Murray and Kohorn 1991) revealed that these strains either lack or have significantly lower amounts of LHCII and LHCI in their thylakoid membranes. Therefore, Chl *b*-less mutants of higher plants have a highly truncated auxiliary light-harvesting antenna of PSII and a smaller PSI antenna size (Ghirardi et al. 1986; Harrison et al. 1993; Terao and Katoh 1996). In contrast to higher plants, Chl *b*-less strains of the green alga *Chlamydomonas reinhardtii*,

Abbreviations: Chl = chlorophyll; CP24, CP26 and CP29 = chlorophyll proteins of 24, 26 and 29 kDa, respectively;  $F_v$ ,  $F_{\text{max}}$  = variable and maximum fluorescence yield, respectively; LHC = light-harvesting complex; P700 = electron donor pigment of PSI;  $Q_A$  = primary electron acceptor of PSII; TAP = Tris-acetate-phosphate medium; TABP = TAP medium supplemented with sodium bicarbonate; TBP = Tris-bicarbonate-phosphate medium

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when grown on acetate in the light, appear to contain in their thylakoid membrane all apoproteins of the LHC (Picaud and Dubertret 1986; Allen and Staehelin 1994). However, it was reported that trimeric LHCII does not assemble and that the PSII Chl antenna size is truncated in the Chl *b*-less *C. reinhardtii* (Michel et al. 1983; Picaud and Dubertret 1986; Allen and Staehelin 1994).

The interplay between the presence of Chl *b* and the source of carbon during cell growth in the regulation of *Lhc* gene expression is not understood. The presence of acetate in the medium of light-grown *C. reinhardtii* is reported to down-regulate transcription of *Lhcb* genes (Kindle 1987). It was also reported that low levels of CO<sub>2</sub> up-regulate the transcription of *Lhc* genes (Soman-chi et al. 1998). Therefore, differentially regulated transcription and expression of *Lhcb* genes would be expected when cells are grown under different carbon conditions. Different levels of LHC would affect the Chl antenna size of the photosystems and the organization and function of the photosynthetic apparatus, depending on the primary carbon source available during cell growth.

In the present work, we investigated the organization and function of the photochemical apparatus in *Chlamydomonas reinhardtii* cultivated in the presence of either acetate, a mixture of acetate and bicarbonate, or bicarbonate alone in the growth medium. Since Chl *b*-less mutants of higher plants have been used successfully as model organisms in the study of the assembly of the light-harvesting complex, we extended these studies to include the organization and function of the photochemical apparatus in a Chl *b*-less mutant of *Chlamydomonas reinhardtii*. This strain was derived via DNA insertional mutagenesis in which the chlorophyll *a* oxygenase gene was affected (Tanaka et al. 1998). The advantage of tagged genetic transformation for the generation of mutants is that genes responsible for a given property can be later isolated. This work shows that it is possible to employ genetic transformation of green algae for the isolation of strains with a truncated Chl antenna size. Our results show that the organization and function of the photochemical apparatus in *C. reinhardtii* are strongly influenced by the carbon source during cell growth. In addition, we show that the light-harvesting Chl antenna size of PSI and PSII are not affected in the same manner by the absence of Chl *b*.

## Materials and methods

**Growth conditions of the algae.** *Chlamydomonas reinhardtii* wild-type strain *cw15* and the chlorophyll *b*-less mutant *chs3* (Tanaka et al. 1998) were grown in liquid cultures in flat 1-L Roux bottles at 22 °C under continuous illumination at 200 μmol photons m<sup>-2</sup> s<sup>-1</sup> of cool-white fluorescent light. Cultures were stirred to ensure uniform illumination and to prevent cell settling. Cells were grown to the mid-logarithmic phase either photoheterotrophically in Tris-acetate-phosphate medium (TAP, pH 7.0) (Gorman and Levine 1965), photomixotrophically in TAP medium supplemented with 25 mM sodium bicarbonate and 40 mM Tris-HCl, pH 7.4 (TABP medium), or photoautotrophically in modified 40 mM Tris-HCl-phosphate medium (pH 7.4) supplemented with 25 mM sodium bicarbonate (TBP medium).

**Cell count and Chl determination.** Cell density was monitored by counting the number of cells per milliliter of culture using a Neubauer ultraplane hemacytometer. Cells or thylakoid membranes were extracted in 80% acetone and debris was removed by centrifugation at 10,000 *g* for 5 min. The absorbance of the supernatant was measured with a Shimadzu UV-160U spectrophotometer. The Chl (*a* and *b*) concentration of the samples was determined according to Arnon (1949), with equations corrected as in Melis et al. (1987).

**Thylakoid membrane isolation.** Cells were harvested by centrifugation at 1,000 *g* for 3 min at 4 °C. Pellets were resuspended in 1–2 ml of growth medium and stored frozen at –80 °C until all samples were ready for processing. Samples were thawed on ice and diluted with ice-cold sonication buffer containing 50 mM Tricine (pH 7.8), 10 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.2% polyvinylpyrrolidone 40, 0.2% sodium ascorbate, 1 mM aminocaproic acid, 1 mM aminobenzamidine and 100 μM phenylmethylsulfonylfluoride (PMSF). Cells were broken by sonication in a Branson 250 Cell Disrupter operated at 4 °C for 30 s (pulse mode, 50% duty cycle, output power 5). Unbroken cells and starch grains were removed by centrifugation at 3,000 *g* for 4 min at 4 °C. The thylakoid membranes were collected by centrifugation of the supernatant at 75,000 *g* for 30 min at 4 °C. The thylakoid membrane pellet was resuspended in a buffer containing 50 mM Tricine (pH 7.8), 10 mM NaCl, 5 mM MgCl<sub>2</sub> for spectrophotometric measurements, or 250 mM Tris-HCl (pH 6.8), 20% glycerol, 7% SDS and 2 M urea for protein analysis. Solubilization of thylakoid membrane proteins was carried out for 30 min at room temperature, a procedure designed to prevent the formation of protein aggregates during denaturation. Samples were centrifuged in a microfuge for 4 min to remove unsolubilized material, β-mercaptoethanol was added to yield a final concentration of 10% and the samples were stored at –80 °C.

**Analysis by SDS-PAGE and Western blotting.** Samples were brought to room temperature prior to loading for electrophoresis and diluted accordingly to yield equal Chl concentrations. Gel lanes were loaded with an equal amount of Chl (2 nmol Chl per lane). The SDS-PAGE analysis was carried out according to Laemmli (1970), with resolving gels containing 12.5% acrylamide, at a constant current of 9 mA for 16 h. Gels were stained with 1% Coomassie brilliant blue R for protein visualization. Electrophoretic transfer of the SDS-PAGE-resolved proteins onto nitrocellulose was carried out for 3–5 h at a constant current of 800 mA, in transfer buffer containing 50 mM Tris, 380 mM glycine (pH 8.5), 20% methanol and 1% SDS. Identification of thylakoid membrane proteins was accomplished with polyclonal antibodies raised against the LHC complex of *Zea mays*, kindly provided by Dr. R. Bassi (Di Paolo et al. 1990; Bassi et al. 1992). Cross-reaction with the antibodies was visualized by a chromogenic reaction with anti Ig-G secondary antibodies conjugated with alkaline phosphatase (BioRad) and it was quantified by densitometry. For densitometry, an HP-ScanJet 6100C optical scanner was employed, on-line with a PowerMacintosh/G3 computer. DeskScanII software in combination with Adobe Photoshop were used for scanning of the western blots, whereas NIH Image version 1.6 software was employed for the deconvolution and quantification of the bands.

**Chlorophyll fluorescence, oxygen evolution and spectrophotometric analyses.** The initial ( $F_0$ ), variable ( $F_v$ ) and maximum ( $F_{max}$ ) chlorophyll fluorescence yield of intact cells was measured at 690-nm (combination of CS 2-60, CS 2-64 Corning filters and 690-nm interference filter). Actinic excitation of the cultures was provided by green light (CS 4-96 and CS 3-69 Corning filters; intensity of 75 μmol photons m<sup>-2</sup> s<sup>-1</sup>) (Melis and Hart 1980). For these measurements an aliquot from the culture was incubated in the dark for 10 min prior to the measurement and the chlorophyll fluorescence was recorded in the absence or presence of 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU; 2.5 μM final concentration).

Oxygen evolution activity of the cultures was measured at 22 °C with a Clark-type oxygen electrode illuminated with a slide projector lamp. Yellow actinic excitation was provided by a CS 3-69 Corning cut-off filter in combination with an Ealing 35-5453 VIQ5-8 filter. An aliquot of 5 ml cell suspension (2 µM Chl) was transferred to the oxygen-electrode chamber. To ensure that oxygen evolution was not limited by the carbon source available to the cells, 100 µl of 0.5 M sodium bicarbonate solution (pH 7.4) was added to the suspension prior to the oxygen-evolution measurements. Measurement of the light-saturation curve of photosynthesis was implemented with the oxygen electrode, beginning with the registration of dark respiration in the cell suspension, and followed by measurements of the rate of oxygen evolution in steps at 36, 70, 140, 260, 360, 500, 620, 1,750 and 2,600 µmol photons m<sup>-2</sup> s<sup>-1</sup>. Registration of the rate (slope) of oxygen evolution at each light intensity step was recorded for about 2.5 min.

The concentration of the photosystems in thylakoid membranes was estimated spectrophotometrically from the amplitude of the light *minus* dark absorbance difference signal at 700 nm (P700) for PSI, and 320 nm (Q<sub>A</sub>) for PSII (Melis and Brown 1980). The functional light-harvesting Chl antenna size of PSI and PSII was measured from the kinetics of P700 photo-oxidation and Q<sub>A</sub> photoreduction, respectively (Melis 1989).

## Results

*Organization of the photosynthetic apparatus in the wild type and Chl b-less mutant.* Cells of the wall-less strain *cw15* of *Chlamydomonas reinhardtii* (referred to as the wild type in this work) showed a Chl *a*/Chl *b* ratio of 2.7:1 when grown in the presence of acetate as the sole carbon source (Table 1). When grown in the presence of a mixture of acetate and bicarbonate (TABP medium), or bicarbonate alone (TBP medium), the Chl *a*/Chl *b* ratio of the cells was slightly higher at about 3.1:1. Table 1 also shows the Chl content of the wild-type cells under the three growth conditions. The Chl content of the cells was slightly lower when the growth medium contained bicarbonate (TABP or TBP media) than acetate alone (TAP). In the Chl *b*-less mutant, the Chl content of the cells did not change as a function of the

growth medium. Interestingly, and in spite of the lack of Chl *b*, the Chl content of the mutant was relatively high compared to that of the wild type. This result suggests a higher chloroplast concentration of PSI and PSII units in the mutant than in the wild type (see below).

Table 2 presents the thylakoid membrane content in Q<sub>A</sub> (PSII) and P700 (PSI). Quantitation of the functional PSII and PSI reaction centers was obtained from the light-induced absorbance change at 320 (Q<sub>A</sub>) and 700 nm (P700), respectively (Melis and Brown 1980). The wild type contained about 1.8 mmol Q<sub>A</sub> per mol Chl in the TAP-grown cells, a value that increased to about 2.8 mmol Q<sub>A</sub> per mol Chl when bicarbonate was present in the growth medium. The wild type had about 2.1 mmol P700 per mol Chl in the TAP-grown cells, a value that decreased to about 1.9 (TABP) and about 1.6 mmol P700 per mol Chl in the TBP-grown cells. Translated on a per-cell basis, the concentration of Q<sub>A</sub> was fairly constant and at about 6.6 × 10<sup>-18</sup> mol Q<sub>A</sub>/cell, independent of the carbon source (Table 2). However, the amount of PSI was substantially lower in the TABP- and TBP-grown cells. The dissimilar dependence of PSII (Q<sub>A</sub>) and PSI (P700) content on the carbon source during cell growth made for substantially different PSII/PSI ratios in the thylakoid membrane of these samples. A wild-type PSII/PSI ratio of 0.86:1 in TAP increased to 1.48:1 in TABP and was 1.75:1 in TBP. Interestingly, a quantitatively and qualitatively similar dependence of the PSII/PSI ratio was also observed in the Chl *b*-less mutant (Table 2).

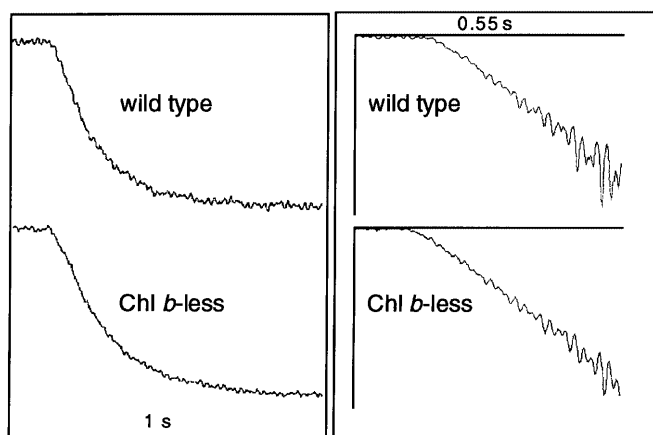
Table 2 also shows that, on a per-cell basis, the Q<sub>A</sub> and P700 contents in the Chl *b*-less mutant were greater (by a factor of about 2) than those in the wild type, irrespective of the carbon source during growth. These results are consistent with the higher Chl content in mutant vs. wild type (Table 1) and show that a truncated Chl antenna size in the mutant may have been compensated by a correspondingly higher density of PSI and PSII units in the mutant thylakoids. The results in Table 2 further suggest that, irrespective of the pigment

**Table 1.** Chlorophyll content and Chl *a*/Chl *b* ratios in the wild type (*cw 15*) and the Chl *b*-less mutant of *C. reinhardtii* grown in various media (see *Materials and methods*). Values represent means ± SD

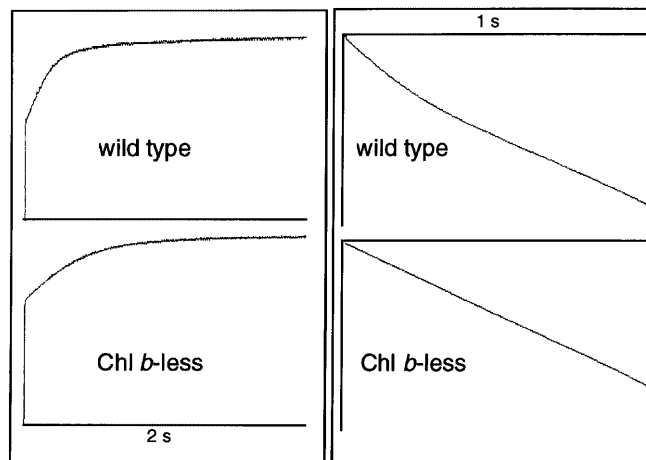
Parameter	Wild type			Chl <i>b</i> -less		
	TAP	TABP	TBP	TAP	TABP	TBP
Chl <i>a</i> /Chl <i>b</i>	2.7 ± 0.14	3.2 ± 0.2	3.0 ± 0.12	–	–	–
mol Chl/cell (×10 <sup>-15</sup> )	3.5 ± 1.1	2.4 ± 0.1	2.4 ± 0.5	3.6 ± 0.6	4.0 ± 0.6	3.8 ± 1.1

**Table 2.** Photochemical apparatus characteristics of wild type and Chl *b*-less strain of *C. reinhardtii* (*n* = 3–5)

Parameter	Wild type			Chl <i>b</i> -less		
	TAP	TABP	TBP	TAP	TABP	TBP
Q <sub>A</sub> /total Chl (mmol:mol)	1.83 ± 0.06	2.76 ± 0.23	2.86 ± 0.09	3.0 ± 0.27	3.1 ± 0.03	4.1 ± 0.6
P700/total Chl (mmol:mol)	2.14 ± 0.13	1.87 ± 0.1	1.63 ± 0.17	3.1 ± 0.24	2.3 ± 0.08	2.2 ± 0.16
Q <sub>A</sub> /cell (mol/cell × 10 <sup>-18</sup> )	6.4	6.6	6.9	10.8	12.4	15.6
P700/cell (mol/cell × 10 <sup>-18</sup> )	7.5	4.5	3.9	11.2	9.2	8.4
PSII/PSI	0.86	1.48	1.75	0.97	1.35	1.86



**Fig. 1.** Light-induced absorbance change measurements. *Left panel*, kinetics of P700 photooxidation ( $\Delta A_{700}$ ) with thylakoid membranes of the wild type and a Chl *b*-less mutant of *C. reinhardtii*. *Upper trace*, wild type; *lower trace*, Chl *b*-less mutant. *Right panel*, corresponding semilogarithmic plot of the  $\Delta A_{700}$  kinetics



**Fig. 2.** Chlorophyll fluorescence induction kinetics. *Left panel*, Chl fluorescence induction traces of the wild type (*upper*) and a Chl *b*-less mutant (*lower*). *Right panel*, corresponding semilogarithmic plots of the area over the fluorescence induction curve

content of the photosynthetic apparatus, the PSII/PSI ratio is highest when the cells are grown in the presence of bicarbonate (TBP medium) and that growth in acetate (TAP) tends to suppress the functional PSII/PSI ratio. The effect of the mutation on the functional Chl antenna sizes of PSII and PSI was further addressed in detail (see below).

*Photosystem chlorophyll antenna size.* Estimates of the functional Chl antenna sizes of PSI and PSII were obtained with the so-called kinetic and spectrophotometric method (Melis and Anderson 1983). In this approach, Chl molecules are functionally assigned to PSI and PSII in direct proportion to the rate of light absorption/utilization by the two photosystems, measured from the kinetics of P700 oxidation and  $Q_A$  reduction in isolated and DCMU-poisoned thylakoids (Melis 1989).

Figure 1 (left panels) shows examples of light-induced changes in the absorbance of the reaction center P700 at  $\lambda = 700$  nm, occurring as a result of P700 photooxidation in thylakoid membranes of the wild type and the Chl *b*-less mutant grown on TAP. Figure 1 (right panels) also shows the respective semilogarithmic plots of the  $\Delta A_{700}$  kinetics, revealing single exponential functions of time with rate constants  $K_{PSI}$  of  $9.0 \text{ s}^{-1}$  for the wild type and  $7.5 \text{ s}^{-1}$  for the Chl *b*-less mutant. The slower P700 photooxidation kinetics for the Chl *b*-less mutant suggests a slightly smaller PSI Chl antenna size than in the wild type.

Figure 2 (left panels) shows examples of light-induced fluorescence induction kinetics, the variable part of which reflects the photoreduction of  $Q_A$  (Melis and Duysens 1979) in the thylakoid membranes of the wild type and the Chl *b*-less mutant grown on TAP. The fluorescence induction kinetics of the wild-type strain were faster than those of the Chl *b*-less mutant, suggesting a larger PSII Chl antenna size for the former. Figure 2 (right panels) also shows the respective semi-

logarithmic plots of the area over the fluorescence induction kinetics. This parameter (area over fluorescence) is directly proportional to the amount of  $Q_A$  that becomes photoreduced (Melis and Duysens 1979; Melis 1989). The analysis (Fig. 2, right panels) revealed biphasic  $Q_A$  reduction kinetics for the wild type, reflecting a PSII heterogeneity and the existence of two populations of PSII (PSII $\alpha$  and PSII $\beta$ ) with significantly different Chl antenna sizes (Melis 1991; Lavergne and Briantais 1996). In the Chl *b*-less mutant,  $Q_A$  photoreduction occurred as a single exponential function of time with significantly slower kinetics, suggesting lack of PSII heterogeneity and the occurrence of a uniform and small Chl antenna size for the mutant (Ghirardi et al. 1986).

From the measured kinetics of P700 photooxidation and “area over fluorescence induction” we determined the functional chlorophyll antenna size of PSI and PSII, respectively, for the wild type and Chl *b*-less mutant (Table 3). In the wild type grown in the presence of acetate (TAP and TABP), PSII $\alpha$  accounted for about 50% of the total PSII, whereas it accounted for only about 30% of the total PSII in cells grown in the presence of bicarbonate as the sole carbon source (TBP). Table 3 also shows values for the respective functional Chl antenna sizes (N) of the photosystems. In the wild type grown with TAP or TABP, PSII $\alpha$  contained about 300 Chl molecules (*a* and *b*) ( $N_{PSII\alpha} = 300 \pm 28$ ), whereas PSII $\beta$  contained about 130 Chl molecules ( $N_{PSII\beta} = 130 \pm 11$ ). The PSII $\alpha$  Chl antenna size for the samples grown on TBP ( $N_{PSII\alpha} = 474 \pm 49$ ) was significantly larger than for that of cells grown on the other media. However, the larger Chl antenna size, along with the relatively smaller fraction of PSII $\alpha$  centers in these samples, suggests a dissociation of PSII $\beta$  centers from the statistical pigment bed of the LHClI normally associated with PSII $\alpha$  (Melis and Duysens 1979), leaving behind a peripheral light-harvesting Chl antenna to be shared by fewer PSII centers. This dissociation could be an artifact occurring during the

**Table 3.** Chlorophyll antenna sizes (N) of PSII and PSI in the wild type and Chl *b*-less mutant of *C. reinhardtii*. The concentration of the various forms of PSII is given as a percentage of total PSII in the thylakoid membrane. The antenna size of PSII and PSI is given

as the number of Chl molecules functionally associated with a photosystem. The value of  $N_{\text{PSII}\alpha}$  in parenthesis (325) is based on the assumption of  $\text{PSII}\alpha/\text{PSII}\beta = 50:50$ . The values given are the mean  $\pm$  SD

Parameter	Wild type			Chl <i>b</i> -less		
	TAP	TABP	TBP	TAP	TABP	TBP
PSII $_{\alpha}$	42 $\pm$ 7.4	53 $\pm$ 10.5	29 $\pm$ 9.5	–	–	–
PSII $_{\beta}$	58 $\pm$ 7.4	47 $\pm$ 10.5	72 $\pm$ 9.5	–	–	–
$N_{(\text{PSII}\alpha)}$	322 $\pm$ 7	284 $\pm$ 49	474 $\pm$ 49 (325)	–	–	–
$N_{(\text{PSII}\beta)}$	127 $\pm$ 3	141 $\pm$ 3	119 $\pm$ 3	–	–	–
$N_{(\text{PSII})}$	–	–	–	93 $\pm$ 3	107 $\pm$ 9	88 $\pm$ 7
$N_{(\text{PSI})}$	291 $\pm$ 17	217 $\pm$ 8	204 $\pm$ 4	245 $\pm$ 14	284 $\pm$ 6	289 $\pm$ 12

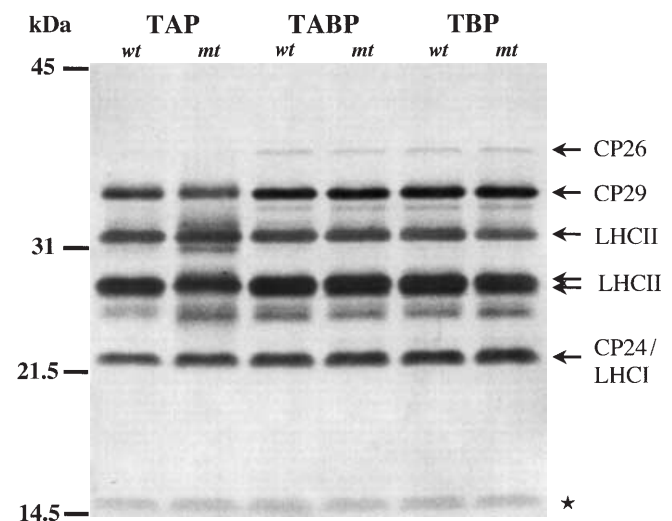
isolation of the thylakoid membranes. Indeed, if one assumed a PSII $_{\alpha}$ /PSII $_{\beta}$  ratio of about 50:50 in the TBP samples, then the calculated Chl antenna size of PSII $_{\alpha}$  ( $N_{\text{PSII}\alpha}$ ) would be about 325 Chl molecules, i.e. similar to that of cells grown on TAP or TABP. On the basis of this assumption, it would appear that the average PSII functional Chl antenna size ( $N_{\text{PSII}}$ ) is fairly constant and independent of the carbon source in the growth medium. In the wild type, the PSI Chl antenna size appeared to decline somewhat from  $N_{\text{PSI}} = 291 \pm 17$  in the TAP-grown cells to  $N_{\text{PSI}} = 204 \pm 4$  in the cells grown in TBP media.

In contrast to the wild type, PSII in the Chl *b*-less mutant did not display Chl antenna size heterogeneity, a characteristic also seen in the Chl *b*-less mutant of barley (Ghirardi et al. 1986). The PSII Chl antenna size in the mutant ( $N_{\text{PSII}} = 96 \pm 10$ ) was substantially smaller than that of the wild type. However, at about 96 Chl *a* molecules per reaction center, it is significantly larger than the minimum Chl antenna size of the PSII-core, which is known to contain only about 37 Chl *a* molecules (Manodori et al. 1984; Glick and Melis 1988). This difference suggests that, in the Chl *b*-less *C. reinhardtii* mutant, a number of the auxiliary LHC proteins are assembled and functionally associated with the PSII-core complex.

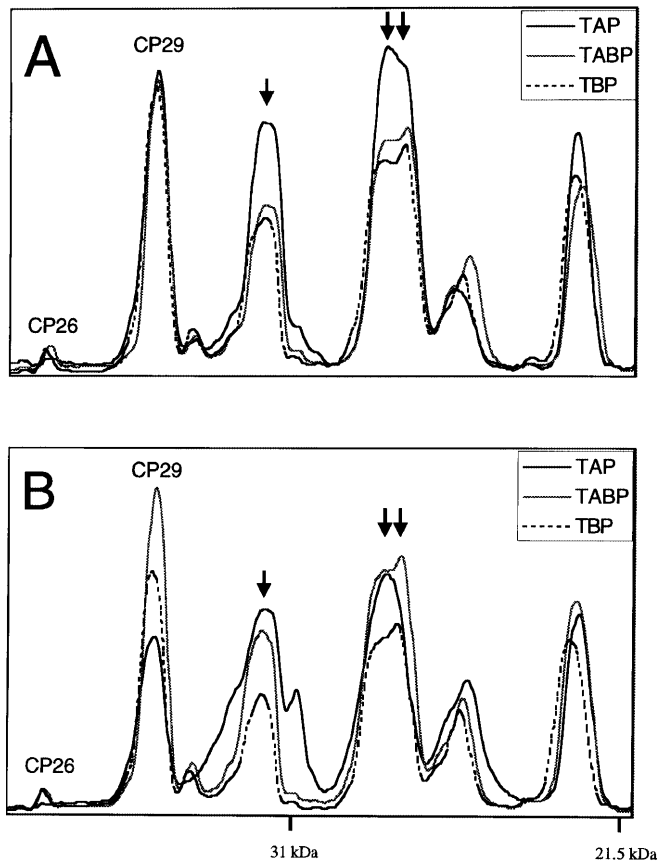
Surprisingly, the PSI Chl antenna size in the Chl *b*-less mutant ( $N_{\text{PSI}} = 245\text{--}290$  Chl *a* molecules per reaction center) was essentially as large as that of the wild type. Thus, the absence of Chl *b* appears not to have had a pronounced effect on the Chl antenna size of PSI, irrespective of the growth conditions (Table 3). This observation suggests that LHCI in *C. reinhardtii* assembles fully and functions normally in the total absence of Chl *b*. It may be concluded that Chl *a* can effectively substitute for Chl *b* in the LHCI of this green alga.

*Composition of LHC polypeptides in the wild type and Chl b-less mutant.* The analysis of the functional Chl antenna size in the Chl *b*-less mutant suggested a significant presence of LHCI and LHCII proteins, which assemble and function irrespective of Chl *b*. This observation warrants further analysis of the LHC apoprotein composition in the wild type and Chl *b*-less mutant. Western blot analysis of the protein composi-

tion in thylakoid membranes of *C. reinhardtii* (Fig. 3) revealed the presence of several LHCII (Bassi and Wollman 1991; Allen and Staehelin 1994) and LHCI proteins (Bassi et al. 1992). The LHCII apoproteins were distinguished according to their abundance in the thylakoid membrane into major (LHCII) and minor (the so-called CP24, CP26 and CP29). The polyclonal antibodies used in this study cross-reacted with most of the LHC proteins of *C. reinhardtii* (Bassi et al. 1992). Several distinct protein bands were discerned (Fig. 3), with arrows indicating the position of the apoproteins for CP26, CP29, the major LHCII as well as that of a composite band consisting of CP24 and LHCI. The minor protein band indicated by a star at about 15 kDa most likely originates from partial degradation of a LHC (Allen and Staehelin 1994). It is evident from the results in Fig. 3 that, irrespective of the growth conditions, the wild type and Chl *b*-less mutant qualitatively



**Fig. 3.** Western blot analysis of electrophoretically separated thylakoid membrane proteins of the wild type (*wt*) and Chl *b*-less mutant (*mt*) of *C. reinhardtii* grown on acetate (TAP), a mixture of acetate and bicarbonate (TABP), or bicarbonate (TBP) as the source of carbon to the cells. Lanes were loaded on an equal-Chl basis (2 nmol Chl per lane) and probed with polyclonal LHC antibodies. The electrophoretic mobility positions of the various LHC apoproteins of PSII are shown. Star indicates the presence of a probable LHC degradation product



**Fig. 4A,B.** Densitometric scans of Western-blot lanes from the wild type (A) and Chl *b*-less mutant (B) as shown in Fig. 3. Superimposed scans were normalized on the basis of equal  $Q_A$  loading. The peaks of CP26 and CP29 are indicated. Arrows show the positions of the major LHCII apoproteins

contain essentially the same LHC apoproteins in their thylakoid membranes. Quantitatively, the abundance of the various LHC forms depends on the carbon source used for cell growth.

A quantitative comparison of the relative abundance of light-harvesting proteins associated with PSII in the wild type and Chl *b*-less mutant under the different growth conditions was obtained upon densitometric scanning of the lanes in Fig. 3. Figure 4A shows results for the wild type grown in TAP, TABP or TBP, normalized to the amount of  $Q_A$  in the respective sample (equal PSII content). Table 4 presents a numerical analysis of the combined results, normalized to the CP29 per  $Q_A$  content of the wild type grown in TAP (100%). In the analysis of Table 4, valid comparisons can be made horizontally in each one of the rows, but not vertically in the various columns. It is evident from the results in Fig. 4A and Table 4 that wild-type *C. reinhardtii* thylakoids contain approximately equal amounts of CP29 per  $Q_A$  under the various growth conditions. However, the major LHCII content appeared to be somewhat lower in the presence of bicarbonate (TABP and TBP media) relative to that in TAP. Since the Chl antenna sizes of PSII were largely independent of the carbon source in the growth medium

**Table 4.** Relative amounts of LHC proteins of PSII and PSI as measured by densitometry of Western blots similar to those shown in Fig. 3. Protein band densities were estimated on the basis of equal  $Q_A$  loading and normalized to the amount of CP29 measured in the wild type, TAP-grown cells

LHC	Wild type			Chl <i>b</i> -less		
	TAP	TABP	TBP	TAP	TABP	TBP
CP26	2	4	4	1	3	3
CP29	100	86	91	55	81	65
LHCII	94	62	54	68	54	32
LHCII	180	145	139	114	129	91
LHCI/CP24	71	66	70	56	64	54

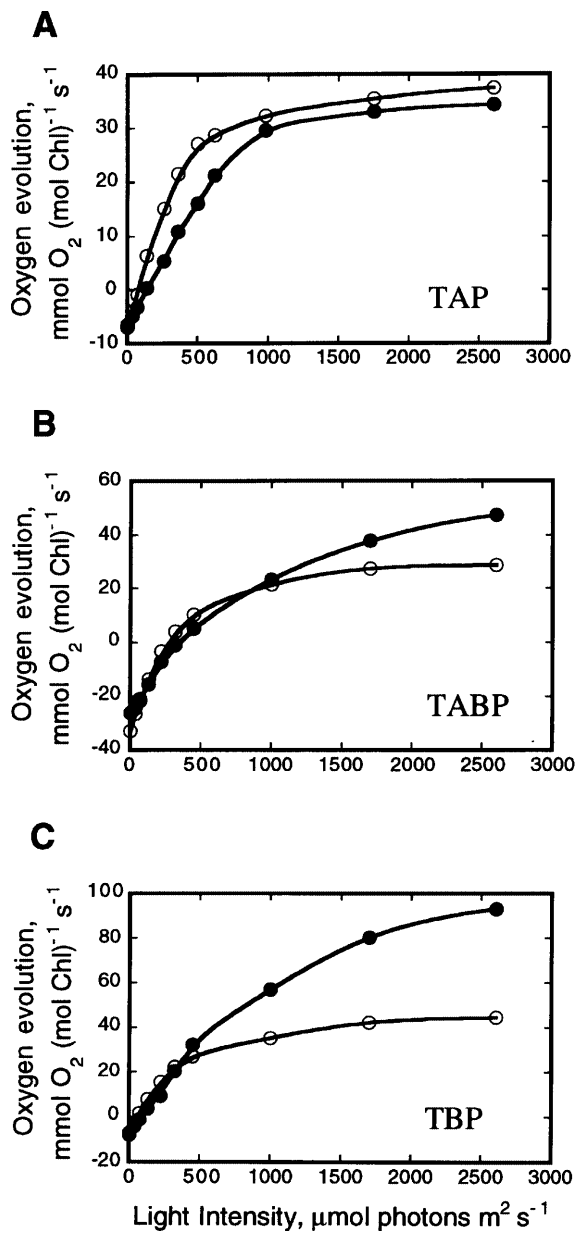
(Table 3), the results indicate the presence of LHCII apoproteins that are not functionally associated with PSII in the thylakoid membrane of the TAP-grown cells.

Figure 4B shows the corresponding results for the Chl *b*-less mutant. In this case, the relative amount of CP29 per  $Q_A$  was lower than that of the wild type, albeit somewhat variable under the different growth conditions. Further, the relative amount of LHCII per  $Q_A$  was significantly lower than that of the wild type. This decrease was more pronounced in cells grown in TBP medium (Table 4).

*Measurements of photosynthetic capacity.* Information about the capacity and efficiency of photosynthesis can be obtained from the light-saturation curve (the so-called *photosynthesis versus irradiance* curve), in which the rate of  $O_2$  evolution is measured and plotted as a function of the actinic light intensity. In these measurements, the rate of  $O_2$  evolution first increases linearly with irradiance and then levels off as the saturating irradiance ( $I_S$ ) is approached (Neale et al. 1993). The slope of the initial linear increase provides information about the photon-use efficiency ( $\Phi$ ) of photosynthesis (Bjorkman Demmig 1987; Neale 1993), whereas the light-saturated rate ( $P_{max}$ ) provides a measure of the capacity of photosynthesis for the particular sample (Powles and Critchley 1980). Figure 5 shows light-saturation curves of photosynthesis for the wild type and Chl *b*-less mutant, grown in media with different sources of carbon.

When grown on TAP (Fig. 5A), the wild type and Chl *b*-less mutant had similar  $P_{max}$  values at 39 and 34  $mmol O_2 (mol Chl)^{-1} s^{-1}$ , respectively (Table 5). However, the slope of the initial linear increase for the Chl *b*-less mutant ( $\Phi = 0.05$ ), which provides information about the photon-use efficiency of photosynthesis, was only about half that for the wild type ( $\Phi = 0.091$ ). These results indicate a similar photosynthetic capacity for the wild type and Chl *b*-less mutant grown on acetate but, at the same time, a strongly reduced photon-use efficiency for the mutant.

When grown on TABP (Fig. 5B), the wild type had a  $P_{max}$  (34  $mmol O_2 (mol Chl)^{-1} s^{-1}$ ) similar to that in TAP-grown cells. However, the  $\Phi$  value ( $\Phi = 0.137$ ) was greater than that measured in TAP ( $\Phi = 0.091$ , Table 5). In TABP medium, the Chl *b*-less mutant showed a higher  $P_{max}$  (48  $mmol O_2 (mol Chl)^{-1} s^{-1}$ ) than that



**Fig. 5A–C.** Light-saturation curves of photosynthesis in the wild type (*open circles*) and a Chl *b*-less mutant (*closed circles*) of *C. reinhardtii*. Rates of oxygen evolution on a per-chlorophyll basis were measured as a function of irradiance for cells grown in acetate (A), acetate/bicarbonate (B), or bicarbonate (C)

measured in TAP. Moreover, the  $\Phi$  value ( $\Phi = 0.086$ ) was also significantly greater than that measured in TAP (0.05). Thus, both types of measurement indicate greater photochemical efficiency in the presence of bicarbonate than in the presence of acetate as the sole carbon source. Interestingly, when grown in TABP, both wild type and mutant exhibited strongly enhanced rates of dark respiration, compared to those measured in acetate only or bicarbonate only media (Fig. 5). This unusual observation corroborates results in a recent study, which showed a 4-fold increase in the rate of dark respiration upon addition of acetate to a  $\text{CO}_2$ -grown culture of *C. reinhardtii* (Endo and Asada 1996).

When grown on TBP, the wild type had a  $P_{\text{max}}$  of  $39 \text{ mmol O}_2 (\text{mol Chl})^{-1} \text{ s}^{-1}$ , i.e. similar to that in TAP-grown or TABP-grown cells (Fig. 5C). The photon-use efficiency ( $\Phi$ ) was high ( $\Phi = 0.125$ ) as in cells grown on acetate/bicarbonate (TABP) media. The photon-use efficiency of the Chl *b*-less mutant ( $\Phi = 0.091$ ) was slightly lower than that of the wild type and almost twice that measured in the TAP-grown mutant (Table 5). Moreover, the Chl *b*-less mutant grown on TBP had a greatly enhanced  $P_{\text{max}}$  value of  $97 \text{ mmol O}_2 (\text{mol Chl})^{-1} \text{ s}^{-1}$  (Table 5). On a per-Chl basis, this light-saturated rate was approximately 2.5 times greater than that of the wild type. Under these conditions, the light saturation curve of photosynthesis for the mutant showed a saturating irradiance significantly higher than that of the wild type, consistent with the differences observed in the Chl antenna size of PSII. Taken together, the results in Fig. 5A–C suggest that PSII determines the properties of the light-saturation curve of photosynthesis in TBP-grown cells. In TAP-grown cells, and to some extent in TABP-grown cells, the rate and capacity of photosynthesis are defined (and probably limited) not by PSII but either by PSI or by the carbon fixing reactions in the chloroplast (see below).

Table 5 also shows the in-vivo  $F_v/F_{\text{max}}$  ratio, which is a measure of the photochemical efficiency of PSII in the chloroplast (Butler and Kitajima 1975). It is evident that  $F_v/F_{\text{max}}$  of the wild type depends on the growth medium of the cells. In the wild type, the presence of bicarbonate improves the photochemical efficiency of PSII ( $F_v/F_{\text{max}} = 0.73$ ) over that in the presence of acetate as the sole carbon source ( $F_v/F_{\text{max}} = 0.65$ ). We conclude, that PSII operates with a lower photochemical efficiency

**Table 5.** Oxygen exchange rates and photon-use efficiency ( $\Phi$ ) in the photosynthetic apparatus of *C. reinhardtii* wild type and the Chl *b*-less mutant. The values shown represent the mean  $\pm$  SD

Parameter measured	Wild type			Chl <i>b</i> -less		
	TAP	TABP	TBP	TAP	TABP	TBP
Respiration ( $\text{mmol O}_2 (\text{mol Chl})^{-1} \text{ s}^{-1}$ )	$6 \pm 1.1$	$33 \pm 1.8$	$6.5 \pm 2.1$	$7 \pm 2.1$	$26 \pm 1.6$	$7 \pm 3.6$
$P_{\text{max}}$ ( $\text{mmol O}_2 (\text{mol Chl})^{-1} \text{ s}^{-1}$ )	$39 \pm 7.8$	$34 \pm 6.0$	$39 \pm 8.8$	$34 \pm 2.2$	$48 \pm 2.8$	$97 \pm 31.1$
Photon-use efficiency ( $\Phi$ , arbitrary units)	0.091	0.137	0.125	0.05	0.086	0.091
$F_v/F_{\text{max}}$	$0.65 \pm 0.04$	$0.73 \pm 0.02$	$0.73 \pm 0.02$	$0.56 \pm 0.02$	$0.55 \pm 0.03$	$0.57 \pm 0.04$

when acetate is the sole carbon source. Interestingly, cells of the Chl *b*-less mutant displayed a lower  $F_v/F_{max}$  ratio of about 0.56, independent of the carbon source (Table 5). This lower PSII photochemical efficiency of the mutant is probably a consequence of the replacement of Chl *b* by Chl *a* in the minor LHCII in this mutant (see below).

## Discussion

Earlier studies from this laboratory with the green alga *Dunaliella salina* demonstrated a novel method for maximizing solar-use efficiencies and photosynthetic productivity in microalgal mass cultures by minimizing the number of the light-harvesting antenna pigments of the photosystems (Neidhardt et al. 1998; Melis et al. 1999). Small Chl antenna sizes in *Dunaliella salina* were generated physiologically, upon continuous exposure of the cells to irradiance stress (Smith et al. 1990). Spontaneous mutants with a truncated Chl antenna size have not been identified in green algae as this property does not confer a competitive advantage to the algae in the wild. In higher plants, however, there have been numerous reports of Chl-deficient and Chl *b*-less mutants (Thorner and Highkin 1974; Terao and Katoh 1989; Murray and Kohorn 1991; Harrison and Melis 1992; Harrison et al. 1993; Krol et al. 1995; Falbel and Staehelin 1996). These proved invaluable in the study of the assembly and organization of the photosynthetic apparatus. In this report, we provide a detailed analysis of the organization and function of the photosynthetic apparatus in a Chl *b*-less mutant of *Chlamydomonas reinhardtii*, generated by DNA insertional mutagenesis (Tanaka et al. 1998). We report a number of similarities and distinct differences in the Chl antenna assembly, size, organization and function in the Chl *b*-less *C. reinhardtii* and that of higher plants.

The Chl *b*-less mutant of *C. reinhardtii* had a significantly truncated Chl antenna size for PSII but no reduction in the Chl antenna size for PSI (Table 3). In spite of the significant loss of Chl from the light-harvesting antenna of PSII, we noted that the Chl content of the mutant cells was equal to, or even greater than that of the wild type (Table 1). More important, the  $Q_A$  and P700 contents of the mutant cells were, on the average, twice as high as those of the wild type (Table 2). This observation suggests the presence and functioning of a greater number of photosynthetic units per chloroplast in the mutant, probably a response of the cells by which to mitigate against the lower light-harvesting capacity introduced by the smaller Chl antenna size in the thylakoid membrane. In higher plants, a distinct aspect of the acclimation response of the photosynthetic apparatus to Chl deficiency in general, and lack of Chl *b* in particular, is an upward adjustment of the PSII/PSI ratio in thylakoids (Ghirardi et al. 1986; Glick and Melis 1988; Harrison et al. 1993). Increased PSII/PSI ratios in Chl-deficient and Chl *b*-less mutants were thought to compensate for the disproportionate reduction in the Chl antenna size of PSII and to

restore balanced electron transport between the two photosystems in the chloroplast thylakoids (Melis 1991, 1996). In *C. reinhardtii*, however, independent of the growth conditions, the PSII/PSI ratio in the Chl *b*-less mutant was similar to that of the wild type, i.e. no upward adjustment of the PSII/PSI ratio was observed. It may be concluded that the Chl *b*-less mutant of *C. reinhardtii* lacks the dynamic photosystem stoichiometry compensation mechanism, which functions to restore balanced electron transport by adjustment of the photosystem ratio in thylakoids. In consequence, Chl *b*-less *C. reinhardtii* showed slightly lower photon-use efficiency values ( $\Phi$ ) than the wild type (Table 5, TBP), probably due to the fact that a portion of the light-energy absorbed by PSI could not be efficiently utilized in the linear photosynthetic electron transport process.

To better understand the organization of the photochemical apparatus in the Chl *b*-less mutant of *C. reinhardtii*, we measured the functional Chl antenna sizes of PSII and PSI as well as the composition of the LHC proteins in the thylakoid membrane. Such studies were extended to include measurements with cells grown under a variety of carbon sources, including acetate, a mixture of acetate and bicarbonate, or bicarbonate alone in the growth medium. Results showed that the PSII antenna sizes of the wild type and Chl *b*-less mutant were largely independent of the carbon source used for growth. However, the PSII Chl antenna size of the mutant was only 30% of that measured for PSII $\alpha$  and about 70% of that measured for PSII $\beta$  in the wild type. This is evidence that pigment deficiency in a green alga can be generated by DNA insertional mutagenesis and can result in a permanently truncated Chl antenna size of PSII.

The PSII antenna size found in the Chl *b*-less mutant ( $N \approx 96$  Chl molecules) was, nevertheless, significantly larger than that of the PSII-core antenna ( $N \approx 37$  Chl molecules, Glick and Melis 1988). Our polypeptide analysis confirmed the presence of CP26, CP29 and a portion of the major LHCII proteins in the thylakoid membrane of the Chl *b*-less mutant (Fig. 3). Obviously, there is stable integration of these LHC polypeptides in the thylakoid membrane of *C. reinhardtii* in the absence of Chl *b*, consistent with previous reports on this matter (Michel et al. 1983; Picaud and Dubertret 1986; Chunaev et al. 1991; Allen and Staehelin 1994; Plumley and Schmidt 1995). In contrast to PSII, the Chl antenna size of PSI in the Chl *b*-less mutant was similar to that of the wild type. In agreement, LHCI proteins that were present in the wild type were also found to comparable levels in the Chl *b*-less mutant. It is concluded that the PSI auxiliary light-harvesting Chl antenna can fully assemble and be functionally connected with the reaction center P700, even in the absence of Chl *b*.

This work provided information about the impact of the carbon source during growth on the photosynthetic capacity and photon-use efficiency of *C. reinhardtii*. Acetate-grown cells of the wild type exhibited a lower PSII photochemical efficiency, measured as  $F_v/F_{max}$  in vivo and, concomitantly, lower overall photosynthetic electron transport efficiency ( $\Phi$ ) than cells grown in the

presence of bicarbonate (Table 5). A lower  $F_v/F_{max}$  in vivo in the presence of acetate as the sole carbon source might originate from an acetate-induced partial reduction of the plastoquinone pool (and hence of  $Q_A$ ) in the thylakoid membrane. Similarly, growth strictly in the presence of acetate as the sole carbon source may also impose limitation in the capacity of photosynthesis at the carbon-fixation level (low levels and/or activity of Rubisco), evidenced in the case of the mutant by the relatively suppressed level of  $P_{max}$  (Fig. 5, TAP). However, when bicarbonate is present in the growth medium, such limitations are alleviated, and cells exhibit maximum photosynthetic efficiencies and productivities. Noteworthy in this respect is the observation of a  $P_{max}$  that is 2.5-fold greater in the Chl *b*-less mutant than in the wild type (Fig. 5, TBP), suggesting that mutants with a truncated Chl antenna size are good candidates for mass culture of algae in which a high photosynthetic efficiency and productivity is required (Melis et al. 1999). This notion is also consistent with recent results with pigment (phycocyanin)-deficient cyanobacteria, which exhibited higher photosynthetic productivities when compared to those of the normally pigmented wild type (Nakajima and Ueda 1997; Nakajima and Ueda 1999).

*In summary*, direct experimental evidence is provided in this work to show that transformation of green algae can be used as a tool by which to generate mutants exhibiting a permanently truncated Chl antenna size and a higher (per Chl) photosynthetic productivity.

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## References

- Allen KD, Staehelin LA (1994) Polypeptide composition, assembly and phosphorylation patterns of the photosystem II antenna system of *Chlamydomonas reinhardtii*. *Planta* 194: 42–54
- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24: 1–15
- Bassi R, Wollman F-A (1991) The chlorophyll-*a/b* proteins of photosystem II in *Chlamydomonas reinhardtii*. *Planta* 183: 423–433
- Bassi R, Soen SY, Frank G, Zuber H, Rochaix J-D (1992) Characterization of chlorophyll-*a/b* proteins of photosystem-I from *Chlamydomonas-reinhardtii*. *J Biol Chem* 267: 25714–25721
- Bjorkman O, Demmig B (1987) Photon yield of  $O_2$  evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* 170: 489–504
- Butler WL, Kitajima M (1975) Fluorescence quenching in photosystem II of chloroplasts. *Biochim Biophys Acta* 376: 116–125
- Chunaev AS, Mirnaya ON, Maslov VG, Boschetti A (1991) Chlorophyll *b*- and lorenzoanthin-deficient mutants of *Chlamydomonas reinhardtii*. *Photosynthetica* 25(2): 291–301
- Di Paolo ML, Dal Belin Peruffo A, Bassi R (1990) Immunological studies on chlorophyll-*a/b* proteins and their distribution in thylakoid membrane domains. *Planta* 181: 275–286
- Endo T, Asada K (1996) Dark induction of the non-photochemical quenching of chlorophyll fluorescence by acetate in *Chlamydomonas reinhardtii*. *Plant Cell Physiol* 37: 551–555
- Falbel TG, Staehelin LA (1996) Partial blocks in the early steps of the chlorophyll synthesis pathway: a common feature of chlorophyll *b*-deficient mutants. *Physiol Plant* 97: 311–320
- Ghirardi ML, McCauley SW, Melis A (1986) Photochemical apparatus organization in the thylakoid membrane of *Hordeum vulgare* wild type and chlorophyll *b*-less *chlorina-f2* mutant. *Biochim Biophys Acta* 851: 331–339
- Glick RE, Melis A (1988) Minimum photosynthetic unit size in System-I and System-II of barley chloroplasts. *Biochim Biophys Acta* 934: 151–155
- Gorman DS, Levine RP (1965) Cytochrome *f* and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci USA* 54: 1665–1669
- Harrison MA, Melis A (1992) Organization and stability of polypeptides associated with the chlorophyll *a-b* light-harvesting complex of photosystem-II. *Plant Cell Physiol* 33: 627–637
- Harrison MA, Nemson JA, Melis A (1993) Assembly and composition of the chlorophyll *a-b* light-harvesting complex of barley (*Hordeum vulgare* L.): immunochemical analysis of chlorophyll *b*-less and chlorophyll *b*-deficient mutants. *Photosynth Res* 38: 141–151
- Jansson S (1994) The light-harvesting chlorophyll *a/b*-binding proteins. *Biochim Biophys Acta* 1184: 1–19
- Kindle KL (1987) Expression of a gene for a light-harvesting chlorophyll *a/b*-binding protein in *Chlamydomonas reinhardtii*: effect of light and acetate. *Plant Mol Biol* 9: 547–563
- Krol M, Spangfort MD, Huner NPA, Oquist G, Gustafsson P, Jansson S (1995) Chlorophyll *a/b* binding proteins, pigment conversions, and early light-induced proteins in a chlorophyll *b*-less barley mutant. *Plant Physiol* 107: 873–883
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685
- Lavergne J, Briantais J-M (1996) Photosystem II heterogeneity. In: Ort DR, Yocum F (eds) *Advances in photosynthesis, vol 4. Oxygenic photosynthesis: the light reactions*. Kluwer, Dordrecht, The Netherlands, pp 265–287
- Manodori A, Alhadeff M, Glazer AN, Melis A (1984) Photochemical apparatus organization in *Synechococcus* 6301 (*Anacystis nidulans*). Effect of phycobilisome mutation. *Arch Microbiol* 139: 117–123
- Melis A (1989) Spectroscopic methods in photosynthesis: photosystem stoichiometry and chlorophyll antenna size. *Philos Trans R Soc Lond B* 323: 397–409
- Melis A (1991) Dynamics of photosynthetic membrane composition and function. *Biochim Biophys Acta* 1058: 87–106
- Melis A (1996) Excitation energy transfer: Functional and dynamic aspects of *Lhc (cab)* proteins. In: Ort DR, Yocum CF (eds) *Advances in photosynthesis, vol 4. Oxygenic photosynthesis: the light reactions*. Kluwer, Dordrecht, The Netherlands, pp 523–538.
- Melis A, Anderson JM (1983) Structural and functional organization of the photosystems in spinach chloroplasts: antenna size, relative electron transport capacity, and chlorophyll composition. *Biochim Biophys Acta* 724: 473–484
- Melis A, Brown JS (1980) Stoichiometry of system I and system II reaction centers and of plastoquinone in different photosynthetic membranes. *Proc Natl Acad Sci USA* 77: 4712–4716
- Melis A, Duysens LNM (1979) Biphasic energy conversion kinetics and absorbance difference spectra of PS II of chloroplasts. Evidence for two different PS II reaction centers. *Photochem Photobiol* 29: 373–382
- Melis A, Hart RW (1980) A laboratory-constructed sensitive difference spectrophotometer for the ultraviolet, visible and far-red region of the spectrum. *Carnegie Yearb* 79: 170–172
- Melis A, Spangfort M, Andersson B (1987) Light-absorption and electron-transport balance between photosystem II and photosystem I in spinach chloroplasts. *Photochem Photobiol* 45: 129–136
- Melis A, Neidhardt J, Benemann JR (1999) *Dunaliella salina* (Chlorophyta) with small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon use efficiencies than normally pigmented cells. *J Appl Phycol* 10: 515–525

- Michel H-P, Tellenbach M, Boschetti A (1983) A chlorophyll *b*-less mutant of *Chlamydomonas reinhardtii* lacking in the light-harvesting chlorophyll *a/b* protein complex but not in its apoproteins. *Biochim Biophys Acta* 725: 417–424
- Murray DL, Kohorn BD (1991) Chloroplasts of *Arabidopsis thaliana* homozygous for the *chl-1* locus lack chlorophyll *b*, lack stable LHCPII and have stacked thylakoids. *Plant Mol Biol* 16: 71–79
- Nakajima Y, Ueda R (1997) Improvement of photosynthesis in dense microalgal suspension by reduction of light harvesting pigments. *J Appl Phycol* 9: 503–510
- Nakajima Y, Ueda R (1999) Improvement of microalgal photosynthetic productivity by reducing the content of light harvesting pigment. *J Appl Phycol* 11: 195–201
- Neale PJ, Cullen JJ, Lesser MP, Melis A (1993) Physiological bases for detecting and predicting photoinhibition of aquatic photosynthesis by PAR and UV radiation. In: Yamamoto HY, Smith CM (eds) *Photosynthetic responses to the environment. Current topics in plant physiology*, vol 8. American Society of Plant Physiologists Publication Series, pp 61–77
- Neidhardt J, Benemann JR, Zhang L, Melis A (1998) Photosystem-II repair and chloroplast recovery from irradiance stress: relationship between chronic photoinhibition, light-harvesting chlorophyll antenna size and photosynthetic productivity in *Dunaliella salina* (green algae). *Photosynth Res* 56: 175–184
- Picaud A, Dubertret G (1986) Pigment protein complexes and functional properties of tetratype resulting from crosses between CP1 and CP2 less *Chlamydomonas* mutants. *Photosynth Res* 7: 221–236
- Plumley FG, Schmidt GW (1995) Light-harvesting chlorophyll *a/b* complexes: interdependent pigment synthesis and protein assembly. *Plant Cell* 7: 689–704
- Powles SB, Critchley C (1980) Effect of light intensity during growth on photoinhibition of intact attached bean leaflets. *Plant Physiol* 65: 1181–1187
- Smith BM, Morrissey PJ, Guenther JE, Nemson JA, Harrison MA, Allen JF, Melis A (1990) Response of the photosynthetic apparatus in *Dunaliella salina* (Green Algae) to irradiance stress. *Plant Physiol* 93: 1433–1440
- Somanchi A, Handley ER, Moroney JV (1998) Effect of low CO<sub>2</sub> growth on *psaE* and *des6* cDNAs in *Chlamydomonas reinhardtii*. In: Garab G (ed) *Photosynthesis: mechanisms and effects*, vol 5. Kluwer, Netherlands, pp 3487–3490
- Tanaka A, Ito H, Tanaka R, Tanaka N, Yoshida K, Okada K (1998) Chlorophyll *a* oxygenase (CAO) is involved in chlorophyll *b* formation from chlorophyll *a*. *Proc Natl Acad Sci USA* 95: 12719–12723
- Terao T, Katoh S (1989) Synthesis and breakdown of the *a* proteins of light-harvesting chlorophyll *a/b* proteins in chlorophyll *b*-deficient mutants of rice. *Plant Cell Physiol* 30: 571–580
- Terao T, Katoh S (1996) Antenna sizes of photosystem I and photosystem II in chlorophyll *b*-deficient mutants of rice. Evidence for an antenna function of photosystem II reaction centers that are inactive in electron transport. *Plant Cell Physiol* 37: 307–312
- Thornber JP, Highkin HR (1974) Composition of the photosynthetic apparatus of normal barley leaves and a mutant lacking chlorophyll *b*. *Eur J Biochem* 41: 109–116