# Limited photosynthetic electron flow but no $CO_2$ fixation in *Chlamydomonas* mutants lacking photosystem I

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**Abstract By measuring $O_2$ and $CO_2$ exchange in mutants of the green alga *Chlamydomonas reinhardtii* in which genes encoding the reaction center of photosystem I (*psaA* or *psaB*) have been deleted, we found that a photosystem II-dependent electron flow using $O_2$ as the final acceptor can be sustained in the light. However, in contrast with recent reports using other *Chlamydomonas* mutants (B4 and F8), we show here that $CO_2$ fixation does not occur in the absence of photosystem I. By deleting the *psaA* gene in both B4 and F8 strains, we conclude that the ability of these mutants to fix $CO_2$ in the light is due to the presence of residual amounts of photosystem I.**



*Key words: Chlamydomonas*; Photosystem I; Photosystem II; Gene deletion; $O_2$ exchange; $CO_2$ exchange

## 1. Introduction

Oxygenic photosynthesis is thought to require the cooperation of two photosystems, photosystem II (PSII) carrying out photolysis of water and supplying electrons to photosystem I (PSI), which in turn reduces ferredoxin. However, some authors have suggested that PSII alone could carry out reduction of ferredoxin and/or $NADP^+$ [1–3], and it has been recently reported that *Chlamydomonas reinhardtii* mutants deficient in PSI could achieve $CO_2$ fixation and photoautotrophic growth [4,5]. In order to obtain conclusive evidence concerning the ability of PSII alone to drive photosynthetic electron transport, an absolute prerequisite is to ensure that no PSI activity is present in the system used. PSII-enriched preparations contain residual amounts of PSI [1] and photosynthetic mutations can exhibit various levels of expressivity. In view of the contradictory results published in the literature on this subject [1–5], we decided to investigate this question with *Chlamydomonas* mutants deleted in the *psaA* or *psaB* genes [6], whose products make up an essential part of the PSI reaction center and, together with the PsaC protein, bind all of its electron transfer cofactors [7,8]. The use of deletions in structural genes avoids the pitfalls of reversion or variable expressivity, which are often encountered with mutations in genes that govern the expression of structural genes.

## 2. Materials and methods

### 2.1. Algal strains and growth conditions

The *psaA* and *psaB* deletion mutants (referred to as *psaA*Δ and *psaB*Δ) were obtained by plastid transformation of the 137c [9] wild type strain (WT) as described [6]. F8 and B4 strains were obtained from E. Greenbaum and L. Mets, respectively. Deletion of *psaA* (third exon) and *psaC* in these strains was performed with the same protocol [6]. Algae were grown heterotrophically at 23°C in liquid Tris-acetate-phosphate (TAP) medium [9] in low light fluences for all manipulations. Spot tests were initiated by placing 12 μl of each log phase culture onto agar plates of either TAP or high salt minimal (HSM) medium [9]. The plates were incubated in low (0.5 μmol photons $m^{-2}$ $s^{-1}$), medium (50 μmol photons $m^{-2}$ $s^{-1}$), or high (500 μmol photons $m^{-2}$ $s^{-1}$) light fluences either aerobically or anaerobically for 4 days. Anaerobiosis was imposed using the BioMérieux (Marcy-l'Etoile, France) 'Generbag Anaer System' according to the manufacturer's instructions.

### 2.2. Gas exchange measurements

Algal cultures were harvested by low speed centrifugation, washed, and resuspended in minimal medium [9]. 1.5 ml of the suspension was placed in the measuring chamber of a mass spectrometer. $O_2$ and $CO_2$ exchange of the algae were measured by mass spectrometry of gases withdrawn directly from liquid cultures. Dissolved gases were introduced into the ion source of the mass spectrometer (model MM 14-80, VG instruments, Cheshire, UK) through a teflon membrane [10]. For $O_2$ exchange measurements, the sample was sparged with $N_2$ to remove $^{16}O_2$, and $^{18}O_2$ (95% $^{18}O$ isotope content; Euriso-Top; France) was then introduced to reach an $O_2$ concentration close to the equilibrium with air. The use of $^{18}O$-enriched $O_2$ allowed the in vivo determination of $O_2$ evolution by PSII (originating from the photolysis of water, which is not enriched) in the presence of $O_2$-consuming processes such as mitorespiration, chlororespiration or photorespiration [11]. $CO_2$ exchange measurements were performed following $NaH^{13}CO_3$ addition (0.3 mM final concentration; 99% $^{13}C$ isotope content; Euriso-Top; France) in the dark before recording $CO_2$ exchange. Light was supplied by a fiber optic illuminator (Schott, Mainz, Germany) and neutral filters were used to vary light intensity. All gas exchange measurements were performed at 25°C.

### 2.3. Immunoblotting analysis

Liquid cultures were harvested and membranes were prepared as described [12]. SDS-polyacrylamide gel electrophoresis and immunoblotting were performed according to standard procedures using a polyclonal antibody raised against the N-terminus of PsaA.

## 3. Results

### 3.1. $O_2$ and $CO_2$ exchange

A continuous light-dependent production of $O_2$ was measured in the *psaA*Δ and *psaB*Δ mutants (Fig. 1A,B), as had been previously observed in another PSI-deficient mutant [2]. At very low light fluences photosynthetic $O_2$ evolution was comparable to WT, but it quickly saturated with increasing light (Fig. 1A). The maximum $O_2$ evolution rate was 130 nmol $O_2$ $min^{-1}$ $mg^{-1}$ chlorophyll, a value representing about 20% of the mitochondrial respiration rate and about 4% of the maximal net photosynthesis measured in WT cells. The $O_2$ uptake rate increased in parallel with $O_2$ evolution (Fig. 1B),

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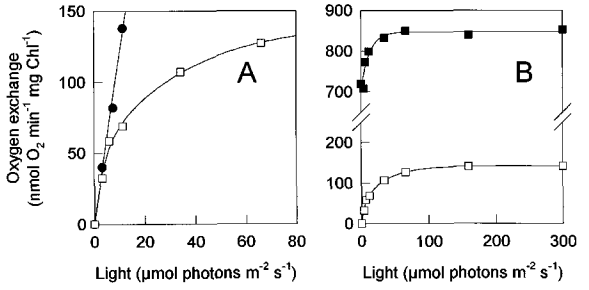


Fig. 1. Effect of photon flux density on $O_2$ exchange rates measured using $^{18}O$-enriched $O_2$ and mass spectrometry in WT (A) and in *psaA*Δ mutant cells (A,B). ●, $O_2$ evolution in the light measured in WT cells; □, $O_2$ evolution in the light measured in the *psaA*Δ mutant; ■, $O_2$ uptake in the light measured in the *psaA*Δ mutant. The same patterns were obtained with the *psaB*Δ, F8-*psaA*Δ, B4 and B4-*psaA*Δ mutants.

suggesting that reducing equivalents generated by PSII (measured as $O_2$ evolution) are used to reduce molecular $O_2$ rather than other final electron acceptors such as $CO_2$. Photosynthetic $CO_2$ uptake was measured by mass spectrometry in the presence of $^{13}C$-enriched $CO_2$, which allows the detection of $CO_2$ fixation rates as low as 1% of the light-saturated $CO_2$ fixation rate in the WT strain (Fig. 2A). However, no light-induced decrease in the $[^{13}CO_2]$ signal was observed in the *psaA*Δ or *psaB*Δ strains, indicating that $CO_2$ fixation did not occur in these mutants (Fig. 2A).

The results shown in Fig. 2 stand in stark contrast to those reported by Greenbaum et al. [4] and Lee et al. [5], in which light-induced $O_2$ evolution and $CO_2$ uptake, as well as photoautotrophic growth, were observed in certain PSI-deficient *Chlamydomonas* strains. The mutants used in those studies, B4 and F8, contain nuclear mutations that block the *trans*-splicing of the *psaA* mRNA [4,13,14], whereas *psaA*Δ and *psaB*Δ contain deletions of the coding sequences of the PSI reaction center proteins (the *psaA*Δ mutation is actually a deletion of the third exon, which encodes the C-terminal 88% of PsaA [6,15]). Two possibilities can be envisaged to explain this discrepancy. First, it is possible that B4 and F8 are able to synthesize small amounts of PSI, which would be difficult to detect by biophysical or biochemical techniques, but sufficient for photosynthetic $CO_2$ fixation. Alternatively, it might be that the genetic background of the WT strain (137c) used for deletion of *psaA* and *psaB* genes is lacking some component(s) required for PSI-independent photosynthesis, which would presumably be present in B4 and F8.

### 3.2. *Photoautotrophic growth and immunodetection of psaA gene products*

In order to distinguish between these hypotheses, we deleted the *psaA* gene from the plastid genome of both B4 and F8 strains, and examined their ability to grow photoautotrophically and fix $CO_2$. While F8 could grow on minimal media, this ability was lost in all of the independent F8-*psaA*Δ transformants (Fig. 3). Likewise, F8 retains some $CO_2$-fixation capacity (about 20% of WT), but the F8-*psaA*Δ strains were unable to take up $CO_2$ (Fig. 2B). Small amounts of PsaA protein (ca. 10% of WT) were immunologically detectable in the F8 strain and disappeared upon deletion of *psaA* (Fig. 3). B4 and B4-*psaA*Δ transformants behaved similarly in that they could neither grow photoautotrophically (Fig. 3) nor fix $CO_2$ (Fig. 2B), nor did they express detectable amounts of PsaA (Fig. 3). In fact, we have never seen light-induced $CO_2$ fixation in any of the *psaA*Δ mutants, even when assayed anaerobically for longer time periods (data not shown).

We performed several control experiments in order to ensure that the lack of photosynthetic growth observed in the gene deletion mutants was due to the loss of PSI rather than secondary effects. Particle bombardment into the chloroplast can also insert DNA into the nucleus, but these insertions are random. As we examined several independent transformants, the loss of photosynthesis is not likely due to secondary nuclear mutations. Moreover, reintroduction of wild-type *psaA* into F8-*psaA*Δ mutants restored photoautotrophic growth (data not shown). Another possibility was that the DNA used to replace *psaA* was somehow affecting the expression of other gene(s) required for PSI-independent photosynthesis. However, we also deleted the gene for PsaC, a small extrinsic protein containing the terminal electron acceptors $F_A$ and $F_B$

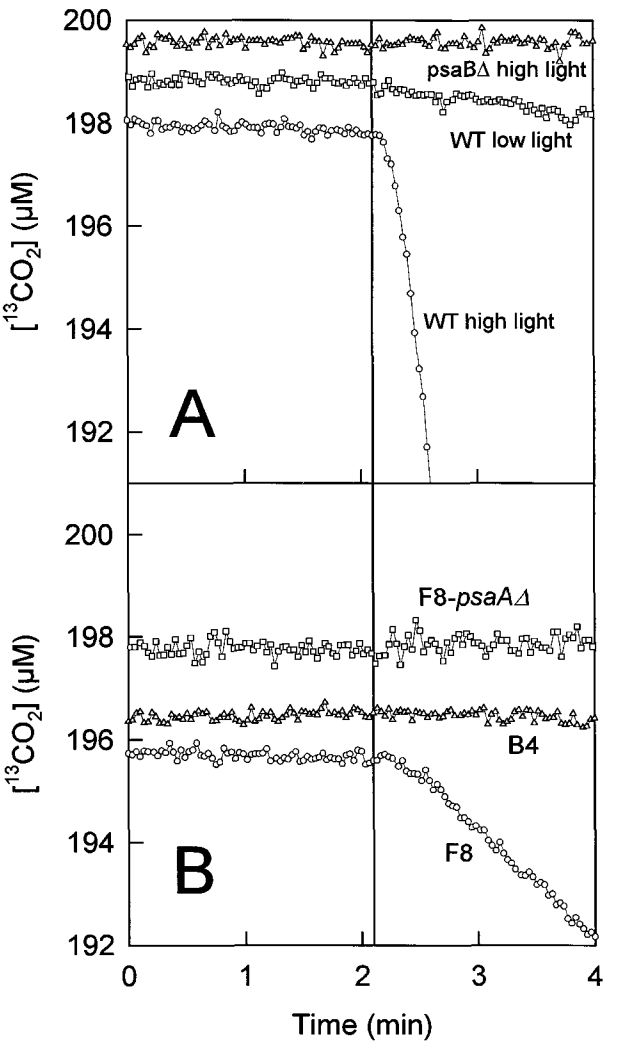


Fig. 2. $CO_2$ exchange during dark/light transitions measured by mass spectrometry using $^{13}C$-enriched $CO_2$ in different *Chlamydomonas* mutants. Light was turned on when indicated by the vertical line, Chl. concentration was 20 μg $ml^{-1}$. A: *psaB*Δ mutant and WT. WT $CO_2$ consumption in low light (3 μmol photons $m^{-2}$ $s^{-1}$) was 1.2% of the consumption rate at saturating light intensity (300 μmol photons $m^{-2}$ $s^{-1}$). Similar results were obtained with the *psaA*Δ strain. B: $CO_2$ exchange during dark/light transitions in the B4, F8, and F8-*psaA*Δ mutants at 300 μmol photons $m^{-2}$ $s^{-1}$.

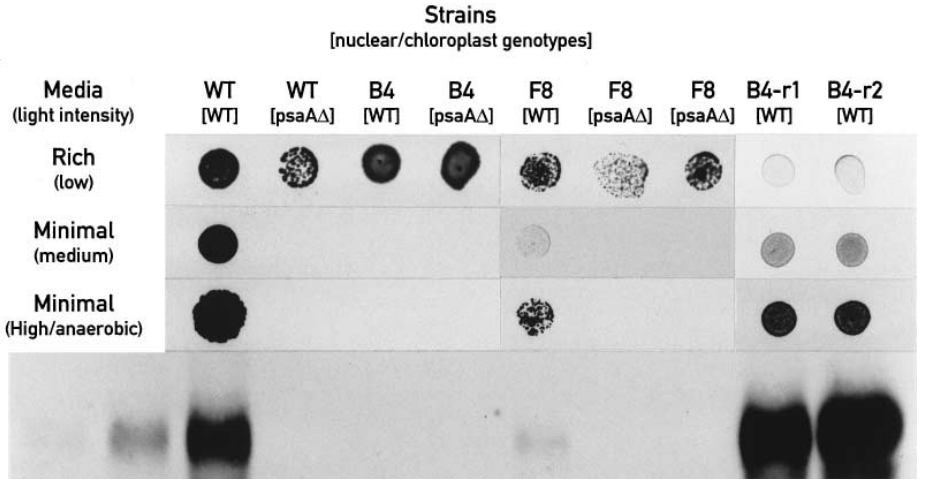


Fig. 3. Photoautotrophic growth and immunoblot analysis of the *psaA* gene product in various *Chlamydomonas* mutant strains. 'Rich' refers to TAP medium, which allows growth of all strains in low light, while minimal medium (HSM) demands photoautotrophic growth. The third row shows spots grown on minimal medium under hight light flux and anaerobic conditions, which relieve the stress caused by illumination of $PSI^-$ strains. Two independent F8-*psaA*Δ transformants are shown. B4-r1 and B4-r2 are revertants of B4; note that the spots of these strains on the rich plates appear light when compared to the others in the row as they were incubated for only two days. The last row is the result of an immunoblot to measure the amount of the PsaA polypeptide present in each strain. Membrane proteins (25 μg per lane) prepared from the cultures were immunoblotted using an anti-PsaA antibody. The leftmost two lanes are serial three-fold dilutions of the WT extract into the *psaA*Δ extract (thus, 11% and 33% of WT) used for quantitation of the amount of detectable PsaA.

[7], both in our wild-type strain [6] and in F8 (data not shown). In both cases, photoautotrophic growth ceases upon loss of *psaC*. The *psaA* (third exon) and *psaC* genes are far from each other in the chloroplast genome [9], and the piece of DNA used to delete *psaC* was different from that used to delete *psaA* and *psaB* [6].

Although we observed a significant light-induced net production of $O_2$ in F8, the F8-*psaA*Δ, B4, and B4-*psaA*Δ strains had the same $O_2$ exchange characteristics as those found for *psaA*Δ in Fig. 1 (data not shown). We were initially puzzled that we could not observe photoautotrophic growth of B4. During several attempts to grow B4 photoautotrophically, we obtained photoautotrophic isolates, all of which had regained the ability to express PsaA (Fig. 3). B4-r1 was isolated from a photoautotrophic culture of B4 provided by Dr. E. Greenbaum. B4-r2 is a photoautotrophic isolate isolated from B4-*psaA*Δ before the deletion had become homoplasmic (subsequent PCR analysis indicated that the *psaA* gene was still present in B4-r2). We have never been able to obtain photoautotrophic revertants of the *psaA*Δ, F8-*psaA*Δ or B4-*psaA*Δ strains, even after plating $>10^9$ cells on HSM in medium light (aerobically or anaerobically) from several independent cultures.

## 4. Discussion

The data described above are consistent with the hypothesis that the photoautotrophic growth and $CO_2$ fixation observed in the B4 and F8 mutants by Greenbaum and colleagues [4,5] required the presence of PSI. We found no evidence to support the hypothesis that differences in photosynthetic ability in the absence of PSI are due to differences in genetic background. The effect of *psaA* inactivation in these strains leads us to conclude that the F8 strain is able to make small amounts of PSI and that the B4 strain is subject to reversion events that restore the ability to synthesize PSI. The small amounts of PSI present in F8, estimated by Western blot analysis to be about 10–15% of the WT, should be sufficient to sustain both linear electron transport activity and photosynthetic $CO_2$ fixation at the observed rate (about 20% of WT). The possibility to sustain oxygenic photosynthesis in the presence of low amounts of PSI might also be explained by the high turnover rate of PSI, as suggested by Boïchenko [16]. At present, we can only speculate as to what occurred in the case of B4. It may be that B4 is able to revert to low levels of PsaA expression, or that infrequent reversion events create mixed populations of cells, some of which can perform photosynthesis. We conclude that photosynthetic $CO_2$ fixation does not occur in *Chlamydomonas* in the absence of PSI.

Interestingly, a substantial electron flow from PSII to $O_2$ can be sustained in the light in the absence of PSI. Electron carriers involved in this pathway remain to be determined. Chlororespiration might be involved [17,18], which would explain the lack of $CO_2$ fixation, since no soluble form of reducing power would be generated by PSII activity. Another possibility, suggested in a previous study [2], is that reducing equivalents produced within chloroplasts are transferred to mitochondria and oxidized by the respiratory chain. Indeed, interactions between chloroplastic and mitochondrial electron transport chains have already been observed in *Chlamydomonas* cells [11,19,20] and also in higher plants [21,22]. Different mechanisms able to generate transportable forms of reducing power by PSII have been proposed, invoking the low-potential pheophytin acceptor of PSII [1,3] or a NAD(P)H-dehydrogenase complex [2,23] functioning in a gradient-dependent reverse mode, as it does in photosynthetic bacteria [24,25]. In the first case, one would expect some $CO_2$ fixation to occur since both reducing power and an electrochemical gradient would be produced by PSII. In the second case, the electrochemical gradient would be used to generate reducing power through an energy-dependent reaction and would not be available for ATP production, thus explaining the absence of $CO_2$ fixation in PSI-deficient mutants. The actual mechanism used by the cell, whether one of these or something as

yet unconsidered, will be an interesting topic for future research.

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