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A single mutation that causes phosphatidylglycerol deficiency impairs synthesis of photosystem II cores in *Chlamydomonas reinhardtii*

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Two mutants of *Chlamydomonas reinhardtii*, *mf1* and *mf2*, characterized by a marked reduction in their phosphatidylglycerol content together with a complete loss in its Δ^3-*trans* hexadecenoic acid-containing form, also lost photosystem II (PSII) activity. Genetic analysis of crosses between *mf2* and wild-type strains shows a strict cosegregation of the PSII and lipid deficiencies, while phenotypic analysis of phototrophic revertant strains suggests that one single nuclear mutation is responsible for the pleiotropic phenotype of the mutants. The nearly complete absence of PSII core is due to a severely decreased synthesis of two subunits, D1 and apoCP47, which is not due to a decrease in translation initiation. Trace amounts of PSII cores that were detected in the mutants did not associate with the light-harvesting chlorophyll *a*/*b*-binding protein antenna (LHCII). We discuss the possible role of phosphatidylglycerol in the coupled process of cotranslational insertion and assembly of PSII core subunits.

Keywords: photosystem II; phosphatidylglycerol; D1 synthesis; *Chlamydomonas*; thylakoid.

The utilization of light energy during photosynthesis to split water molecules and generate reducing species and ATP requires highly organized multimolecular complexes. These complexes contain numerous proteins from chloroplast or nucleo-cytosolic origin and various associated cofactors including chlorophyll, carotenoid pigments and redox components [1]. These large complexes are embedded in a membrane containing a high proportion of glycolipids, including monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) [2]. As reported by Joyard *et al.* [3], thylakoid membranes do not contain phosphatidylcholine, and phosphatidylglycerol (PtdGro or PG) is the only phospholipid that is present in photosynthetic membranes of cyanobacteria and eukaryotes. A particular fatty acid, Δ^3-*trans* hexadecenoic acid; C16:1(3t), esterified in the *sn*-2 position of glycerol, is present among PG found in chloroplast membranes but it is absent from the other eucaryotic cell membranes or from bacterial membranes.

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Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol (PtdGro); C16:1(3t), Δ^3-*trans*-hexadecenoic acid; PSI, photosystem I; PSII, photosystem II; LHC, light-harvesting chlorophyll protein complex; WT, wild-type strain; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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Thylakoid membranes, similarly to mitochondrial inner membranes, are very rich in proteins. The lipid to protein mass ratio is low, especially in appressed regions where PSII is located [4]. As a consequence, a large amount of lipid molecules are directly exposed at the periphery of large protein complexes and the proximal lipidic environment of the protein surface is likely to be involved in the structural and functional organization of integral membrane proteins [5]. This view is consistent with the high degree of lateral and transversal heterogeneity that characterizes the distribution of lipids and their fatty acids along thylakoid membranes [2].

In the eukaryotic alga *Chlamydomonas reinhardtii*, mutants *mf1* and *mf2* were described previously as being deficient in PG with a total loss of its C16:1(3t)-containing species [6]. They display alterations in the organization of their light-harvesting antenna with a spectacular loss in the oligomeric forms of light-harvesting chlorophyll *a*/*b*-binding protein (LHCII), which is responsible for their low yield of fluorescence [6,7]. They are also unable to grow photoautotrophically because they lack PSII activity [7,8]. Two revertant strains, one from *mf1* (*pmf1*) and the other from *mf2* (*pmf2*), selected for the restoration of phototrophic growth, partially recovered a normal PG-C16:1(3t) content [8]. These results suggested that PG-C16:1(3t) may have a specific effect on the expression of PSII-related proteins. However, while the two *mf* mutants grown in the presence of exogenously added PG-C16:1(3t) recover oligomeric LHCII, pointing to a specific role of this lipid in the supramolecular organization of LHCII *in vivo* [9–11], they did not recover any significant PSII activity [8], an observation that questioned the relation between PG-C16:1(3t) deficiency and PSII inactivation.

Here, we developed a more thorough genetic approach to demonstrate the correlation between recovery of PSII activity and restoration of higher levels of C16:1(3t)-containing PG. We show that impaired formation of the PSII core complex in *mf1* and *mf2* results from a considerable decrease in the rate of translation of the D1 and apoCP47 subunits of the PSII core. The marginal amounts of PSII cores produced in these strains can not associate in LHCII–PSII supercomplexes. These results point to a critical function of PG at an early step in the biogenesis of PSII cores.

Materials and methods

Strains, cell growth and genetic analysis

C. reinhardtii mf1 and *mf2* mutant strains were described previously [6,7,10] as well as two phototrophic revertants (*pmf1* and *pmf2*) and were selected, respectively, from *mf1* and *mf2* strains [8]. Fl39 is a nuclear mutant completely deficient in PSII activity [12]. The FUD7 strain bears a deletion of the chloroplast *psbA* gene encoding the D1 subunit of the PSII reaction center [13]. The wild-type strain (WT) used in this work was derived from the 137c strain [14]. Cells were grown at 25 °C in tris/acetate/phosphate medium at 7–10 μmol photons·m^{-2}·s^{-1} cool fluorescent light or in minimum medium at 40–60 μmol photons·m^{-2}·s^{-1} cool fluorescent light [14]. Induction of gametes, crosses and tetrad analysis were performed as described previously [14]. Fluorescence induction kinetics of dark-adapted cells were recorded on cells grown in solid or liquid tris/acetate/phosphate medium as described previously [15].

Construction of the 5′ *psbA–petA* strain

A DNA fragment containing the promoter, 5′ UTR and the first 30 codons of the *psbA* gene was amplified by PCR using oligonucleotide primers Aprom (forward): 5′-CGC ATC GAT GGA TCC TGC CAC TGA CGT CCT ATT TTA ATA CTC C-3′, and Acod (reverse): 5′-CGC GGA TCC ATG GAA TCG ATG TAT AAA CGG TTT TCA GTT GAA GT-3′, and the *Eco*RI restriction fragment of the chloroplast genome R14 [16] as a template. The resulting DNA fragment was then digested by *Cla*I and *Nco*I (two restriction sites generated by the oligonucleotides used), and cloned into vector B_TFFF [17] digested with the same enzymes to form plasmid p(bAC)FFF. The *aadA* cassette conferring spectinomycin resistance [18] excised with *Eco*RV and *Sma*I was then cloned in reverse orientation with respect to the *petA* coding region in p(bAC)FFF linearized with *Hin*cII to yield plasmid pihK(bAC)FFF. This plasmid was used for transformation of a WT, *mt+* strain according to Boynton *et al.* [19]. Transformants in which the endogenous *petA* gene was replaced by homologous recombination by the 5′*psbA–petA* chimera were selected for spectinomycin resistance, brought to homoplasmy and assessed for the presence of the chimeric 5′ *psbA–petA* gene in the chloroplast genome.

Pulse-labelling experiments

Pulse-labelling experiments were carried out according to Kuras and Wollman [20]. In the experiments presented in Fig. 5B, cells equivalent to 1 mg of chlorophyll were washed in 50 mM Tris/HCl pH 7.8, 10 mM NaCl, 1 mM EDTA, resuspended in the same medium containing 0.5 μM of the protease inhibitor Pefabloc (Fluka, Switzerland) and briefly disrupted by sonication. Cell extracts were centrifuged at 600 ***g*** for 2 min, the white-grey pellet discarded and the supernatant centrifuged again at 36 000 ***g*** for 45 min to sediment all the green material.

Cell fractionation

Cell membranes, highly enriched in thylakoids, were prepared from French press-disrupted cells following the method of Chua and Bennoun [21] including differential centrifugation of cell extracts and flotation in sucrose layer gradients. Minor changes were introduced in the molarity of sucrose layers to take into account the lower density of the thylakoid membranes of *mf1* and *mf2*, and thus 1.15 M instead of 1.3 M sucrose was used in the layer above the 1.8 M sucrose-containing initial membrane material. For blue-native PAGE experiments, cell samples equivalent to 1 mg of chlorophyll were washed in 10 mM Hepes pH 7.6, 2 mM EDTA, resuspended in 2 mL of 5 mM Hepes, 1 mM EDTA, 0.5 μM Pefabloc, and briefly disrupted by sonication. Cell extracts were centrifuged at 700 ***g*** for 2 min, the white-grey pellet discarded and the supernatant centrifuged again at 36 000 ***g*** for 25 min to sediment all the green material. The pellet was further resuspended in 0.7 mL of 5 mM Hepes, 1 mM EDTA, and loaded on two layers consisting of 1.4 mL of 1.1 M and 1.2 mL of 1.5 M sucrose in 5 mM Hepes, 1 mM EDTA, then centrifuged in a Beckmann SW60 rotor at 270 000 ***g*** for 20 min. Thylakoid membranes were harvested at the interface of the two sucrose layers.

Blue-native PAGE

Purified thylakoid membranes were prepared from cells disrupted with ultrasound and diluted in 50 mM Bistris, 0.75 M amino-*n*-caproic acid, 0.5 mM Na_2EDTA, 20% (v/v) glycerol, pH 7, to a final chlorophyll concentration of 0.3 mg·mL^{-1}. Membrane suspensions were solubilized with dodecylmaltoside (1%, w/v) at 4 °C for 20 min and centrifuged at 36 000 ***g*** for 4 min. The supernatants were supplemented with Serva blue G-250 (final concentration 0.25%, w/v) prior to loading on the gel. Blue-native electrophoreses were performed according to the general method of Schägger *et al.* [22] with minor modifications. The separating gel consisted of a 4–13% (w/v) acrylamide gradient whereas the stacking gel was 4% (w/v) acrylamide. Final concentrations of Bistris and amino-*n*-caproic acid in gel buffer were 25 mM and 250 mM, respectively. Cathodic buffer (50 mM tricine, 15 mM Bistris) was supplemented with 0.012% (w/v) Serva blue G-250. Electrophoresis (using glass plates of 10 × 12 cm) was run overnight (4 °C, 110 V) with replacement of the blue cathodic buffer by a new colourless buffer for two additional hours. Fragments of green bands or full-length thin strips were excised from the gel, briefly rinsed with water and frozen when not used immediately. Before the second dimension denaturing electrophoresis, strips were incubated for 30 min in 125 mM Tris pH 6.8, 50 mM

dithiothreitol, 20% (v/v) glycerol, 4% (w/v) SDS, heated at 70 °C for 2 min and further analysed on 9–18% (w/v) acrylamide gradient gels.

Denaturing gel electrophoresis

Electrophoresis in the presence of SDS was performed using 9–18% (w/v) acrylamide gradient gels as reported previously [23]. Polyacrylamide gels (12–18%, w/v) containing 8 M urea were performed according to de Vitry *et al.* [24].

TMBZ staining of electrophoresis gels

After separation of whole cell proteins on denaturing 12–18% (w/v) polyacrylamide/urea gels, covalently bound cytochromes were stained with 3,3′,5,5′-tetramethylbenzidine (TMBZ) according to Thomas *et al.* [25].

Lipid analysis

Aliquots of cells (150 μg chlorophyll) were harvested by centrifugation, fixed in boiling ethanol for 5 min and lipids were extracted with chloroform according to Bligh and Dyer [26]. Individual lipids were separated by thin layer chromatography on silica gel 60 plates using the solvent system chloroform/acetone/methanol/acetic acid/water (50/20/10/10/5, v/v/v/v/v) and lipid spots detected with iodine vapour [6]. Fatty acid methyl esters were prepared by transesterification in methanol/BF_3, recovered with *n*-pentane, dissolved in methanol and analysed by capillary gas-liquid chromatography using a 50 m long, 0.25 mm diameter CP-wax 52 column. Heptanoic acid was used as an internal standard.

Results

The absence of functional PSII and lack of PG-C16:1(3t) result from a single nuclear mutation

To assess the relation between PG-C16:1(3t) deficiency and PSII inactivation, we undertook a genetic analysis of the *mf1* and *mf2* strains. First, we analysed segregation of the two phenotypes on colonies arising from the four products of meiosis (tetrads) from zygotes obtained in *mf2* × WT crosses. All 24 tetrads presented a 2 : 2 Mendelian segregation for PSII deficiency, as characterized by their fluorescent induction kinetics of dark-adapted cells. Two colonies had a wild-type phenotype [$PSII^+$] with several phases of fluorescence rise and decay that led to a steady-state level well below the F_{max} level reached upon inhibition of photosynthetic electron flow in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Fig. 1). The two others displayed a DCMU-insensitive steady state fluorescence yield from the onset of illumination typical of a PSII-deficient phenotype [$PSII^-$]. This showed that the PSII deficiency in the *mf2* strain most probably resulted from a single nuclear mutation. The progeny from five of these tetrads was used for lipid analysis. The content in PG-C16:1(3t) together with the content in total PG (relative to chlorophyll) displayed a 2 : 2 segregation in each tetrad, however the mean values for total PG content indicated the contribution of a

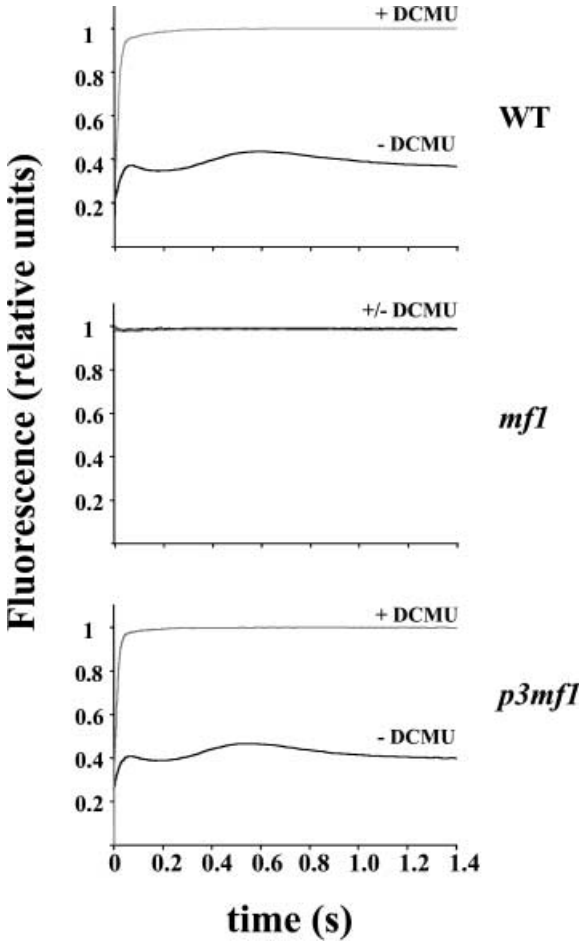

Fig. 1. Fluorescence induction kinetics of dark-adapted cells of the WT, *mf1* and revertant *p3mf1* strains. Cells were grown on liquid tris/acetate/phosphate medium. The F_o level of the fluorescence response was not calibrated. Kinetics were recorded with dark-adapted cells that were either treated or untreated with 10 μM DCMU (that inhibits electron flow from PSII) and thus in treated cells the F_{max} level is reached. Fluorescence induction kinetics from *mf2* and [$PSII^-$] members of tetrads from the cross WT × *mf2* were identical to that of the *mf1* strain.

Table 1. PG and C16:1(3t) contents in tetrads from the cross *mf2* x WT. Content of PG is expressed in μg of PG total fatty acids per mg chlorophyll and C16:1(3t) content is expressed in percent of PG total fatty acids. Data of one tetrad and the mean values from the [$PSII^-$] and [$PSII^+$] clones of the five tetrads analyzed are displayed with SD.

Clone from tetrad	PG	C16:1(3t)
One tetrad		
1 [$PSII^-$]	51.4	0.6
2 [$PSII^-$]	83.8	0.3
Mean of five tetrads	60 ± 16.2	< 0.6
One tetrad		
3 [$PSII^+$]	130.3	11.6
4 [$PSII^+$]	123.3	23.2
Mean of five tetrads	127.6 ± 51.6	14.8 ± 5

Table 2. PG and C16:1(3t) contents in WT, *mf* mutants and *pmf* revertant strains. Content of PG is expressed in μg of PG total fatty acids per mg chlorophyll and C16:1(3t) content is expressed in percent of PG total fatty acids. For WT, *mf1*, *p3mf1*, *mf2* and *pmf2* cells grown in TAP medium, data are the mean of two to four independent cultures and SD are indicated; for the other strains only one determination was made. Strains *mf1* and *mf2* are unable to grow in minimum medium. ND, not determined.

	Strain									
Lipid	WT	*mf1*	*p1mf1*	*p2mf1*	*p3mf1*	*p4mf1*	*p5mf1*	*p6mf1*	*mf2*	*pmf2*
TAP medium										
PG	103.4 ± 7.9	49 ± 18.5	55	64	64.6 ± 16.7	85.3	ND	ND	34.8 ± 5.1	63.3 ± 3.1
C16:1(3t)	19.6 ± 3.2	< 0.66	7.3	6	8.6 ± 5.8	4.7	ND	ND	< 0.8	15.8 ± 3.1
Minimum medium										
PG	154.7	–	80	98	80.7	94	108	64.7	–	ND
C16:1(3t)	26.7	–	10	8.2	11.5	8.5	7.4	7.3	–	ND

complex genetic context. Because, in each tetrad, all [PSII⁻] progeny (and only these) were severely deficient in PG-C16:1(3t) and partially deficient in PG (Table 1), this demonstrated the cosegregation previously observed from 32 [PSII⁻] clones from a random progeny [8]. Thus, lipid and PSII alterations result from a single or two tightly linked mutation(s). The latter hypothesis is rather unlikely as phototrophic revertant strain (*pmf2*) derived from *mf2*, showed a joint reversion of PG-C16:1(3t) deficiency [8]. For strain *mf1*, we could not use a similar strategy based on tetrad analysis, as this strain is completely sterile. This prompted us to analyse more revertant strains selected from *mf1* (six revertants named *p1mf1* to *p6mf1*) on the basis of photosynthetic growth (hence PSII activity, as deduced from their fluorescence induction kinetics; Fig. 1). They all showed partial restoration of their C16:1(3t) and total PG content whether they were grown in mixotrophic or phototrophic conditions (Table 2). This demonstrated in a statistically valuable way the conclusion previously drawn from only one *pmf1* revertant strain [8]. Thus, a single nuclear mutation is responsible for both the lipid and PSII defects in the two strains *mf1* and *mf2*. The sterility of the *mf1* strain prevented us from determining whether these two mutations were allelic or not.

Loss of PSII core complexes in the *mf1* and *mf2* mutants

We then investigated the molecular basis for PSII deficiency, by looking at the polypeptide pattern in thylakoid preparations from the two *mf* mutants. The apoCP47 and apoCP43 polypeptides that form the core antenna of PSII were highly deficient as compared to the WT, whereas they were partially restored in the *pmf* revertant strains (Fig. 2). As observed in most PSII mutants identified so far in *C. reinhardtii* [1], the strong decrease of apoCP47 and apoCP43 was accompanied by a similar decrease in polypeptides D1 and D2 (data not shown).

It should be noted that polypeptides from the LHCII antenna (P11, P16 and P17) or from the minor antenna complexes (CP26 and CP29 [27]) accumulated significantly in the two mutants *mf1* and *mf2*, although some limited change in their amount was observed. With the use of a polyclonal antiserum raised against LHCII from maize, we detected the presence of P11, P16 and P17 in *mf1* and *mf2* thylakoids (data not shown), together with four to five

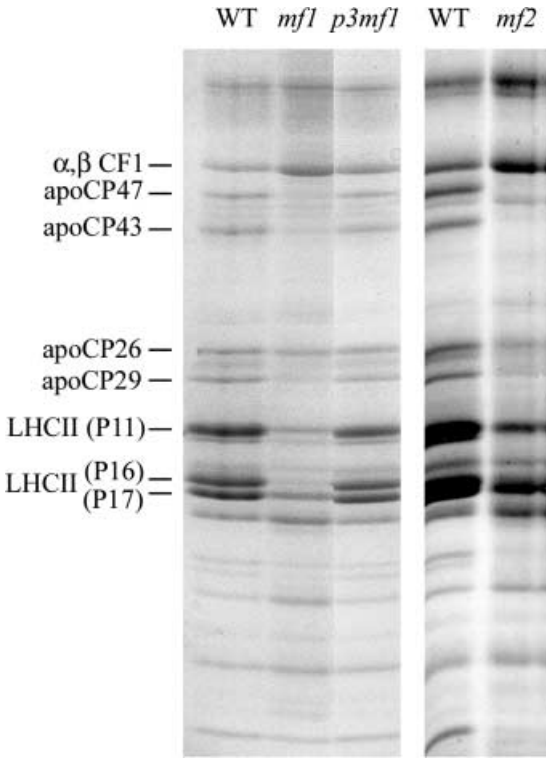

Fig. 2. Thylakoid membrane polypeptides from WT, *mf1*, *mf2* and revertant *p3mf1* strains after electrophoresis on 9–18% (w/v) SDS/PAGE with Coomassie blue staining. The three tracks on the left were loaded with a thylakoid suspension equivalent to 7 μg chlorophyll, and the two on the right with a thylakoid suspension equivalent to 10 μg chlorophyll. Other revertant *pmf* strains display similar patterns to that of *p3mf1*.

immunoreactive polypeptides of lower molecular mass that were absent from WT membranes and presumably resulted from the degradation of LHCII polypeptides [11]. The position of CP29 and CP26 in our gel system was identified by mass spectroscopy analysis of the individual bands excised from the gels (data not shown).

These data unveil a marked deficiency in PSII core complexes in *mf* mutants whereas the peripheral PSII antenna was not altered to the same extent.

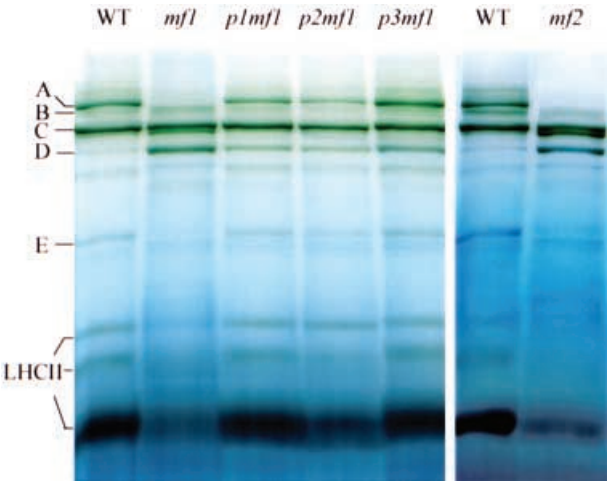

Fig. 3. Blue-native gel electrophoresis analysis of the pigment–protein complexes in thylakoids from WT, *mf1*, *mf2* and three revertant *pmf1* strains. Samples equivalent to 16 μg chlorophyll were loaded in each track. The upper part of the gel resolves PSII oligomers (band A), core complex monomers (band E) and PSI oligomers (bands B–D). The lower part of the gel resolves LHCII proteins.

Organization of the residual amount of PSII in *mf* mutants

To test whether the minor amounts of PSII core subunits still accumulating in *mf* mutants could be found in PSII supramolecular assemblies, we used blue-native gel electrophoresis. Dodecylmaltoside-solubilized thylakoids of the WT strain displayed several green bands (Fig. 3; A–E) migrating above those containing free antennae. The free antenna was recovered in three bands obviously reduced in *mf1* and *mf2* but largely restored in all revertant *pmf1* strains (Fig. 3). From their polypeptide composition, showing the presence of apoCPI and LHCI (Fig. 4), the three green bands B, C and D can be assigned to PSI–LHCI complexes. They were present in all strains (Fig. 3), although their relative ratios were altered in strains *mf1* and *mf2*, exhibiting an increase in band D and splitting of band C. All of these bands correspond to various forms of PSI supercomplexes (Fig. 4). Thus, the lack of PG-C16:1(3t) modifies the state of oligomerization of PSI–LHCI supercomplexes in *C. reinhardtii* [7,9] as in *Arabidopis thaliana* [28]. Band A corresponds to dimeric PSII core complexes with their associated antenna [29] and contains the core complex polypeptides apoCP47, apoCP43, the minor antenna complex CP26, CP29 and the LHCII antenna polypeptides (Fig. 4). Band A was completely missing in *mf1* and *mf2* membranes but partially restored in thylakoids from all *pmf1* strains tested (Fig. 3). In the WT strain, small amounts of PSII core subunits were visible in band E migrating just above the cytochrome b_6–f complex, which represents monomeric PSII without antenna polypeptide (Fig. 4). Band E was clearly seen in the green pattern from *pmf1* strains (Fig. 3). Inspection of two-dimensional gels reveals that traces of apoCP47 and apoCP43 were also present but restricted to band E in the two mutants *mf1* (data not shown) and *mf2* (Fig. 4) and were not associated with any LHCII antenna polypeptides.

We conclude that the small amount of PSII core complexes that accumulate in these mutants cannot form stable supercomplexes with the peripheral antenna or that these supercomplexes did not resist electrophoretic separation. The absence of such associations is, however, consistent with the previous data obtained from fluorescence spectra giving evidence for connection of LHCII to PSI in *mf1* and *mf2* [6,7].

The lack of PSII cores in *mf1* and *mf2* is due to a decreased synthesis of two PSII core subunits

The strongly reduced accumulation of PSII could result from a defect in the synthesis of any of the major PSII core subunits or from a post-translational degradation process. To address that point, cells of two progeny from the *mf2* × WT cross (a [PSII$^-$] and a [PSII$^+$] member from the same tetrad) were pulse labelled with [^{14}C]acetate for 5 min in the presence of cycloheximide. As controls, we also labelled FUD7 cells, deleted for the *psbA* gene encoding D1

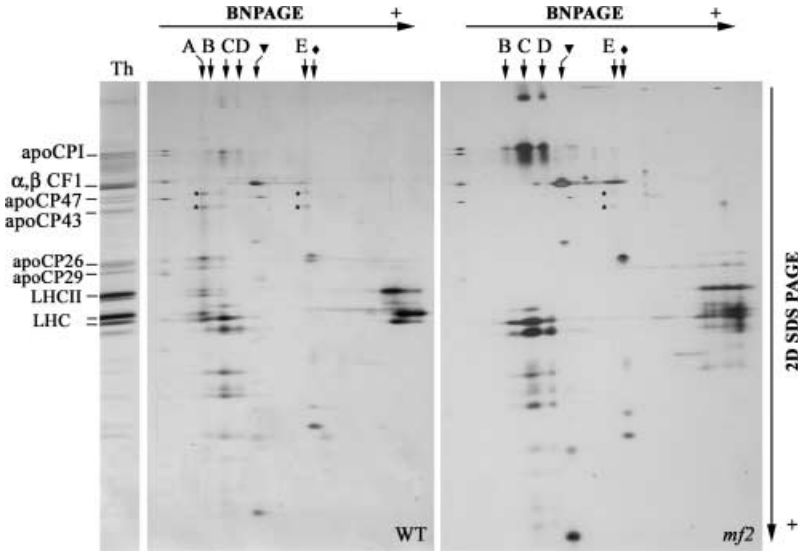

Fig. 4. Two-dimensional separation of polypeptides from WT and *mf2* chlorophyll-binding complexes, resolved by blue-native gel electrophoresis in the first dimension. After electrophoresis, the gels were silver stained. A–E represent the positions of green bands (as shown in Fig. 3); stars designate the positions of apoCP43 and apoCP47 belonging to the core complex of PSII. Note that a PSII band whose composition is close to that of band A (PSII–LHCII) is visible just next to band C (PSI–LHCI) in the WT profile. ▼, subunits of the CF0–CF1 ATPsynthetase; ◆ position of cytochrome b_6–f complexes.

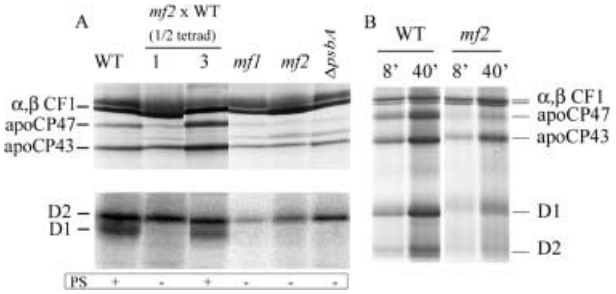

Fig. 5. Protein pulse labelling experiments in the *mf1* and *mf2* strains. (A) Short pulse labelling (for 5 min with 5 μCi·mL^{-1} [^{14}C]acetate) of PSII core subunits in a half-tetrad (one [PSII$^-$] and one [PSII$^+$] progeny) from the cross *mf2* × WT as well as in *mf1*, *mf2*, WT and FUD7 cells. Labelled polypeptides were separated by electrophoresis on 9–18% (w/v) SDS/PAGE (for a better resolution of apoCP47 and apoCP43; upper panel) or 12–18% (w/v) polyacrylamide/urea gels (for a high resolution of D2 and also D1; lower panel). PS+, [PSII$^+$] strains (WT nuclear background); PS–, [PSII$^-$] strains (*mf2* nuclear background). (B) Comparison of short and longer pulse-labelling experiments performed on the WT and *mf2* strains under 60 μmol photons·m^{-2}·s^{-1}. Samples were harvested from a single culture, 8 and 40 min after addition of 1.2 μCi·mL^{-1} [^{14}C]acetate and analysed by electrophoresis on 9–18% (w/v) SDS/PAGE. Note the clear identification of D1 labelling after 40 min pulse-labelling. Results obtained with the *mf1* strain were similar.

(ΔpsbA), as well as the *mf1*, *mf2* and WT cells. As observed in Fig. 5A, the absence of D1 in the FUD7 mutant causes a decreased synthesis of apoCP47 but not of D2 and apoCP43 as previously reported [24]. A similar situation was observed in the *mf* mutants and [PSII$^-$] progeny. Synthesis of D1 and apoCP47 were barely detectable (Fig. 5A), whereas synthesis of D2 and apoCP43 remained similar to those in the WT strain. We noted that labelling of D1 and apoCP47 were clearly detectable in 40 min pulses in *mf1* and *mf2* strains (Fig. 5B), suggesting that the low labelling observed in 5 min pulses is not due to rapid degradation of the neosynthesized proteins. A similar result was also obtained with the *mf1* strain (data not shown). The tracks from the [PSII$^+$] and [PSII$^-$] progeny were identical to those from their WT and *mf* parents, respectively. These changes in synthesis of PSII subunits followed the same segregation, as that observed for PSII activity and PG content.

Translation initiation of the *psbA* mRNA is not affected in the *mf2* strain

The dramatic decrease in D1 synthesis in *mf1* and *mf2* strains did not correlate with any significant changes in the accumulation of *psbA* mRNA, as revealed by RNA-filter hybridization experiments (data not shown). This indicates the likelihood of a translational defect of D1 synthesis occuring in these strains. To determine if translation initiation of the *psbA* mRNA was compromised, we inserted a chimeric gene, containing the *petA*-coding region (encoding cytochrome *f*) translated under the control of the 5′ UTR of *psbA* in place of the endogenous *petA* gene. The transformant, hereafter refered to as strain 5′*psbA–petA*, *mt*+ (Fig. 6A), was subsequently crossed with the *mf2, mt*– strain, to compare the expression of the chimeric gene in

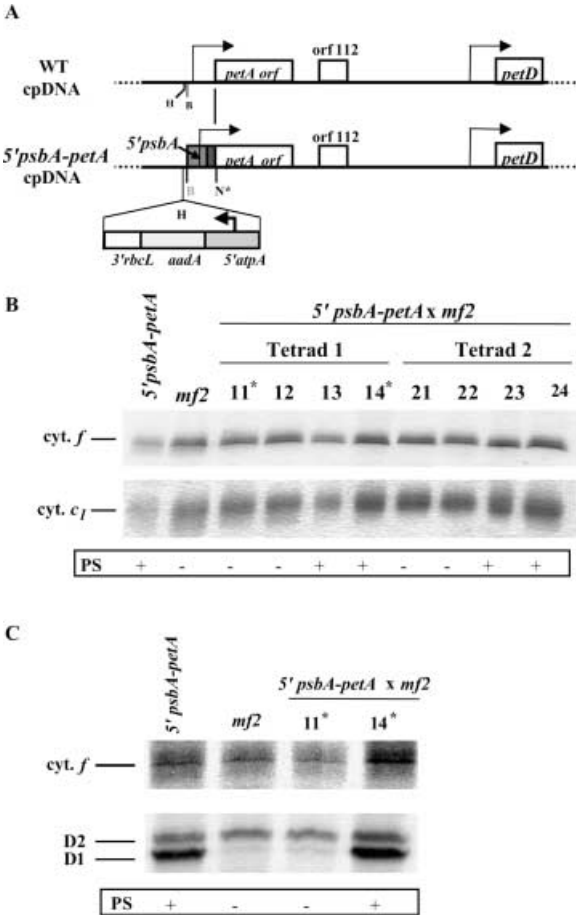

Fig. 6. Translation of the chimeric 5′*psbA–petA* gene in WT and *mf2* nuclear context. (A) Schematic maps of the *petA* gene in WT and *5′psbA–petA* strains. Relevant restriction sites (B, *Bgl*II; N*, an *Nco*I site introduced by site directed mutagenesis around the *petA* initiation codon for cloning purposes; H, *Hin*cII) are indicated. (B) Accumulation of cytochrome *f* in the parents and offspring of two tetrads from the cross *5′psbA–petA* × *mf2*. PS+, [PSII$^+$] strains (WT nuclear background); PS–, [PSII$^-$] strains (*mf2* nuclear background). Whole cell proteins were separated on denaturing 12–18% (w/v) polyacrylamide/urea gels and stained with TMBZ; cyt *f*, cytochrome *f*; cyt c_1, mitochondrial cytochrome *c* (loading control). (C) Short pulse labelling experiments (5 min with 5 μCi·mL^{-1}) for chloroplast-encoded proteins in the parental strains and in two members of the first tetrad presented above (B, *). Progeny number 11 has the *mf2* nuclear background while progeny number 14 has a WT nuclear background.

either the WT or *mf2* nuclear background. The whole progeny of that cross carries the chimeric gene because of the uniparental inheritance of the chloroplast genome transmitted from the *mt*+ parent [14]. Two members of each tetrad also inherited the *mf2* nuclear background and were identified, using a fluorescence induction kinetics screen, by their [PSII$^-$] phenotype, whereas the other two members displayed [PSII$^+$] phenotype. Accumulation of cytochrome *f* was then assayed by TMBZ staining on whole cell extracts from 5′*psbA–petA* and *mf2* parental strains and from two tetrads of that cross (Fig. 6B). We observed no significant changes in the accumulation of cytochrome *f* between the members of the two tetrads tested. We assessed

directly the rate of synthesis of cytochrome *f* in the two genetic backgrounds by 5 min pulse-labelling experiments performed on the parental strains 5′*psbA–petA* and *mf2* and on two members of tetrad 1. From fluorescence induction kinetics, member 11 and member 14 have *mf2* and WT nuclear backgrounds, respectively. The expected near-absence of synthesis of the D1 protein is clearly visible in the *mf2* and number 11 strains, whereas it is WT-like in the 5′*psbA–petA* and number 14 strains (Fig. 6C). In contrast, synthesis of cytochrome *f*, driven by the chimeric 5′*psbA–petA* transcript, was similar in all strains tested, after corrections for ^{14}C incorporation among the various strains. Thus, the nuclear *mf2* background had no effect on the translation rate of this chimeric gene. We conclude that the *mf2* mutation does not act on the 5′ UTR of the *psbA* transcript.

Discussion

A single nuclear mutation causes both the absence of functional PSII and the lack of PG-C16:1(3t)

The *mf1* and *mf2* mutants were originally screened as unusual PSII mutants because they lack variable fluorescence – a signature of the absence of PSII [15] – but have a low (instead of a high) fluorescence yield. This unusual feature was attributed to a major change in the supramolecular organization of the peripheral antenna. The absence of LHCII oligomers in these strains leads to the accumulation of LHCII monomers that presumably transfer their excitation energy to PSI [7]. It was subsequently proven that changes in supramolecular organization of the peripheral antenna were due to the absence of one particular fatty acid, C16:1(3t), which probably causes a decrease in the overall content in PG [9]. Addition of PG-C16:1(3t) to the growth medium allowed the recovery of oligomeric LHCII [10,11]. However, in contrast to the changes in the state of antenna oligomerization, the PSII defect was almost insensitive to the addition of exogenous PG-C16:1(3t) [8], raising the possibility that PG-C16:1(3t) deficiency was not responsible for PSII inactivation. The extensive genetic analysis performed in the present study nevertheless defines a single nuclear mutational event that governs both phenotypic characters, as suggested by preliminary data from El Maanni *et al.* [8]. All phototrophic revertants isolated from *mf1* and *mf2* also recovered some PG-C16:1(3t), leading to an increase in total PG content. The lack of PG-C16:1(3t) is not a mere consequence of PSII deficiency, as PSII deficient mutants of *C. reinhardtii* such as *Fl39* (a nuclear mutant) or FUD7 (Δ*psbA*) do not present such lipid alterations (data not shown). Thus, the deficiency in PG caused by the absence of the PG-C16:1(3t) form in *mf1* and *mf2* thylakoid membranes is responsible for the near-complete absence of PSII core complexes. The two mutants hardly accumulated any PSII subunits. By blue-native PAGE, we detected only trace amounts of PSII core complexes, comprised of subunits apoCP47 and apoCP43. None were associated with peripheral antenna, even though our electrophoretic method preserved core–antenna complex associations as shown by the presence of PSI–LHCI and PSII–LHCII in the WT pattern. In contrast, PSII–LHCII complexes were observed in revertant *pmf1* cells. Thus PG deficiency in *mf1* and *mf2* mostly targets PSII-containing supercomplexes, although it also affects the relative distribution of different types of PSI–antenna supercomplexes [9]. Because PG was reported to be directly involved in PSI functional organization, based on the study of crystals of trimeric PSI from *Synechococcus elongatus* [30], a detailed analysis of the early steps in PSI electron transfer in the *mf* mutants would be required before one can draw conclusions on the role of PG in PSI supramolecular organization in *C. reinhardtii*.

Phosphatidylglycerol, photosynthesis and PSII biogenesis

The *hf2* nuclear mutant of *C. reinhardtii* displays a severe defect in SQDG, the other thylakoid-specific anionic lipid [31]. Despite a partial alteration of PSII activity, it contained the same amount of PSII core components as the WT strain [32]. Thus, impaired PSII biogenesis in *mf1* and *mf2* mutants does not simply result from a decrease in thylakoid anionic lipids. Several studies emphasized the specific role of PG in photosynthesis, in particular for PSII in cyanobacteria and higher plants [33–36]. The requirement of PG in photosynthesis was recently established *in vivo* by the isolation of two mutants of *Synechocystis* defective in the PG biosynthesis pathway. These were inactivated in the genes responsible for the last step of CDP-diacylglycerol synthesis [37] or phosphatidyl-glycerol-3-phosphate synthesis [38]. They both depended on PG supplementation for phototrophic growth. The withdrawal of PG from the culture medium correlated with alterations in PSII activity [37]. Recently, PG was shown to be essential for the dimerization step of PSII core monomers in the *pgsA* mutant of *Synechocystis* bearing a disruption of the phosphatidyl-glycerol-3-phosphate synthase gene [39].

Photosynthetic mutants fully devoid of PG have not been described up to now in eukaryotes. An *A. thaliana* mutant deficient in phosphate accumulation was found to be partially PG-deficient; its growth rate or photosynthetic parameters were WT-like in two different light conditions but its contents in SQDG and DGDG were increased [40]. In contrast, a pale green mutant of *A. thaliana* with impaired photosynthesis was found to bear a mutation in the gene encoding plastidic phosphatidylglycerolphosphate synthase, leading to a reduced PG content [41]. Disruption of the *PGP1* gene by T-DNA insertion in *A. thaliana* illustrated the importance of PG for the biogenesis of thylakoid membranes [42,43]. Thus the essential function of PG for photosynthetic viability, as demonstrated in cyanobacteria, can probably be extended to photosynthetic eukaryotes even if the molecular mechanism(s) mediated by PG remain(s) to be determined.

Here we show that the two PG-deficient *mf1* and *mf2* mutants of *C. reinhardtii* accumulate only trace amounts of PSII core monomers that are unable to oligomerize in PSII–LHCII supercomplexes. In this alga, LHCII mutants with high PSII activity are easily recovered [44]. The loss in LHCII–PSII core supercomplexes, therefore, should not be responsible for the decreased content in PSII cores, pointing to an effect of PG in PSII core biogenesis. The large but partial PG deficiency occuring in *mf1* and *mf2* includes the total loss of one PG form that contains the C16:1(3t) fatty acid. Thus, it is reasonnable to assume that this fatty acid plays a prominent role in the contribution of PG to PSII

biogenesis. Consistent with this view, the higher ratio of PG to PSII in spinach preparations of dimeric PSII reaction center than in monomeric PSII [45] can be interpreted in light of the results from treatments with phospholipase A2, that decrease the PG-C16:1(3t) content and lead to monomerization of dimeric PSII reaction centers. Conversely dimerization of PSII reaction centers *in vitro* requires the presence of PG-C16:1(3t). An *A. thaliana* mutant, totally deficient in PG-C16:1(3t) did not display any significant alteration of its photosynthetic properties but showed a concomitant increase in PG-C16:0, which may be compensatory in this case [28]. As reported for the formation of the trimeric LHCII antenna in *C. reinhardtii*, the fatty acid C16:1(3t) could increase the efficiency of PG for PSII core biogenesis in the situation of active synthesis determined by the high growth rate of this alga [46]. Altogether, these observations argue for a critical role of C16:1(3t)-containing PG in the biogenesis of PSII core complexes and their subsequent oligomerization in *C. reinhardtii*.

PG plays no part in translation initiation of D1 but could contribute to its cotranslational insertion

The drastic decrease in PSII core content in the *mf1* and *mf2* mutants could be attributed to a higher susceptibility of the cores to proteolytic degradation or to their lower rate of synthesis. When we studied the rates of synthesis of the individual PSII subunits by 5 min pulse-labelling experiments, we observed that synthesis of D1 and apoCP47 were barely detectable in the *mf1* and *mf2* mutants, although the mRNA levels for these two subunits were similar to those observed in the WT strain. Due to the control by epistasy of synthesis (CES) process [1], D1 and apoCP47 display concerted rates of synthesis. In the absence of D1, the rate of synthesis of apoCP47 is strongly reduced, while the rates of synthesis of both D1 and apoCP47 drops when D1 cannot assemble within PSII complexes, for example as a result of the lack of D2 [24]. The decreased synthesis of D1 (and, as a consequence, of apoCP47) in the *mf* mutants was thus consistent with an impaired PSII assembly. We observed recently that the CES behaviour of D1 (its much lower rate of translation when it cannot assemble within PSII) resulted from a translational regulation that depends on the 5′ UTR of *psbA* (L. Minai, F.-A. Wollman and Y. Choquet, unpublished results). We thus tested whether the rate of translation of a chimeric reporter gene harbouring the coding region of *petA* translated under the control of the 5′ UTR of *psbA* was decreased when it was expressed in *mf2* strain. Much to our surprise, the level of synthesis of its protein product (cytochrome *f*) in the *mf2* nuclear background was identical to that observed in a WT nuclear background. We are therefore bound to conclude that the reduced synthesis of D1 is not due to a defect in translation initiation but to a subsequent step that could be either elongation, termination, membrane insertion or very rapid cotranslational degradation of the D1 protein. However D1 synthesis in the *mf* strains, which is hardly detectable in 5 min pulse-labelling experiments, is still easily detectable in longer pulses, arguing against a rapid degradation of the polypeptide. It is difficult to discriminate further between these alternatives at present, because chimeric genes made of the coding sequence of *psbA* translated under the control of unrelated 5′ UTRs are only very poorly expressed (L. Minai, unpublished results).

The inability of D1 to react with crosslinkers was postulated to be due to a particular stability of its conformation mediated by saturated fatty acids of boundary lipids [47]. Later, PG was proposed to anchor D1 into the thylakoid membranes of cyanobacteria by a strong interaction with the hydrophobic part of the molecule [35]. If this binding occurs at an early step in D1 synthesis, i.e. during the process of the cotranslational insertion of the D1 protein into the thylakoid membrane, then one could imagine that the absence of PG leads to a drop in translational elongation of the D1 protein. Indeed anionic phospholipids were shown to contribute to the coupled translation–insertion of some protein subunits of the electron transport chain from inner mitochondrial membranes [48]. A null *PGS1* mutant of *S. cerevisiae*, in which the content of PG and cardiolipid could be controlled by modulating the expression of a plasmid-introduced *PGS1* gene, was also used to demonstrate that these anionic phospholipids have a mandatory function in the translation of cytochrome *b* and the three largest subunits of cytochrome oxidase [49]. On the other hand, the activity of the SecYEG translocase in bacteria is strictly dependent *in vitro* on the presence of PG in *E. coli* and *Bacillus subtilis* [50]. Therefore, the requirement for PG could arise at the level of the Sec translocon through which D1 is inserted in the thylakoid membranes [51–53]. We note, however, that translocation of the other Sec passenger proteins, such as cytochrome *f*, was not altered in the *mf* mutants, an observation that does not support a prominent role of PG-C16:1(3t) in Sec translocation across thylakoid membranes. PG could still contribute to cotranslational insertion of D1 in the thylakoid membranes through a number of other steps that have not been properly characterized yet.

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References

1. Wollman, F.A., Minai, L. & Nechushtai, R. (1999) The biogenesis and assembly of photosynthetic proteins in thylakoid membranes. *Biochim. Biophys. Acta* **1411**, 21–85.
2. Siegenthaler, P.A. (1998) Molecular organization of acyl lipids in photosynthetic membranes of higher plants. In *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenthaler, P.A. & Murata, N., eds), pp. 119–144. Kluwer Academic Publishers, Dordrecht, the Netherlands.
3. Joyard, J., Maréchal, E., Miège, C., Block, M.A., Dorne, A. & Douce, R. (1998) Structure, distribution and biosynthesis of glycerolipids from higher plant chloroplasts. In *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenthaler, P.A. & Murata, N., eds), pp. 21–52. Kluwer Academic Publishers, Dordrecht, the Netherlands.

4. Ivancich, A., Horvath, L.I., Droppa, M., Horvath, G. & Farkas, T. (1994) Spin label EPR study of lipid solvation of supramolecular photosynthetic protein complexes in thylakoids. *Biochim. Biophys. Acta* **1196**, 51–56.
5. Fyfe, P.K., McAuley, K.E., Roszak, A.W., Isaacs, N.W., Cogdell, R.J. & Jones, M.R. (2001) Probing the interface between membrane proteins and membrane lipids by X-ray crystallography. *Trends Biochem. Sci.* **26**, 106–112.
6. Maroc, J., Trémolières, A., Garnier, J. & Guyon, D. (1987) Oligomeric form of the light-harvesting chlorophyll a + b protein complex CPII, phosphatidylglycerol, Δ^3-*trans*-hexadecenoic acid and energy transfer in *Chlamydomonas reinhardtii*, wild-type and mutants. *Biochim. Biophys. Acta* **893**, 91–99.
7. Garnier, J., Maroc, J. & Guyon, D. (1987) Characterization of new strains of photosynthetic mutants of *Chlamydomonas reinhardtii*. IV. Impaired excitation energy transfer in three low fluorescent mutants. *Plant Cell Physiol.* **28**, 1117–1131.
8. El Maanni, A., Dubertret, G., Delrieu, M.J., Roche, O. & Trémolières, A. (1998) Mutants of *Chlamydomonas reinhardtii* affected in phosphatidylglycerol metabolism and thylakoid biogenesis. *Plant Physiol Biochem.* **36**, 609–619.
9. Garnier, J., Wu, B., Maroc, J., Guyon, D. & Trémolières, A. (1990) Restoration of both an oligomeric form of the light-harvesting antenna CPII and a fluorescence state II – state I transition by Δ^3-*trans*-hexadecenoic acid-containing phosphatidylglycerol, in cells of a mutant of *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta* **1020**, 153–162.
10. Trémolières, A., Roche, O., Dubertret, G., Guyon, D. & Garnier, J. (1991) Restoration of thylakoid appression by Δ^3-*trans*-hexadecenoic acid-containing phosphatidylglycerol in a mutant of *Chlamydomonas reinhardtii*. Relationships with the regulation of excitation energy distribution. *Biochim. Biophys. Acta* **1059**, 286–292.
11. Dubertret, G., Mirshahi, A., Mirshahi, M., Gerard-Hirne, C. & Tremolieres, A. (1994) Evidence from *in vivo* manipulations of lipid composition in mutants that the Δ^3-*trans*-hexadecenoic acid-containing phosphatidylglycerol is involved in the biogenesis of the light-harvesting chlorophyll a/b-protein complex of *Chlamydomonas reinhardtii*. *Eur. J. Biochem.* **226**, 473–482.
12. Maroc, J. & Garnier, J. (1981) Gel electrophoresis of chloroplast membranes of mutants of *Chlamydomonas reinhardtii* which have impaired photosystem II function and lack photosynthetic cytochromes. *Biochim. Biophys. Acta* **637**, 473–480.
13. Bennoun, P., Spierer-Herz, M., Erickson, J., Girard-Bascou, J., Pierre, Y., Delosme, M. & Rochaix, J.D. (1986) Characterization of photosystem II mutants of *Chlamydomonas reinhardtii* lacking the *psbA* gene. *Plant Mol Biol.* **6**, 151–160.
14. Harris, E. (1989) *The Chlamydomonas Sourcebook. A Comprehensive Guide to Biology and Laboratory Use*. San Diego Academic Press, San Diego, CA, USA.
15. Bennoun, P. & Delepelaire, P. (1982) Isolation of photosynthesis mutants in *Chlamydomonas*. In *Methods in Chloroplast Molecular Biology* (Edelman, M., Hallick, R.B. & Chua, N.-H., eds), pp. 25–38. Elsevier Biomedical Press, Amsterdam, New York, Oxford.
16. Rochaix, J.D. (1978) Restriction endonuclease map of the chloroplast DNA of *Chlamydomonas reinhardtii*. *J. Mol. Biol.* **126**, 597–617.
17. Rimbault, B., Esposito, D., Drapier, D., Choquet, Y., Stern, D.B. & Wollman, F.A. (2000) Identification of the initiation codon for the *atpB* gene in *Chlamydomonas* chloroplasts excludes translation of a precursor form of the β subunit of the ATP synthase. *Mol. Gen. Genet.* **264**, 486–491.
18. Goldschmidt-Clermont, M. (1991) Transgenic expression of aminoglycoside adenine transferase in the chloroplast: a selectable marker of site-directed transformation of *Chlamydomonas*. *Nucleic Acids Res.* **19**, 4083–4089.
19. Boynton, J.E., Gillham, N.W., Harris, E.H., Hosler, J.P., Johnson, A.M., Jones, A.R., Randolph-Anderson, B.L., Robertson, D., Klein, T.M. & Shark, K.B. (1988) Chloroplast transformation in *Chlamydomonas* with high velocity microprojectiles. *Science* **240**, 1534–1538.
20. Kuras, R. & Wollman, F.A. (1994) The assembly of cytochrome b6/f complexes: an approach using genetic transformation of the green alga *Chlamydomonas reinhardtii*. *EMBO J.* **13**, 1019–1027.
21. Chua, N.H. & Bennoun, P. (1975) Thylakoid membrane polypeptides of *Chlamydomonas reinhardtii*: wild-type and mutant strains deficient in photosystem II reaction center. *Proc. Natl Acad. Sci. USA* **72**, 2175–2179.
22. Schägger, H., Cramer, W.A. & von Jagow, G. (1994) Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis. *Anal. Biochem.* **217**, 220–230.
23. Pineau, B., Gérard-Hirne, C. & Selve, C. (2001) Carotenoid binding to photosystem I and II of *Chlamydomonas reinhardtii* cells grown under weak light or exposed to intense light. *Plant Physiol. Biochem.* **39**, 73–85.
24. de Vitry, C., Olive, J., Drapier, D., Recouvreur, M. & Wollman, F.A. (1989) Posttranslational events leading to the assembly of photosystem II protein complex: a study using photosynthesis mutants from *Chlamydomonas reinhardtii*. *J. Cell Biol.* **109**, 991–1006.
25. Thomas, P.E., Ryan, D. & Levin, W. (1976) An improved staining procedure for the detection of the peroxidase activity of cytochrome P-450 on sodium dodecyl sulfate polyacrylamide gels. *Anal Biochem.* **75**, 168–176.
26. Bligh, E.G. & Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917.
27. Bassi, R. & Wollman, F.A. (1991) The chlorophyll-a/b proteins of photosystem II in *Chlamydomonas reinhardtii*. Isolation, characterization and immunological cross-reactivity to higher plant polypeptides. *Planta* **183**, 423–433.
28. McCourt, P., Browse, J., Watson, J., Arntzen, C.J. & Somerville, C.R. (1985) Analysis of photosynthetic antenna function in a mutant of *Arabidopsis thaliana* (L.) lacking *trans*-hexadecenoic acid. *Plant Physiol.* **78**, 853–858.
29. Thidolm, E., Lindström, V., Tissier, C., Robinson, C., Schröder, W.P. & Funk, C. (2002) Novel approach reveals localization and assembly pathway of the PsbS and PsbW proteins into the photosystem II dimer. *FEBS Lett.* **513**, 217–222.
30. Jordan, P., Fromme, P., Witt, H.T., Klukas, O., Saenger, W. & Krauss, N. (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature* **411**, 909–917.
31. Sato, N., Tsuzuki, M., Matsuda, Y., Ehara, T., Osafune, T. & Kawaguchi, A. (1995) Isolation and characterization of mutants affected in lipid metabolism of *Chlamydomonas reinhardtii*. *Eur. J. Biochem.* **230**, 987–993.
32. Sato, N., Sonoike, K., Tsuzuki, M. & Kawaguchi, A. (1995) Impaired photosystem II in a mutant of *Chlamydomonas reinhardtii* defective in sulfoquinovosyl diacylglycerol. *Eur. J. Biochem.* **234**, 16–23.
33. Murata, N., Higashi, S.I. & Fujimura, Y. (1990) Glycerolipids in various preparations of photosystem II from spinach chloroplasts. *Biochim. Biophys. Acta* **1019**, 261–268.
34. Kruse, O., Radunz, A. & Schmid, G.H. (1994) Phosphatidylglycerol and β-carotene bound onto the D1 core peptide from photosystem II in the filamentous cyanobacterium *Oscillatoria chalybea*. *Z. Naturforsch.* **49c**, 115–124.
35. Kruse, O. & Schmid, G.H. (1995) The role of phosphatidylglycerol as a functional effector and membrane anchor of the D1 core peptide from photosystem II particles of the cyanobacterium *Oscillatoria chalybea*. *Z. Naturforsch.* **50c**, 380–390.

36. Duchêne, S., Smutny, J. & Siegenthaler, P.A. (2000) The topology of phosphatidylglycerol populations is essential for sustaining photosynthetic electron flow activities in thylakoid membranes. *Biochim. Biophys. Acta* **1463**, 115–120.
37. Sato, N., Hagio, M., Wada, H. & Tsuzuki, M. (2000) Requirement of phosphatidylglycerol for photosynthetic function in thylakoid membranes. *Proc. Natl Acad. Sci. USA* **97**, 10655–10660.
38. Hagio, M., Gombos, Z., Varkonyi, Z., Masamoto, K., Sato, N., Tsuzuki, M. & Wada, H. (2000) Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis. *Plant Physiol.* **124**, 795–804.
39. Sakurai, I., Hagio, M., Gombos, Z., Tyystjarvi, T., Paakkarinen, V., Aro, E.M. & Wada, H. (2003) Requirement of phosphatidylglycerol for maintenance of photosynthetic machinery. *Plant Physiol.* **133**, 1376–1384.
40. Härtel, H., Essigmann, B., Lokstein, H., Hoffmann-Benning, S., Peters-Kottig, M. & Benning, C. (1998) The phospholipid-deficient pho1 mutant of *Arabidopsis thaliana* is affected in the organization, but not in the light acclimation, of the thylakoid membrane. *Biochim. Biophys. Acta* **1415**, 205–218.
41. Xu, C., Härtel, H., Wada, H., Hagio, M., Yu, B., Eakin, C. & Benning, C. (2002) The pgp1 mutant locus of *Arabidopsis* encodes a phosphatidylglycerolphosphate synthase with impaired activity. *Plant Physiol.* **129**, 594–604.
42. Hagio, M., Sakurai, I., Sato, S., Kato, T., Tabata, S. & Wada, H. (2002) Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana*. *Plant Cell Physiol.* **43**, 1456–1464.
43. Babiychuk, E., Muller, F., Eubel, H., Braun, H.P., Frentzen, M. & Kushnir, S. (2003) Arabidopsis phosphatidylglycerophosphate synthase 1 is essential for chloroplast differentiation, but is dispensable for mitochondrial function. *Plant J.* **33**, 899–909.
44. Olive, J., Wollman, F.A., Bennoun, P. & Recouvreur, M. (1981) Ultrastructure of thylakoid membranes in *C. reinhardtii*: evidence for variations in the partition coefficient of the light-harvesting complex-containing particles upon membrane fracture. *Arch. Biochem. Biophys.* **208**, 456–467.
45. Kruse, O., Hankamer, B., Konczak, C., Gerle, C., Morris, E., Radunz, A., Schmid, G.H. & Barber, J. (2000) Phosphatidylglycerol is involved in the dimerization of photosystem II. *J. Biol. Chem.* **275**, 6509–6514.
46. Dubertret, G., Gérard-Hirne, C. & Trémolières, A. (2002) Importance of trans-Δ^3-hexadecenoic acid-containing phosphatidylglycerol in the formation of the trimeric light-harvesting complex in *Chlamydomonas*. *Plant Physiol. Biochem.* **40**, 829–836.
47. Adir, N. & Ohad, I. (1988) Structural properties of the D1 and surrounding photosystem II polypeptides as revealed by their interaction with cross-linking reagents. *J. Biol. Chem.* **263**, 283–289.
48. Chang, S.C., Heacock, P.N., Mileykovskaya, E., Voelker, D.R. & Dowhan, W. (1998) Isolation and characterization of the gene (CLS1) encoding cardiolipin synthase in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273**, 14933–14941.
49. Ostrander, D.B., Zhang, M., Mileykovskaya, E., Rho, M. & Dowhan, W. (2001) Lack of mitochondrial anionic phospholipids causes an inhibition of translation of protein components of the electron transport chain. A yeast genetic model system for the study of anionic phospholipid function in mitochondria. *J. Biol. Chem.* **276**, 25262–25272.
50. van der Does, C., Swaving, J., van Klompenburg, W. & Driessen, A.J. (2000) Non-bilayer lipids stimulate the activity of the reconstituted bacterial protein translocase. *J. Biol. Chem.* **275**, 2472–2478.
51. Nilsson, R., Brunner, J., Hoffman, N.E. & van Wijk, K.J. (1999) Interactions of ribosome nascent chain complexes of the chloroplast-encoded D1 thylakoid membrane protein with cpSRP54. *EMBO J.* **18**, 733–742.
52. Zhang, L., Paakkarinen, V., van Wijk, K.J. & Aro, E.M. (1999) Co-translational assembly of the D1 protein into photosystem II. *J. Biol. Chem.* **274**, 16062–16067.
53. Zhang, L., Paakkarinen, V., Suorsa, M. & Aro, E.M. (2001) A SecY homologue is involved in chloroplast-encoded D1 protein biogenesis. *J. Biol. Chem.* **276**, 37809–37814.