Posttranslational Events Leading to the Assembly of Photosystem II Protein Complex: A Study Using Photosynthesis Mutants from *Chlamydomonas reinhardtii*

Catherine de Vitry,* Jacqueline Olive,* Dominique Drapier,* Michel Recouvreur,† and Francis-André Wollman*

*Service de Photosynthèse, Institut de Biologie Physico-Chimique, 75005 Paris, France; and †Laboratoire de Microscopie Electronique, Institut Jacques Monod, Université Paris VII, 75005 Paris, France

Abstract. We studied the assembly of photosystem II (PSII) in several mutants from *Chlamydomonas reinhardtii* which were unable to synthesize either one PSII core subunit (P6 [43 kD], D1, or D2) or one oxygen-evolving enhancer (OEE1 or OEE2) subunit. Synthesis of the PSII subunits was analyzed on electrophoretograms of cells pulse labeled with [14C]acetate. Their accumulation in thylakoid membranes was studied on immunoblots, their chlorophyll-binding ability on nondenaturing gels, their assembly by detergent fractionation, their stability by pulse-chase experiments and determination of in vitro protease sensitivity, and their localization by immunocytochemistry.

In *Chlamydomonas*, the PSII core subunits P5 (47 kD), D1, and D2 are synthesized in a concerted manner while P6 synthesis is independent. P5 and P6 accumulate independently of each other in the stacked membranes. They bind chlorophyll soon after, or concomitantly with, their synthesis and independently of the presence of the other PSII subunits. Resistance to degradation increases step by step: beginning with assembly of P5, D1, and D2, then with binding of P6, and, finally, with binding of the OEE subunits on two independent high affinity sites (one for OEE1 and another for OEE2 to which OEE3 binds). In the absence of PSII cores, the OEE subunits accumulate independently in the thylakoid lumen and bind loosely to the membranes; OEE1 was found on stacked membranes, but OEE2 was found on either stacked or unstacked membranes depending on whether or not P6 was synthesized.

Photosystem II (PSII) is a major protein complex of the photosynthetic apparatus in oxygen-evolving species. Light-harvesting chlorophyll–protein complexes (LHCs) transfer excitons to PSII cores where primary photochemistry occurs. PSII complexes (PSII cores with oxygen-evolving enhancer [OEE] subunits) are able to carry out the oxidation of water.

The PSII core comprises five main intrinsic chloroplast-encoded subunits P5, P6, D1, D2, and cytochrome b$_{599}$ (59). Their molecular masses vary slightly from one species to another. Two subunits of 47–50 and 43–47 kD, called P5 and P6 in *Chlamydomonas reinhardtii* or by their molecular mass in higher plants (respectively encoded by psbB and psbC genes), bind most of the PSII core chlorophylls (58) and form the core antenna (9, 44). The chlorophyll–P5 and chlorophyll–P6 complexes—called, respectively, CPIII and CPIV in *C. reinhardtii* or CP47 and CP43 in higher plants—can be separated by electrophoresis at 4°C (13, 21). D1 and D2 of 32-35 kD (encoded, respectively, by psbA and psbD genes [18, 48, 64]) cooperate in the binding of the primary reactants (44) and show sequence homologies with the subunits L and M of the reaction center from purple bacteria (39, 53). Three extrinsic polypeptides encoded by nuclear genes (12, 60) are involved in oxygen evolution; OEE1 (29–33 kD) stabilizes the association of manganese ions with thylakoid membranes, whereas OEE2 (20–24 kD) and OEE3 (16–18 kD) sequester calcium and chloride ions next to the oxygen-evolving site (for review see 42). Additional PSII subunits of low molecular mass have been identified (11) and some of them might have a role in oxygen evolution (35). Thus, a functional oxygen-evolving PSII complex requires the assembly of numerous polypeptides (for review see 47), which are encoded either in the chloroplast or in the nucleus, and the binding of ~50 cofactors, among which are chlorophylls, carotenoids, pheophytins, plastoquinones, a nonheme iron, and manganese ions. Once inserted and processed in the unstacked thylakoid membrane regions, the PSII subunits undergo a lateral translocation to the stacked regions where most of the mature PSII complexes accumulate (36).

In this work we studied PSII assembly using several mutants of *C. reinhardtii*. This unicellular green alga can grow heterotrophically on acetate and is thereby appropriate for

---

1. Abbreviations used in this paper: LHC, light-harvesting complex; OEE, oxygen-evolving enhancer; PSII, photosystem II; WT, wild type.
the isolation and growth of photosynthesis mutants (for review see 46). PSII mutants offer the opportunity to analyze how various polypeptide deficiencies alter PSII assembly. Such an approach has been used mainly to study pretranslational and translational events in the process of assembly (28, 47). In this work, we focus more specifically on posttranslational events. We have undertaken a comparative analysis of three types of PSII mutants: mutants unable to synthesize either one reaction center subunit (D1 or D2), one core antenna subunit (P6), or one OEE subunit (OEE1 or OEE2). We characterized PSII biogenesis in each strain by the synthesis, insertion into the membrane, turnover, partial assembly, binding of cofactors, phosphorylation, and translocation to the gronal regions of the various PSII subunits. This mutant-based analysis led us to distinguish four main steps in the process of PSII assembly in the thylakoid membranes.

Materials and Methods

Cell Culture Conditions and Mutants

Wild-type (WT) and PSII mutants of *C. reinhardtii* were grown at 300 lux in Tris-acetate-phosphate medium, pH 7.2, to a density of 2-4 × 10⁶ cells/ml. Partial characterization of the mutants has been previously reported for (a) the PSII core nuclear mutant F34 (12); (b) the PSII core chloroplast mutants FUD34 (20), FUD7 (4), and FUD47 (19); (c) the OEE nuclear mutants BF25 (3, 37) and FUD44 (38); and (d) the F54-14 double mutant lacking photosystem I and chloroplast ATP synthase (15). The nuclear mutant FUD39 lacking OEE2, isolated and generously provided by P. Bennoun (Centre National de Recherche Scientifique, Paris, France), is characterized for the first time in this paper.

Protein Isolation

Thylakoid membranes were isolated as described (10). They were either prepared for electrophoresis by solubilization in 1% SDS at a chlorophyll concentration of 1 mg/ml or solubilized in 1.25% digitonin/1% Triton X-100 at a chlorophyll concentration of 1.2 mg/ml to purify PSII particles. Control PSII core particles were isolated from mutant F54-14 and purified by sucrose density gradient centrifugation (15); an identical treatment was performed on the PSII mutants to test the state of PSII assembly.

PAGE

SDS-PAGE was run according to Laemmli (32) as modified (13) using 7.5-15% polyacrylamide gradients or 12-18% polyacrylamide gradients containing 8 M urea (45). Study of the binding of chlorophyll to newly synthesized P5 and P6 was performed on two-dimensional gels (first, 7.5-15% polyacrylamide gradient gel with 8 M urea at room temperature). Polypeptides were stained by Coomassie brilliant blue R-250 or detected by autoradiography of the dried gels using industrial P films (Agfa-Gevaert, Rueil-Malmaison, France).

Antibody Preparation, Electroblotting, and Hybridization

To obtain α-OEE2 and α-OEE3 antisera, we purified the proteins as follows. Thylakoid membranes from the F54-14 mutant were incubated in 50 mM Tris, pH 11, at 4°C with continuous stirring for 30 min. Polypeptides released in the supernatant were precipitated in presence of 10% TCA, and the resulting pellet was washed twice in ice-cold 80% acetone, dried, and solubilized in the presence of 1% SDS for two successive preparative SDS-gel electrophoresis (first gel, 12-18% polyacrylamide gradient containing 8 M urea; second gel, 7.5%-15% polyacrylamide gradient). OEE2 and OEE3 were electroeluted from the second preparative gel (27). Rabbits were given subcutaneous injections of the purified proteins in Freund's complete adjuvant; after 4, 8, and 16 wk, each rabbit was given booster injections of purified OEE2 or OEE3 with poly(A).poly(U) as adjuvant (25). α-P5, α-P6, and α-OEE1 antibodies were obtained by courtesy of N. H. Chua (Rockefeller University, New York). Antiserum prepared against PSII parti-
subunits in the thylakoids of such mutants can, however, be assessed on immunoblots. In Fig. 1B the amounts of PSII core subunits accumulated in the WT of C. reinhardtii (lane 1) are compared with PSII mutants lacking synthesis of either P6 (lanes 2 and 3), D1 (lane 4), or D2 (lane 5). About 10% of the WT amount of P5 was found in the thylakoid membranes of the two mutants lacking P6 (lanes 2 and 3) whereas it was barely detectable in the mutants lacking D1 or D2 (lanes 4 and 5). We note that the \( \alpha \)-P5 antibody recognized a main band in the position of P5 and a minor one slightly below P6 (Fig. 1A). That the latter band is due to a genuine product of P5, probably a degradation product, and not to a faint cross-reaction with P6 is supported by its absence in the mutants showing little P5 but rather high P6 accumulation (see overexposed immunoblots in Fig. 1B, lanes 4 and 5).

About 10% of the WT amount of P6 was detectable in the mutants lacking D1 or D2 (lanes 4 and 5). Although P6 was totally undetectable in the FUD34 mutant (lane 3), some accumulation of P6 could still be detected in the F34 mutant (lane 2). Some accumulation of D1 and D2 (the unphosphorylated form D2.2, but not the phosphorylated form D2.1) could be detected in the thylakoid membranes of the two mutants lacking P6 (lanes 2 and 3). In contrast, D2 was undetectable in the mutant lacking D1 (lane 4) but trace amounts of D1 were detectable in the mutant lacking D2 (lane 5). Another PSII reaction center subunit, cytochrome b559 (polypeptide L8), could be observed on SDS-urea gels after silver staining; some accumulation of cytochrome b559 was detected in the absence of the PSII core antenna subunit P6 (F34 mutant), but not in the absence of the reaction center subunit D2 (FUD47 mutant) (data not shown).

### Synthesis of the PSII Core Subunits: Independence of P6 Synthesis

The rates of synthesis of the polypeptides encoded in the chloroplast were detected in 5-min pulse-labeling studies performed in the presence of \(^{14}C\)acetate after addition of an inhibitor of cytoplasmic translation. Such experiments are shown in Fig. 2A, lane 0, where one can compare the rates of synthesis of the PSII core subunits in three PSII mutants (panels 3–5) and in the WT (panel 1). As previously reported: (a) synthesis of P6 was totally absent in the chloroplast mutant FUD34 (panel 3) and hardly detectable in the nuclear mutant F34 (data not shown; 14, 20) (The conflicting claim of Jensen et al. [28] that F34 showed high levels of P6 synthesis but lacked D1 arose from additional mutations in the strain used by these authors [Girard-Bascou, J., Y. Pierre, and P. Delepelaire, manuscript in preparation]); (b) there was no synthesis of D1 in the FUD7 mutant (panel 4) which has a deletion in the psbA gene (4); and (c) there was no synthesis of D2 in the FUD47 mutant (panel 5) which is inactivated in the psbD gene (19). That some synthesis of D1 still occurred in this mutant is better seen in 45-min pulse-labeling experiments (Fig. 2B, panel 5).

Thus, each of these PSII mutants was lacking synthesis of only one of the PSII core subunits. However, examination of the rates of synthesis of the other PSII core subunits indicated additional modifications in some mutants. In the absence of D1, the rate of D2 synthesis was unaffected while most of P5 synthesis was prevented (FUD7 mutant; panel 4). The control of D1 over P5 synthesis and conversely of P5 over D1 synthesis was reported in other C. reinhardtii mutants by Jensen et al. (28). In the absence of D2, most of the D1 and P5 synthesis was prevented (FUD47 mutant; panel 5) as noted by Erickson et al. (19). In this case, the decreased rate in P5 synthesis might be a consequence of the lower synthesis of D1.

In contrast, P6 synthesis occurred independently. This was indicated by similar rates of P6 synthesis in the WT and in mutants lacking D1 or D2 (cf. panels 4, 5, and 1) and conversely by similar rates of synthesis of P5, D1, and D2 in the WT and in mutants lacking P6 (cf. panels 3 and 1). The synthesis of polypeptide L8, which is probably a cytochrome b559 apoprotein (59), was unaffected in all mutants (data not shown).
**Assembly of the PSII Core Subunits: A P5/D1/D2/L8 Step**

To determine the state of assembly between the PSII core subunits remaining in these mutants, we followed the procedure for PSII particle purification from *C. reinhardtii* as described (15). Detergent-solubilized thylakoid membranes from cells pulse labeled in the presence of an inhibitor of cytoplasmic translation were loaded on a sucrose gradient. The distribution of the PSII subunits was analyzed among the gradient fractions after ultracentrifugation. The content of chloroplast-encoded polypeptides in such fractions is shown for the FUD34 mutant in Fig. 3 A. Most of the α and β subunits of the ATP synthase were found at the bottom part of the gradient (lane 5) whereas cytochrome f and cytochrome b₆ were located in the middle of the gradient (lanes 15-19). PSII subunits P5, D1, D2, and L8 (a cytochrome b₅₉ subunit) were found in the fractions immediately above those containing the ATP synthase subunits (lanes 7-10). P6 is totally absent in the FUD34 mutant (Fig. 1). Densitometric scanning of labeled PSII core subunits in each fraction from the various PSII mutants and from the control strain are shown in Fig. 3 B. The codistribution of the PSII core subunits in the control gradient illustrates their assembly in a complex. We observed that each of the PSII core subunits from the mutants lacking in P6 (FUD34 and F34) showed the same distribution and were found at sucrose densities close to that of the control PSII particles. In addition, the PSII subunits from such fractions could be coprecipitated by α-P5 antibodies (data not shown). We attribute the slight displacement towards lower densities of the PSII fractions in the F34 gradient to the higher contamination by LHCs (visible on Coomassie blue-stained electrophoretograms; data not shown), which is of lower density than PSII particles (15). These results indicate that the PSII subunits can assemble in a P5/D1/D2/L8 complex in the mutants lacking P6.

In contrast, the mutants lacking D1 or D2 (FUD7 and FUD47) displayed an erratic distribution of the remaining PSII core subunits, suggesting the absence of PSII assembly. However, we cannot exclude weak interactions between the remaining PSII subunits in such mutants, which would be disrupted by detergent solubilization.

**The Binding of Chlorophyll to the PSII Core Subunits: An Early Event**

To examine the binding of chlorophyll to the PSII core antenna subunits, we looked for the presence of the chlorophyll-binding forms of P5 and P6 (CPIII and CPIV, respectively). In the WT (Fig. 4), CPIII and CPIV can be detected as spots off the diagonal in two-dimensional gel electrophoresis (arrows); newly synthesized products encoded by the chloroplast were selectively detected using cells pulse labeled for 45 min in presence of an inhibitor of cytoplasmic translation. CPIII and CPIV were heat sensitive and were not detected when the sample was heated before the first dimension electrophoresis (compare WT and WT heated). In the mutants lacking P6 (F34), only CPIII was detected. In the mutants lacking D1 or D2 (FUD7 and FUD47), CPIV was easily detected; CPIII could be detected in overexposed autoradiograms only (Fig. 4, FUD47 inset) owing to the impaired synthesis of P5 (Fig. 2). These results indicate that the binding of chlorophyll to P5 and P6 occurred in <45 min and independently of PSII core assembly. We similarly detected CPIII and CPIV in these mutants in shorter pulses (5 min); a subsequent chase of 50 min performed in the absence of protein synthesis was not accompanied by an increased conversion of P5 or P6 in CPIII and CPIV (data not shown). Thus binding of chlorophyll to P5 and P6 is an early event in the process of insertion into the membrane of the core antenna subunits.

Also detectable in Fig. 4 is the existence of a native form of D2 (D2n; an “in” diagonal spot indicated by arrows at the center of two-dimensional gels) in addition to its denatured form (D2d). D2n shared three characteristics with chlorophyll-binding complexes: (a) a modified electrophoretic mobility compared with the denatured form; (b) a heat sensitivity (compare WT and WT heated or FUD7 and FUD7 heated); and (c) an acetone sensitivity (data not shown). Whether D2n contains chlorophyll or another cofactor requires additional study.
Figure 3. Newly synthesized polypeptide pattern of fractions after sucrose gradient centrifugation of solubilized membranes of PSII core mutants (45-min pulse labeling). (A) Autoradiogram of FUD34 mutant fractions. α and β are ATP synthase subunits. Cytochromes of the bc1 complex are shown (cyt.b6 and cyt.f). Dashed line points to degradation product of P5 (as shown in Fig. 1). Analysis was by SDS-7.5–15% polyacrylamide gradient gel electrophoresis. (B) Same as A after densitometric scanning of autoradiograms of pulse-labeled fractions for P6 (o), D1 (●), D2 (○), and L8 (▲) and of autoradiograms after immunoblotting for P5 (●).
The Sensitivity of PSII Core Subunits to Proteases Is Increased in the Mutants

That several PSII subunits showed unchanged rates of insertion but no accumulation in the mutants’ membrane suggested that they would be more susceptible to proteolytic degradation. Therefore we assessed the trypsin sensitivity of the newly synthesized PSII core subunits. In the WT (Fig. 5 A), a fraction of P5 and D1 was cleaved by trypsin whereas P6 and D2 showed little trypsin sensitivity. In contrast, P5, P6, and D2 were all trypsin sensitive in the FUD7 mutant lacking D1. As shown by immunoblotting, the whole pool of P6 accumulated in the FUD7 mutant was trypsin sensitive (Fig. 5 B). In the FUD34 mutant lacking P6 (Fig. 5 A), D1 and D2 were more sensitive to trypsin than in the WT. However, when the protease concentration was increased up to 50 mg/ml, P6, D1, and D2 were all trypsin sensitive. In addition, whereas P5 was totally digested by trypsin in the FUD34 mutant, it was only converted to a slightly lower molecular mass form in the WT (Fig. 6 C). Thus we observed in vitro an increasing sensitivity to proteases along with a decreasing ability of the PSII core subunits to assemble in the thylakoid membranes.

Turnover of the PSII Core Subunits: Turnover of D2 Increases in the Absence of D1

We then addressed the question as to whether the above results could be correlated in vivo with an increased turnover of the PSII core subunits in the absence of assembly. A 4-h pulse-chase experiment is shown in Fig. 2. The lack of accumulation of D2 in the mutant lacking D1 correlated well with the increased turnover of D2 in this mutant as compared with that in the WT (cf. panels 4 and J).

Surprisingly, in all the other instances the lack of accumulation of the PSII subunits, which showed rates of synthesis...
similar to that in the WT, could not be correlated with an increase in turnover within the time course of our chase experiment.

**PSII Core Subunits Are Localized Predominantly in the Stacked Regions of the Thylakoid Membranes in the Mutants**

We have previously used postembedding immunogold labeling on thin sections from broken cells to show that most of the mature PSII protein complexes were found in the stacked regions of the thylakoid membranes of the WT (55). In addition, significant labeling of the thylakoid membranes of PSII mutants could be obtained with antibodies directed against PSII subunits (54). Therefore, it was possible to investigate whether unassembled or partially assembled PSII core subunits had the property of segregating in the stacked membrane regions. The localization of P5 and P6 was assessed by this technique. As shown in Fig. 6, the labeling of the thylakoid membranes was much weaker in the mutants (B and D) than in the WT (A and C). The unstacked membrane regions were limited on one side by swollen lumen (clear to electrons) and on the other side by stroma (dense to electrons) while the stacked membrane regions were localized in the piles of membranes limited by two swollen lumen. For P6, the densities of labeling in the stacked membrane regions were 1.4 particles/μm in the mutants lacking D1 or D2 vs. 8.1 in the WT, with a background labeling of 0.1 particles/μm estimated on the thylakoid membranes of the FUD34 mutant which is totally deficient in P6. For P5, the densities of labeling in the stacked membrane regions were 0.7 particles/μm in the mutants lacking P6 vs. 7.5 in the WT, with a background labeling of 0.3 particles/μm estimated on the thylakoid membranes of the FUD47 mutant which is highly deficient in P5. A quantitative estimation of the extent of segregation of P5 and P6 between the stacked and unstacked membrane regions is given in Table I. According to the representation we have used, a uniform distribution would yield values of about one, whereas distributions favoring stacked or unstacked membrane regions would give values, respectively, below and above one. Table I shows that P5 and P6 were predominantly in the stacked membrane regions in the mutants but not sequestered as strictly as in the WT case.

**Localization of the OEE Subunits on the Thylakoid Membrane**

As reported previously in *C. reinhardtii* (22, 30), mutant cells, unable to accumulate the PSII core because of the absence of synthesis of P6, D1, or D2, displayed an unaltered
Figure 6. Immunogold labeling of α-PSII core antenna subunit antibodies in WT and PSII core mutants. α-P6 antibody (1/200) on WT (A) and FUD7 mutant (B) and α-P5 antibody (1/50) on WT (C) and F34 mutant (D). Arrows indicate unstacked membranes. Bar, 0.25 μm.

accumulation of the OEE subunits in their mature form (Fig. 7 B). However, we observed that purified thylakoid membranes from these mutants were depleted of OEE subunits but had the same content of P6 as whole cells. This was indicative either of a nonthylakoidal localization of the OEE subunits detected in whole cells or of their loose binding to the thylakoid membranes, causing loss during the purification.
Table 1. Immunogold Labeling Densities

| Subunit | WT     | F34 and FUD34 | FUD7 and FUD47 |
|---------|--------|---------------|----------------|
|         | d/a,  | d/a,          | d/a,           |
| P5      | 0.26 ± 0.13 | 0.49 ± 0.11 | -              |
| P6      | 0.18 ± 0.02 | -             | 0.61 ± 0.15    |
| OEE1    | 0.15 ± 0.03 | 0.21 ± 0.07   | 0.25 ± 0.08    |
| OEE2    | 0.34 ± 0.07 | 1.65 ± 0.59   | 0.54 ± 0.09    |

Ratio of immunogold labeling densities in the unstacked over the stacked regions (d/a) in WT and PSII mutant thylakoid membranes.

Immunocytochemical analysis of the OEE subunits showed that they were chloroplast localized in the mutants as in the WT. This is exemplified for OEE2 at low magnification in Fig. 8 A: most of the gold particles were found in the chloroplast, while the cytoplasm, nucleus, and mitochondria were devoid of labeling. However, the α-OEE2 antiserum produced some extrachloroplastic labeling which was next to the plasma membrane in the mutants as in the WT. At higher magnification (Fig. 8, B–E), one observes that α-OEE1 and α-OEE2 antisera labeled the thylakoid membranes of the mutants as heavily as in the WT. We then calculated the labeling densities in the stacked and unstacked regions of the thylakoid membranes in each mutant. Table I shows that OEE1 was found mainly in the stacked regions. Surprisingly the localization of OEE2 depended on the synthesis of P6: OEE2 was largely retained in the unstacked membrane regions in the two mutants lacking P6, whereas it was found in the stacked membrane regions in the two mutants showing P6 synthesis but lacking either D1 or D2.

Subunits OEE1 and OEE2/OEE3 Bind at Two Different Sites on the PSII Core Complex; Stabilization of PSII Core by OEE Binding

We compared three low-fluorescent nuclear mutants deficient in oxygen evolution that were partly characterized previously (37, 38). As illustrated by immunoblots of cells in Fig. 9 A, mutants BF25 and FUD39 specifically lacked synthesis of OEE2, whereas the FUD44 mutant lacked synthesis of OEE1.

Examination of the content of OEE subunits in thylakoid membranes of these mutants (Fig. 9 A) revealed that there was no membrane binding of OEE3 in the absence of OEE2, whereas OEE1 binding still occurred. On the other hand, the absence of OEE1 in the FUD44 mutant did not prevent membrane binding of OEE2 and OEE3. These results clearly demonstrate the existence of two binding sites: one for OEE1 and another for OEE2 to which OEE3 binds.

The amounts of OEE2 and OEE3 retained on the thylakoid membranes from the FUD44 mutant were, however, smaller than in the WT (Fig. 9 A) and may be accounted for by the decreased content of PSII core subunits in this mutant (Fig. 9 B). The OEE2-lacking FUD39 mutant also showed such a decrease in P6, D1, and D2, whereas the OEE2-lacking BF25 did not. Spectroscopic estimation of PSII centers, based on the PSII charge separation detected at 515 nm (63), confirmed that the FUD44 and FUD39 mutants contained...
Figure 8. Immunogold labeling of α-OEE subunit antibodies in WT and PSII core mutants. (A) α-OEE2 antiserum 1/25 in F34. Most of the gold particles are concentrated on the chloroplast (c) and are absent from the nucleus (n), mitochondria (m), and cytoplasm (cy); some extrachloroplastic labeling appears near the plasma membrane. (B-D) α-OEE2 antiserum 1/25. Most of the gold particles are on the stacked membranes in FUD47 (C) as in WT (D), while in F34 (B) most of them (arrowheads) are present on the unstacked membranes (arrows). (E and F) α-OEE1 antibody 1/100 in WT (E) and FUD34 (F). Most of the gold particles are on the stacked membranes. For quantification, see Table I. Bars: (A) 0.5 μm; (B–F) 0.3 μm.
only 30% of the PSI/primary activity found in the WT on a chlorophyll basis as compared with ~65% in the BF25 mutant. Since the rate of synthesis of the PSI core subunits in these mutants was similar to that in WT (data not shown), we conclude that the decrease in their PSI content is indicative of a lower PSI/core stability.

The α-P6 immunoblots of Fig. 9 B revealed a lower molecular mass form of P6 in cells and in purified thylakoid membranes of OEE-lacking mutants, particularly in the absence of OEE2. It was taken as indicative of an interaction between P6 and OEE2 (and to a lesser extent OEE1), in the absence of which partial cleavage of P6 may occur.

### Discussion

The comparative analysis of several PSII mutants unable to synthesize either a reaction center subunit, a core subunit, or an OEE subunit gave new insights on the processes of synthesis, accumulation, and assembly of the subunits of the PSII protein complex in the thylakoid membranes. The main characteristics of these PSII mutants are summarized in Tables II and III.

#### Translational and Posttranslational Control of the Accumulation of the PSII Core Subunits

We have observed in vitro that the trypsin sensitivity of several PSII core subunits was increased in the thylakoid membranes of PSII mutants that failed to accumulate the PSII core complex. This was indicative of the exposure of proteolytic sites on the PSII subunits, which were masked in the WT thylakoid membranes. Indeed, in the absence of D1 synthesis, we observed an increased turnover of D2 in vivo in the time range of our pulse–chase experiments (4 h). A similar increase in D2 turnover in the absence of either D1 or P5 synthesis was reported by Jensen et al. (28). However, we failed to observe an increased turnover of P5 and D2 in the absence of P6 synthesis as well as of P6 in the absence of D1 or D2 synthesis. An increased turnover of P6 has been reported in other PSII mutants from *C. reinhardtii* or maize (28, 33). However, in both cases the contrast between the amounts of labeled P6 in the mutants and in the WT was visible only 8–16 h after the end of the pulse labeling. Our pulse-labeling conditions, in the presence of an inhibitor of cytoplasmic translation, limited the chase length to 4 h (see Materials and Methods).

Still, we observed that exogenous, and in some instances endogenous, proteases have an increased access to the PSII core subunits remaining in the thylakoid membranes of the mutants that fail to accumulate the complex. A change in the interactions between the PSII subunits could contribute in two ways to an increased protease sensitivity: polypeptide regions normally engaged in (a) the interactions with cofactors or (b) between the subunits of the mature complex may become accessible to proteases in the absence of proper complex assembly. Indirect effects are also conceivable, resulting for instance from conformational changes or enhancement of molecular movements in the absence of assembly.

Besides this posttranslational control on the accumulation of the PSII subunits, it should be mentioned that, in several *C. reinhardtii* mutants unable to synthesize one PSII subunit, deficiencies in some of the other PSII subunits may be caused by a decrease in their rate of synthesis. Such a translational

### Table II. Characteristics of Mutants Unable to Synthesize a PSII Core Subunit

| Strain | P5 | P6 | D1 | D2 | L8 | OEE | Detected synthesis | Accumulation | Detected assembly | Chlorophyll binding |
|--------|----|----|----|----|----|-----|---------------------|--------------|-------------------|---------------------|
| F34    | +  | (e) + | + | + | + | (+) | (+) | (e) | e | e | e | + | Yes | Yes |
| FUD34  | + | - | + | + | + | (+) | (+) | - | e | e | ND | + | Yes | Yes |
| FUD7   | (+) | + | - | + | + | e | (+) | - | - | ND | + | No | Yes |
| FUD47  | (+) | + | (+) | - | + | e | (+) | (e) | - | + | ND | + | No | Yes |

Polypeptide synthesis and accumulation were indicated: (−) absent; ([e]) traces; (e) very low; ([+] impaired; (+) level similar to the WT; and (ND) not determined. The ability of the PSII core subunits to assemble (Detected assembly) and of P5 or P6 to bind chlorophyll (Chlorophyll binding) are presented.

* OEE subunits are almost totally lost after thylakoid purification.
control of one PSII subunit over another has been reported for D2 over D1 by Erickson et al. (19) and for D1 over P5 by Jensen et al. (28). Translational control is also apparent in our study of the FUD7 mutant, where the absence of D1 synthesis impaired synthesis of P5 (4), and the FUD47 mutant, where the absence of D2 synthesis impaired that of D1 and probably as a consequence that of P5. Interestingly, P6 synthesis was unaltered in these two mutants as was the synthesis of P5, D1, and D2 in the F34 and FUD34 mutants which were unable to synthesize P6. The independence between P6 and D2 synthesis indicates that their respective genes psbC and psbD, which are well separated in the plastid genome of *C. reinhardtii* (46), are expressed independently. It is also the case in the cyanobacterium *Synechocystis* (56) although these two genes overlap as in most plant genomes (2, 8, 24). Therefore this independence of P6 synthesis seems to be a general feature in PSII biogenesis.

### Biogenesis of D1 and D2 Subunits

The sequence homologies between D1, D2, and the L and M subunits of the bacterial reaction center (39, 53) and, more recently, the purification of D1/D2 photoactive particles (44) have demonstrated that D1 and D2 are the two subunits of the PSII reaction center that bind the primary reactants. The lack of assembly of the PSII core subunits in the mutants unable to synthesize either D1 or D2 suggests that assembly of the PSII reaction center is a prerequisite for PSII core assembly. Our study points out some differences between D1 and D2 in the process of PSII core biogenesis. Traces of D1 accumulated in the mutant unable to synthesize D2, whereas no D2 accumulated in the mutant unable to synthesize D1. Indeed there was an increased proteolytic disposal of D2 in the absence of D1. However, as noted above, D1 synthesis was drastically decreased in the absence of D2 whereas the reverse situation did not occur. Thus stoichiometric accumulation of the PSII reaction center subunits appears to depend both on a translational control of D1 by D2 and on a posttranslational control of D2 by D1. It has been reported that there was an increased turnover of D1 during photoinhibition without significant changes in the turnover of D2 (31). Presumably, in this case, rapid replacement of D1 occurs through insertion into the membrane of the new polypeptides triggered by the D2 present in the thylakoid membranes with which they would subsequently assemble.

### Biogenesis of P5 and P6

The two core antenna subunits P5 and P6 are quite homologous with ~30% conserved amino acids in their membrane-spanning regions (2, 24). They bind the same amounts of chlorophyll and the resulting chlorophyll–protein complexes have very similar spectral characteristics (9). We have observed in vivo that binding of chlorophyll is an early event in the biogenesis of these subunits. It was detected within the first 5 min of insertion into the membrane of P5 and P6 and occurred independently of PSII core assembly. This is much faster than other posttranslational modifications, such as PSII phosphorylation which occurs with a half-time of ~30 min (12). In barley, photoconversion of protochlorophyllide into chlorophyll paralleled the synthesis of PSII core antenna subunits, suggesting that binding of chlorophyll is concurrent with polypeptide synthesis and controls the translation (29). We conclude from the early binding of chlorophyll that the low stability of P5 and P6 in the PSII mutants has to be accounted for by a primary process rather than the lack of binding of chlorophyll.

We reported previously that P6 can be more easily detached than P5 from the rest of the PSII core (58). Our present study reveals additional differences between the two core antenna subunits. Whereas we, and others (4, 28), observed a translational control of P5 by D1, it is apparent from our experiments that synthesis of P6 occurs totally independently. In addition, we have shown that P5 can assemble with D1 and D2 (and L8) in the absence of P6. Such a partial assembly could probably allow the primary charge separation to occur as suggested by the detection of some primary charge separation in a *Synechocystis* mutant inactivated in psbC (56). Assembly of the PSII core therefore appears to occur in two steps: (a) assembly of P5, D1, and D2 and then (b) assembly with P6. Since we observed that the two building blocks, P6 and P5/D1/D2, can segregate independently in the stacked membrane regions, it is likely that the second step of assembly occurs in the stacked regions whereas the first step might occur in the unstacked regions where the subunits are inserted cotranslationally (23).

### Biogenesis of the OEE Subunits

The OEE subunits were processed to their mature form and accumulated in the PSII core mutants as in the WT. Such an observation was reported in *C. reinhardtii* (22, 47) and (for OEE1) in maize, grown in the dark or under far red light, which failed to accumulate PSII cores (51). In agreement with the conclusion that OEE1 was accumulated in the lumen of far red–grown maize thylakoids because it was protected from trypsin in right side out vesicles (51), we observed by immunogold labeling that the three OEE subunits were found on the thylakoid membranes of the PSII mutants as in the case of the WT. They were not floating in the lumen of the thylakoids, but stuck to the membranes. However, they interacted only loosely with the membranes since they were lost after purification of the thylakoids. These observations

---

### Table III. Characteristics of Mutants Unable to Synthesize an OEE Subunit

| Strain | Detected synthesis | Accumulation | Membrane bound* |
|--------|--------------------|--------------|-----------------|
|        | PSII | OEE1 | OEE2 | OEE3 | PSII | OEE1 | OEE2 | OEE3 | PSII | OEE1 | OEE2 | OEE3 |
| FUD44  | +    | -    | +    | +    | (+)  | -    | +    | +    | -    | (+)  | ( +) | ( +) |
| BF25   | +    | +    | -    | +    | (+)  | +    | -    | +    | (+)  | -    | +    | (+)  |
| FUD39  | +    | +    | -    | +    | (+)  | +    | -    | +    | (+)  | -    | +    | (+)  |

Polypeptide synthesis and accumulation are indicated: (−) absent; ([+] impaired; and (+) level similar to WT.

* OEE subunits when membrane bound are proportional to PSII accumulation.

† Similar for the PSII core subunits P5, P6, D1, D2, and L8.
suggest that the proteolytic activity on the luminal side of the membranes is lower than that on the stromal side; it has been shown, for instance, that in absence of the rubisco large subunits, the unassembled small subunits are rapidly degraded in the stroma (49).

Surprisingly, OEE1 and OEE2 were not uniformly distributed along the membranes of the mutants. They were found in the stacked membrane regions in mutants lacking synthesis of either D1 or D2, whereas OEE2, but not OEE1, was retained in the unstacked membrane regions in the two mutants lacking P6 synthesis. This observation excludes that the apparent membrane binding of the OEE subunits viewed after immunogold labeling would originate from an artificial cross-linking between membrane proteins and the OEE subunits floating in the lumen because of the embedding procedure. On the other hand, it points to the existence of binding sites of low affinity for OEE1 in the stacked membrane regions. These may be some of the purported OEE binding proteins proposed by Ljungberg et al. (34) or some lipids specifically found in the stacked membrane regions (43). The localization of OEE2 in the stacked membrane regions in the mutants lacking D1 or D2, and not in the mutants lacking P6, suggests a specific interaction between OEE2 and P6 which is, however, difficult to understand owing to the substoichiometric amounts of P6 in these mutants. Such an interaction is, however, consistent with the protection of P6 against proteolytic degradation in the presence of OEE2 (a degradation product of P6 was particularly abundant in mutants lacking OEE2).

Analysis of nuclear mutants lacking synthesis of either OEE1 or OEE2 gave further insight on the organization of the OEE subunits on the thylakoid inner surface. The immunoblots performed on the thylakoid membranes from these mutants revealed a stoichiometric binding on the PSII core of OEE2 and OEE3 in the absence of OEE1. In contrast, OEE3 binding was lost in the absence of OEE2 whereas OEE1 stoichiometric binding still occurred. Thus OEE1 and OEE2 have distinct binding sites on the PSII core, and OEE3 interacts with the PSII core by binding to OEE2. Such an interaction between OEE2 and OEE3 has been observed in reconstitution experiments (40). However, similar experiments have led to the opposite conclusion that the binding site of OEE2 is located on OEE1 (41). This latter view is clearly not supported by the present study.

The binding sites of the OEE subunits on the PSII core may involve several of its subunits. A cross-link between P5 and OEE1 was observed by two groups (5, 17) whereas experiments based on protease sensitivity were consistent with an interaction between OEE1 and P6 (26). In a mutant of Scenedesmus, which accumulates PSII cores but lacks the COOH-terminal processing of D1, there was an impaired binding of OEE2 and OEE3 (16). Therefore the OEE2 binding site may implicate D1, in addition to P6, as we discussed above.

Mayfield et al. (37, 38) reported that OEE1, but not OEE2, was required for PSII core stability in the thylakoid membranes. We have shown here that the amounts of PSII cores accumulated by the two mutants lacking synthesis of OEE2 were widely different, but always below that in the WT. These observations indicate that OEE2 also contributes to PSII stability. The mechanism by which the absence of an OEE subunit decreases the stability of the PSII cores in the membrane remains to be elucidated.

**Conclusion**

We have focused on various factors which may control the stability of the PSII subunits in *C. reinhardtii*. Among these parameters were (a) their ability to assemble in the thylakoid membranes; (b) the binding of chlorophyll or of phosphate groups, which may stabilize their proper conformation; and (c) their change in lateral distribution in the thylakoid membranes, which may control the exposure of the polypeptides to endogenous proteases. These aspects of PSII biogenesis were compared in several PSII mutants that failed to accumulate the PSII protein complex. All of them still showed binding of chlorophyll to the residual PSII core subunits. Therefore the lack of accumulation of the PSII protein complexes in the PSII mutants cannot be accounted for by a change in binding of chlorophyll to the PSII core subunits. We can also exclude that the instability of the PSII subunits was due to the absence of PSII phosphorylation since nonphosphorylated PSII core does accumulate in an LHC-deficient mutant (57, 59). A change in lateral distribution could be involved in a decreased stability of the PSII cores since we observed that P5 and P6 were less strictly sequestered in the stacked membrane regions in these mutants than in the WT. But the major contribution to the lower stability of PSII subunits in the mutants seems to arise from their failure to assemble properly. Indeed, we have observed an increased sensitivity to trypsin of the PSII core subunits with the decrease in PSI assembly in the mutants. In some instances, turnover studies demonstrated that endogenous proteases have an increased access to the PSII subunits in such mutants. The results discussed in this paper led us to distinguish four main steps in the process of PSII unit assembly (Fig. 10) that correspond to an increased stability of the PSII subunits.

In step one, the PSII core subunits, which are translated on thylakoid-bound ribosomes in the unstacked membrane regions (36), are synthesized in a concerted manner for P5/D1/D2 and independently for P6, with early binding of chlorophyll to P5 and P6. The OEE subunits are imported in the chloroplast, processed, and translocated independently in the thylakoid lumen where they can interact loosely with the membrane.

In step two, which occurs probably soon after step one, presumably in the unstacked membrane regions, partial stabilization of the PSII core subunits occurs by assembly of P5, D1, and D2.

In step three, further stabilization occurs by assembly of P6 with the P5/D1/D2 moiety of the PSII core, probably in the stacked membrane regions. The PSII core then associates with the OEE subunits through two independent binding sites of high affinity: one for OEE1 and another for OEE2 that may involve P6 (OEE3 binds via OEE2). This association (PSII complex) increases the stability of the PSII core.

Step four describes the assembly of the active PSII complex with its peripheral antenna, the LHC, leading to the formation of the PSII unit. The rationale for this step relies on previous studies. It is known that the PSII complex and the LHC are accumulated independently in the thylakoid membranes. However, some posttranslational modifications of PSII subunits seem to involve the presence of the LHC. Delepelaire has shown that there are slow posttranslational modifications of P6, D2, and two small integral membrane PSII subunits, L5 and L6 (12), that are due to polypeptide phosphorylations (14, 59). We observed previously that such...
Figure 10. Proposed model of PSII assembly. (Step one) Synthesis of PSII core subunits is concerted for D2, D1, and P5 and is independent for P6. OEE subunits are translocated independently. (Step two) PSII core assemblies in two steps: assembly of P5/D1/D2 (in stroma lamellae?) and assembly of (P5/D1/D2)/P6 (in stacked regions?) which stabilizes the reaction center. (Step three) Fully active PSII complex (PSII core and OEE subunits) assembles with further stabilization. (Step four) PSII unit (PSII complex and LHC) assembles with concomitant PSII phosphorylation. Schematic polypeptide structures are used in steps one and two; they are based on the predicted membrane-spanning segments and on the NH2-terminal localization. The bracket indicates the concerted synthesis of D2, D1, and P5. The chlorophyll bound to the PSII core antenna and to the LHC is symbolized by diamonds. Interaction sites between OEE subunits and the PSII core are drawn taking into account the various reports on OEE binding sites (5, 16, 17, 26). Proper OEE binding is not a prerequisite for PSII core (as well as for PSII unit) assembly and is therefore drawn in dashed lines.

a PSII phosphorylation was lacking in the absence of PSII core accumulation (14) as well as in the absence of LHC accumulation (57, 59). We have then suggested that this phosphorylation is associated with the formation of the PSII unit. Such a PSII phosphorylation process may, in addition, facilitate the regulation of LHC-PSII interactions during state transitions (1, 50, 59, 61).

We thank J. Lavergne and J.-L. Popot for critical reading of the manuscript and H. Couratier for the high quality of the photographs.

This work was supported by the Centre National de la Recherche Scientifique.

Received for publication 18 November 1988 and in revised form 21 April 1989.

References

1. Allen, J. F., J. Bennett, K. E. Steinback, and C. J. Arntzen. 1981. Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature (Lond.). 291: 25-29.

2. Alt, J., J. Morris, P. Westhoff, and R. G. Herrmann. 1984. Nucleotide sequence of the clustered genes for the 44 kD chlorophyll a apoprotein and the “32 kD”-like protein of the photosystem II reaction center in the spinach plastid chromosome. Curr. Genet. 8:597-606.

3. Bennoun, P., B. A. Diner, F.-A. Wollman, G. Schmidt, and N.-H. Chua. 1981. Thylakoid polypeptides associated with photosystem II and particles. In Photosynthesis III. G. Akoyunoglou, editor. Balabanhan International Science Service, Philadelphia. 839-849.

4. Bennoun, P., M. Spierer-Herz, J. Erickson, J. Girard-Bascou, Y. Pierre, M. Delosme, and J.-D. Rochaix. 1985. Characterization of photosystem II mutants of Chlamydomonas reinhardtii lacking the psbA gene. Plant Mol. Biol. 6:151-160.
5. Bricker, T. M., W. R. Odom, and C. B. Queirolo. 1988. Close association of the 33 kDa extrinsic protein with the appronote of CP43 in photosystem II. FEMS (Fed. Eur. Biochem. Soc.) Lett. 231:111-117.
6. Burnett, W. N. 1981. Western blotting: electrophoretic transfer of proteins from sodium dodecyl sulfate polyacrylamide gels to modified nitrocellulose paper. Annu. Rev. Biochem. 50:85-91.
7. Carlemalm, E., R. Marvati, and W. Villiger. 1982. Resin development for electron microscopy and an analysis of embedding at low temperature. J. Microsc. 128:122-143.
8. Chisholm, D., and J. G. K. Williams. 1988. Nucleotide sequence of psbC, the gene encoding CP-43 chlorophyll a-binding protein of photosystem II in the cyanobacterium Synechocystis. Plant Mol. Biol. 10:293-301.
9. Choquet, Y., C. de Vitry, P. Delepelaire, F.-A. Wollman, and P. Tape. 1988. Spectroscopic characterization of the PSII chlorophyll-protein complexes. Biochim. Biophys. Acta. 934:389-395.
10. Chua, N.-H., and P. Bennoun. 1975. Thylakoid membranes polypeptides of Chlamydomonas reinhardtii: wild-type and mutants strains deficient in photosystem II reaction center. Proc. Natl. Acad. Sci. USA. 72:2175-2179.
11. Delepelaire, P. 1984. Partial characterization of the biosynthesis and in processing of the 32 kDa chloroplast protein. Biochim. Biophys. Acta. 761:111-115.
12. Delepelaire, P., and N.-H. Chua. 1979. Lithium dodecyl sulfate/polyacrylamide gel electrophoresis of thylakoid membranes at 4°C: characterization of two additional chlorophyll a-protein complexes. Proc. Natl. Acad. Sci. USA. 76:111-115.
13. Delepelaire, P., and F.-A. Wollman. 1985. Correlations between fluorescence and phosphorylation changes in thylakoid membranes of Chlamydomonas reinhardtii in vivo: a kinetic analysis. Biochim. Biophys. Acta. 809:277-283.
14. Diner, B. A., and F.-A. Wollman. 1980. Isolation of highly active photosystem II particles from a mutant of Chlamydomonas reinhardtii. Eur. J. Biochem. 107:21-52.
15. Diner, B. A., D. F. Ries, B. N. Cohen, and J. G. Metz. 1988. COOH-terminal processing of polypeptide D1 of the photosystem II reaction center of Scenedesmus obliquus is necessary for the assembly of the oxygen-evolving complex. J. Biol. Chem. 263:8972-8980.
16. Enami, I., K. Sato, and S. Katoh. 1987. Cross-linking between the 33 kDa extrinsic protein and the 47 kDa chlorophyll-carrying protein of the PSII reaction center core complex. FEMS (Fed. Eur. Biochem. Soc.) Lett. 128:266-161.
17. Erickson, J. M., M. Rahire, and J.-D. Rochaix. 1984. Chlamydomonas reinhardtii gene for the 32,000 mol. wt. protein of photosystem II contains four large introns and is located entirely within the chloroplast repeat. EMBO (Eur. Mol. Biol. Organ.) J. 3:701-706.
18. Erickson, J. M., M. Rahire, P. Malnoe, J. Girard-Bascou, Y. Pierre, P. Bennoun, and J.-D. Rochaix. 1986. Lack of the D2 protein in a Chlamydomonas reinhardtii psbD mutant affects photosystem II stability and expression of the COOH terminal. J. Biol. Chem. 261:1113-1117.
19. Girard-Bascou, J. 1988. Thèse d’état. Contribution à l’étude génétique de l’appareil photosynthétique et de l’usage vert de Chlamydomonas reinhardtii. Université Paris Sud, Orsay, France. 140 pp.
20. Greer, B. R., D. Cam, and J. Van Houten. 1982. The chlorophyll-protein complexes of Atnabulata: a novel chlorophyll a/b complex which forms oligomers. Biochim. Biophys. Acta. 681:248-255.
21. Greer, K. L., F. G. Plumley, and G. W. Schmidt. 1986. The water oxidation complex of Chlamydomonas: accumulation and maturation of the largest subunit in photosystem II mutants. Plant Physiol. 82:114-120.
22. Hirsch, D., and A. Michaels. 1985. The chloroplast 32 kDa protein is synthesized on thylakoid-bound ribosomes in Chlamydomonas reinhardtii. FEBS (Fed. Eur. Biochem. Soc.) Lett. 187:240-244.
23. Holschuh, K., W. Bottomley, and P. R. Whitfeld. 1984. Structure of the spinach chloroplast genes for the D2 and 44 kDa reaction-center proteins of photosystem II and for RNA-Ser(UGA). Nucleic Acids Res. 12:8819-8834.
24. Holschuh, K., W. Bottomley, and P. R. Whitfeld. 1984. Structure of the spinach chloroplast genes for the D2 and 44 kDa reaction-center proteins of photosystem II and for RNA-Ser(UGA). Nucleic Acids Res. 12:8819-8834.
25. Hovanessian, A. G., J. Galabru, Y. Riviere, and J.-L. Montaguier. 1988. The chloroplast-encoded D1 protein of photosystem II: genetic and molecular analysis. EMBO (Eur. Mol. Biol. Organ.) J. 7:521-526.
26. Hovanessian, A. G., J. Galabru, Y. Riviere, and J.-L. Montaguier. 1988. The chloroplast-encoded D1 protein of photosystem II: genetic and molecular analysis. EMBO (Eur. Mol. Biol. Organ.) J. 7:521-526.
27. Hovanessian, A. G., J. Galabru, Y. Riviere, and J.-L. Montaguier. 1988. The chloroplast-encoded D1 protein of photosystem II: genetic and molecular analysis. EMBO (Eur. Mol. Biol. Organ.) J. 7:521-526.
28. Jensen, K. H., D. L. Herrin, F. G. Plumley, and G. W. Schmidt. 1986. The water oxidizing complex of photosystem II: genetic and molecular analysis. Trends Biochem. Sci. 10:122-124.
29. Kyle, D. J., I. Ohad, and C. J. Arntzen. 1984. Membrane protein damage and repair: selective loss of a chloroplast-encoded chlorophyll-binding protein during translation in plant chloroplast biogenesis. J. Biol. Chem. 261:11138-11145.
30. Kuchka, M. R., S. P. Mayfield, and J.-D. Rochaix. 1988. Nuclear muta-
of the photosystem II core complex in genetically engineered mutants of the cyanobacterium Synechocystis sp. PCC 6803. Photosynth. Res. 17:97–113.

57. de Vitry, C., and F.-A. Wollman. 1988. Changes in phosphorylation of thylakoid membrane proteins in light-harvesting complex mutants from Chlamydomonas reinhardtii. Biochim. Biophys. Acta. 809:277–283.

58. de Vitry, C., F.-A. Wollman, and P. Delepelaire. 1984. Function of the polypeptides of the photosystem II reaction center in Chlamydomonas reinhardtii. Biochim. Biophys. Acta. 767:415–422.

59. de Vitry, C., B. A. Diner, and Y. Lemoine. 1987. Chemical composition of photosystem II reaction centers (PSII); phosphorylation of PSII polypeptides. In Progress in Photosynthesis Research. J. Biggins, editor. Martinus Nijhoff publishers, Dordrecht, Netherlands. 2:105–108.

60. Westhoff, P., C. Jansson, I. Kelin-Hippas, R. Berzborn, C. Larsson, and S. G. Bartlett. 1985. Intracellular coding sites of polypeptides associated with photosynthetic oxygen evolution of photosystem II. Plant Mol. Biol. 4:137–146.

61. Wollman, F.-A., and P. Delepelaire. 1984. Correlation between changes in light energy distribution and changes in thylakoid membrane polypeptide phosphorylation in Chlamydomonas reinhardtii. J. Cell Biol. 98:1–7.

62. Wollman, F.-A., and B. A. Diner. 1980. Cation control of fluorescence emission, light scatter and membrane stacking in pigment mutants of Chlamydomonas reinhardtii. Arch. Biochem. Biophys. 201:646–659.

63. Wollman, F.-A., J. Olive, P. Bennoun, and M. Recouvrer. 1980. Organization of the photosystem II centers and their associated antennae in the thylakoid membranes: a comparative ultrastructural, biochemical and biophysical study of Chlamydomonas wild-type and mutants lacking in photosystem II reaction centers. J. Cell Biol. 87:728–735.

64. Zurawski, G., H. J. Bohnert, P. R. Whitfeld, and W. Bottomley. 1982. Nucleotide sequence of the gene for the Mr 32,000 thylakoid membrane protein from Spinacea oleracea and Nicotiana debneyi predicts a totally conserved primary translation product of M, 38,950. Proc. Natl. Acad. Sci. USA. 79:7699–7703.