Role of the RCII-D1 Protein in the Reversible Association of the Oxygen-evolving Complex Proteins with the Lumenal Side of Photosystem II*

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The nuclear-encoded proteins of the oxygen-evolving complex (OEC) of photosystem II are bound on the luminal side of the thylakoid membrane and stabilize the manganese ion cluster forming the photosystem II electron donor side. The OEC proteins are released from their binding site(s) following light-induced degradation of reaction center II (RCII)-D1 protein in Chlamydomonas reinhardtii. The kinetics of OEC proteins release correlates with that of RCII-D1 protein degradation. Only a limited amount of RCII-D2 protein is degraded during the process, and no loss of the core protein CP43 and CP47 is detected. The release of the OEC proteins is prevented when the photoinactivated RCII-D1 protein degradation is retarded by addition of 3-(3,5-dichlorophenyl)-1,1-dimethylurea or by a high PQH2/PQ ratio prevailing in membranes of the plastocyanin-deficient mutant Ac 208. The released proteins are not degraded but persist in the thylakoid lumen for up to 8 h and reassociate with photosystem II when new D1 protein is synthesized in cells exposed to low light, thus allowing recovery of photosystem II function. Reassociation also occurs following D1 protein synthesis in darkness when RCII activity is only partially recovered. These results indicate that (i) the D1 protein participates in the formation of the luminal OEC proteins binding site(s) and (ii) the photoinactivation of RCII-D1 protein does not alter the conformation of the donor side of photosystem II required for the binding of the OEC proteins.

The nuclear encoded 33-, 23-, and 16-kDa polypeptides of the oxygen-evolving complex (OEC) stabilize the manganese cluster of photosystem II (PSII) (reviewed by Ghanotakis and Yocum (1990) and Vermaas et al. (1993)). These proteins are synthesized in the cytosol and are translocated across the chloroplast outer envelope to the luminal space of the thylakoid membranes (Brock et al., 1995). The translocation process which involves participation of bipartite transit sequences and processing of the precursor proteins, has been previously investigated in detail (reviewed in Robinson and Klösgen (1994)). The OEC proteins are bound to PSII on the luminal side of the thylakoid membrane and can be released from the binding site(s) by high Tris concentration at alkaline pH as well as rebinding under appropriate conditions including presence of chloride and calcium ions and light (Vermaas et al., 1993; Tamura et al., 1989). The amount of these proteins in mature differentiated thylakoids is not stoichiometric with the PSII content and apparently an excess of the OEC proteins exists in the form of a free pool (Ettlinger and Theg, 1991).

The OEC proteins are present in thylakoids of etiolated barley (Hashimoto et al., 1993) as well as in Chlamydomonas mutant cells lacking a functional PSII (Rochaix and Erickson, 1988). The Chlamydomonas y-1 mutant does not synthesize chlorophyll and lacks thylakoid membranes when grown in darkness. In these cells as well as in the wild type, OEC proteins accumulate in the dark and their levels vary only slightly during greening (Malone et al., 1988).

While the levels of the OEC proteins seem to be independent of their functional association with an assembled PSII unit, it is possible that the unbound proteins turn over at rates higher than those of the proteins associated with the thylakoid membrane (Palomares et al., 1993). Results of in vitro experiments in which isolated thylakoids were exposed to photoactivation and degradation of the RCII-D1 protein demonstrated release of the bound manganese (Virgin et al., 1988; Hundal et al., 1990) in agreement with the fact that the carboxyl end of the RCII-D1 protein is involved in the stabilization of the manganese cluster of PSII (Metz et al., 1986; Diner et al., 1988). Under these conditions the OEC proteins disassociate from the remaining components of PSII concomitant with the manganese release. Thus the question arises what is the fate of the OEC proteins during the process of light-induced photoactivation of PSII and degradation of the RCII-D1 protein in vivo. If the free OEC proteins turn over faster than those associated with a functional PSII, one would expect an increase in the turnover of the OEC proteins following degradation of the RCII-D1 protein in vivo. Furthermore, it is not clear whether the release of the OEC proteins occurs only as a result of the D1 protein degradation and disassembly of the RCII or the release is already induced by an alteration of the RCII donor side following irreversible photoactivation of photosystem II. In the present work we have investigated the fate of the OEC proteins during the process of photoactivation. RCII-D1 protein degradation, and recovery of activity of the PSII complex in control and mutants of the unicellular green alga Chlamydomonas reinhardtii. The results demonstrate that the level of the OEC proteins remains constant during photoactivation and subsequent degradation of the RCII-D1 protein in the absence of cytosolic protein translation activity. The OEC proteins remain bound to the luminal side of the membranes following irrevers-
ible photoinactivation of photosystem II, but are released from the binding site(s) following degradation of the RCII-D1 protein and reassociate with the membrane binding site(s) following synthesis and assembly of new RCII-D1 protein.

MATERIALS AND METHODS

Cell Growth, Photoinhibition, and Recovery Experiments—C. reinhardtii y-1 (control) and mutant cells Ac500 and T44 were grown in semicontinuous or batch cultures in a mineral medium containing sodium acetate as a carbon source as described previously (Ohad et al., 1967). Ac500 is a plastocyanin-less mutant (Quinn et al., 1993). Chlamydomonas T44 is a temperature-sensitive mutant and does not accumulate PSI core proteins when grown at 37°C (Reisman et al., 1986).

Cells were harvested in the late exponential phase of growth. The cells were washed by centrifugation in fresh growth medium and were resuspended in the same medium at a final chlorophyll concentration of 30 μg·mL⁻¹. Photoinhibition and recovery were carried out as described before (Zer and Ohad 1995). To prevent chloroplast protein synthesis, chloramphenicol (10 μg·mL⁻¹; Sigma) was added as indicated at a final concentration of 200 μg·mL⁻¹ (Schuster et al., 1988). After photoinhibition, the cells were pelleted by centrifugation and the pellet was extracted by addition of 10 μL cold acetone and broken by passing through an ice cooled French pressure cell operated at 600 p.s.i. The cell homogenate was freed from cell debris and unbroken cells by centrifugation at 10,000 × g for 2 min, and the thylakoid-enriched membrane fraction was sedimented by centrifugation at 15,000 × g for 20 min at 4°C. For total cell protein preparation, cells were pelleted by centrifugation, and the pellet was extracted by 90% (v/v) cold acetone in water to remove pigments and lipids. The denatured proteins were solubilized in sample buffer for electrophoretic separation. Equal amounts of protein were loaded on the gel, normal-ized on a chlorophyll basis (equivalent to 1 mol of chlorophyll per lane). The protein pattern was resolved by SDS-PAGE according to Laemmli (1970). After polyacrylamide gel electrophoresis, the separated polypeptides were electrotransferred to nitrocellulose membranes for immunodetection using monoclonal polyclonal antibodies (Hundal et al., 1990; Zer et al., 1994) and the enhanced chemiluminescence method (ECL). Exposure was carried out for different times to ensure linearity of the response and quantification of the immunoblots carried out by a computer-programmed densitometric scanning using NIH Image programs.

Fluorescence Kinetics Measurements—Photosynthetic electron flow was measured by fluorescence kinetics measurements carried out using a homemade, computer-assisted fluorimeter as described before (Schuster et al., 1984; Zer et al., 1994).

RESULTS

Light-dependent Degradation of RCII-D1 Protein Induces Release of OEC Proteins from the Thylakoid Membranes—Thylakoids isolated from Chlamydomonas cells exposed to photoinhibitory light (2,500 μmol·m⁻²·s⁻¹) for 4.5 h and thus having lost a considerable amount of the RCII-D1 protein, contain drastically reduced levels of the OEC proteins as compared with thylakoids isolated from control cells. However, the 33- and 23-kDa OEC protein content in the total cell protein extract of photoinhibited cells remains constant, and only a minor decrease is observed in the 16-kDa OEC protein level (Fig. 1). Thylakoids isolated by the same procedure from control cells evolve oxygen using artificial electron acceptors as expected if the oxygen-evolving complex is properly assembled (not shown; see also Schuster et al. 1988)). We have shown before that isolated Chlamydomonas thylakoids open during mild sonica-tion and close when the sonication ceases (Regitz and Ohad 1976). Release of the soluble unbound or loosely bound lumenal proteins from the isolated thylakoids may thus occur during breaking of Chlamydomonas cells and temporary opening of the membranes by passing the cell suspension through the French press. This is indicated by release of most of the lume

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**FIG. 1.** Correlation between the light-induced degradation of RCII-D1 protein and the release of OEC proteins from the lumenal binding site of the membranes. Cells were photoinhibited at 2500 μmol·m⁻²·s⁻¹ for 4.5 h in the presence of chloramphenicol. Samples of thylakoid membranes (lanes A) and of total cell proteins (lanes B) from control cells (C) and photoinhibited cells (PI) were separated by SDS-PAGE. Immunoblots were incubated with anti-OEC-33, anti-OEC-23, anti-OEC-16, anti-D1, or anti-plastocyanin (PC) antibodies.
maintains a high ratio of PQH2/PQ in the light (Gong and Ohad, 1994) and thus exhibits a high PQH2/PQ significantly retarded in the site. The degradation of photoinactivated RCII-D1 protein is mediated by binding of various ligands at the QB quinone binding site. It has been demonstrated before that the cleavage of photoinactivated RCII-D1 protein is regulatory light at 37°C was significantly reduced only in the T44 cells (Fig. 3). These results indicate that the release of OEC-33 from the membrane binding site(s) is related primarily to the disassembly of the PSII complex. It has been demonstrated before (Gong and Ohad, 1991) as long as the photoinactivation process is not degraded (Fig. 4). Similar results were obtained when estimating the OEC-33 content of thylakoids isolated from control (C) and photoinhibited (PI) cells by immunodecoration.

**Fig. 2.** Kinetics of light induced degradation of RCII-D1 protein and the dissociation of OEC-33 from PSII. Cells were photoinhibited for 3 h at 2000 μmol·m⁻²·s⁻¹ in the presence of chloramphenicol. Samples were taken at 0.75, 1.5, and 3 h. The samples were assayed by immunoblotting using anti-D1 and anti-OEC-33 polyclonal antibodies. Densitometric quantification, average of three independent experiments are shown. □, D1 protein; △, OEC-33 protein. The correlation coefficient between the degradation of the D1 protein and the release of OEC-33 is 0.99.

Photoinactivation of RCII-D1 Protein Is Not Sufficient to Induce Release of OEC-33 from the Thylakoid Membranes—Photoinactivation of RCII precedes the degradation of the RCII-D1 protein (Ohad et al., 1994). The question thus arises whether release of the protein from the binding site(s) on the luminal side of the membrane is induced by the process of photoinactivation. Alternatively, the release of OEC-33 can be a consequence of the RCII-D1 protein degradation and disassembly of the PSII complex. It has been demonstrated before that the cleavage of photoactivated RCII-D1 protein is regulated by binding of various ligands at the QB quinone binding site. The degradation of photoinactivated RCII-D1 protein is significantly retarded in Chlamydomonas mutants impaired in plastoquinol oxidation and thus exhibiting a high PQH2/PQ ratio when exposed to light (Zer et al., 1994). Similar results are obtained by addition of DCMU, a quinone analog competing with plastoquinone binding at the Qb site (Jansen et al., 1993; Gong and Ohad, 1995; Zer and Ohad, 1995). These experimental systems could thus be used to answer the above question. The C. reinhardtii Ac208 mutant lacks plastocyanin and thus maintains a high ratio of PQH2/PQ in the dark (Gong and Ohad, 1991). We found that OEC-33 is not released from the thylakoid fraction isolated from Ac208 cells photoactivated as described before (Gong and Ohad 1991) as long as the photo-inactivated RCII-D1 protein is not degraded (Fig. 4). Similar results were obtained when estimating the OEC-33 content of thylakoids isolated from the control phenotype Chlamydomonas y-1 cells photoactivated in the presence of DCMU (Fig. 5) as well as for the 23- and 16-kDa OEC proteins (data not shown). These results demonstrate that the binding of OEC proteins to the thylakoid membrane is not affected by the photoinactivation process per se. The dissociation occurs only when the RCII-D1 protein is degraded resulting in partial disassembly of the PSII complex in vivo (Adir et al., 1990).

Free OEC-33 Reassociates with the Thylakoid Membrane following Synthesis of RCII-D1 Protein—It is well established that recovery of PSII activity in photoactivated Chlamydomonas cells requires replacement of the degraded RCII-D1 protein and thus depends on a de novo chloroplast protein synthesis (Prasil et al., 1992; Leitsch et al., 1994; Zer et al., 1994). Since OEC-33 remains in the photoactivated cells it is possible that the protein may reassociate with the thylakoid membranes during the recovery process. Alternatively, the free OEC-33 may be slowly degraded and recovery of activity may require a de novo synthesis of OEC-33. To distinguish between these possibilities, Chlamydomonas cells were photoactivated and released from a photoactivated thylakoid membrane at 2500 μmol·m⁻²·s⁻¹ for 5 h with the addition of chloramphenicol. Samples were taken at times at indicated, and membrane preparations were assayed for the presence of OEC-33 and D1 proteins by immunodecoration (Panel A); Panel B, densitometric quantification of the immunoblots: squares, D1; triangles, OEC-33; closed symbols, y-1; open symbols, Ac208. Photoinactivation was measured as loss of variable fluorescence in whole cells and was more than 90% after 3 h of light exposure (not shown) as reported before (Zer et al., 1994).

**Fig. 3.** Correlation between dissociation of OEC-33 and light-induced degradation of RCII proteins in the ts T44 mutant cells. Chlamydomonas y-1 and T44 cells were photoinhibited at 2000 μmol·m⁻²·s⁻¹ for 1.5 h at 37°C without the addition of chloramphenicol. The level of CP47, CP43, OEC-33, D2, and D1 protein was assayed in thylakoid membranes isolated from control (C) and photoinhibited (PI) cells by immunodecoration.

**Fig. 4.** Photoinactivation of RCII-D1 protein is not sufficient to induce dissociation of OEC-33 from PSII if the degradation of the D1 protein is prevented by an increase in the ratio PQH2/PQ. y-1 and Ac208 cells were photoinhibited at 2500 μmol·m⁻²·s⁻¹ for 5 h with the addition of chloramphenicol. Samples were taken at times as indicated, and membrane preparations were assayed for the presence of OEC-33 and D1 proteins by immunodecoration (Panel A); Panel B, densitometric quantification of the immunoblots: squares, D1; triangles, OEC-33; closed symbols, y-1; open symbols, Ac208. Photoinactivation was measured as loss of variable fluorescence in whole cells and was more than 90% after 3 h of light exposure (not shown) as reported before (Zer et al., 1994).
results (Fig. 6) show that the total OEC-33 content of the cells did not change significantly during the duration of the entire experiment (12 h). OEC-33 was released from the thylakoid fraction isolated from photoinactivated cells in which the RCII-D1 protein was degraded. Reassociation of OEC-33 with the thylakoid membranes was found only in thylakoids isolated from cells allowed to synthesize chloroplast proteins and from cells allowed to recover activity in presence of cycloheximide, which inhibited cytosolic protein synthesis by more than 90% (not shown). Since the nuclear encoded OEC-33 protein level associated with the thylakoid membrane fraction increased in spite of the presence of cycloheximide, this protein could not be synthesized de novo and thus must have been present in the thylakoid lumen of the intact cell. These results clearly indicate that OEC-33 is not degraded nor damaged when dissociated from the membrane fraction and can rebind in a functional form during the process of PSII reassembly as indicated by reactivation of electron flow (see below).

It has been reported that the degraded RCII-D1 protein can also be replaced by newly synthesized protein in cells allowed to recover from photoinhibitory light treatment in darkness (Zer et al., 1994; Gong and Ohad, 1995). However, the RCII reassembled under such conditions recover only partially photosynthetic electron flow activity (Huse and Nilsen, 1989; Gong and Ohad, 1995). To test whether OEC-33 may reassociate with the inactive RCII reassembled in the dark, cells were photoinactivated in high light treatment (not shown). This finding supports the proposed explanation of the release of the free OEC proteins as being the result of transient mechanical rupture of the thyla-

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**DISCUSSION**

In this work we took advantage of the fact that the thylakoid membranes of the green alga C. reinhardtii are temporally opened during cell breakage and release the free soluble proteins located in the luminal space of the membrane as demonstrated by the release of plastocyanin. Plastocyanin release occurs irrespective of the light treatment to which the cells have been subjected prior to breakage. The release of this protein loosely bound to the membrane, which participates in electron transfer as a free mobile carrier (Haehnel et al., 1989) is expected if the thylakoid vesicles are broken open during the process of cell homogenization. The OEC proteins were present in the luminal space of the thylakoids before the mechanical breakage of the cells as indicated by immunolocalization of the OEC-33 polypeptide in both cells fixed prior to or after the high light treatment (not shown). This finding supports the proposed explanation of the release of the free OEC proteins as being the result of transient mechanical rupture of the thyla-

**Fig. 5. OEC-33 does not dissociate from the thylakoid membranes obtained from cells photoinactivated in presence of DCMU.** Cells were photoinhibited at 1800 μmol·m⁻²·s⁻¹ for 9 h in the presence of chloramphenicol, with or without addition of DCMU. Samples were taken at times as indicated, and thylakoid membranes were isolated and assayed by immunodecoration for the content of OEC-33 and D1 proteins (Panel A). Panel B, densitometric quantification of the immunoblots: squares, D1 protein; triangles, OEC-33; closed and open figures represent cells incubated in the absence or presence of DCMU, respectively. Photoinactivation was more than 90% after 3 h of light exposure as assayed by measurements of variable fluorescence.

**Fig. 6. Reassociation of OEC-33 with PSII requires chloroplast protein synthesis.** Chlamydomonas γ-1 cells were photoinhibited at 2000 μmol·m⁻²·s⁻¹ for 4 h (P1). At the end of photoinhibition the cells were washed and incubated for recovery at 25 μmol·m⁻²·s⁻¹ for 8 h. Recovery was carried out in the presence or absence of chloramphenicol (Cap) or cycloheximide (Chi). Thylakoid membranes were isolated, and the OEC-33 and D1 protein content was assayed by immunodecoration. C, control.

**Fig. 7. Association of OEC-33 with the thylakoid membranes following recovery from photoinhibition in the dark or low light.** Cells were photoinhibited at 2500 μmol·m⁻²·s⁻¹ for 3 h (P1). At the end of photoinhibition the cells were washed and incubated for recovery at low light (LL) or in the dark for 4 or 8 h as indicated. Panel A, immunoblots of membrane preparations. Panel B, photosynthetic activity measured by variable fluorescence kinetics (Fv/Fo) as described under “Materials and Methods.” ⃰, recovery in low light; □, recovery in the dark.
koids. However, the OEC proteins are freed from the binding site(s) only in thylakoids in which the RCII-D1 protein has been degraded, but not in those in which the PSI1 is not affected by a high light treatment. Similarly, the OEC proteins are not released from thylakoids in which the PSI1 has been photodamaged and irreversibly inactivated while the degradation of the RCII-D1 protein was prevented as it is the case in the mutants defective in plastiquinoid oxidation (Zer et al., 1994), or in wild type cells photoactivated in the presence of DCMU (Gong and Ohad, 1991; Zer and Ohad, 1995).

The results presented in this work demonstrate that the RCII-D1 protein is involved in the reversible association of the OEC proteins with the donor side of the PSII complex exposed on the luminal thylakoid surface. This conclusion is supported by the fact that the kinetics of OEC-33 dissociation from the luminal side of the thylakoids follows closely that of the light induced degradation of the RCII-D1 protein. Under the conditions used in this work to induce the degradation of the RCII-D1 protein, only partial degradation of the RCII-D2 protein was detected. It was shown that similarly, CP47 and CP43, chlorophyll-binding proteins of the PSI1 core, are also relatively stable during photoactivation. Furthermore, under the conditions of light-induced degradation of RCII-D1 protein in vivo the RCII disassembly is only partial and the CP43 and RCII-D2 protein as well as the cytochrome b_{559} subunits remain associated (Adir et al., 1990). Thus it appears that the binding of the OEC protein on the donor side of PSI1 is related to the presence of an assembled PSI1 core complex containing the RCII-D1 protein as part of the complex.

The results demonstrating that the process of photodamage in itself is not sufficient to cause the release of the OEC proteins from the binding site, indicate that the organization of the core polypeptides segments forming the PSI1 donor side on the luminal face of the thylakoid membranes is not sufficiently altered to induce the dissociation of the OEC proteins and presumably also of the manganese cluster forming the oxygen evolving complex. It was previously reported that the photoactivated RCII-D1 protein must be degraded before newly synthesized precursor pD1 protein can be integrated and stabilized in a reassembled PSI1, indicating that the photoactivated RCII-D1 protein in this case is still assembled in a core complex (Adir et al., 1990; Zer et al., 1994). The results of this work indicate that the photoactivated RCII-D1 protein, which has lost its ability to host a functional acceptor side (Gong and Ohad, 1995; Zer and Ohad, 1995), retains its structural organization in the complex maintaining the OEC binding site(s) of the PSI1 on the donor side. Thus the reassociation of OEC proteins with the thylakoid membranes during recovery from photoinhibition corresponds to synthesis of new RCII-D1 protein and its reassembly in a PSI1 complex.

Newly synthesized RCII-D1 protein does not accumulate in differentiated chloroplasts unless the preexisting RCII-D1 protein in RCII is degraded during light exposure. However, newly synthesized D1 protein is stabilized and accumulates in darkness following preceding photodamage of RCII and ensuing degradation of the RCII-D1 protein in darkness (Zer and Ohad, 1995; Gong and Ohad 1995). However, in this case the reasssembled PSI1 is not functional in electron transfer from water to plastocyanin. The lack of the reactivation of PSI1 recovered in darkness is not directly related to the binding of manganese, which was shown to require light following in vitro dissociation of the manganese and OEC proteins due to Tris washing at high pH (Tamura et al., 1989). This conclusion is based on the fact that reactivation of electron transfer following resynthesis and integration of RCII-D1 protein in the dark is prevented in both wild type Chlamydomonas and Scenedesmus cells which can form active photosynthetic membranes during growth in darkness as well as in the Scenedesmus LF-1 mutant (Gong and Ohad 1995) in which the manganese cluster is not assembled due to the lack of processing of the precursor pD1 protein. However, the LF-1 cells contain a partially active PSI1 capable of charge separation and electron transfer from the secondary donor Yz to PQ (Taylor et al., 1988; Guenther et al., 1990). This activity is not recovered in the photoactivated LF-1 mutant cells following synthesis of the RCII-D1 protein in the darkness (Gong and Ohad, 1995). OEC-33 reassociates with the luminal side of the membranes following recovery of the degraded RCII-D1 protein proportional to the amount of the new RCII-D1 protein synthesized during the recovery process irrespective of whether the recovery of RCII occurred in the light or in darkness. This indicate that binding of the OEC-33 per se does not require light in this case. The fact that the inactive complex is assembled in a way sufficient to bind OEC-33 indicates that the light requirement for the activation of the PSI1 assembled in the darkness following photoactivation and RCII-D1 protein degradation is not necessarily related to the process of manganese and OEC-33 protein binding. The role of light in this process remains to be elucidated.

The question arises as to what may be the control system preventing additional OEC proteins synthesis during the high light treatment and the recovery process. The data we have presented here do not exclude de novo synthesis and turnover of a small amount of OEC proteins below the detection level by immunoassays. However, it is clear that no significant amounts of the OEC proteins are synthesized nor degraded during the photoinhibition and recovery process. Preliminary results indicate that the level of the OEC-33 message is not significantly altered during the above processes. Thus the control mechanism preventing excessive accumulation of the OEC translation products remains to be established.

The persistence of the OEC-33 proteins during the turnover of the RCII-D1 protein may fulfill an important physiological function. During the process of light-induced turnover of the RCII-D1 protein as well as during recovery from severe photoinhibition and massive degradation of the protein, it is essential that newly synthesized precursor pD1 protein may become part of a functional complex containing the OEC proteins as soon as it is integrated and processed (Prasil et al., 1992). In the absence of OEC protein binding, the in vivo reassembled PSI1 will lack the activity of the donor side, resulting in rapid photoactivation and loss of its RCII-D1 protein at rates considerably faster than in normal functional PSI1 (Wang et al., 1992; Ohad et al., 1994; Rova et al., 1994; Gong and Ohad, 1995). Thus, the persistence of the OEC proteins during the turnover of the RCII-D1 protein ensures fast reactivation and recovery, i.e. maintenance of photosynthetic activity even in high light, exposed cells as long as the rate of the precursor pD1 protein synthesis matches that of the RCII-D1 protein degradation.

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REFERENCES

Adir, N., Shochat, S., and Ohad, I. (1990) J. Biol. Chem. 265, 12563–12568
Brode, I. W., Mills, J. D., Robinson, D., and Robinson, C. (1995) J. Biol. Chem. 270, 1657–1662
Diner, B. A., Ries, F. D., Cohen, B. N., and Metz, J. G. (1988) Biochim. Biophys. Acta 972–980
Ettlinger, W. F., and Theg, S. M. (1991) J. Biol. Chem. 266, 321–328
Ghanotakis, D. F., and Yocum, C. F. (1990) Annu. Rev. Plant Physiol. Plant Mol.
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Biol. 41, 255–276
Gong, H., and Ohad, I. (1991) J. Biol. Chem. 266, 21293–21299
Gong, H., and Ohad, I. (1995) Biochim. Biophys. Acta 1228, 181-188
Guenther, J. E., Nemson, J. A., and Melis, A. (1990) Photosynth. Res. 24, 35–46
Hawthorn, W., Ratjaczek, R., and Robenek, H. (1989) J. Cell Biol. 108, 1397–1405
Hashimoto, A., Akasaka, T., and Yamamoto, Y. (1993) Biochim. Biophys. Acta 1183, 397–407
Hundal, T., Virgin, I., Styring, S., and Andersson, B. (1990) Biochim. Biophys. Acta 1017, 235–241
Huse, H., and Nilsen, S. (1989) Photosynth. Res. 21, 171–179
Jansen, M. A. K., Depka, B., Trebst, B., and Edelman, M. (1993) J. Biol. Chem. 268, 21242–21252
Laemmli, U. K. (1970) Nature 227, 680–685
Leitsch, J., Schnettger, B., Critchley, C., and Krause, G. H. (1994) Planta 194, 15–21
Malnoe, P., Mayfield, S. P., and Rochaix, J. D. (1988) J. Biol. Chem. 263, 609–616
Metz, J. G., Pakrasi, H. B., Seibert, M., and Arntzen, C. J. (1986) FEBS Lett. 205, 269–274
Ohad, I., Siekevitz, P., and Palade, G. E. (1967) J. Cell Biol. 35, 521–552
Ohad, I., Keren, N., Zer, H., Gong, H., Mar, T. S., Gal, A., Tal, S., and Domovich, Y. (1994) in Photoinhibition from the Molecule to the Field (Baker, N., ed) pp. 161-177, Bios Scientific Publishers, Oxford, UK
Palomares, R., Herrmann, R. G., and Oelmüller, R. (1993) Eur. J. Biochem. 217, 345–352
Prasili, O., Adir, N., and Ohad, I. (1992) in The Photosystems: Structure, Function and Molecular Biology (Barber, J., ed) pp. 295–348, Elsevier, Amsterdam, The Netherlands
Quinn, J., Li, H. H., Singer, J., Morimoto, B., Mets, L., Kindle, K., and Merchant, S. (1993) J. Biol. Chem. 268, 7832–7841
Reisman, S., Michaels, A., and Ohad, I. (1986) Biochim. Biophys. Acta 849, 41–50
Regitz, G., and Ohad, I. (1976) J. Biol. Chem. 251, 247–252
Robinson, C., and Klösgen, R. (1994) Plant Mol. Biol. 26, 15–24
Rochaix, J. D., and Erickson, J. (1988) Trends Biochem. Sci. 13, 56–59
Rova, M., Fenzzen, L. G., Fredriksson, P. O., and Styring, S. (1994) Photosynth. Res. 35, 75–83
Schuster, G., Owens, G. C., Cohen, Y., and Ohad I. (1984) Biochim. Biophys. Acta 767, 596–605
Schuster, G., Timberg, R., and Ohad I. (1988) Eur. J. Biochem. 177, 403–410
Tamura, N., Inoue, Y., and Cheniae, G. M. (1989) Biochim. Biophys. Acta 976, 173–181
Taylor, M. A., Nixon, P. J., Todd, C. M., Barber, J., and Bowyer, J. R. (1988) FEBS Lett. 235, 109–116
Vermaas, W. F. J., Styring, S., Schröder, W. P., and Andersson, B. (1993) Photosynth. Res. 36, 249–263
Virgin, I., Styring, S., and Andersson, B. (1988) FEBS Lett. 233, 408–412
Wang, W. Q., Chapman, D. J., and Barber, J. (1992) Plant Physiol. 99, 16–20
Zer, H., and Ohad, I. (1995) Eur. J. Biochem. 231, 448–453
Zer, H., Prasili, O., and Ohad, I. (1994) J. Biol. Chem. 269, 17670–17676
