

Comparative Analysis of the Biogenesis of Photosystem II in the Wild-Type and Y-1 Mutant of *Chlamydomonas reinhardtii*

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Abstract. Expression of the genes of the photosystem II (PSII) core polypeptides D1 and D2, of three proteins of the oxygen evolving complex of PSII and of the light harvesting chlorophyll *a/b* binding proteins (LHCP) has been compared in wild-type (wt) and in the *y-1* mutant of *Chlamydomonas reinhardtii*. Since wt, but not *y-1* cells produce a fully developed photosynthetic system in the dark, comparison of the two has allowed us to distinguish the direct effect of light from the influence of plastid development on gene expression. The PSII core polypeptides and LHCP are nearly undetectable in dark-grown *y-1* cells but they accumulate progressively during light induced greening. The levels of these proteins in wt are the same in the light and the dark. The amounts of the proteins of the oxygen evolving complex do not change appreciably in the light or in the dark for both wt and *y-1*. Steady state levels of chloroplast mRNA encoding the

core PSII polypeptides remain nearly constant in the light or the dark and are not affected by the developmental stage of the plastid. Levels of nuclear encoded mRNAs for the oxygen evolving proteins and of LHCP increase during light growth in wt and *y-1*. In contrast to wt, synthesis of LHCP proteins is not detectable in *y-1* cells in the dark but starts immediately after transfer to light, indicating that LHCP synthesis is controlled by a light-induced factor or process. While the rates of synthesis of D1 and D2 are immediately enhanced by light in wt, this increase occurs only after a lag in *y-1* and thus must be dependent on an early light-induced event in the plastid. These results show that the biosynthesis of PSII is affected by light directly, by the stage of plastid development, and by the interaction of light and events associated with plastid development.

THE photosystem II (PSII)¹ complex of higher plants and algae plays a key role in photosynthesis since it is involved in the primary charge separation across the thylakoid membrane and in water splitting and oxygen evolution. PSII consists of a membrane-embedded core with its associated light-harvesting complex and oxygen evolving complex. The PSII core contains at least six different polypeptides: P5 (47-kD polypeptide), P6 (43-kD polypeptide) D1, D2, and two apo-cytochromes b559 (27, 32, 43). It has been shown recently that the reaction center of PSII consists of D1, D2 and the apocytocchromes b559 (1). All the polypeptides of the PSII core are encoded by the chloroplast genome and translated on chloroplast ribosomes (for review cf. reference 3). In contrast the three proteins of the oxygen evolving complex OEE1, OEE2, and OEE3 are coded for by nuclear genes, translated on cytoplasmic ribosomes and imported

into the chloroplast (41). The same is true for the light-harvesting chlorophyll *a/b* proteins (LHCP) which contain the antenna chlorophylls and carotenoids of the PSII reaction center (7, 37). It is thus apparent that the biosynthesis of photosystem II depends on the cooperation of the protein synthesizing systems from the nucleocytoplasmic and chloroplast compartments.

In higher plants light has a profound effect on the development of the plastids (39). Dark grown plants lack chlorophyll and contain undifferentiated plastids called etioplasts. The synthesis of chlorophyll, the differentiation of etioplasts into chloroplasts and the rapid increase in the steady-state levels of mRNA encoding chloroplast proteins all occur simultaneously upon illumination of dark grown plants. It is therefore difficult to determine whether changes in the expression of genes involved in photosynthesis during dark-light transitions are caused directly by light or whether they result from developmental changes induced by light. One advantage of using *C. reinhardtii* for these studies is the possibility of distinguishing between these two alternatives because (a) wild type (wt) strains of *C. reinhardtii* are able to synthesize chlorophyll and a fully functional photosynthetic apparatus in the dark and (b) mutants exist, like *y-1*, which mimic higher plants in that they are unable to synthesize chlorophyll or de-

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1. *Abbreviations used in this paper:* PSII, photosystem II; LHCP, light harvesting chlorophyll *a/b* binding proteins; TAP, Tris-acetate-phosphate medium; wt, wild type.

velop functional chloroplasts in the dark but do so in the light. Dark grown *y-1* cells contain a partially differentiated plastid that lacks a functional photosynthetic membrane system and resembles etioplasts from higher plants. Upon illumination chlorophyll synthesis resumes and the thylakoid membranes are fully reconstituted within 6–8 h (28, 29). During this greening phase no cell division occurs.

By comparing the accumulation and synthesis of PSII polypeptides and the levels of mRNAs encoding them in the dark and during the ensuing light phase in wild-type (wt) and *y-1*, we have been able to distinguish between the effect of light per se and the effect of light-induced plastid development on the expression of the PSII genes. The results indicate that the control of the expression of these genes is rather complex and that both light directly, and light-induced plastid development have profound effects at the transcriptional, translational, and posttranslational level.

Materials and Methods

Strains and Growth Conditions

wt 137c and the yellow mutant *y-1* were used. Cells were grown for 7 d in the dark in liquid Tris-acetate-phosphate medium (TAP), pH 7.0 (10). 2 d before harvest the cells were diluted to 5×10^5 /ml and collected at a final concentration of $1-2 \times 10^6$ cells/ml. Cell manipulations which required darkness were done under green safe light.

Protein Isolation, PAGE Electrophoresis, and Antibody Hybridization

wt cells were grown either in the dark or under constant light to a density of $1-2 \times 10^6$ cells/ml and 50 ml from each cell culture were collected at 8,000 g for 10 min. *y-1* cells (250 ml) were grown in the dark to 1×10^6 cells/ml for 7 d. The cell culture was transferred into light and 50 ml of cells were collected by centrifugation at 8,000 g for 10 min after 0 (dark), 1.5, 3, 6, or 24 h in the light. Protein isolation and sample preparation (26), PAGE, protein blotting, antibody hybridization, stripping, and reuse of the filters were as described (24, 25).

Northern Hybridizations

1 l of wt and *y-1* cells were grown in the dark to a density of 1×10^6 cells/ml and 0.75×10^6 cells/ml, respectively. 200 ml of the cells were kept in dark, while the rest of the cultures were transferred to light and 200 ml samples were collected and processed after 0 (dark), 1.5, 6, 12, 24, and 30 h of illumination. The cells kept in dark were harvested with the sample from the 30-h time point. The cells were harvested by centrifugation at 8,000 g for 10 min, resuspended into 3 ml 6 M guanidine-hydrochloride and frozen at -70°C . RNA isolation, electrophoresis on denaturing agarose gels, and transfer onto nitrocellulose filters were done as described (14). Each lane on the agarose gel contained 4 μg of total RNA. The filters were prehybridized and hybridized as described (21).

The probes used for chloroplast genes were: LS, internal Hind III fragment of the gene of the large subunit of ribulose 1,5 biphosphate carboxylase subcloned into pUC19 (9). P5, Bam HI fragment Ba10 cloned into pBR313, containing the gene of the 47-kD PSII core polypeptide (30, 31). P6, Eco RI fragment R9 cloned into pCRI, containing a large portion of the gene of 43-kD PSII core polypeptide (11, 31). D1, Eco RI fragment R14 cloned into pCRI containing a large portion of the gene of D1 (11) and D2, cloned internal Sau3A fragment of the gene of D2 (12). The probes used for nuclear genes were: SS a cDNA clone of the small subunit of ribulose 1,5 biphosphate carboxylase (15), LHCP a cDNA clone of LHCP (Soen, S., unpublished observations); and OEE1, OEE2 and OEE3, cDNA clones of the OEE1, OEE2 and OEE3 proteins (26).

Pulse Labeling of Proteins with [^{14}C]Acetate

Cells were grown to a density of 1×10^6 cells/ml and transferred to medium lacking acetate 1 h before labeling. In some cases cycloheximide was added 10 min before the addition of label. 200 ml of wt and *y-1* cells

were grown in the dark. After the cells had been transferred for 1 h into medium lacking acetate in the dark, 50 μCi of [^{14}C]acetate was added to a batch of 40 ml of cells and incubation was continued for 10 min in the dark. The other 40 ml cell cultures were labeled in the same manner immediately after transfer of the cells into light, and after 1.5, 6, and 24 h in the light. At the end of the labeling period the cell cultures were adjusted to 50 mM acetate by adding 2 M nonradioactive acetate, harvested quickly by centrifugation at 10,000 g for 2 min, resuspended into 0.5 ml 0.8 M Tris-HCl, pH 8.3, 0.4 M sucrose 1% β -mercaptoethanol, and frozen immediately at -70°C . The cells were allowed to thaw and were sonicated three times for 10 s, centrifuged at 24,000 rpm in a Beckman SW60 rotor for 30 min at 4°C . The pellet (membrane fraction) was resuspended into 200 μl of 0.8 M, Tris-HCl, pH 8.3, 0.4 M sucrose, 1% β -mercaptoethanol and the supernatant (soluble fraction) was saved.

Pulse-labeling of Proteins in the Presence of Rifampicin

wt cells were grown in the dark as described above. At a density of 1×10^6 cells/ml, the cells were collected by centrifugation at 8,000 g for 10 min, resuspended into 160 ml acetate free media. Rifampicin was added to 80 ml of cell culture to a final concentration of 250 $\mu\text{g}/\text{ml}$. The stock solution of rifampicin was 7.5 mg/ml in 10 mM KH_2PO_4 (pH 4.5). 40 ml untreated and 40 ml rifampicin-treated cells were kept in the dark. In the same way 40 ml untreated and 40 ml treated cells were transferred into light. After 1 h the dark-grown and light-grown cells were pulse-labeled with [^{14}C]acetate and processed as described above. Since rifampicin at the concentration used absorbs light and to have the same light regimes for the treated and untreated cultures, the latter received the equivalent amount of inactivated rifampicin. The drug was inactivated by autoclaving before being added to the culture, a treatment that did not alter significantly the color of the rifampicin solution. The light intensity behind the culture flask was 2,000 lux.

In Vivo Labeling of RNA with $^{32}\text{PO}_4$ in the Presence of Rifampicin

400 ml of TAP media containing only 0.03 mM PO_4 was inoculated with wt cells to a density of 0.5×10^6 cells/ml. 24 h later 2×200 ml of cells were collected by centrifugation and resuspended into 2×200 ml TAP lacking PO_4 (TAP- PO_4). Rifampicin was added to one of the cultures to a final concentration of 250 $\mu\text{g}/\text{ml}$. After 1 h the two cultures were collected by centrifugation and one was resuspended into 20 ml TAP- PO_4 and the other into 20 ml TAP- PO_4 + 250 $\mu\text{g}/\text{ml}$ rifampicin. 200 μCi of $^{32}\text{PO}_4$ (carrier-free) were added to each culture. After 30 min of labeling, the cells were collected by centrifugation, resuspended into 2.5 ml 6 M guanidine hydrochloride and RNA was isolated as described (14).

Dot Blot Hybridizations

20 μg of plasmid DNA were linearized and precipitated. Plasmids containing regions of the D1, D2, and LHCP genes were used (cf. Northern hybridization for details). A plasmid containing the 3' region of the chloroplast 23S ribosomal RNA gene was also used. Each plasmid DNA was resuspended into 20 μl H_2O , heated to 100°C for 5 min, and cooled in ice. 0.7 M NaOH was added to a final concentration of 0.35 M and the samples were incubated at room temperature for 15 min. The solution was neutralized with 40 μl 2 M ammonium acetate and immediately chilled in ice. 10 μg of each plasmid DNA were spotted onto 2 filters of nitrocellulose and hybridized to RNA labeled in vivo with $^{32}\text{PO}_4$ either in the presence or absence of rifampicin as described (21).

Results

Accumulation of PSII Proteins in wt and *y-1* Cells

Cells from the mutant *y-1* were grown for 7 d (corresponding to 14 generations) in the dark, transferred into light, and collected at various times: before transfer into light, and after 1.5, 3, 6, and 24 h of illumination. As a control wt cells were grown under the same conditions as *y-1* in constant light and constant dark. Membrane and soluble protein extracts from cells collected at the different time points were subjected to PAGE and the amount of specific proteins measured by West-

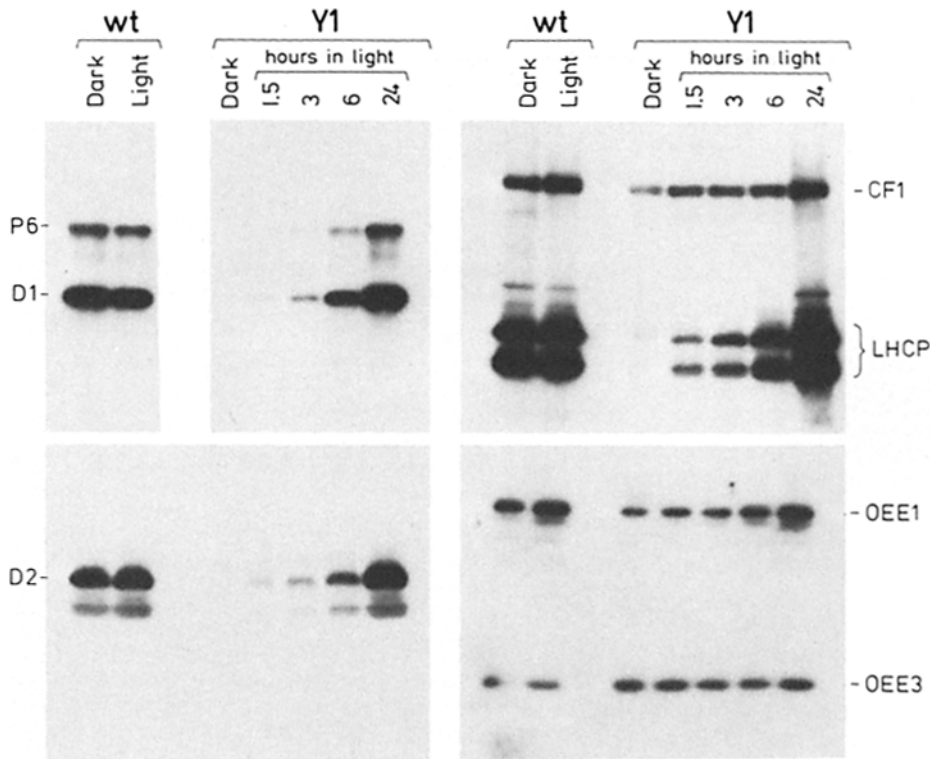


Figure 1. Accumulation of PSII core (P6, D1, D2), peripheral (OEE1, OEE3) and antenna proteins (LHCP) in wt (*wt*) and *y-1* (*Y1*) cells during the dark and after light phase of 1.5, 3, 6, and 24 h of illumination. CF1 indicates the α and β subunits of ATP synthase. After electrophoretic fractionation on 7.5–12.5% SDS-polyacrylamide gels the proteins were transferred to CNBr activated paper. The protein blots were allowed to react with antisera specific for the indicated proteins. The proteins were visualized by autoradiography after labeling of the antigen-antibody complexes with ^{125}I -labeled *Staphylococcus aureus* protein A. Because the D2 antibody was made against gel purified D2 protein that was slightly contaminated with polypeptides of similar mobility, additional bands besides D2 react with this antibody.

ern blotting using as probes antibodies against the major protein of the LHCP, the core PSII proteins D1, D2, P5, P6 (membrane fraction) and the three proteins from the oxygen evolving complex OEE1, OEE2, OEE3 (soluble fraction). The results shown in Fig. 1 indicate that the proteins examined in wt cells grown in the dark or in the light accumulate to similar levels, although slightly reduced levels of OEE1 in dark- vs. light-grown wt cells, can be seen.

The accumulation of several of the PSII proteins in dark-grown *y-1* cells is strikingly different from wt (Fig. 1). Proteins D1, D2, and P6 from the PSII core are undetectable in the dark and accumulate progressively during the greening phase. Similar results were obtained with P5 (data not shown). LHCP follows a similar pattern except that minute amounts of the protein are detectable in the dark. A common feature of these polypeptides is that they are part of chlorophyll-protein complexes (27, 37) and their accumulation parallels the accumulation of chlorophyll. Other membrane-associated polypeptides such as the α and β subunits of ATP synthase (marked as CF1 in Fig. 1) accumulate in dark grown *y-1* cells and their levels increase only slightly during the greening phase. Similar results were obtained with the two subunits of ribulose biphosphate carboxylase whose levels in dark- and light-grown *y-1* cells were undistinguishable (data not shown).

Although the polypeptides of the oxygen evolving system are part of the PSII complex, OEE1 and OEE3 accumulate in the dark in the *y-1* mutant as they do in wt and their levels vary only slightly during the greening phase (Fig. 1). The same pattern was found for the OEE2 protein (data not shown).

Synthesis of PSII Proteins in wt and *y-1* Cells

The results of the previous section indicate that several pro-

teins accumulate progressively during the greening of *y-1*. To determine whether this is due to increased synthesis or increased stability of the protein, the rate of protein synthesis was measured by pulse-labeling both wt and *y-1* cells with [^{14}C]acetate (Fig. 2). Cells were labeled for 10 min in the dark (lanes 1 and 6), immediately after transfer to light (lanes 2 and 7), and after 1.5 h (lanes 3 and 8), 6 h (lanes 4 and 9) and 24 h (lanes 5 and 10) of illumination. The labeled cell extracts were separated into membrane and soluble fractions before electrophoresis on polyacrylamide gels and the proteins visualized by autoradiography.

An increase in the rate of synthesis of D1 and of the three LHCP proteins can be seen during the light phase in both wt and *y-1*. Smaller increases are also observed for labeled bands which comigrate with P5 and P6 (Figs. 2 and 3). Since D2 comigrates with one of the LHCP polypeptides, the increase in the rate of synthesis of D2 during the greening phase was confirmed by pulse-labeling cells in the presence of cycloheximide (Fig. 3). Comparison of the rate of synthesis of membrane proteins in wt and *y-1* reveals two major differences. First, LHCP synthesis is detectable in dark grown wt cells, but not in dark-grown *y-1* cells (Fig. 2). In the latter LHCP synthesis appears to be light dependent since it occurs immediately after transfer of *y-1* cells to the light (Fig. 2, lane 7). We have verified that newly synthesized LHCP, as measured in a 10-min pulse is not detectable in the soluble fraction of dark-grown *y-1* cells (data not shown). Second, the pattern of D1 and D2 synthesis is different in wt and *y-1* cells. While D1 and D2 synthesis increases several fold upon exposure of wt to light, such an increase is not observed in *y-1* (Figs. 2 and 3). In these cells a significant rise of the rate of synthesis of D1 and D2 occurs later. Although we detect this increase after 1.5 h of illumination it may occur earlier, but certainly not as rapid as the increase of LHCP synthesis (Fig. 2).

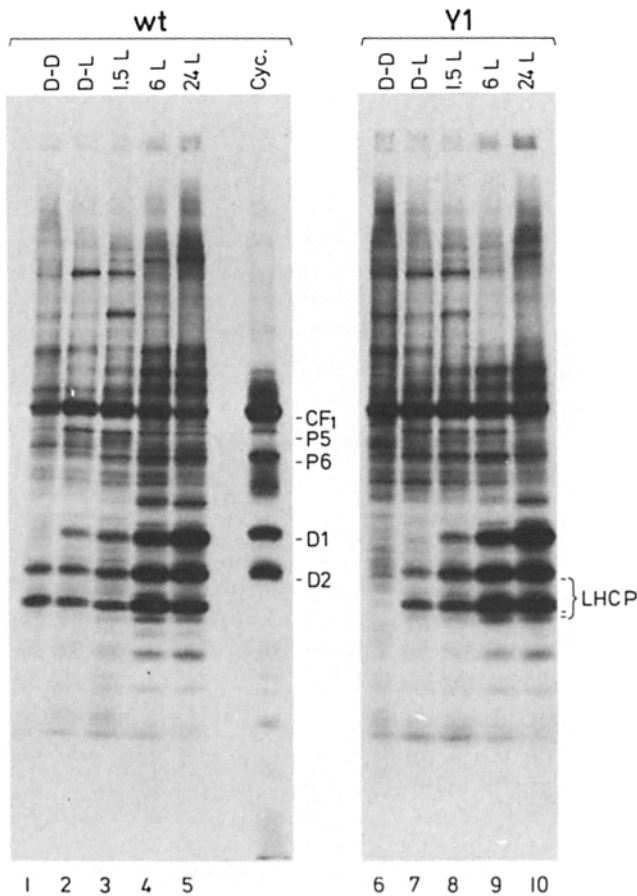


Figure 2. In vivo synthesis of membrane proteins of wt (*wt*) and *y-1* (*Y1*) cells during the dark and after light phase of 0, 1.5, 6, and 24 h of illumination. Cells were labeled for 10 min with [¹⁴C]acetate at the times indicated. Membrane proteins were separated electrophoretically on gels and autoradiographed. The middle lane (Cyc.) displays chloroplast membrane protein from cells labeled in the presence of cycloheximide.

mRNA Accumulation of PSII Proteins in wt and *y-1*

To determine whether the increased accumulation and synthesis of PSII proteins during the light phase of these dark-light transitions was due to an increase in mRNA levels, their steady state mRNA levels were determined. Total RNA from wt and *y-1* cells which had been grown in the dark or transferred into light for 1.5, 6, 12, 24, and 30 h was fractionated by electrophoresis on denaturing agarose gels, and blotted to nitrocellulose. The filters were hybridized with cloned chloroplast DNA fragments encoding LS, P5, P6, D1, and D2 and with cloned nuclear cDNAs encoding LHCP, OEE1, OEE2, OEE3, and SS.

The chloroplast mRNA levels in wt and *y-1* do not vary significantly in dark grown and illuminated cells (Fig. 4) although the D1 and D2 mRNAs are slightly reduced at the end of the light phase in wt. Larger changes occur in the levels of nuclear encoded mRNA (Fig. 5). Transfer of wt and *y-1* from the dark to the light is followed by an increase in the mRNA levels of LHCP, OEE1, and OEE2 and OEE3. Such an increase is not observed for the mRNA of SS (Fig. 5). Surprisingly the level of the mRNAs of the nuclear-encoded PSII proteins in dark-grown cells is substantially lower in wt

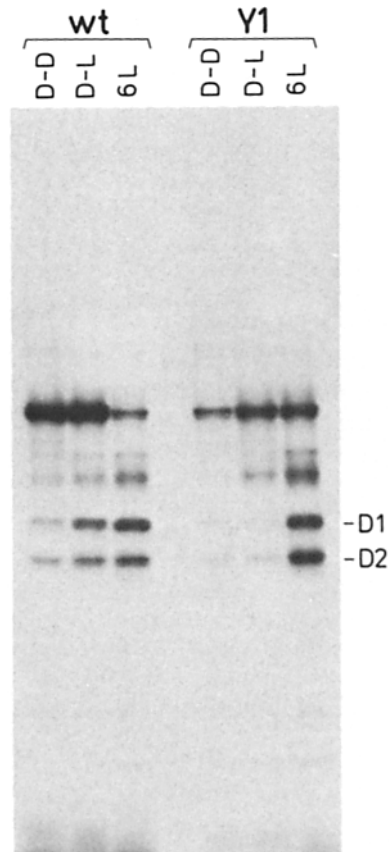


Figure 3. In vivo synthesis of chloroplast membrane proteins of wt (*wt*) and *y-1* (*Y1*) cells during the dark and after light phase of 0 and 6 h of illumination. Conditions were as in Fig. 2 except that the cells were labeled in the presence of cycloheximide.

than in *y-1*. The drop in mRNA levels at 30 h in the light which is observed in wt, but not in *y-1* is very likely due to the fact that the wt has a higher growth rate and reaches stationary phase at this time point. It is known that RNA from stationary cells is more susceptible to degradation during extraction (our unpublished observations).

Since the levels of the chloroplast mRNAs of D1 and D2 do not change after exposure to light (Fig. 4) and since the rate of synthesis of these proteins increases dramatically under these conditions (Figs. 2, 3) one may conclude that the regulation of expression of these genes occurs at the translational level. However if transcription and translation are coupled in the chloroplast as in prokaryotic organisms, only newly synthesized mRNA would be translated and responsible for the increase in protein synthesis.

To test this possibility wt cells were pulse-labeled with [¹⁴C]acetate in the dark or after 1 h of illumination in the presence of rifampicin which has been shown to selectively inhibit the transcription of the chloroplast ribosomal RNA genes in *C. reinhardtii* (35, 36). No differences could be detected between untreated cells and cells treated with rifampicin (Fig. 6). In particular the strong stimulation of D1 synthesis in the light occurs in both cultures indicating that the synthesis of D1 and presumably D2 in the light is regulated at the translational level and that chloroplast transcription and translation are not necessarily coupled. To be certain

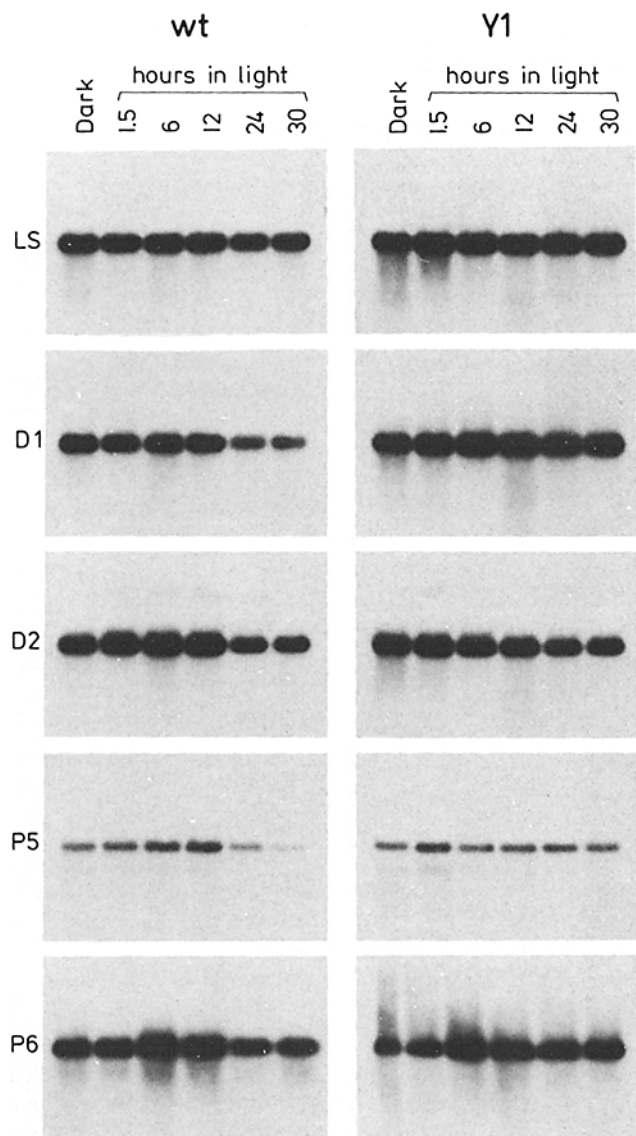


Figure 4. Levels of chloroplast mRNAs from wt (*wt*) and *y-1* (*Y1*) cells during the dark and after light phase of 1.5, 6, 12, and 24 h of illumination. Total RNA was fractionated on denaturing formaldehyde gels and blotted to nitrocellulose membranes. The filters were hybridized with labeled cloned DNA fragments containing coding sequences of LS, D1, D2, P5, and P6 (for details see Materials and Methods).

that rifampicin inhibits not only the transcription of the chloroplast ribosomal genes but also the transcription of chloroplast protein genes, RNA was labeled *in vivo* with $^{32}\text{PO}_4$ in the presence and absence of rifampicin and hybridized on dot blots to plasmids containing gene sequences of D1, D2, chloroplast 23S rRNA and LHCP. While the transcription of the chloroplast-encoded genes was inhibited in the presence of rifampicin this was not the case for the nuclear-encoded LHCP gene (data not shown). The results of this study are summarized in Table I. While the chloroplast mRNA levels are not affected by either light or the developmental stage of the plastid, nuclear mRNAs of the PSII components are influenced by light in a similar fashion in wt and *y-1*. Table

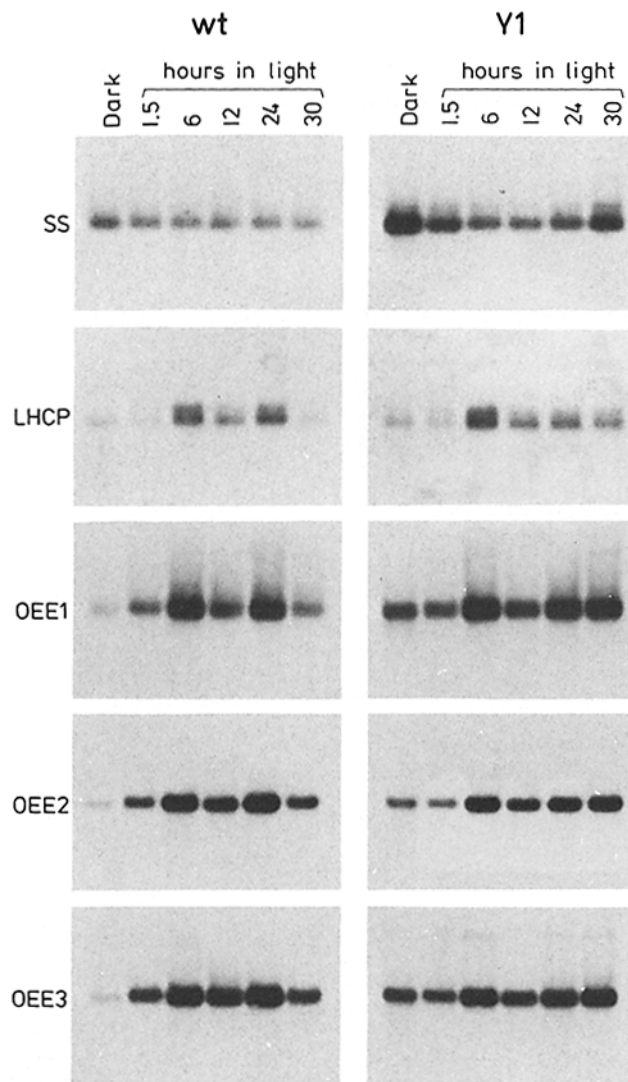


Figure 5. Levels of nuclear mRNAs from wt and *y-1* (*Y1*) cells during the dark and after light phase of 1.5, 6, 12, and 24 h of illumination. Total RNA was processed as described in Fig. 4. The filters were hybridized with labeled cloned cDNAs encoding SS, LHCP, OEE1, OEE2, and OEE3.

I also shows the selective stimulation of synthesis of D1, D2, and LHCP when the cells are transferred to light.

Discussion

In this study we have compared the accumulation and rate of synthesis of several PSII proteins and the steady state levels of their corresponding mRNAs in wt and *y-1* mutant cells of *C. reinhardtii*. Since the wt but not the *y-1* mutant is able to synthesize a fully functional photosynthetic membrane in the dark, this system allows one to distinguish the effects of light per se from the influence of light-induced plastid development on gene expression.

Accumulation of the PSII Core Polypeptides is Conditioned by the Chloroplast Developmental Stage

Ohad et al. (28, 29) have shown that after six generations in

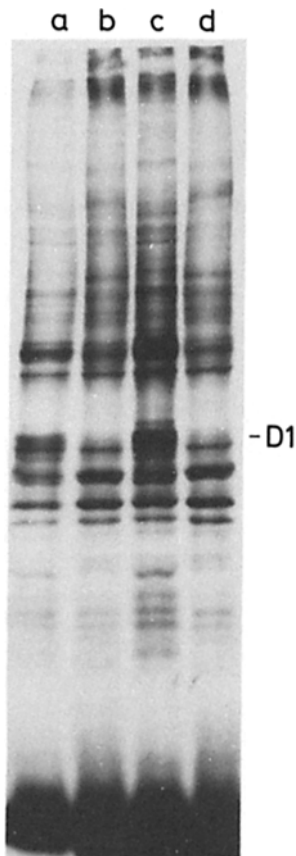


Figure 6. Synthesis of membrane proteins of *wt* in the light (lanes *a* and *c*) and in the dark (lanes *b* and *d*). Cells were labeled with [¹⁴C]acetate for 15 min without (lanes *a* and *b*) or with active rifampicin (lanes *c* and *d*). Membrane proteins (equal counts per lane) were separated electrophoretically on gels and autoradiographed. The position of the D1 polypeptide is indicated.

the dark *y-1* cells contain less than 2% of the chlorophyll of cells grown in light, an observation we have confirmed. The chloroplast lamellar system is extremely reduced and photosynthetically inactive under those conditions. After transfer to light there is a lag period of ~2 h before chlorophyll starts to accumulate. Reconstitution of functionally active thylakoid membranes closely follows chlorophyll formation (28, 29). We have shown that this also holds for the stable appearance of the chloroplast-encoded PSII core polypeptides D1, D2, P5, and P6 and for the nuclear-encoded LHCP. A common feature of these proteins is that they are chlorophyll apoproteins or at least closely associated with chlorophyll (8, 37). A close coupling between chlorophyll synthesis and chlorophyll-apoprotein accumulation has also been reported in higher plants and it has been proposed that chlorophyll may stabilize these proteins in the membrane (2, 4, 5, 23, 34, 40).

Since no difference in the amounts of these proteins could be detected between light- and dark-grown wild-type cells, it can be concluded that it is not light per se which directly affects the accumulation of the proteins but that the latter is conditioned by the developmental stage of the chloroplast.

Although the three proteins of the oxygen-evolving complex belong to the PSII complex, their patterns of accumulation differ considerably from those of the PSII core polypeptides. In both *wt* and *y-1* the levels of these proteins in dark- and light-grown cells are nearly equal. The accumulation of these proteins thus occurs in the absence of chlorophyll production or PSII core accumulation.

Table I. Summary of Protein Accumulation, Protein Synthesis and mRNA Accumulation in Wild-type and *y-1* Cells of *C. reinhardtii* during Dark-Light Transitions

	Chloroplast-encoded genes									
	LS		D1		D2		p5		p6	
	wt	<i>y1</i>	wt	<i>y1</i>	wt	<i>y1</i>	wt	<i>y1</i>	wt	<i>y1</i>
PA	→	→	→	↗	→	↗	→	↗	→	↗
PS	→	→	↗	↗	↗	↗	ND	ND	ND	ND
M	→	→	→	→	→	→	→	→	→	→

	Nuclear-encoded genes									
	SS		LHCP		OEE1		OEE2		OEE3	
	wt	<i>y1</i>	wt	<i>y1</i>	wt	<i>y1</i>	wt	<i>y1</i>	wt	<i>y1</i>
PA	→	→	→	↗	→	→	→	→	→	→
PS	→	→	↗	↗	ND	ND	ND	ND	ND	ND
M	→	→	↗	↗	↗	↗	↗	↗	↗	↗

(↗) Increasing after transfer into light; (→) constant after transfer into light.

ND, not determined.

PA, steady state protein level.

PS, protein synthesis.

M, mRNA level.

Translational and Posttranslational Regulation of the Synthesis of Chloroplast-encoded PSII Core Polypeptides

Our studies confirm that neither light nor the developmental stage of the plastid have any significant effect on the steady-state levels of the chloroplast-encoded mRNAs of the PSII core polypeptides (22). However the rates of synthesis of D1 and D2 change considerably during the dark-light transition in both *wt* and *y-1*.

Comparison of the protein pulse-labeling patterns (Figs. 2 and 3) in *wt* and *y-1* reveals that the synthesis of D1 and D2 is stimulated by light and that this stimulatory effect depends on the developmental stage of the chloroplast. This can be seen in Figs. 2 and 3 which show that while the rates of synthesis of D1 and D2 increase immediately after transfer of *wt* cells to the light, this increase occurs after a lag in *y-1* cells.

It has been reported that etioplasts of 5-d old dark-grown barley seedlings are unable to synthesize the chloroplast-encoded PSI and PSII chlorophyll apoproteins (the latter corresponding to P5 and P6 in our study) and that illumination selectively induces the synthesis of the proteins after a short lag (23, 40). Together these results suggest that the synthesis of these proteins is linked to an early event in light-induced plastid development.

It is noteworthy that the increased rate of synthesis of the

D1 and D2 proteins in wt during the light phase is not accompanied by corresponding increases in the levels of these proteins (cf. Figs. 1 and 2). One can infer that the turnover of these proteins in wt is higher in the light than in the dark. This confirms earlier results indicating increased turnover in the light for D1 in *Spirodela* (13) and *C. reinhardtii* (42).

The expression of several chloroplast genes appears to be regulated at the translational or even posttranslational level since the corresponding mRNA levels do not change significantly during dark-light transitions. This has been shown for the chlorophyll apoproteins of PSI and PSII in barley (2), for D1 in barley (2) and in *Spirodela* (4) and for LS in pea (19). However, the possibility has to be considered that even if the steady state levels of the mRNAs remain constant, a transcriptional control could still operate if transcription and translation are coupled in the chloroplast. We have ruled out this possibility by showing that the pattern of pulse-labeled proteins of cells treated with rifampicin, an inhibitor of chloroplast transcription in *C. reinhardtii*, are indistinguishable from untreated cells.

Regulation of Gene Expression of Nuclear-Encoded Polypeptides Associated with PSII

The LHCP protein has been extensively studied in higher plants and shown to be encoded by a small multigene family (6, 10). The transcription of this gene is controlled by phytochrome (1, 38) and chlorophyll is required for stabilization of the protein and for its correct insertion into the thylakoid membrane (2, 34). One or several plastid derived factors or signals appear to be necessary for the light stimulated transcription of LHCP (3, 24). In *C. reinhardtii* the transcript levels of the LHCP mRNA vary in a wave like pattern during the cell cycle being high and low in the light and dark phases, respectively (33). The transcript level appears to be controlled both by light and by a chlorophyll synthesis intermediate which may act as a negative effector (17, 20). Our results show that the levels of LHCP mRNA in wt cells subjected to a dark-light transition follow a similar pattern as in higher plants. Upon transfer from dark to light the level of LHCP mRNA rises to reach a maximum after 6 h of illumination (Fig. 5). Similar patterns are also observed with the mRNAs encoding the proteins of the oxygen evolving complex, but not with the mRNA of SS which remains constant. The patterns of accumulation of these nuclear mRNAs are similar in y-1 and wt cells during the light phase. One significant difference is that in dark-grown y-1 cells the nuclear mRNA levels are consistently higher than in dark-grown wt cells. One possibility is that a repressor which is acting in the dark in wt is missing or reduced in y-1. It is also possible that these differences in mRNA levels between wt and y-1 result from some pleiotropic effects of the y-1 mutation, especially since it has been reported that the accumulation of any of several intermediates in the chlorophyll synthesis pathway can reduce the levels of LHCP mRNA (20). Analysis of other conditional chlorophyll deficient mutants may provide further insights into this problem. It is noteworthy that the increase of the mRNA levels of the proteins of the oxygen evolving complex in y-1 during the light phase is not followed by a corresponding rise in these proteins. (cf. Figs. 1 and 5). The significance of this observation is not clear.

Comparison of the rate of synthesis of LHCP in wt and y-1 cells reveals at least two different kinds of regulation. First, the enhanced rate of protein synthesis after 6 h of illumination (Fig. 2) can be correlated with an increase in LHCP mRNA (Fig. 5) suggesting transcriptional control. A significant increase in the amount of translatable mRNA of LHCP after transfer of y-1 cells to the light has also been reported previously (18). Second, in wt cells LHCP is synthesized in the dark and there is no direct effect of light on translation since its rate of synthesis remains constant for at least 1.5 h of illumination (Fig. 2). In contrast LHCP synthesis is not detectable in dark-grown y-1 cells in either the membrane or soluble fraction. However the synthesis proceeds at the same rate as in wt after transfer of the cells to the light (Fig. 2). This suggests that a factor or signal controlling LHCP translation is missing in dark-grown y-1 cells. The appearance of this factor does not depend on a functional photosynthetic system because LHCP synthesis starts immediately after illumination of y-1 cells. These results can be interpreted in two ways. The first is that a factor or signal controlling LHCP translation which is missing in dark-grown y-1 cells is induced by light. The appearance of this factor does not depend on a functional photosynthetic membrane since LHCP synthesis starts immediately after illumination. It has also been suggested that in *Lemna gibba* translation of LHCP may require a light-induced product or process (34). The other possibility is that LHCP is still synthesized in dark-grown y-1 cells but that it turns over very rapidly because it is unable to integrate stably into the membrane. As soon as illumination starts a change occurs, e.g. conversion of the existing protochlorophyllide pool into chlorophyllide which allows for a rapid insertion and stabilization of LHCP into the membrane (2, 4, 5, 34). The short pulses (10 min) that we used should have allowed us to detect LHCP unless its turnover rate was extremely rapid, as it has been shown that LHCP synthesis can be detected in chl b mutants of barley strains in which the apoprotein never accumulates (2).

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