Light regulation of photosystem I (PSI) biogenesis was studied in a unicellular green alga, *Chlamydomonas reinhardtii*. When *Chlamydomonas* cells were transferred from darkness to the light, mRNAs for both nuclear- and chloroplast-encoded PSI subunits were induced in concert. This light induction was inhibited by photosynthetic electron transport (PET) inhibitors, 3-(3,4 dichlorophenyl)-1,1-dimethylurea and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone, but not by an uncoupler, carbonyl cyanide m-chlorophenylhydrazone. This indicated that PET plays a pivotal role in the light induction of PSI subunit mRNAs, but that phosphorylation is not necessary. When we irradiated the *Chlamydomonas* cells with PSI-light (695 nm) or PSII-light (644 nm), which makes the plastoquinone pool oxidative and reductive, respectively, PSII-light caused the accumulation of PSI proteins more abundantly than did PSI-light. However, there was no difference for the PSI subunit mRNA levels between these light sources. From these results, we conclude that PET plays dual roles in the regulation of PSI biogenesis in *Chlamydomonas*: when cells are illuminated, PET first induces the PSI subunit mRNAs irrespective of the redox state of the intersystem electron carriers, and then their redox state fine-tunes PSI biogenesis at translational and/or post-translational steps to fulfil the chromatic adaptation.

**Keywords**: Chlamydomonas — Chromatic adaptation — Photosynthetic electron transport — Photosystem I — Redox regulation.

Abbreviations: CCCP, carbonyl cyanide m-chlorophenylhydrazone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; Fm, maximal level of chlorophyll fluorescence; PET, photosynthetic electron transport; PQ, plastoquinone; Redox, reduction/oxidation.

**Introduction**

Photosystem I (PSI) is a light-driven plastocyanin-ferredoxin oxidoreductase in the thylakoid membrane of chloroplasts, and consists of subunits encoded by both the chloroplast and nuclear genomes (Obokata et al. 1993, Scheller et al. 1997, Webber and Bingham 1999). Genes for PSI subunits are activated in response to light stimuli in both etiolated seedlings and in green photosynthetically active cells (Rodermel and Bogorad 1985, Brunner et al. 1991, Salvador et al. 1993, Yamamoto et al. 1995). During the de-etiolation process of angiosperms, phytochrome signals regulate the abundance of PSI subunit mRNAs (Zhu et al. 1985, Neuhaus et al. 1993, Brunner et al. 1991), and the light conversion of protochlorophyllide to chlorophyllide triggers the accumulation of P700 apoproteins (Eichacker et al. 1990, Kim et al. 1994). In contrast, light induction of PSI subunits in green photosynthetically competent cells is less understood.

Photosynthesis reactions lead to the reduction/oxidation (redox) of various intracellular molecules. Recently, the redox control of photosynthesis-related genes was reported in cyanobacteria (Alfonso et al. 2000, Li and Sherman 2000), green algae (Danon and Mayfield 1994, Escoubas et al. 1995, Maxwell et al. 1995), and higher plants (Karpinski et al. 1997, Pfannschmidt et al. 1999, Zhang et al. 2000, Oswald et al. 2001). These findings support the notion that photosynthesis serves as an environmental sensor, whereby information concerning light intensity and quality are converted to redox signals and transduced to respective genes (Huner et al. 1998, Foyer and Noctor 1999). So far, the redox state of the plastoquinone (PQ) pool has been shown to regulate the nuclear genes for the apoproteins of light-harvesting complexes (Escoubas et al. 1995, Maxwell et al. 1995) and for ascorbate peroxidase (Karpinski et al. 1997).

Redox state of the Cyt b/f complex appeared to regulate the transcription of a PSI gene, psaE (Bissati and Kirilovsky 2001).

We are interested in the regulatory mechanism of PSI biogenesis in photosynthetically competent cells. To address this question, we used a unicellular green alga, *Chlamydomonas reinhardtii*, as a model organism, because even in darkness it contains a developed chloroplast, and it is also much easier to treat with photosynthesis inhibitors than higher plants. The present knowledge about the redox control of photosynthesis genes, as described above, led us to speculate that the redox-state of the intersystem electron transport, possibly the PQ pool and/or the Cyt b/f complex, may regulate the light induction of PSI subunit mRNAs. However, our study with herbicides and monochromatic light irradiation revealed that light induction of PSI subunit mRNAs is triggered by the photosynthetic electron flow, irrespective of the redox state of the intersystem electron transport, and that chromatic regulation of PSI biogenesis operates at post-mRNA levels, presumably during translational and/or assembly processes of PSI subunits.

**Fig. 1** Light responses of PSI subunit mRNAs. *Chlamydomonas* cells were cultured in a regimen of 16 h light/8 h dark to a logarithmic phase, and mRNA levels of the respective genes were determined by Northern hybridization at indicated times of light period (open circle); similarly for those cells kept in prolonged darkness (closed circle). 0 h indicates the onset of light illumination. The left panels show the chloroplast-encoded genes, *psaA, psaB,* and *psaC,* and the right panels represent the nuclear-encoded genes of *psaD, psaE, psaF, psaG,* and *psaH.* *Cblp* is a constitutively expressed gene encoding G-protein β subunit-like polypeptide (Schloss 1990).
Redox regulation of PSI genes

Results

Light response of PSI genes in C. reinhardtii

When C. reinhardtii cells were transferred from darkness to the light, mRNA levels of both chloroplast- and nuclear-encoded PSI genes started to increase after 2 h (Fig. 1). The magnitude of light induction was generally greater for the nuclear-encoded genes than for the chloroplast genes. When the cells were kept in prolonged darkness, the mRNA levels continued to decrease. It is interesting that the mRNA abundance of the subunits important for the photosynthetic electron flow (psaA, B, D and F) peaked at 6 h of illumination, whereas those for peripheral subunits (psaE, G and H) increased at least until 8 h.

Photosynthesis is involved in the light induction of PSI genes

To examine the effect of light on PSI genes, we chose psaE and psaB as the models of nuclear and chloroplast genes, respectively. The product of psaE, the PsaE subunit, is involved in the interaction of PSI with ferredoxin, whereas the psaB product, PsaB, is a component of the reaction center core.
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2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), which inhibits electron transport from PQ to the Cyt b$_6$f complex (Trebst 1980). When Chlamydomonas cells were treated with DBMIB in the light, although the reduction of the cellular ATP level was similar to that of CCCP treatment (Table 1), both psaE and psaB mRNA levels were greatly decreased (Fig. 3, the right panels). At the same time, the mRNA level of a control gene, Cblp (G-protein β subunit-like polypeptide; Schloss 1990) showed little reduction (Fig. 3). Since light induction of psaB and psaE mRNAs was inhibited by DBMIB and DCMU, but not by CCCP, we concluded that the photosynthetic electron flow, but not photophosphorylation, plays a pivotal role in the regulation of PSI gene expression.

Redox state of the plastoquinone pool is not involved in the light induction

In order to obtain further insight into how PET induces the PSI subunit mRNAs, we examined whether the redox state of the PQ pool is responsible for this induction. PQ is an electron carrier between PSII and PSI, and the redox state of the PQ pool was shown to regulate the transcription of the chloroplast-encoded PSI genes in mustard (Pfannschmidt et al. 1999).

DCMU and DBMIB inhibit the electron flow at the oxidative and reductive sides of PQ, respectively (Trebst 1980). As shown in Fig. 2 and 3, both herbicides inhibited the light induction of psaB and psaE mRNAs, indicating that the redox state of the PQ pool is not involved in this scenario. We should be careful in interpreting the inhibitor effects: a complete block of the PET by DBMIB, for instance, is known to cause oxidative stress in chloroplasts (Irihimovitch and Shapira 2000). To avoid any side effects, we treated the Chlamydomonas cells with lower concentrations of DBMIB (0.5–2.0 μM) under dim light. These concentrations did not affect the cellular ATP level (Fig. 4A), indicating that the inhibition of the electron transport was only partial. DBMIB at 0.5–2 μM showed no effect, while 4 μM DBMIB inhibited psaE and psaB mRNA accumulation (Fig. 4B, C). It is therefore unlikely that the redox state of the PQ pool is involved in this light induction.

DBMIB is an antagonist of plastoquinol, inhibiting the Cyt b$_6$f complex-dependent protein kinase activity by binding to the Q$_o$ site of this complex (Vener et al. 1997). Therefore, it might be possible that the apparent inhibitory effect of DBMIB on light induction was caused by the blocking of this kinase activity. To confirm that the light induction of PSI genes was independent of the redox state of the PQ pool, we attempted to alter the redox state, not by inhibitors but by illumination with monochromatic light.

Light emitting diode (LED) light sources of 695 nm and 644 nm were designed to preferentially excite chlorophyll a
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and chlorophyll b, respectively, thus more efficiently activating PSI or PSII (Melis et al. 1996). As shown in Fig. 5, irradiation of *Chlamydomonas* cells with 644-nm light decreased the chlorophyll fluorescence of PSII (Fig. 5, Fm/II), whereas that of 695 nm increased the fluorescence signal (Fig. 5, Fm/I). This response is a characteristic of state transition; the 644-nm light induced the transition to state II, and the 695-nm light induced the transition to state I (Haldrup et al. 2001). This indicates that the 644-nm light and 695-nm light preferentially activated PSII and PSI, respectively, and caused the reduction and oxidation of the intersystem electron transport carriers (Allen 1992). Hereafter, we refer to the 644-nm light and 695-nm light as PSII-light and PSI-light, respectively.

By using these light sources, we examined the effect of the redox state of the intersystem electron carriers on the light induction of PSI genes again. When the cells were irradiated with PSI-light or PSII-light at 10 W m\(^{-2}\) for 6 h from the beginning of the light period, both light treatments induced the *psaE* and *psaB* mRNAs to a similar extent, although the overall level of *psaE* mRNA was lower than in white light (Fig. 6). To test whether full induction of *psaE* mRNA required wavelengths that were missing in these monochromes, we superimposed dim white light (0.5 W m\(^{-2}\)) on the monochromatic light, and examined the effect of PSI-light and PSII-light. As shown in Fig. 6, additional illumination with dim white light restored *psaE* expression to the level found in white light. Again, also under these light conditions, PSI-light and PSII-light induced the *psaB* and *psaE* mRNAs similarly. These results indicate that light induction of *psaB* and *psaE* mRNAs occurs irrespective of the redox state of the intersystem electron carriers.

**Chromatic regulation of PSI biogenesis operates at translational and/or post-translational steps**

In most photosynthesizing organisms, including *Chlamydomonas*, PSI biogenesis is regulated in response to irradiated light quality, leading to the chromatic adaptation of the photosystem stoichiometry (Kim et al. 1993, Murakami and Fujita 1993, Fujita 1997, Murakami et al. 1997, Pfannschmidt et al. 1999). Since PSI subunit mRNA levels were not regulated in response to our PSI-light or PSII-light (Fig. 6), we tested whether chromatic adaptation really occurred under our experimental conditions.

*Chlamydomonas* cells were cultured under the PSI-light or PSII-light for 24 h, after which the abundance of the PSI core...
The effects of PSI-light (PSI-L) or PSII-light (PSII-L) irradiation on the cellular abundance of PsaA/B proteins. (A) Lithium lauryl sulfate PAGE profiles of the total membrane fractions from the wild-type strain of Chlamydomonas cells. Asterisks indicate PsaA/B proteins. The cells were exposed to PSI-light (5 W m\(^{-2}\), approx. 29 μE m\(^{-2}\) s\(^{-1}\)) or PSII-light (5 W m\(^{-2}\), approx. 27 μE m\(^{-2}\) s\(^{-1}\)) for 24 h, then 5 mg of total membrane proteins were extracted, resolved by lithium lauryl sulfate PAGE (15%) at 4°C, and stained with Coomassie Brilliant Blue. (B) Lithium lauryl sulfate PAGE profiles of the PsbA-deficient strain, FUD7, treated as in (A). (C) The psaE and psaB mRNA levels of the wild-type cells irradiated with PSI-light (left) or PSII-light (right) as in (A).

subunits was compared. As shown in Fig. 7A, the PsaaA/B proteins (asterisk) were more abundant in PSII-light than in PSI-light. Thus, chromatic regulation of PSI genes actually occurred in our experimental system. Since PSI subunit mRNA levels did not change under these conditions (Fig. 7C), translational and/or post-translational processes should be crucial for this regulation.

We then tested whether PET is involved in this regulation by subjecting a PSII mutant strain FuD7, which lacks the psbA gene (Bennoun et al. 1986), to the PSI-light or PSII-light treatment. As shown in Fig. 7B, the membrane protein composition of FUD7 did not change through these treatments, indicating that PSII activity is indispensable for the chromatic regulation of PSI biogenesis.

**Discussion**

**Photosynthetic electron flow induces the PSI mRNAs, irrespective of the redox state of the plastoquinone pool**

This study provided two important insights into the light regulation of PSI biogenesis. The first is that the accumulation of PSI subunit mRNAs is induced by photosynthetic electron flow. Previous studies on the light induction of PSI genes were mostly focused on the greening process of etiolated plants, and revealed that the phytochrome-derived signals are involved in this response (Zhu et al. 1985, Brunner et al. 1991, Neuhaus et al. 1993). In contrast, the light induction of PSI genes in green, photosynthetically active cells is not well understood. The unicellular green alga *C. reinhardtii* has developed a chloroplast even in the dark, and using this alga we revealed that at least 40% of the light-induced accumulation of PSI mRNAs is mediated by photosynthesis-dependent mechanisms (Fig. 2).

This light induction was disturbed by the photosynthesis electron transport inhibitors, DCMU and DBMIB, but not by an uncoupler, CCCP, indicating that the photosynthetic electron flow, but not photophosphorylation, plays a pivotal role in this regulation.

Electron carriers between PSI and PSII, e.g. PQ and the Cyt b\(_6\)/f complex, were shown to be redox sensors and to adjust photosystem stoichiometry to light quality during the long-term photoacclimation process (Murakami and Fujita 1993, Pfannschmidt et al. 1999, Tullberg et al. 2000). This adjustment is fully or partially dependent on the PSI biogenesis in cyanobacteria, green algae and higher plants (Kim et al. 1993, Murakami and Fujita 1993, Murakami et al. 1997, Pfannschmidt et al. 1999, Tullberg et al. 2000). Pfannschmidt et al. (1999) showed that reduction of PQ by PSII-light and also by DBMIB treatment induced the transcription of the chloroplast-encoded *psaAB* gene in mustard seedlings. To examine whether this is also the case for the PSI subunit mRNAs in *Chlamydomonas*, we altered the redox state of the PQ pool by photosynthesis inhibitors and by irradiation with monochromatic light. Unexpectedly, reduction of the PQ pool by DBMIB application gave a significant inhibitory effect on the light induction of *psaB* and *psaE* mRNAs (Fig. 3, 4). Moreover, these mRNAs were induced by both PSI-light and PSII-light, which oxidized and reduced the PQ pool, respectively (Fig. 6). From these results, we conclude that the light induction of PSI subunit mRNAs occurs, irrespective of the redox state of the PQ pool.

As this light induction occurs irrespective of the redox state of intersystem electron carriers, its physiological significance cannot be ascribed to the photosystem stoichiometry adjustment. This induction mechanism allows PSI genes to be expressed only when PET is active. This means that PSI gene expression is regulated by positive feedback. This feedback regulation appears to be important, especially for unicellular organisms including *Chlamydomonas*, because they carry a limited amount of nutrients, and hence economical growth and cell proliferation would be critical for their survival.

PET induced the mRNAs of the nuclear-encoded PSI genes as well as those of the chloroplast genes. Thus, the induction signal(s) generated by PET should be transduced from the chloroplast to the nucleus.

This signal transduction mechanism is the subject of future study. The light induction showed little sensitivity to light intensity, and occurred even in dim light where the rate of PET was less than 2% of the maximum level (Fig. 2). In addi-
tion, as described above, this light induction was affected little by light quality (Fig. 6). Thus, the signal transduction mechanism of this response should be different from those of previously reported redox regulations of light-intensity response (Escoubas et al. 1995, Karpinski et al. 1997) or light-quality response (Pfannschmidt et al. 2001).

Dual roles of PET in PSI biogenesis

The second important finding in this study is that the chromatic regulation of PSI genes should occur at translational or post-translational steps in Chlamydomonas (Fig. 7). As in cyanobacteria and higher plants, Chlamydomonas regulates the photosystem stoichiometry in response to the environmental light quality (Melis et al. 1996, Fujita 1997). Murakami et al. (1997) demonstrated that this adaptation is achieved by de novo synthesis of the PSI complex, rather than by its degradation. However, the step of the PSI biogenesis that is critical for this chromatic regulation was unknown. Our study demonstrates that irradiation with PSII-light or PSII-light causes differences in the accumulation of PSI subunits, but not of PSI mRNAs (Fig. 7A, C). Taking these results and the report of Murakami et al. (1997) into account, translation and/or the assembly process of PSI subunits should be the critical target for chromatic regulation, at least in Chlamydomonas.

The chromatic response of PSI biogenesis was lost in the psbA-deficient mutant (Fig. 7C). This indicates that linear PET driven by PSII is indispensable for this regulation. The sensor of this chromatic response could be the redox state of the PQ pool and/or the Cyt b6/f complex, as reported for higher plants and cyanobacteria (Murakami and Fujita 1993, Pfannschmidt et al. 1999, Tullberg et al. 2000).

From this study, we conclude that Chlamydomonas cells have complex redox regulatory systems of PSI biogenesis; at least one regulatory system is insensitive to the redox state of the intersystem components, while another is responsive to it. When Chlamydomonas cells are illuminated, the photosynthetic electron flow first turns on the accumulation of PSI subunit mRNAs, and then the redox state of the intersystem electron carriers fine-tunes the PSI biogenesis during translational and/or post-translational steps to fulfill the chromatic adaptation.

Materials and Methods

Algal strains and culture conditions

A wild-type strain of C. reinhardtii, ec125 (+), and a psbA deletion mutant, Fd7 (Bennoun et al. 1986), were cultured in HS medium (Harris 1989) containing 14.6 mM sodium acetate under a regimen of 16 h light (white fluorescent tubes 10 W m⁻²) and 8 h dark at 25°C. The cells at a logarithmic growth phase were harvested, kept at −80°C, and subjected to RNA extraction.

Photosynthesis inhibitors

The PET inhibitors, DCMU and DBMIB, and an uncoupler, CCCP, were added to the Chlamydomonas cell cultures 0.5–1.0 h before the start of the light period unless otherwise indicated. The final concentrations of DCMU, DBMIB and CCCP were 10 µM, 0.5–6 µM, and 2–4 µM, respectively.

Monochromatic light treatment

Experiments with monochromatic light were basically designed according to Allen (1992) and Melis et al. (1996). Monochromatic light was irradiated, using LEDs with their peak wavelengths at 695 nm (PSI-light; NR312, Stanley Electric Co. Ltd., Tokyo) or 644 nm (PSII-light; TLRI90P, Toshiba Semiconductor, Co. Ltd., Tokyo), and their bandwidths were 30 nm and 18 nm, respectively. The state transitions from state 2 to state 1 by PSII-light irradiation, and from state 1 to state 2 by PSII-light irradiation, were monitored with a pulse amplitude modulation fluorimeter (PAM 101/103, Heinz Walz, Effeltrich, Germany) (Haldrup et al. 2001, Matsuoka and Obokata 2001), using logarithmic growing cells acclimated to the background white light (<0.5 W m⁻²) for 5 min. The intensity of the monochrome light was adjusted to 10 W m⁻² (approx. 58 µE m⁻² s⁻¹ for PSII-light and approx. 54 µE m⁻² s⁻¹ for PSI-light) or 5 W m⁻². Light intensity was measured by a Kettering radiometer model 4909 (Springfield Jarco Instruments, OH, U.S.A.).

Determination of PET rate

The relative rate of PET was calculated according to the following equation: Electron transport rate = (PSII quantum yield) × (incident flux density of photosynthetically active radiation) (Schreiber et al. 1998). PSI quantum yield in the logarithmic growing cells was measured with PAM 101/103 after dark adaptation for 5 min. The incident flux of photosynthetically active radiation was measured by the Kettering radiometer.

RNA extraction and Northern analysis

Total RNA was extracted essentially according to Shepherd et al. (1983) with some modification. The cells were lysed in a cold RNA extraction buffer containing 100 mM Tris-HCl (pH 8.5), 400 mM LiCl, 10 mM EGTA and 5 mM EDTA, supplemented with 1/10 volume of 20% SDS and 1/100 volume of 2 µg ml⁻¹ proteinase K. After phenol–chloroform extraction, RNA was purified by precipitation with 1 M LiCl and finally dissolved in H₂O. RNA samples were denatured in 53% formamide containing 6.8% formaldehyde at 65°C for 10 min, electrophoresed in 6.7% formaldehyde-containing agarose gel in MOPS buffer, and blotted to Hybond N⁺ (Amersham International plc, Amersham, U.K.), followed by Northern hybridization according to Church and Gilbert (1984). Hybridization probes for psaD, psaB, and psaC were prepared by PCR from the total genomic DNA of C. reinhardtii, and those for psaD, psaE, psaF, and Chlp were from cDNA clones (Franzén et al. 1989a, Schloss 1990, Farah et al. 1993), using gene-specific primers as follows: psaD, GGTGTCCTCGAATGCGTATCAGTCCCAACGGATGAT; psaB, CCAGCTTTTCCGACTGTCAGAAG and GGAATCACACAGAAGCCAAATAGTGA; psaC, GTTGCAGCTGCTTCTGTCATAG and GCCCATACTCTCTTGTGCTCTCTTCT; psaD, GGGCCGCTGCCACTGCG and TCTGCGCCACTGCGGTTGTA; psaE, CTGGCCCCTTCATCCCAACCA and ACCCGCTCGTCTCCCTGTC; psaF, CCTTCCATGCGGCGCTGCTCG and CACGGTGCAGTCCGTCCTGCTC; Chlp, ACCCCCTGCTCAACCCCTGCTG and CTGACGCTGATGGTCTGCTCCA. The probe for Chlp was a 0.6-kbp EcoRI fragment (Franzén et al. 1989b), and the psaF probe was a 0.9-kbp EcoRI fragment of plGF 28–3 (Franzén et al. 1989b). Probes were labeled with [α-³²P]dCTP by random priming (Ausbel et al. 1989). Autoradiographic images were analyzed using a Fujix BAS-2000 MacBAS bio-imaging analyzer (Fuji Photo Film Co. Ltd., Tokyo).
Determination of the cellular ATP level

An amount of 1×10⁶ cells was harvested and disrupted by agitation with glass beads in 5% trichloroacetic acid. After freezing and thawing twice, cell debris was removed by centrifugation, and the supernatant was diluted and neutralized by adding Tris-acetate to a final concentration of 0.1 mM. The ATP concentration of the neutralized solution was measured using the Enli™ ATP assay system (Promega Corporation, Madison, WI, U.S.A.) and Luminescencer JAB-2100 (ATTO, Tokyo).

Isolation of total membrane proteins and lithium lauryl sulfate gel electrophoresis

Harvested Chlamydomonas cells were suspended in ice-cold washing solution containing 0.1 M Na₂CO₃, 0.1 M DTT and 1 mM phenylmethylsulfonyl fluoride. After freezing and thawing, the total membrane fractions were recovered by centrifugation at 15,000×g for 10 min, and washed three times. The obtained membrane fractions were dissolved with lithium lauryl sulfate to a final concentration of 2%, and were then subjected to electrophoresis in 15% polyacrylamide gel at 4°C according to Delepeila and Cha (1982).

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