Light-Intensity-Dependent Expression of Lhc Gene Family Encoding Light-Harvesting Chlorophyll-a/b Proteins of Photosystem II in Chlamydomonas reinhardtii

Haruhiko Teramoto, Akira Nakamori, Jun Minagawa and Taka-aki Ono

Laboratory for Photobiology (1), Photodynamics Research Center, The Institute of Physical and Chemical Research, Sendai 980–0845, Japan

Excessive light conditions repressed the levels of mRNAs accumulation of multiple Lhc genes encoding light-harvesting chlorophyll-a/b (LHC) proteins of photosystem (PS)II in the unicellular green alga, Chlamydomonas reinhardtii. The light intensity required for the repression tended to decrease with lowering temperature or CO₂ concentration. The responses of six LhcII genes encoding the major LHC (LHCII) proteins and two genes (Lhcb4 and Lhcb5) encoding the minor LHC proteins of PSII (CP29 and CP26) were similar. The results indicate that the expression of these Lhc genes is coordinately repressed when the energy input through the antenna systems exceeds the requirement for CO₂ assimilation. The Lhc mRNA level repressed under high-light conditions was partially recovered by adding the electron transport inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), suggesting that redox signaling via photosynthetic electron carriers is involved in the gene regulation. However, the mRNA level was still considerably lower under high-light than under low-light conditions even in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Repression of the Lhc genes by high light was prominent even in the mutants deficient in the reaction center(s) of PSII or both PSI and PSII. The results indicate that two alternative processes are involved in the repression of Lhc genes under high-light conditions, one of which is independent of the photosynthetic reaction centers and electron transport events.

Photosynthesis is regulated at various levels in response to fluctuating light intensity under various ambient temperature and nutrient conditions. The proper responses to the various environmental cues are necessary for photosynthetic plants to use light energy efficiently and to protect themselves from photoinhibitory damage caused by excessive irradiance (Aro et al., 1993; Long et al., 1994; Osmond, 1994). Excessive light energy absorbed by chlorophyll is dissipated by non-radiative processes (Crofts and Yerkes, 1994; Horton et al., 1996; Gilmore, 1997) and is properly distributed between two photosystems (PS) by state transition (Allen, 1995; Gal et al., 1997), whereas the energy input is regulated by changes in the size of the light-harvesting antenna systems to modulate the optical cross section.

Light-harvesting chlorophyll a/b (LHC)II proteins, which are major components of light-harvesting antennae of PSII in higher plants and green algae, typically change their abundance in response to the intensity of irradiance (Anderson et al., 1988, 1995). Under stress and intense light, enhanced amounts of reactive oxygen species will react with proteins and lipids, not only in chloroplasts but also in the cytosol, and will induce various types of photodamage. Therefore, the quality and quantity control of the LHC protein complex is required to avoid photodamage by alleviating excitation energy pressure. Although the LHC protein complex could be controlled by various mechanisms including pigment synthesis, the repression of the Lhc genes under stressful light conditions must be an important antistress response of plants. However, little is known about the mechanism of how the excessive light intensity is sensed and how the signal is transduced to change gene expression. One proposal is that the redox state of the photosynthetic electron transport carrier(s) between the two PS in green algae monitors the energy balance because such carriers will be over-reduced if the energy input exceeds the requirement for the dark reaction. The abundance of LHCII protein and/or mRNA decreases with the increase of the reduced QA population probed by chlorophyll a fluorescence in Chlorella vulgaris (Maxwell et al., 1995a) and Dunaliella salina (Maxwell et al., 1995b). Expression of the LHCII gene in Dunaliella tertiolecta is enhanced by interrupting electron transfer from QA to QB with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and it is repressed by inhibiting the oxidation of plastoquinol with 2,5-dibromo-3-methyl-6-isopropyl-
expression.

The light-intensity-dependent control of LHCII gene expression. On the other hand, studies of some higher plants have shown that the redox state of the photosynthetic electron carriers is not strictly correlated with LHCII gene expression (Gray et al., 1996; Montané et al., 1998).

The light-harvesting antennae comprise several homologous LHC proteins encoded by a nuclear gene family (Jansson, 1994, 1999; Green and Durnford, 1996). Although the exact reasons for the multiplicity of the LHC proteins have not been elucidated, each LHC protein may have distinct functions in light harvesting, such as the optimization of light energy transfer and the dissipation of excessive light. Studies to date on the response of Lhc genes to light intensity have mainly focused on the gene encoding the most abundant LHC (LHCII) protein. Whether each Lhc gene is regulated independently or whether they are all coordinately regulated in response to the light intensity remains unknown. To understand the light-dependent regulation of the entire antenna system, comprehensive studies on the light response of all Lhc genes are required.

The unicellular green alga Chlamydomonas reinhardtii has been extensively applied as a model experimental system for studies of photosynthesis. The composition of LHC proteins in this alga has been best characterized in algal species (Bassi and Wollman, 1991; Bassi et al., 1992; Allen and Staehelin, 1994). We characterized the Lhc gene family encoding the LHC proteins of PSI using the C. reinhardtii expressed sequence tag (EST) databases (Teramoto et al., 2001). The results revealed that this alga has at least six genes encoding the major LHC (LHCII) proteins and two genes for the minor LHC proteins (CP29 and CP26). The highly homologous LHCII proteins in C. reinhardtii cannot be assigned to any of the three proposed types in higher plants (Lhcb1-Lhcb3), but they can be classified into four distinct types. Type I is encoded by the three genes: Lhcb1-1.1, Lhcb1-1.2, and Lhcb1-1.3. Types II, III, and IV are encoded by Lhcb1-2, Lhcb1-3, and Lhcb1-4, respectively. Therefore, C. reinhardtii should provide a promising experimental system with which to study regulation of Lhc gene expression under various environmental conditions.

The present study uses quantitative reverse transcriptase (RT)-PCR to examine the amounts of the multiple Lhc mRNAs that accumulate in C. reinhardtii cells exposed to various intensities of light at various temperatures and under different CO2 conditions. The Lhc mRNA levels were also examined in the presence of the PSI inhibitor DCMU and in mutants deficient in the PSI or both the PSI and PSI reaction center(s). We discuss the mechanisms involved in the light-intensity-dependent control of Lhc gene expression.

RESULTS

Light-Intensity-Dependent Expression of Lhc Genes at Various Temperatures

Figure 1 shows profiles of DNA amplification by PCR in the RT-PCR analysis of Lhcb1-4 and Lhcb4 encoding LHCII and CP29 protein, respectively. C. reinhardtii cells cultured mixotrophically under continuous light (5 μE m⁻² s⁻¹) were dark-adapted for 12 h at 26°C and then transferred to various light intensities (0, 50, 100, or 200 μE m⁻² s⁻¹) for 6 h at 22°C. Total RNA was analyzed by RT-PCR to quantify the mRNA. Relative mRNA abundance revealed by electrophoresis of the RT-PCR products (Fig. 1A) was more precisely quantified by analyzing the kinetics of the PCR reaction using a real-time PCR assay system (Fig. 1, B and C). The amounts of Lhcb1-4 and Lhcb4 mRNAs were significantly larger under low light (50 μE m⁻² s⁻¹), as revealed by the appearance of the PCR products at fewer PCR cycles, than in the dark or under high light (200 μE m⁻² s⁻¹). In contrast, light intensity barely affected the amount of

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18S rRNA (Fig. 1, A and D), which was used as the internal standard in the quantitative RT-PCR analysis described below.

Figure 2 shows the effects of light intensity on the levels of LhcII-4 and Lhcb4 mRNAs that accumulated at various temperatures. The abundance of Lhc mRNA was normalized with respect to that of 18S rRNA and then presented as values relative to that under dark conditions, where effects of temperature on the amounts of the Lhc mRNAs could be minimized. Figure 2A shows that the mRNA level of LhcII-4 in the cells exposed to low (50 μE m⁻² s⁻¹) and medium (100 μE m⁻² s⁻¹) light intensity at 26°C was increased 8- to 10-fold compared with that in the cells kept in darkness. However, the level in the cells exposed to high light (200 μE m⁻² s⁻¹) only doubled. These findings indicate that the mRNA level is significantly enhanced by illumination at low and medium intensity but the light-dependent enhancement is repressed to be low at high intensity. Low-light exposure at 22°C similarly enhanced the mRNA level, but the level under medium light was one-fourth of that under low light, and the mRNA level was not enhanced under high light. In contrast to the findings at 22°C and 26°C, low light barely enhanced the mRNA level at 18°C. The level under medium and high light at 18°C was even lower than that in darkness. It is of note that no visible symptom of photodamage was observed and the cells proliferated well under the high-light conditions at 18°C. These results indicate that the threshold light intensity required to repress the mRNA accumulation decreased as the temperature lowered. The cells at 18°C may perceive the light at 200 μE m⁻² s⁻¹ as being more intense than 200 μE m⁻² s⁻¹ at 26°C. Figure 2B shows that the mRNA level of Lhcb4 responded to light intensity and temperature in a similar manner to that of LhcII-4, although the mRNA level was slightly higher under 100 μE m⁻² s⁻¹ than under 50 μE m⁻² s⁻¹ at 26°C.

Figure 3 compares the responses of the mRNA levels of four Lhc genes (LhcII-1.1, LhcII-1.3, LhcII-3, and LhcII-4) encoding the major LHC (LHCCI) proteins and two (Lhcb4 and Lhcb5) encoding the minor LHC proteins (CP29 and CP26) to light intensities at three different temperatures. The levels of mRNA accumulation are shown relative to those under low light (50 μE m⁻² s⁻¹). The responses of the mRNAs of the tested Lhc genes to light intensity and the effects of temperature on their responses were essentially similar. These results suggest that these Lhc genes are coordinately regulated under common signal transduction pathways. The relative mRNA levels for Lhcb4 and Lhcb5 were slightly but reproducibly higher than those for the LhcII genes under high light (200 μE m⁻² s⁻¹) at all the tested temperatures. This suggests that the genes of the minor LHC proteins were less sensitive to down-regulation by high light than those of the major LHC proteins.

**Effects of CO₂ on Light-Intensity-Dependent Expression of Lhc Genes**

Figure 4 shows the effects of the CO₂ concentration on the light-dependent changes in the mRNA levels of the Lhc genes. The cells were photoautotrophically cultured in HS minimal medium under low light (50 μE m⁻² s⁻¹) at 26°C bubbled with either air (low CO₂) or 5% (v/v) CO₂ in air (high CO₂), then exposed to very high light (1,000 μE m⁻² s⁻¹) for 6 h. The mRNA level for all the tested Lhc genes (LhcII-1.1, LhcII-1.3, LhcII-3, LhcII-4, Lhcb4, and Lhcb5) in the cells exposed to very high light under the low CO₂ concentration was about 20% of that in the cells incubated under low light. These changes are compatible with the results obtained under high light (200 μE m⁻² s⁻¹; Figs. 2 and 3), where the cells were dark-adapted before exposure to the various light conditions with no air-bubbling. The mRNA levels under low light were not significantly affected by the CO₂ concentration (data not shown). In contrast to low CO₂ conditions, exposure to high light under high CO₂ conditions elevated the mRNA level to 7- to
30-fold of that under low light. These results indicate that limitation of the supply of carbon source is the primary cause of the decreased Lhc mRNAs levels with high light under our experimental conditions.

Effects of DCMU on Light-Intensity-Dependent Expression of Lhc Genes

The results so far indicated that the Lhc genes are down-regulated when light energy absorbed by the light-harvesting system exceeds the requirement of the photosynthetic dark reactions, which are retarded by a decrease of temperature or a limited CO₂ supply. Under the excessive light conditions, the photosynthetic electron carriers subsequent on the acceptor side of PSII are in an over-reduced state. Therefore, the redox state of one of the electron carriers may be used to sense the energy imbalance under excessive light conditions that would down-regulate the Lhc genes. To examine this notion, we examined light-intensity-dependent changes in the Lhc mRNAs levels in the presence of the herbicide, DCMU, which inhibits electron transfer from reduced QA to QB. C. reinhardtii cells were cultured in the dark for 12 h at 26°C in the presence or absence of 10 μM DCMU, then transferred to low (50 μE m⁻² s⁻¹) or high light (200 μE m⁻² s⁻¹) for 6 h at 22°C. Photosynthetic oxygen evolution was almost completely inhibited in the presence of 10 μM DCMU (data not shown).

Figure 5 shows the results of quantitative RT-PCR analysis of the six Lhc genes described above and LhcbII-1.2 and LhcbII-2. The level of the LhcbII-1.1 mRNA was approximately 50-fold lower under high light than under low light in the absence of DCMU. The presence of DCMU reduced the mRNA level by only 20% in the cells exposed to low light, indicating that
the mRNA accumulates under low light even when electron transport at the Q<sub>B</sub> site is interrupted. In contrast, DCMU caused a 10-fold increase in the mRNA level in the cells transferred to high light. These findings are consistent with the view that excessive electron donation by PSII reduces the Lhc mRNA level. We emphasized, however, that the mRNA level enhanced by DCMU under high light was still significantly lower than that under low light. This indicates that the level of Lhcb4 mRNA accumulation can respond to high light even when electron delivery from PSII is inhibited by DCMU. The effect of DCMU on the responses of the other Lhc genes to light intensity was similar, although the extent of the responses varied among those genes. With respect to the Lhcb4 and Lhcb5 genes, the mRNA levels were relatively high under high light in the presence and absence of DCMU. The levels under high light reached 50% to 60% of those under low light in the presence of DCMU. Figure 5 also shows the mRNA levels of the CRY1 and psbA genes. The nuclear gene CRY1 encodes the ribosomal protein S14 (Nelson et al., 1994), and psbA is a chloroplast gene encoding the D1 protein of the reaction center of PSII (Erickson et al., 1984). In contrast to the levels of the Lhc mRNAs, those of both the CRY1 and psbA mRNAs were relatively higher under high light than under low light. DCMU did not affect the CRY1 mRNA level but enhanced that of the psbA mRNA. The enhancement of the psbA mRNA level by DCMU is compatible with the fact that the oxidized PQ pool can induce psbA expression in chloroplasts and cyanobacteria (Pfannschmidt et al., 1999; Li and Sherman, 2000). As an alternative, this may be ascribed to the inhibitory effect of DCMU on mRNA degradation in chloroplasts (Salvador and Klein, 1999), although such effect is not observed for the psbA gene. However, why the mRNA level is enhanced by high light remains unclear, but an increased turnover rate of the D1 protein may be involved.

**Figure 5.** Effects of DCMU on light-intensity-dependent changes in accumulation of Lhc mRNAs. *C. reinhardtii* cells dark-adapted for 12 h at 26°C were exposed to low (50 μE m<sup>-2</sup> s<sup>-1</sup>) or high (200 μE m<sup>-2</sup> s<sup>-1</sup>) light for 6 h at 22°C with or without DCMU. Quantitative RT-PCR data for Lhcb1-1, Lhcb1-2, Lhcb1-3, Lhcb1-2, Lhcb1-3, Lhcb1-4, Lhcb5 mRNAs and CRY1 and psbA mRNAs are presented. Abundance of mRNA relative to values under low light without DCMU is shown after normalization with respect to that of 18S rRNA. Results from two independent experiments were similar, and mean values are presented.

**Figure 6.** Light-intensity-dependent changes in accumulation of Lhc mRNAs in mutants deficient in the photosynthetic reaction center(s). Wild-type strain C-9 (WT), a strain deficient in PSI reaction center, ac-u-e (-PSII), and a strain deficient in both PSI and PSII reaction centers, FUD7-ΔpsaA (-PSI and -PSII) were dark-adapted for 12 h at 26°C and then exposed to low (50 μE m<sup>-2</sup> s<sup>-1</sup>) or high (200 μE m<sup>-2</sup> s<sup>-1</sup>) light for 6 h at 22°C. Quantitative RT-PCR data for Lhcb1-4, Lhcb4, and CRY1 mRNAs are presented. Abundance of mRNA in each strain relative to values under low light is shown after normalization with respect to that of 18S rRNA. Each value is mean with standard error. Points indicate means and bars indicate standard errors of three independent experiments.
similar to that of the wild type, although the extent of changes was somewhat different. The mRNA levels of these two Lhc genes in the mutants were comparable with those of the wild type under low light (data not shown). These results indicate that as in the wild-type strain, Lhcb1-4 and Lhcb4 mRNA accumulation was repressed by high light in these PS-deficient mutants. On the other hand, the level of CRY1 mRNA was not significantly affected by changes in light intensity in either the mutants or in wild-type cells.

**DISCUSSION**

*C. reinhardtii* has at least six genes corresponding to the major LHC (LHCII) proteins and two corresponding to the minor LHC proteins (CP29 and CP26) according to an analysis of the EST databases (Teramoto et al., 2001). Lhcb1-1.2, LhcbII-2, and Lhcb5 could be expressed at much lower levels than the other LhcII genes if the relative number of EST clones for each gene reflects the differences in expression levels for multiple Lhc genes. The present results showed that levels of all of the tested Lhc mRNAs similarly responded to the light intensity when *C. reinhardtii* cells were exposed to light for 6 h. The mRNA levels were markedly increased by the exposure to low light, but this induction was repressed under high light. The levels were down-regulated at lower light intensities at lower temperatures or under conditions of a limited CO2 supply, suggesting that the mRNA levels are repressed when the photon energy absorbed by PS exceeds the energy used by the CO2 assimilation reactions. The results showed that all of the tested Lhc genes are coordinately regulated under excessive and low light conditions. Multiple products of these genes are thought to be assembled into a PSII antenna complex. Therefore, the coordinate regulation of the Lhc mRNAs levels may contribute to an efficient control of antenna size in response to changes in some environmental factors.

The present results showed that the mRNA levels for the Lhcb4 and Lhcb5 genes encoding the minor LHC proteins (CP29 and CP26) tended to be less responsive to light intensity than those of the major LHC proteins (Fig. 3). Results from cells acclimated to various light conditions for a longer time suggest that Lhcb5 is regulated by light in a less strict manner compared with LhcbI-1.1 (Minagawa et al., 2001). The major and minor LHC proteins differ in terms of pigment contents and protein-protein interaction (Green and Durnford, 1996; Horton et al., 1996). Therefore, the differential expression between these two types of LHC genes may reflect their functional specificity in an antenna complex. It is, therefore, reasonable that the genes for the inner antennae (CP29 and CP26) are somewhat differently regulated than the rest of the peripheral antennae (LHCII). Schrager et al. (2001) reported that a gene product for CP29 was specifically present in the cDNA library generated from *C. reinhardtii* cells starved for nutrients. The result may be compatible with the less strict repression of Lhcb4 under excessive light conditions observed in this study. The present study examined the expression of eight genes for LHC proteins of PSII, which have been found based on the analysis of *C. reinhardtii* EST databases (Teramoto et al., 2001). However, it is of note that genes expressed at very low levels or under specific conditions may not be detected from the EST databases. Several Lhc genes, presumably for LHCII proteins, were recently deposited in GenBank under accession numbers AF330793 (Mussgnug and Kruse), AF479777, AF479778, and AF479779 (Elrad, Niyogi and Grossman). Detailed characteristics of these genes have not been reported.

Escoubas et al. (1995) reported that LHCII gene expression under high light is enhanced by inhibiting electron transfer from QA to QB with DCMU but is repressed by inhibiting the oxidation of plastoquinone with 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone in *D. tertiolecta*. On the basis of these results, they proposed that the redox state of the PQ pool is involved in the light-intensity-dependent expression of Lhc in green algae and that an over-reduced PQ pool is an initial signal for excessive light excitation leading to the down-regulation of gene expression. A similar redox control of gene expression has been proposed for both nuclear and chloroplast genes encoding photosynthetic proteins (Allen, 1995; Pfannschmidt et al., 1999, 2001b; Oswald et al., 2001) and for photosynthetic genes in cyanobacteria (Li and Sherman, 2000; Bissati and Kirilovsky, 2001). Furthermore, a similar redox control mechanism participates in the state I-state II transition operated for the redistribution of absorbed excitation energy between PSI and PSII (Allen, 1995; Gal and Ohad, 1997).

Figure 5 shows that DCMU partially restored the accumulation of Lhc mRNAs, which were repressed under high light. This result could be compatible with the above control mechanism via the redox state of the PQ pool. However, much less Lhc mRNAs accumulated under high light than under low light even in the presence of DCMU, indicating that the regulation mechanism through the redox-state of the pooled PQ cannot simply account for the repression of the Lhc genes under excessive light. The repression of the Lhc mRNAs levels under high light was distinct even in the two mutants deficient in the reaction center(s) shown in Figure 6. This demonstrated that Lhc mRNAs levels can be down-regulated by high light through a mechanism that is independent of the photosynthetic reaction centers. Therefore, a putative redox-independent mechanism is responsible for repressing the Lhc mRNAs levels under excessive light shown in Figures 2 through 4 and under high light in the presence of DCMU shown in Figure 5. However, these data cannot completely exclude the possibility that the high-light repression in the mutants occurs
via a mechanism that is not directly related to the repression found in the wild-type cells.

In general, the PQ pool-dependent redox control mechanism is important for fine-tuning the distribution of excitation energy between the two PS and/or adaptation to physiological light conditions (Pfannschmidt et al., 2001a). When the irradiance exceeds the level that triggers photoinhibitory effects, the function of PSII is impaired. The function of PSII can be restored by the repair cycle including selective degradation of the damaged D1 protein and regeneration of the PSII complex with de novo synthesized D1 protein (Aro et al., 1993; Andersson and Aro, 2001). It has been reported that reactive oxygen species preferentially inhibit the repair process (Nishiyama et al., 2001). If the majority of PSII are photodamaged, the control of the Lhc genes through the redox state of a linear electron transport chain will not work well because the damaged PSII cannot reduce the electron transport carriers sufficiently. Under these situations, the redox-independent mechanism for down-regulation of Lhc genes demonstrated in the present study may be important for minimizing excitation pressure to facilitate the repair of the damaged PSII.

We considered how *C. reinhardtii* cells perceive high light to repress the Lhc genes in the presence of DCMU and in the absence of PS. We note that the PS mutants used in this study retained amounts of antennae that were comparable with those of wild-type cells. The LHC antennae could be a potent source of reactive oxygen species in principle. An excited triplet state of oxygen (1O2*) and 3Chl* formation in the LHC antenna should be restored by the repair cycle including selective degradation of the damaged D1 protein and regeneration of the PSII complex with de novo synthesized D1 protein (Aro et al., 1993; Andersson and Aro, 2001). It has been reported that reactive oxygen species preferentially inhibit the repair process (Nishiyama et al., 2001). If the majority of PSII are photodamaged, the control of the Lhc genes through the redox state of a linear electron transport chain will not work well because the damaged PSII cannot reduce the electron transport carriers sufficiently. Under these situations, the redox-independent mechanism for down-regulation of Lhc genes demonstrated in the present study may be important for minimizing excitation pressure to facilitate the repair of the damaged PSII.

Taking these into consideration, a reactive oxygen species or its reactant with a cellular component may function as an initial signal of excessive light intensity. Many metabolic systems scavenge reactive oxygen species in chloroplasts and the cytosol. Therefore, one of the metabolic species of such systems may be involved in the repression of Lhc mRNAs levels under high light. In fact, several lines of evidence indicate that the expression of some nuclear and plastid genes in plants is controlled by reactive oxygen species. Further studies are required to elucidate the involvement of these factors in the repression of Lhc mRNAs levels under excessive and stressful light conditions.

Up-regulation of Lhc genes accompanying transfer from dark to moderate light is performed not only via photosynthetic reactions but also via a signal transduction mechanism with a specific photoreceptor molecule, such as phytochrome in some plant species (Terzaghi and Cashmore, 1995; Argiello-Astorga and Herrera-Estrella, 1998). Therefore, the enhanced Lhc mRNA accumulation upon exposure to low light in this study could be at least partly ascribed to up-regulation by this type of photoreceptor system. This view is consistent with the results that the addition of DCMU and the deletion of the PS little affected the low-light accumulation. Although phytochrome has not been reported in *C. reinhardtii*, a protein with retinal as chromophore (chlamyopsin; Deininger et al., 1995) and a homolog of cryptochrome (CPH1; Small et al., 1995) have been identified. Kindle (1987) proposed that the light-induced expression of a Lhc gene is controlled by a system with a blue-light receptor rather than by a phytochrome in *C. reinhardtii*, although the photoreceptor has not yet been identified. In Arabidopsis, a blue-light receptor (NPL1) conducts the high-light avoidance response of chloroplasts (Jarlo et al., 2001; Kagawa et al., 2001). Thus, a blue-light receptor might be involved in the high-light repression of the levels of Lhc mRNAs. However, this view is less likely because the light enhancing the Lhc mRNA level turns to repress the level under lower temperatures or less CO2 supply, indicating that the Lhc mRNAs levels do not respond to light intensity itself.
We cannot completely exclude the possibility that the Lhc genes are independently controlled under high light by multiple sensory and signaling processes, in concert with each other.

In conclusion, the present results indicate the mechanism for high-light repression of Lhc genes independent of the function of the both photosynthetic electron transport chain, in addition to the process dependent on the redox state of the photosynthetic electron transport chain. The former redox-independent process is assumed to be functional when plants are exposed to excessive and stressful light, which expedites photoinhibitory effects.

**MATERIALS AND METHODS**

**Strains and Culture Conditions**

The wild-type *Chlamydomonas reinhardtii* strain C-9 was obtained from the IAM culture collection at the University of Tokyo. The PSII-deficient strain and the PSII-PSI double-deficient mutant FUD7-ΔpsaA generated by deleting the *psaA* gene in a PSII-deficient strain FUD7 (Bennoun et al., 1986) was a gift from Dr. K. Redding.

*C. reinhardtii* cells were mixotrophically cultured in Tris-acetate-phosphate medium (Gorman and Levine, 1965) under continuous dim light (5 μmol m⁻² s⁻¹) at 26°C with constant agitation. The cultures were in the mid-logarithmic growth phase (approximately 1 x 10⁶ cells mL⁻¹) when plants were exposed to excessive and stressful light, which expedites photoinhibitory effects.

**Quantitative RT-PCR**

Total RNA was prepared from algal cells using the TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s manual. Using an RNA PCR kit (TaKaRa, Tokyo), single-stranded cDNA was synthesized from 0.5 μg of total RNA with random primers in 20 μL of reaction mixture, and then 1 μL of the cDNA mixture was added as a template in 19 μL of PCR mixture containing each primer (0.2 μmol) and DNA polymerase master mix with SYBR Green I (SYBR Green PCR Master Mix, PE Biosystems, Foster, CA) or LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics, Mannheim, Germany). After heating at 95°C for 10 min, PCR reactions proceeded via 40 cycles of 15 s at 95°C, 5 s at 60°C, and 10 s at 72°C. The amount of the amplified DNA was monitored by fluoroscopy and sequencing of the PCR product. Quantification was based on the theory that the cycle threshold value, which is defined as the cycle number required to obtain a fluorescence signal above the background, correlates inversely with the log of the initial template concentration (Huguchi et al., 1992). The relative abundance of the targeted mRNAs from several samples was determined from a standard curve that was constructed from a set of dilution series of single-stranded cDNA from one sample. To standardize the results, the relative abundance of 18S rRNA was also determined and used as the internal standard.

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**LITERATURE CITED**


