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# REPRESSION OF *Lhcb* GENES FOR CHLOROPHYLL *a/b*-BINDING PROTEINS UNDER HIGH-LIGHT CONDITIONS IN *Chlamydomonas*

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*Lhcb* genes encoding light-harvesting chlorophyll-*a/b* binding (LHC) proteins of photosystem (PS) II were comprehensively characterized using the expressed sequence tag (EST) databases in the green alga, *Chlamydomonas reinhardtii*. The gene family was composed of eight *Lhcb* genes including four new genes, which were isolated and sequenced. The effects of light intensity on the levels of mRNAs accumulation of multiple *Lhcb* genes were studied under various conditions. The results indicate that *Lhcb* genes are coordinately regulated in response to light conditions, and repressed when the input light energy exceeded the requirement for $CO_2$ assimilation. The effects of high light on the expression of the *Lhcb* genes observed in the presence of an electron transport inhibitor, DCMU, and in mutants deficient in photosynthetic reaction centers suggest the presence of two alternative mechanisms for regulating the genes expression under high-light conditions.

**Key words:** photosynthesis, light-harvesting, LHC, gene expression, *Chlamydomonas*

## INTRODUCTION

Under excessive irradiance, PS II is suffered from photoinhibitory damages. Therefore, photosynthetic organisms have developed various mechanisms for protecting themselves from the input of excess light energy, including the dissipation of the absorbed energy by non-radiative process and the decrease of the optical cross section by changes in the size of the light-harvesting antenna system [1]. Light harvesting antennae complex of PS II is composed of major chlorophyll-*a/b* binding (LHC) proteins and minor LHC proteins, of which abundance is changed in response to the intensity of the irradiance. Various LHC proteins probably play some distinct functions for regulating light-harvesting events in the complex [2]. The repression of the *Lhcb* genes under stressful light condition must be important for the quality and quantity control of the LHC protein complex. However, little is known about the mechanism how the excessive light is sensed and how the signal is transduced to change gene expression. Notably, a signal for the excessive light must be transferred from chloroplasts to nucleus where LHC proteins are encoded in eukaryotes [3]. In this paper, we identify the *Lhcb* gene family encoding LHC proteins of PS II using expressed sequence tags (ESTs), and characterize their expressions under excessive light conditions in the green alga *Chlamydomonas reinhardtii*.

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## MATERIALS AND METHODS

The wild type *C. reinhardtii* strain C-9 and 2137 were mixotrophically cultured in TAP medium under continuous dim light (5μE $m^{-1}$ $s^{-1}$) at 26 ℃. The 3' and 5' ends of cDNA of *Lhcb*-related genes in *C. reinhardtii* were identified by 3'-RACE and cap site hunting. The genomic DNA of *Lhcb* was amplified by PCR using specific primer sets for each *Lhcb* gene, cloned, and sequenced. For studying the effects of light intensity on the expression of the *Lhcb* genes, the culture at mid-logarithmic phase were

dark-adapted for 12 hr, then subjected to various conditions. The amounts of mRNA of individual *Lhcb* genes were quantified by reverse transcription (RT)-PCR using a real time PCR monitoring system (Gene Amp 5700:PE Biosystem or LightCycler: Roche Diagnotics). The PS II deficient strain *ac-u-ε* was obtained from *Chlamydomonas* Genetic Center (Duke Univ.) and the PS II/I double deficient strain was a kind gift from Dr. K. Redding.

## RESULTS AND DISCUSSION

*Lhcb* gene family in *C. reinhardtii*. A total of 699 among over 15,000 ESTs related to the *Lhcb* genes were assigned to eight genes, including four new genes [4]. We assigned 615 ESTs to six distinct *LhcII* genes, which encodes a homolog to a major LHC (LHC II) proteins. Sequences of the cDNA and genomic clones of these genes revealed that their coding regions have 80-95% identity but no similarity in their untranslated regions. Database searches also revealed 60 ESTs with high homology to Lhcb4 protein (CP29) and 24 ESTs to Lhcb5 protein (CP26), but no EST for CP24 and PsbS proteins was found. A *Lhcb4* gene was cloned and sequenced.

Figure 1 shows a phylogenetic analysis of the LHC II proteins from *C. reinhardtii* and a higher plant (tomato). Among six LhcII proteins, three proteins show high homology, suggesting their generation by more recent gene duplication, and were classified into one type; Type I (LhcII-1.1, -1.2 and -1.3). Since other three proteins are a little different from Type I protein and they are different from each other to a similar extent, they were classified into three different types: Type II (LhcII-2), Type III (LhcII-3) and Type IV (LhcII-4), respectively. Each type of *C. reinhardtii* LHC II protein is divergent from LHC II

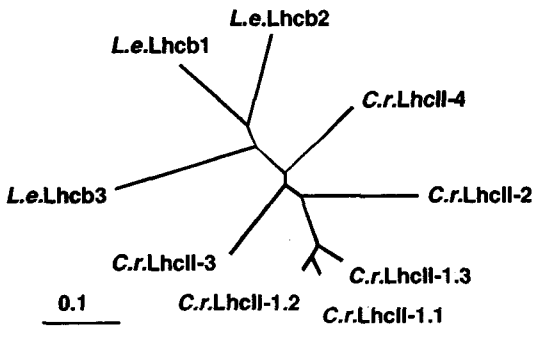


Figure 1. Unrooted phylogenetic tree for Lhc II proteins, including possible transit peptides, from *C. reinharditii* and tomato (*L.e.*).

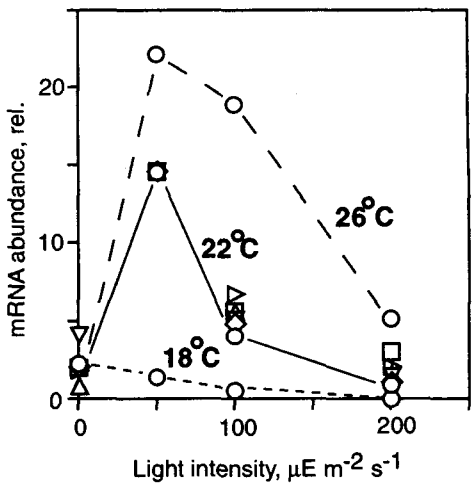


Figure 2. Effects of light intensity and temperature (for *LhcII-4*) on the levels of *Lhcb* genes mRNA. Abundance of mRNA, relative to values at 50 μE $m^{-1}$ $s^{-1}$, of various *Lhcb* genes at 22 ℃ is shown after normalization with respect to that of 18S rRNA. For *LhcII-4*, the mRNA level, relative to values in darkness, was examined at 18, 22 and 16 ℃. *LhcII-4* (○), *LhcII-1.1* (△), *LhcII-1.3* (◇), *LhcII-3* (▽), *Lhcb4* (□) and *Lhcb5* (▷).

proteins of higher plants to the same extent. Furthermore, other LHC II genes from various green algae were not assignable to any type LHC II proteins from *C. reinhardtii* and higher plants. These suggest that an ancestral *LhcII* gene diverged into multiple types after phylogenetic separation of green algae and higher plants, and that this process occurred independently in each algal species.

*Light insensity dependent Lhcb genes expression.* Figure 2 shows the effects of light intensity on the mRNA levels of four *Lhcb* genes encoding the major LCH II proteins (*LhcII-1.1, 1.3, 3* and *4*) and two (*Lhcb4* and *Lhcb5*) encoding the minor LHC proteins (CP29 and CP26) at 22 ℃. The response of the mRNAs to light intensity was very similar among all of the tested *Lhcb* genes. The mRNA levels were markedly increased when the cells were exposed to relatively low light (50 μE $m^{-1}$ $s^{-1}$), but the light-dependent increase in the levels of mRNAs were very small at 100 μE $m^{-1}$ $s^{-1}$, and the levels were not enhanced at 200 μE $m^{-1}$ $s^{-1}$. The results suggest that these *Lhcb* genes are coordinately regulated. In the figure, effects of temperature on the light intensity dependent behavior of the mRNA level are shown for *LhcII-4* gene. The mRNA level was significantly enhanced even at the high light (200 μE $m^{-1}$ $s^{-1}$) when the cells were subjected to light at higher temperature (26 ℃). In contrast, no enhancement was

observed even at low light (50 μE $m^{-1}$ $s^{-1}$) at lower temperature (18 ℃). The levels of mRNAs for other *Lhcb* genes responded to temperature in a similar manner (data not shown). The levels of *Lhcb* genes were enhanced even under high light when the culture was bubbled with 5 % $CO_2$ in air [6], indicating that limitation of the supply of carbon source is the primary cause of the decrease of the *Lhcb* mRNA levels under the high light conditions.

*Effects of DCMU and PSII/PSI mutants on repression of Lhcb gene at high irradiance.* To examine a possible redox-dependent mechanism, in which the redox state of one of the electron carriers on the acceptor side of PS II is used to sense the energy imbalance at high light [5], the change of *Lhcb* mRNA level was studied in the presence of DCMU that inhibits electron transfer from $Q_A$ to $Q_B$. As shown in Figure 3 (left panel), the level of the *LhcII-4* mRNA was markedly lower under high light (H) than under low light (L) in the absence of DCMU. The presence of DCMU reduced the mRNA level by only 15 % at low light (data not shown), but enhanced the level by 3.5-fold at high light. The enhancement effect of DCMU was observed in all examined *Lhcb* genes although the extent of the enhancement (3.5-10 fold) changed depending on the genes [6]. These suggest that excessive electron donation by PS II causes the reduction of the *Lhcb* mRNA levels. However, the mRNA level enhanced by DCMU under high light was significantly lower than that under low light: less

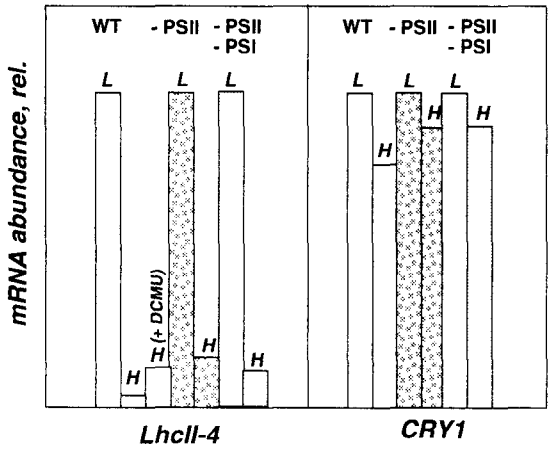


Figure 3 Light intensity-dependent changes in level of *LhcII-4* mRNA in the presence of DCMU and in mutants deficient in PS II reaction center, *ac-u-ε* (– PSII), and both PS II and PS I, FUD7-Δ*psaA* (– PS II, – PS I). Cells were dark-adapted for 12 h at 26 ℃, then exposed to low light (*L*) (50 μE $m^{-1}$ $s^{-1}$) or high light (H) (200 μE $m^{-1}$ $s^{-1}$) for 6 h at 22 ℃. 10 μM DCMU was added before the light exposure when indicated.

than 15 % of the level at low light. Therefore, it is indicated that the levels of *Lhcb* mRNAs can respond to both low and high light intensities even when electron delivery from PS II is inhibited by DCMU. This implies that over-reduction of the photosynthetic electron carriers and some redox component accepting electron from the photosystems is not necessarily required to repress the *Lhcb* mRNAs levels under excessive light.

Figure 3 shows the light-intensity dependent change in the level of *LhcII-4* mRNA in the mutants deficient in PS II (– PS II) and PSII/PSI (– PS II, – PS I). The mRNA levels in these mutants also responded to light intensity in a manner similar to that of the wild type. Figure 3 also shows the mRNA level of the nuclear gene CRY1 encoding the ribosomal protein S14. The mRNA level was not affected much by changes in light intensity in either the mutants or in wild-type cells, and by the presence of DCMU [6]. This indicates that the direct and nonspecific destruction of mRNA molecules and/or damage to nuclear transcriptional machinery are not the cause of the high-light induced repression of the *Lhcb* gene found in the mutants. The present results demonstrated that the presence of the mechanism for high-light repression of *Lhcb* genes independent of the both photosynthetic reaction centers, in addition to the process dependent on the redox sate of the photosynthetic electron transport chain. The former mechanism might be functional when plants are exposed to excessive and stressful light, which expedites photoinhibitory effects.

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