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Light-intensity dependent expression of genes for light-harvesting chlorophyll-*a/b* proteins of photosystem II in *Chlamydomonas reinhardtii*

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Introduction

Several homologous light-harvesting chlorophyll *a/b*-binding (LHC) proteins constitute the antenna system that absorbs photon and transfer its energy to the reaction centers of the photosystems in green algae and higher plants. This system tunes the energy input to other photosynthetic processes in response to the light intensity by changing the antenna size.

Unicellular green algae seem to be of great advantage to studies of acclimation to light. In fact, it has been reported that LHCII protein and/or mRNA abundance changes in response to light intensity in some algal species (Escoubas et al. 1995, Maxwell et al. 1995a, 1995b). However, many of genes for algal LHC proteins have not been identified yet, in contrast to the higher plants *Lhc* genes which have been well established (Green and Durnford 1996). To elucidate the light-dependent regulation of the antenna system, responses of the multiple *Lhc* genes must be studied comprehensively.

In this study, we characterized most of the genes for LHC proteins associated with PSII in a unicellular green alga, *Chlamydomonas reinhardtii*, using the expressed sequence tag (EST) databases, and examined changes in levels of the multiple *Lhc* mRNAs in response to light intensity using quantitative RT-PCR.

Materials and methods

Gene cloning. The 3' end of mRNA was determined using 3'-RACE. Total RNA was prepared from *C. reinhardtii* C-9. Single-stranded cDNA was synthesized from the total RNA using an oligo(dT) primer, and then used as a template for PCR to amplify the 3' end of the target cDNA.

The 5' end of mRNA was determined by cap site hunting. The Cap Site cDNA of *C. reinhardtii* was commercially constructed (Nippon Gene, Tokyo, Japan). The 5'-terminal cap structure of mRNA was replaced with a specific oligoribonucleotide. Single-stranded cDNA was synthesized from the recapped mRNA using random primers, and then used as a template for PCR to amplify the 5' end of the target cDNA.

Genomic DNA was prepared from *C. reinhardtii* 2137, and used as a template for PCR to amplify the target gene. The genomic DNA, digested with *Pst*I and self-ligated, was used as a template for inverse PCR.

The PCR products were cloned into plasmids and sequenced.

Quantitative RT-PCR. Single-stranded cDNA was synthesized from total RNA, and used as a template for a real-time PCR assay to determine the abundance of the target RNA using the LightCycler (Roche Diagnostics, Mannheim, Germany).

Table 1. PSII-LHC related genes in *Chlamydomonas reinhardtii*

Gene	Protein	Related gene (protein) in higher plants	Number of EST clone
LhcII-1.1 (*cabII-1* [a])	LHCII type I [e]	*Lhcb1*/*Lhcb2*/*Lhcb3* (LHCII)	137
LhcII-1.2 (*Lhcb2* [b])	LHCII type I [e]	*Lhcb1*/*Lhcb2*/*Lhcb3* (LHCII)	3
LhcII-1.3	LHCII type I [e]	*Lhcb1*/*Lhcb2*/*Lhcb3* (LHCII)	169
LhcII-2 (*Lhcb3* [c])	LHCII type II [e]	*Lhcb1*/*Lhcb2*/*Lhcb3* (LHCII)	28
LhcII-3	LHCII type III [e]	*Lhcb1*/*Lhcb2*/*Lhcb3* (LHCII)	122
LhcII-4	LHCII type IV [e]	*Lhcb1*/*Lhcb2*/*Lhcb3* (LHCII)	156
Lhcb4		*Lhcb4* (CP29)	60
Lhcb5 [d]	LHCII p10 [d]	*Lhcb5* (CP26)	24

[a] Imbault et al. 1988 (GenBank M24072)
[b] O'Connor et al. (GenBank AF104630)
[c] O'Connor et al. (GenBank AF104631)
[d] Minagawa et al. 1998 (GenBank AB050007)
[e] *Chlamydomonas* LHCII proteins were classified into four types (from Type I to Type IV) in this study.

Results and Discussion

PSII-LHC proteins in C. reinhardtii. Database searches of the *C. reinhardtii* EST library (over 15,000 sequences) revealed that 699 ESTs were related to PSII-LHC proteins in higher plants (Table 1). They were assigned to eight genes, including four new genes (*LhcII-1.3*, *LhcII-3*, *LhcII-4* and *Lhcb4*). We determined the full-length sequences of the new transcripts, and isolated their genomic clones containing the entire coding regions using PCR and inverse PCR. Sequence comparison revealed that six of the *Lhc* genes from *C. reinhardtii* correspond to the major LHC (LHCII) proteins from higher plants, and that the other two genes (*Lhcb4* and *Lhcb5*) correspond to the minor LHC proteins (CP29 and CP26). No ESTs corresponding to another minor LHC protein (CP24) were found. The six LHCII proteins in *C. reinhardtii* cannot be assigned to any of the three types proposed for higher plants (Lhcb1 - Lhcb3), but were classified into four types based on the sequence similarity. The composition of the algal genes for PSII-LHC differs from that of higher plants with respect to the absence of CP24 and the different type-divergence of LHCII proteins. Therefore, the ancestral LHC protein seems to have diverged into LHCII, CP29 and CP26 before the phylogenetic separation of green algae and higher plants, and LHCII have diverged into multiple types thereafter.

Effects of temperature and CO_2 on light intensity-dependent expression of Lhc genes.

Effects of temperature: C. reinhardtii cells, which had been mixotrophically grown in TAP medium under continuous illumination (5 $\mu E\ m^{-2}\ s^{-1}$), were dark-adapted for 12 h at 26 °C, and then exposed to light with various intensity (0, 50, 100 and 200 $\mu E\ m^{-2}\ s^{-1}$) for 6 h at 18 and 26 °C. The *Lhc* mRNA levels were examined by quantitative RT-PCR. The *Lhc* mRNA abundance relative to the maximum at each temperature was plotted after normalization with respect to 18S rRNA abundance (Fig. 1). The mRNA level for *LhcII-4* in the algal cells exposed to low light (50 $\mu E\ m^{-2}\ s^{-1}$) at 26 °C was 10 times higher than in the cells kept in

darkness. However, the level in the cells exposed to higher light (200 μE m^{-2} s^{-1}) was one fourth of the level in the low light-exposed cells. At lower temperature (18 °C), light had more negative effects on the mRNA levels. Similar changes were observed for *Lhcb4*. However, the response to high light was relatively small compared with that observed for *LhcII* mRNA.

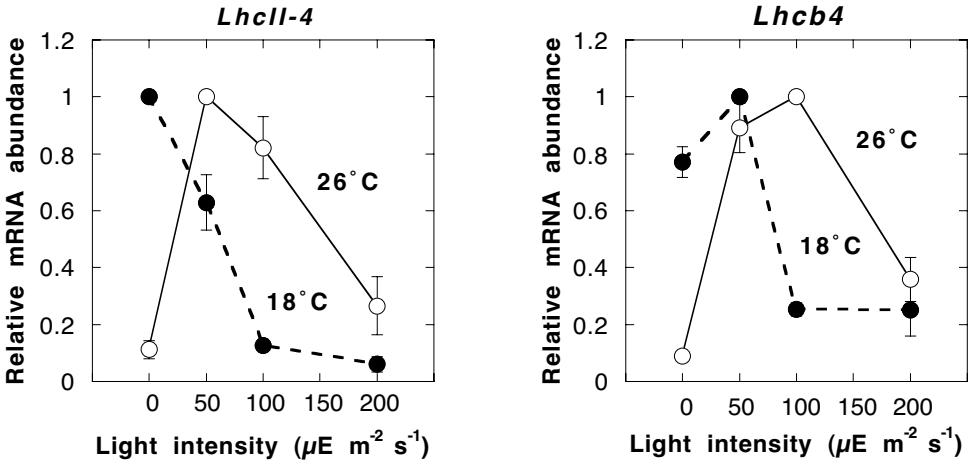

Fig. 1. *Lhc* mRNA levels in the algal cells exposed to light with various intensity at the different temperatures.

Effects of CO_2: C. reinhardtii cells were photoautotrophically grown in HS medium in the low light (50 μE m^{-2} s^{-1}) at 26 °C with bubbling either air or 5% CO_2 enriched air. The mRNA level for *LhcII-4*, *Lhcb4* and *Lhcb5* was 10 times lower in the cells exposed to high light (1,000 μE m^{-2} s^{-1}) for 6 h than in the cells kept in the low light under the low CO_2 conditions (Fig. 2: left panel). On the other hand, the level was 8 to 10 times higher in the high light than in the low light under the high CO_2 conditions (Fig. 2: right panel).

In conclusion, the level of multiple *Lhc* mRNAs changed in response to light intensity, and the response was markedly affected by temperature and CO_2 supply. Since temperature and CO_2 concentration must affect the biochemical dark processes of photosynthesis, the level of *Lhc* mRNAs is probably regulated in response to the imbalance between energy absorbed by light-harvesting antennae and energy used for driving the dark reactions. The regulation of mRNA levels for *Lhc* genes must be involved in changing the antenna size of PSII during acclimation to light intensity.

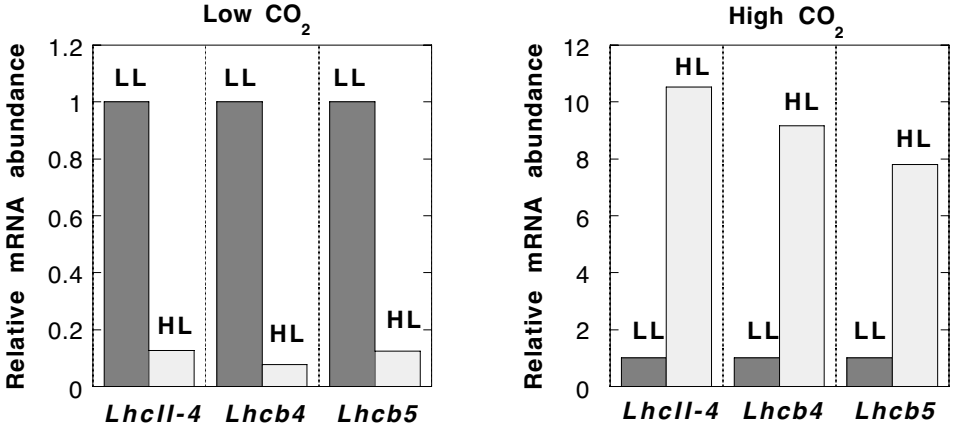

Fig. 2. *Lhc* mRNA levels in the high light-exposed cells relative to the cells kept in the low light at the different CO_2 conditions.

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