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Full Length Research Paper

Light intensity dependent expression of *Lhca* gene family encoding LHCI in *Dunaliella salina*

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In order to compare the expression pattern and function of *Lhca* gene families, the *DsLhca* genes have been cloned, and two novel genes have been isolated by screening a *Dunaliella salina* cDNA library with a DNA probe coding for the conserved first transmembrane helix of this protein. The number of exons in the different *DsLhca* genes was slightly conserved. The deduced pre-proteins of the two novel gene had molecular masses of 24.7 and 30.7 kDa, and were assigned into light-harvesting complex of photosystem I (LHCI) Type I and Type III of higher plants in phylogenetic tree, respectively. The *DsLhca1 and DsLhca3* responded to the light intensity divergently from *DsLhca2*. It indicates that changes in the protein composition of LHCI, depending on the growth conditions, will thus result in an adaptation state of the antenna produced to perform better under different environmental conditions.

Key words: *Dunaliella salina*, *Lhca* gene family, light-harvesting complex of photosystem I (LHCI), environment stress response.

INTRODUCTION

Photosystem I (PSI), the plastocyanin-ferredoxin oxidoreductase, is composed of two moieties: the core complex, responsible for charge separation and electron transport, and the outer light-harvesting complex of photosystem I (LHCI), which extends the light harvesting capacity and ensures photo-protection. Most of the available information on LHCI are derived from higher plants, where LHCI are thought to be composed of four types of LHC proteins (Jansson, 1999), LHCI type I, II, III and IV, encoded by four genes coding for LHCI proteins (*Lhca1*, *Lhca2*, *Lhca3* and *Lhca4* genes), respectively. Lots of experiments have suggested that these proteins can dimerize into LHCI-730 (Types I and IV) and LHCI-680 (Types II and III) complexes (Ballottari et al., 2004; Klimmek et al., 2006). Two additional *Lhca* genes, *Lhca5* and *Lhca6*, have been identified in *Arabidopsis thaliana*, as well as in other plants (Jansson, 1999). They have been thought to be only minor components of higher plant LHCI because of the substoichiometric amounts of their gene products (Morosinotto et al., 2005; Storf et al., 2004).

LHCI in green alga has also been studied. Biochemical, proteomics and genomics studies indicate that the LHCI of *Chlamydomonas reinhardtii* contains approximately seven to nine different Lhca proteins (Elrad and Grossman, 2004; Tokutsu et al., 2004). But these Lhca complexes have very similar physicochemical properties. This complexity has so far hampered the possibility to purify each of them to homogeneity. To study these complexes, the *Lhca* genes from *C. reinhardtii* were cloned and characterized. The results showed that the different properties of the individual Lhca complexes are functional to adapt the architecture of the PSI-LHCI

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Abbreviations: Chl, Chlorophyll(s); ***D. salina,*** *Dunaliella salina*; ***DsLhca*****,** *Lhca* from *Dunaliella salina*; ***DsLhcb,*** *Lhcb* from *Dunaliella salina*; **LHC,** light-harvesting complex(es); **LHCI,** light-harvesting complex of PS I; **LHCII,** light-harvesting complex of PS II; ***Lhca* genes,** genes coding for LHCI proteins; ***Lhcb* genes,** genes coding for LHC proteins in PSII; **PS I,** photosystem I ; **PS II,** photosystem II; **HL,** high light; **LL,** low light.

supercomplex to different environmental conditions of light qualities (Milena et al., 2010).

However, it has not been reported that the *Lhca* gene family in the green alga responds to the light intensity. But expression of genes coding for *Lhcb* in *C. reinhardtii* has been investigated under various light intensity (Tatsuru and Ayumi, 2003; Teramoto et al., 2002). In general, *Lhc* genes are most strongly expressed under LL and down-regulated under HL (Ayumi and Anastasios, 1997). The results indicated that, excessive light conditions repressed the mRNAs levels of these genes and all the tested *Lhcb* genes are coordinately regulated under excessive and low light conditions. It is also found that shift from LL to HL results to a rapid and evanescent reduction in *LhcII-3* mRNA level in *D. salina* (Liang et al., 2007).

D. salina possesses a notable ability to adapt to light stress and is used as a model organism for the study of photosynthesis in the stress. In order to gain information on the properties of *Lhcas* of *D. salina* and to interpret responses to different light intensity in terms of functional adaptation, we have cloned an *Lhca* gene of *D. salina* in our previous study. In this work, two novel genes (*Lhca2* and *Lhca3*) in *D. salina* encoding LHCI polypeptides were cloned and classified by phylogenetic analysis. Then, we examined their expression characteristics by real-time quantitative PCR in LL, HL and darkness conditions, and found that the *DsLhca1 and DsLhca3* responded to the light intensity divergently from *DsLhca2*. It indicates that changes in the protein composition, depending on the growth conditions, will thus results in a special antenna system produced to perform better under different environmental conditions.

MATERIALS AND METHODS

Strain and culture conditions

D. salina strain (strain number 435) was obtained from Institute of Hydrobiology, the Chinese Academy of Science. The cells were grown in an artificial hypersaline medium containing 1.5 M NaCl (Pick et al., 1986). Cultures were grown in flat bottles at 25°C under illumination at 100 µmole photon m^{-2} s^{-1}.

Cloning and sequencing of *DsLhca2* and *DsLhca3* from cDNA library and genomic DNA

Total RNA was prepared from *D. salina* using the TRIZOL (Invitrogen) reagent. The cDNA library was constructed in our previous work. The probe for hybridization was generated by PCR. The cDNA sequence of *DsLhca1* gene (Milena et al., 2010) was used to design the primers. The sense primer was 5'- TTT GAC CCA CTG GGC CTG GGC -3', corresponding to nucleotides 260 to 280 and the antisense primer was 5'- CAC CAG CCT CGT ACC ACT TGG -3', corresponding to nucleotides 390 to 410. For library screening, 200 ng probe was obtained by PCR and purified gel. Then, it was randomly labeled with [α-^{32}P] dCTP (Random Primer DNA Labeling Kit; TaKaRa). The positive clones were sequenced.

To determine the sequence of the *DsLhca2* and *DsLhca3* genes, the genomic DNA of *D. salina* was prepared by CTAB and amplified by PCR using a specific primer set corresponding to the 5' and 3' untranslated regions of each transcript. The oligonucleotides were as follows: 5'-GCA CCA AGG CTT CCC CCA GTT A -3' and 5'-CCT CAT CCC ATA CTC CAT CCA A -3' for *DsLhca2*; 5'- GCA ATC GCT TAG TTC AGG AAA CA-3' and 5'- GCA TCT CCT AGA AAT CCA CTC CA-3' for *DsLhca3*. The amplified DNA was cloned and sequenced.

Analysis of sequences and phylogenetic tree

Sequences were analyzed using Vector NTI 10.0 (Invitrogen). BLASTX was performed in NCBI homepage (http://www.ncbi.nlm.nih.gov/BLAST/). The transmembrane regions and orientation were predicted by TMpred program (Hofmann and Stoffel, 1993). The sequences were aligned and phylogenetically analyzed by the program CLUSTAL X (Thompson et al., 1997) using the Neighbor-Joining method (Saitou and Nei, 1987). The selected *Lhca* genes of plant species were as follows: *C. reinhardtii* [Cr.LhcI-1 (AB122114), Cr.LhcI-2 (AB122115), Cr.LhcI-3 (AB122116), Cr.LhcI-4 (AB122117), Cr.LhcI-5 (AB122118), Cr.LhcI-6 (AB122119), Cr.LhcI-7 (AB122120)], *A. thaliana* [At.Lhca1 (M85150), At.Lhca2.1 (AF134120), At.Lhca3 (U01103), At.Lhca4 (M63931), At.Lhca5 (AF134121), At.Lhca6 (U03395)], *Ostreococcus tauri* [Ot.Lhca1 (AY954737), Ot.Lhca2 (AY954734), Ot.Lhca3 (AY954735), Ot.Lhca4 (AY954729), Ot.Lhca5 (AY954736)].

Real-time quantitative PCR analysis of *Lhca* genes

D. salina cells were cultured as aforementioned (LL conditions, 100 µmole photon m^{-2} s^{-1}). Then the cultures were transferred to dark condition and HL condition (1000 µmole photon m^{-2} s^{-1}) in the logarithmic phase of growth. Total RNA was isolated from cells after 0, 1, 3, 6 and 9 h under each condition using the TRIZOL (Invitrogen) reagent. Real-time quantitative PCR was performed using SYBR RT-PCR Kit (Takara, Japan) The primers for *DsLhca1* were 5'-GTG CCC AGC ACT CTT TTG TAC G-3' (sense) and 5'-GGG TAG CAC AAC TTG GAA CCA TC-3' (antisense). The primers for *DsLhca2* were 5'- TGC TGC AAT GAT CGG AGA CCT-3'(sense) and 5'- CCA GCC GGG AAA ACT GTC AAC-3'(antisense), and the primers for *DsLhca3* were 5'- GCA GTG GAT CAA CGC AGA GTA CG-3'(sense) and 5'- GGT GCT CAT CTT GCT GGT GAG AG-3'(antisense). The fluorescence of the end of each cycle was monitored by iCycler iQ (BIO-RAD, Richmond, CA). The relative abundance of 18S rRNA was also determined and used as the internal standard. Relative gene expression data was analyzed using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

RESULTS

Isolation of *DsLhca* cDNA sequences

The constructed cDNA library can represent 3.07×10^6 mRNA fragment in *D. salina*. Average size of inserted cDNA fragment is about 1.5 kb. Several positive clones were screened with probe *DsLhca1*. Further, two new *Lhca* cDNAs clones were obtained after sequencing, which were named *DsLhca2* and *DsLhca3*, respectively, depending on the order of their isolation. The nucleotide sequences of these two novel genes are deposited in the GenBank database under the accession numbers:

FJ770250 and FJ770251.

Characteristics of the predicted *DsLhcas* polypeptides and the genomic DNA sequences

The mature *Lhca* proteins encoded by *Lhca2* and *Lhca3* are calculated to be composed of 198 and 257 amino acid residues (21.4 and 27.5 kDa), respectively. An alignment of three *DsLhca* and several other *Lhca* polypeptides is shown in Figure 1. *DsLhcas* show the lower degree of homology. The mature *Lhca* polypeptides from *D. salina* have low amino acid sequence identity, just between the ranges over 23.3 to 27.3%. The putative structural elements of *Lhca* proteins including three transmembrane helices (A to C), an amphipathic helix (D) near the C-terminus, and chlorophyll ligands were indicated based on the reported high resolution three-dimensional structure of pea LHCII (Kühlbrandt et al., 1994). All the *DsLhcas* possess the GFDPLG motif in Helix B and the same motif N-terminal in Helix A with the exception of *DsLhca3* which lacks the latter one. The pattern of seven hydrophobic residues between two chlorophyll ligands (Gln and Glu) in Helix C of *DsLhca2* and *DsLhca3* sequences perfectly aligns with the sequences of other LHCs, while this general motif cannot be observed in DsLhca1 and OtLhca5. Another important motif in Helix C includes a conserved Arg residue which is located two residues downstream from the second pigment-binding site (conserved Glu). Conservation of the Arg suggests importance of this residue either for pigment binding or stability of the LHC (Melkozernov and Blankenship, 2003). Eight putative Chl-binding sites are conserved in *DsLhca* polypeptides with the exception of DsLhca1 and OtLhca5 which lack the first binding site in Helix C. This may suggest that these two proteins have a little difference with other *Lhca* proteins in secondary structure.

In order to check whether introns are a general characteristic of all *Lhca* genes in *D. salina*, chromosomal DNA of the genes was amplified by PCR. The number and location of introns of *DsLhcas* were shown in Figure 2. In the 19 introns of these three genes, 13 of them all begin with the sequence GTG, 5 of them begin with GTA, and only one begins with GTC, and all of them end with CAG, exception one, the seventh intron of *DsLhca2* sequence, which end with TAG (data not shown). All of these sequences match the consensus 5' and 3' intron splice sites for mRNA. The 3' 'acceptor' splice sites are also preceded by pyrimidine-rich sequences and all but one (the third intron of *DsLhca1*) do not contain another AG pair closer than 20 nt within the splice site. These are all common features of mRNA 3' splice sites. Half of the splice junctions are within condons, four occurring after the first nucleotide, five occurring after the second nucleotide, and ten between condons. A typical algal polyadenylation signal TGTAAA is present in the 3' UTR-of *DsLhca* with the exception of *DsLhca3*.

Phylogenetic analysis

Lhca protein sequences of the green alga *D. salina* were also compared to those from vascular plants and other algae, to consider the divergence of these genes in the context of phylogenetic separation between the green algae and terrestrial plant. Figure 3 shows a neighbor-joining tree summarizing the relationships of representative *Lhca* proteins from *D. salina*, *Chlamydomonas reinhardtii, Ostreococcus tauri* and *Arabidopsis thaliana*.

Four major clades were observed in phylogenetic tree. Thus these clades were suggested to have emerged before *Chlorophyceae* and *Streptophyta* diverged from each other. One clade includes *DsLhca2* proteins (labeled LHCI Type I), and one includes *DsLhca3* proteins (labeled LHCI Type III). These two branches are sufficiently divergent to be considered in different types. Since AtLhca1 and AtLhca3 encode LHCI Type I and LHCI Type III protein, respectively, which are well conserved among higher plants (Jansson, 1999), *DsLhca2* and *DsLhca3* are the most likely counterparts in *D. salina*. This could be further supported by the facts that both *DsLhca2* and *DsLhca3* proteins conserve a specific feature of the respective 'type' observed in higher plants such as a very short sequence between Helix C and A (Type I) and a six-residue insertion at the beginning of Helix B (Type III). A small clade only included DsLhca1 and OtLhca5 proteins, which cannot be assigned into any type of Lhca proteins in *A. thaliana* and *C. reinhartii*. Another large *Lhca* lineage which includes the rest is sought to be peripheral LHCI proteins.

Expression of *DsLhca genes*

Real-time quantitative PCR was used to analyze the relationship and difference among these three *DsLhca* mRNA expression patterns of *D. salina* under various conditions of light intensity. The results show the changes in *DsLhca* mRNAs levels after a shift from LL to HL or dark conditions (Figure 4). *DsLhca1* (Figure 4A) and *DsLhca2* (Figure 4B) mRNAs were depressed in HL, within the 9-h time course, the level of their mRNAs accumulation under HL reduced to 15 and 13% of those under LL, respectively. The abundance of *DsLhca3* mRNA (Figure 4C) decreased rapidly within the first 6 h following a light shift from LL to HL, and remained at that level for the next 3 h, which account for approximately 30% as low as their mRNA level found in LL-treated cells. However, in the LL to dark shift, the response pattern of *DsLhca* genes had a few differences with that under HL condition. 3 h after transfer to darkness, the amount of *DsLhca1*, *DsLhca2* and *DsLhca3* mRNA decreased to 35, 55 and 42%, respectively, but maintained that level in the

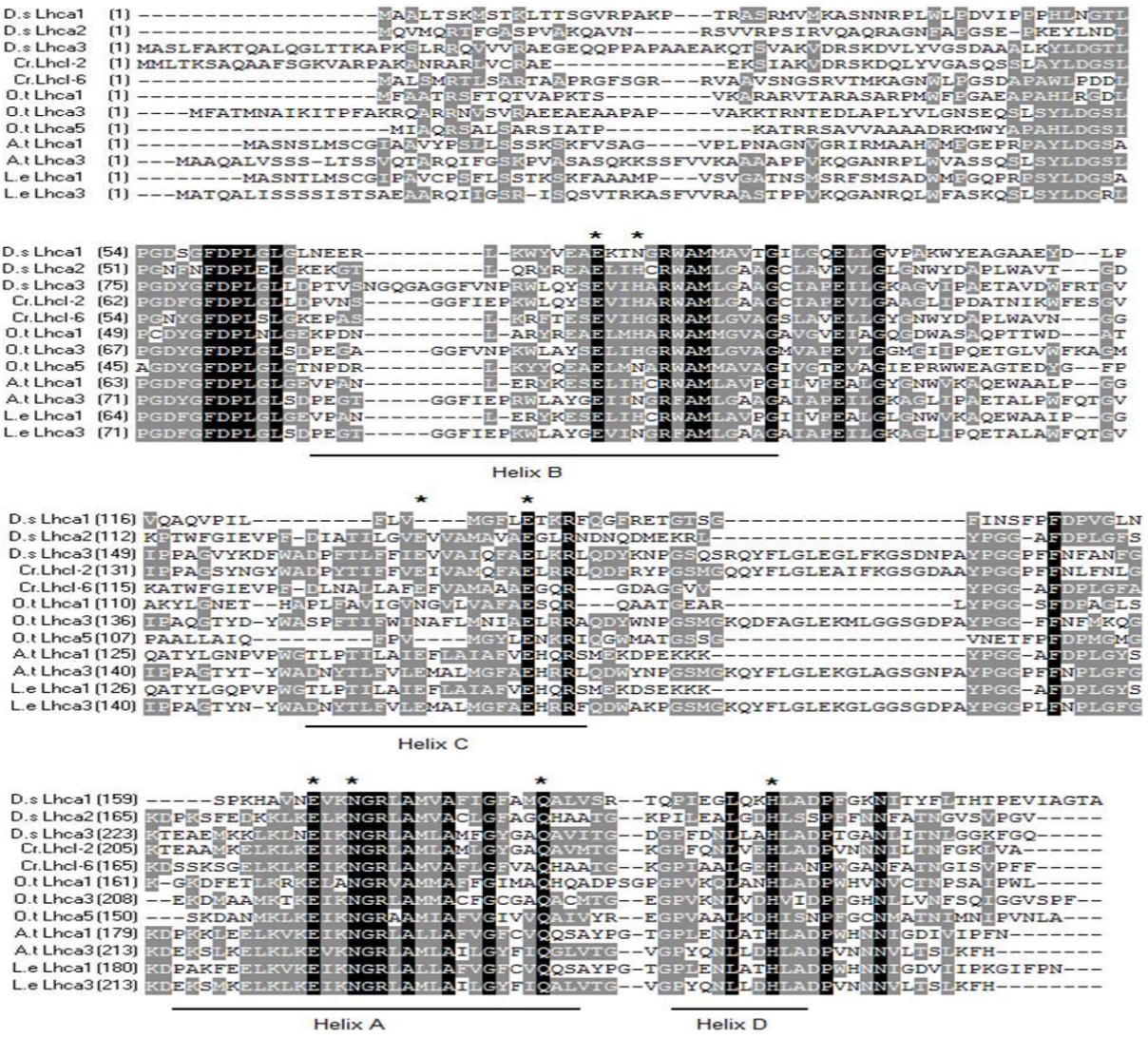

Figure 1. Alignment of amino acid sequences of LHC proteins from *Dunaliella salina*, *Ostreococcus tauri*, *Chlamydomonas reinhardtii*, *Chlamydomonas stellata*, *Porphyridium cruentum*, *Volvox carteri*, *Arabidopsis thaliana* and *Lycopersicon esculentum*. Amino acid sequences were aligned using the Vector NTI. The identical amino acid sequences are black highlighted. The grey areas are conservative and weak similar amino acid sequences. The bold lines indicate the putative ranges of the membrane-spanning (A-C) and amphiphathic (D) helices in *Lhca* genes from *C. reinhardtii*. Their precise positions may vary slightly in the other proteins. Asterisks indicate residues identified as Chlorophyll ligands in LHCII . The underlined regions are motifs in helices.

next 3 h. Subsequently, the level of the *DsLhca2* decreased, and reduced to 31% of those under low light finally. In contrast, the amount of *DsLhca1* and *DsLhca3* mRNAs reverted, and reached up to 71 and 93%, compared to cells kept in LL.

DISCUSSION

Classification of *DsLhca* genes

The isolation and characterization of novel *Lhca* genes in *D. salina* enabled us to carry out a further study on the composition of *Lhca* gene family of *D. salina*. Thus including the previously reported one (*DsLhca1*), *D. salina* has at least three genes encoding the LHCI proteins. As the phylogenetic tree showed, *DsLhca2* and *DsLhca3* should be classified into LHCI Type I and Type III, respectively. These two clades were suggested to have emerged before the separation of algal and vascular plant lineages. DsLhca2 and DsLhca3 proteins might have similar location and function in *D. salina* since LHCI Type I and Type III work as inner antennae for PSI in higher plants. Interestingly, both DsLhca1 and OtLhca5 possess a very short sequence between Helix C and A which is a characteristic feature of the LHCI Type I, but

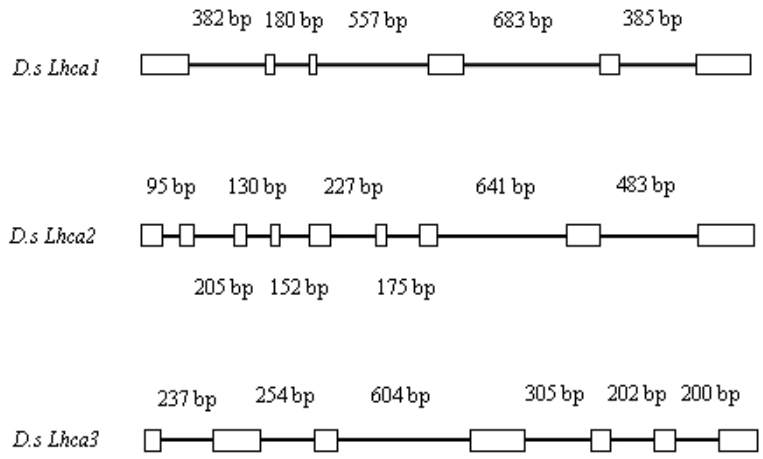

Figure 2. The structure of *DsLhca* genes in *D. salina*. Exons are shown as boxes and the introns as a black line (□: exons; ▬: introns). The intron lengths in nucleotides are indicated and exons are shown to scale.

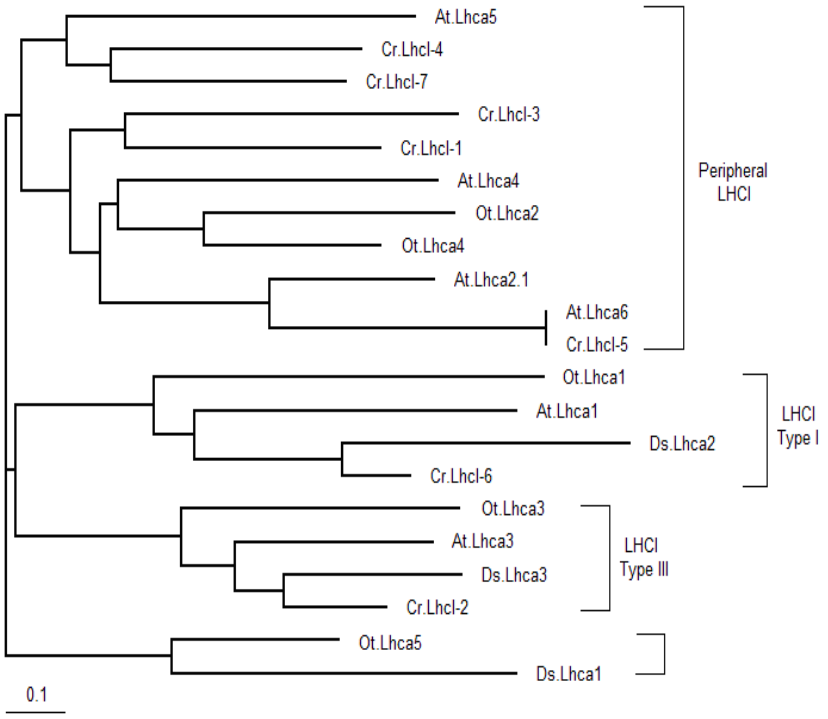

Figure 3. Evolution of plant Lhca. Unrooted phylogenetic tree with bootstrap values (% of 1000 replicates) showing the relationship among the LHC proteins of *Dunaliella salina, Chlamydomonas reinhadtii, Ostreococcus tauri* and *Arabidopsis thaliana* was constructed using the Neighbor-Joining method. Amino acid sequences were aligned using the CLUSTAL X program.

both proteins cannot be assigned into any types of Lhca proteins according to the phylogenetic tree. These two proteins not only bear high relationship in phylogenetic evolution with each other, both of them also lack the first pigment-binding site and a general motif in Helix C. Their high dissimilarity with other Lhca proteins suggests that they may have a unique role in their capability of pigment-binding and using the light with different wavelengths. Although, the significant differences among the primary sequences of LHCs are observed in the region of the N-terminal and the middle parts of the alignments assigned to the second transmembrane helix (Helix C), the Lhca

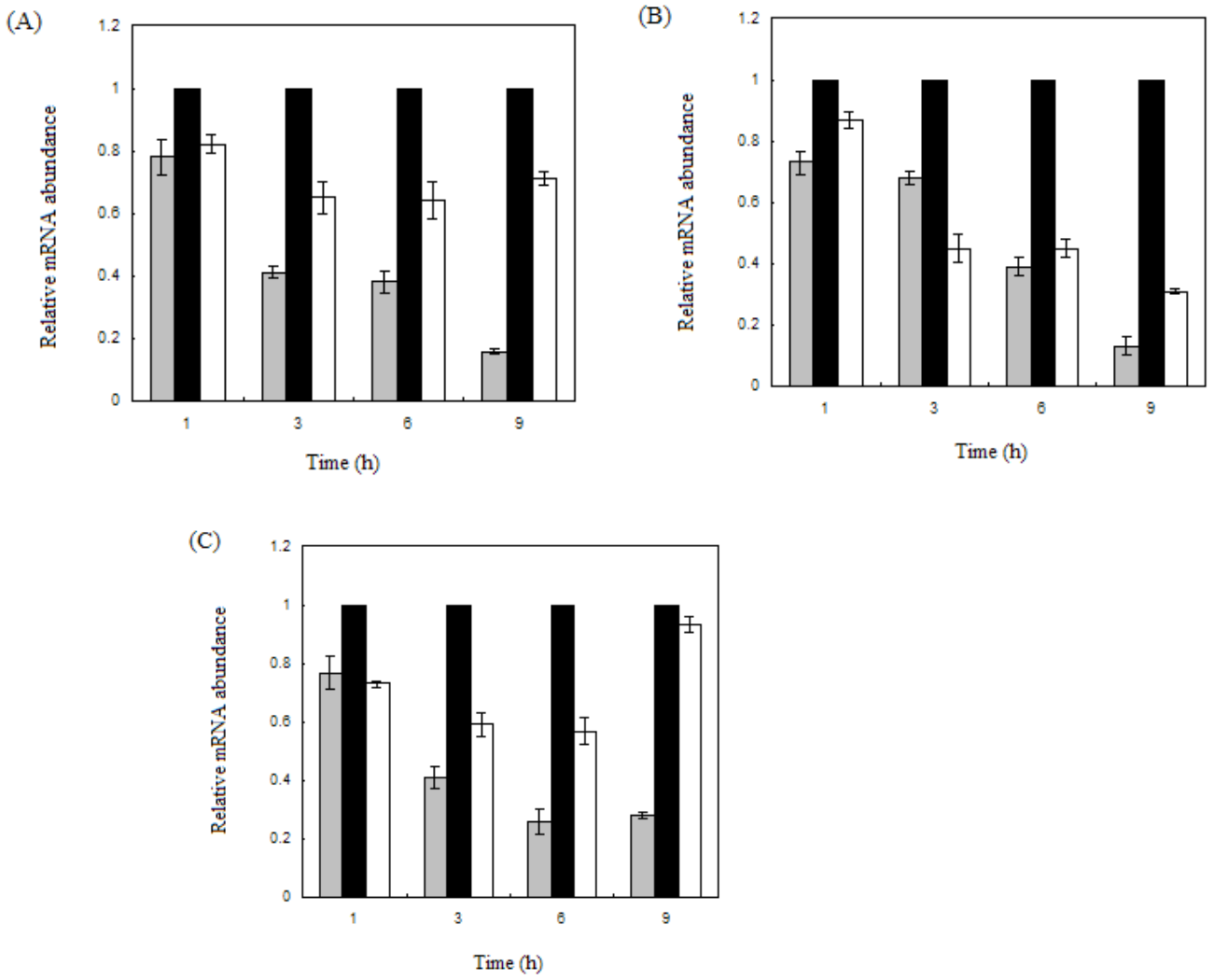

Figure 4. Changes in *DsLhca1* (A), *DsLhca2* (B) and *DsLhca3* (C) mRNAs levels after a shift from LL to HL or darkness conditions. The mRNA abundance of DsLhca genes in LL was used as a control. Gene expression was analyzed by real-time quantitative PCR using a specific primer set for each gene. Data are presented as mean ± standard error; *n* = 3. Low light treatment (■); high light treatment (▩); dark treatment (□).

proteins from kinds of organisms have strong overall conservation of secondary structure, such as three transmembrane helices A to C and one small helix D at the C terminus. It indicates that these secondary structures are fundamental to the role of Lhca proteins in the photoprotection.

While DsLHCb proteins are relatively homogeneous, *DsLhca* proteins are rather heterogeneous and more diverged from DsLHCb. The large diversity of *DsLhca* proteins could indicate that the DsLhca family diversified before the divergence of DsLHCb, this is also supported by observations in other organisms (Hwang and Herrin, 1993). Examination of genomic sequence reveals that the aforementioned three *Lhca* genes in *D. salina* possess 5 to 8 introns, while 5 to 7 introns in *DsLhcb* genes (Wei et al., 2007). This reveals little conservation in the number of exons in the different *DsLhc* genes.

Expression of *DsLhca* gene family in different conditions

The regulation of the *Lhc* genes under different light conditions is an important anti-stress response of photosynthetic organisms. The present results showed that the *DsLhca1 and DsLhca3* responded to the light intensity divergently from *DsLhca2*. The mRNAs levels of *DsLhca1* and *DsLhca3* markedly decreased by exposure to HL, but decreased at first, and then reverted under darkness condition. However, the expression of *DsLhca2* was not merely down-regulated by exposure to HL but darkness. The depression of *DsLhcas* due to algae needs fewer LHC proteins under HL in order to reduce potential damage to the photosynthetic apparatus caused by excessive light energy. The HL-induced depression of *DsLhcas* followed the same pattern as that found in

DsLHCII-3. The mRNA levels of *DsLhca1* and *DsLhca3* reverted under darkness condition. This character has not been observed in *DsLhca1*. It may attribute to the difference of *DsLhcas* in their red form content and thus in their capability of using the far-red light (Milena et al., 2010). The comparability of expression among three *DsLhca* on transcriptional level suggested that changes in the protein composition of LHCI, depending on the growth conditions, will result in an adaptation state of the antenna produced to perform better under different environmental conditions. To understand the light-dependent regulation of the entire antenna system, comprehensive studies on the light response of all *Lhc* genes are required.

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REFERENCES

Ayumi T, Anastasios M (1997). Irradiance-Dependent changes in the Size and Composition of the Chlorophyll a-b Light-Harvesting Complex in the Green Alga *Dunaliella salina*. Plant Cell Physiol., 38(1): 17-24.

Ballottari M, Govoni C, Caffarri S, Morosinotto T (2004). Stoichiometry of LHCI antenna polypeptides and characterization of gap and linker pigments in higher plants Photosystem I. Eur. J. Biochem., 271(23-24): 4659-4665

Elrad D, Grossman AR (2004). A genome's-eye view of the light-harvesting polypeptides of *Chlamydomonas reinhardtii*. Curr. Genet., 45: 61-75.

Hofmann K, Stoffel W (1993). Tmbase-A database of membrane spanning proteins segments. Biol. Chem. Hoppe- Seyler, 374: 166.

Hwang S, Herrin DL (1993). Characterization of a cDNA encoding the 20-kDa photosystem I light-harvesting polypeptide of *Chlamydomonas reinhardtii*. Curr. Genet., 23: 512-517.

Jansson S (1999). A guide to the Lhc genes and their relatives in Arabidopsis. Trends Plant Sci., 4: 236-240

Klimmek F, Sjodin A, Noutsos Christos, Leister D, Jansson S (2006). Abundantly and rarely expressed *Lhc* protein genes exhibit distinct regulation patterns in plants. Plant Physiol., 140: 793-804.

Kühlbrandt W, Wang DN, Fujiyoshi Y (1994). Atomic model of plant light-harvesting complex by electron crystallography. Nature, 367: 614-621.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods, 25: 402-408.

Melkozernov AN, Blankenship RE (2003). Structural Modeling of the Lhca4 subunit of LHCI-730 peripheral antenna in photosystem I based on similarity with LHCII. J. Biol. Chem., 278: 542-551.

Milena M, Manuela M, Francesca P, Stefano C, Roberta C, Roberto B (2010). Functional analysis of Photosystem I light-harvesting complexes (Lhca) gene products of *Chlamydomonas reinhardtii*. Biochimica et Biophysica Acta, 1797: 212-221

Morosinotto T, Ballottari M, Klimmek F, Jansson S Bassi R (2005). The association of the antenna system to photosystem I in higher plants. J. Biol. Chem., 280: 31050-31058.

Pick U, Karni L, Avron M (1986). Determination of ion content and ion fluxes in the halotolerant alga *Dunaliella salina*. Plant Physiol., 81: 92-96.

Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Storf S, Stauber EJ, Hippler M, Schmid VH (2004). Proteomic Analysis of the Photosystem I Light-Harvesting Antenna in Tomato. Biochem., 43: 9214-9224.

Tatsuru M, Ayumi T (2003). Anastasios M, Chlorophyll antenna size adjustments by irradiance in *Dunaliella salina* involve coordinate regulation of chlorophyll a oxygenase (CAO) and *Lhcb* gene expression. Plant Mol. Biol., 51: 757-771.

Teramoto H, Nakamori A, Minagawa J, Ono T (2002). Light-intensity-dependent expression of Lhc gene family encoding light-harvesting chlorophyll-a/b proteins of photosystem II in *Chlamydomonas reinhardtii*. Plant Physiol., 130: 325-333.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res., 24: 4876-4882.

Tokutsu R, Teramoto H, Takahashi Y, Ono TA, Minagawa J (2004). The light-harvesting complex of photosystem I in *Chlamydomonas reinhardtii*: Protein composition, gene structures and phylogenic implications. Plant Cell Physiol., 45: 138-145.

Wei L, Cao Y, Liang X, Liu Y, Deng TT, Bai LH (2007). Cloning and expression study of a gene coding for the major light-harvesting chlorophyll *a/b* protein of photosystem II in green alga *Dunaliella salina*. J. Appl. Phycol., 19: 89-94.