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Cloning and expression analysis of two different *LhcSR* genes involved in stress adaptation in an Antarctic microalga, *Chlamydomonas* sp. ICE-L

**Shanli Mou · Xiaowen Zhang · Naihao Ye ·
Meitao Dong · Chengwei Liang · Qiang Liang ·
Jinlai Miao · Dong Xu · Zhou Zheng**

Abstract Light-harvesting complexes (LHCs) play essential roles in light capture and photoprotection. Although the functional diversity of individual LHCs in many plants has been well described, knowledge regarding the extent of this family in the majority of green algal groups is still limited. In this study, two different *LhcSR* genes, *LhcSR1* and *LhcSR2* from *Chlamydomonas* sp. ICE-L, were cloned from the total cDNA and characterized in response to high light (HL), low light (LL), UV-B radiation and high salinity. The lower F_v/F_m as well as the associated induction of non-photochemical quenching (NPQ), observed under those conditions, indicated that *Chlamydomonas* sp. ICE-L was under stress. Under HL stress, the expression of *LhcSR1* and *LhcSR2* increased rapidly from 0.5 h HL and reached a maximum after 3 h. In LL, *LhcSR2* expression was up-regulated during the first 0.5 h after which it decreased, while the expression of *LhcSR1* decreased gradually from the beginning of the experiment. In addition, the transcript levels of *LhcSR1* and *LhcSR2* increased under UV-B radiation and high salinity. These results showed that both genes were inducible and up-regulated under stress conditions. A higher NPQ was accompanied by the up-regulated *LhcSR* genes, suggesting that *LhcSR* plays a role in thermal energy dissipation. Overall, the results presented here suggest that *LhcSR1* and *LhcSR2* play a primary role in photoprotection in *Chlamydomonas* sp. ICE-L under stress conditions and provide an important basis for investigation of the adaptation mechanism of *LhcSR* in Antarctic green algae.

Keywords *Chlamydomonas* sp. ICE-L · *LhcSR* genes · Cloning · QRT-PCR · Expression analysis

S. Mou · Q. Liang · J. Miao · Z. Zheng (✉)
Key Laboratory of Marine Bioactive Substance,
The First Institute of Oceanography, State Oceanic
Administration, Qingdao 266061, China
e-mail: zhengzhou@fio.org.cn

X. Zhang · N. Ye (✉) · D. Xu
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences,
Qingdao 266071, China
e-mail: yenh@ysfri.ac.cn

M. Dong
Qingdao Agricultural University, Qingdao 266109, China

C. Liang
Qingdao University of Science and Technology, Qingdao
266042, China

Introduction

Light is necessary for photosynthesis, but excess light can cause photoinactivation and even result in cell death (Peers et al. 2009). The light-harvesting complexes (LHCs) of terrestrial plants and green algae play essential roles in light capture and photoprotection (Barber and Andersson 1992; Niyogi 2000). The LHC protein superfamily is a centerpiece of eukaryotic photosynthesis that comprises the LHC family and several families involved in photoprotection, such as LHC-like proteins, subunit S of photosystem II (PSBS), ferrochelatase II and red lineage chlorophyll *a/b* binding (CAB)-like proteins (RedCAP) (Engelken et al. 2010). All LHC proteins contain three membrane-spanning helices, among which helix one and three are homologous and likely originate via internal duplication (Green and Pichersky 1994). These proteins share a consensus sequence known as the generic LHC motif, which is a highly hydrophobic sequence of about 22

amino acids with three invariant charged amino acids [glutamic acid (Glu) and arginine (Arg)], as well as a few highly conserved glycine (Gly) residues (Jansson 1999).

LhcSR was originally identified as a light-induced transcript known as LI818 (Gagné and Guertin 1992; Savard et al. 1996). This element is a stress-related member of the LHC protein superfamily. LhcSR (LI818) is present in many major groups of algae, including green algae (*Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Mesostigma viride*, and *Volvox carteri f. nagariensis*), chlorarachniophytes (*Bigelowiella natans*) and diatoms (*Thalassiosira pseudonana* and *Phaeodactylum tricornutum*); however, it is not found in lycopods (*Selaginella*) or higher plants (Neilson and Durnford 2010; Zhu and Green 2010). Several studies have shown that *LhcSR* transcripts accumulate under environmental conditions known to induce photo-oxidative stress, including deprivation of carbon dioxide (Miura et al. 2004), sulfur (Zhang et al. 2004) or iron (Naumann et al. 2007), high light (HL) (Ledford et al. 2004), and low temperature (Dong et al. 2011). The strong induction that occurs under the aforementioned conditions implies that these proteins play a role in acclimation to photo-oxidizing conditions, and that excess photons must be de-excited to avoid photodamage (Terashima et al. 2011). All of these studies have shown that *LhcSR* are stress responsive genes that play a potential photoprotective role during stressful conditions. With completion of the genome sequence (Merchant et al. 2006) and the availability of EST databases (Shrager et al. 2003), three *LI818* genes have been identified in *C. reinhardtii* and annotated as *LhcSR1*, *LhcSR2* and *LhcSR3*. Recent studies have shown that the LhcSR3 protein is required for energy-dependent quenching (qE) (Peers et al. 2009), which is the component of non-photochemical quenching (NPQ) that operates on a timescale of seconds to minutes as a rapidly inducible, short-term response to changing light intensity (Li et al. 2000).

The discovery that the LhcSR protein family has homologs in the PSII system suggests that these are LHC type proteins that evolved very early and then passed to Chl *a/c*-containing algae through secondary endosymbiosis (Koziol et al. 2007). In diatoms, fucoxanthin and chlorophylls are bound within the light-harvesting antenna complexes by fucoxanthin chlorophyll *a/c* binding proteins (FCPs), which are homologous to the CAB of green algae and higher plants (Green and Durnford 1996). Three FCP proteins encoded by *fcp6*, *fcp7* and *fcp12* in the centric diatom *Cyclotella cryptica* share high similarities with LI818 proteins in green algae (Eppard and Rhiel 1998; Eppard et al. 2000). However, the lack of homologs in red algae poses a problem when explaining the extant distribution of this unique protein (Neilson and Durnford 2010). The LHC protein is present in the moss *Physcomitrella patens*, but absent from the lycopod *Selaginella moellendorffii* and other terrestrial plants, which indicates that a transition to a different mechanism for NPQ occurred during the transition to land, which in this case likely involved PSBS (Peers et al. 2009).

In the Antarctic region, seasonal changes in light intensity, UV-B radiation and high salinity of ice bubbles restrict photosynthesis in polar oceans (Horner et al. 1992). Accordingly, genetic differences in algae found in these regions must explain why they are able to grow in polar waters and sea ice, and why many are low light (LL) and high radiation adapted (Mock and Valentin 2004). Accordingly, elucidating the molecular mechanism of acclimation and regulation of photosynthesis in Antarctic algae is very important. Many interesting studies of polar diatoms have recently been conducted to identify the main environmental factors that influence photosynthesis and growth. Mock and Valentin (2004) studied the psychrophilic diatom *Fragilariopsis cylindrus* and found that the expression of genes for photosystem II (PSII; *psb*A, *psb*C) and carbon fixation (*rbc*L) was down-regulated during reduction of the temperature to the freezing point of seawater in polar seas. However, our knowledge of these globally important polar algae is primarily based on field and ecophysiological investigations, which have a limited potential to explain the molecular mechanisms of photosynthesis regulation (Mock and Valentin 2004).

In the Antarctic Ocean, microalgae are the main biomass primary producers (Horner et al. 1992). As a photosynthetic psychrophilic alga, *Chlamydomonas* sp. ICE-L is a major biomass producer that can thrive in the extreme environments of the Antarctic (Wang et al. 2004). *Chlamydomonas* sp. ICE-L can proliferate and propagate at temperatures of 0–10°C (Liu et al. 2010) and are more resistant to UV-B radiation than mesophilic green algae (Miao et al. 2002). Our experimental set-up involved cloning of the *LhcSR* genes related to photosynthesis and analysis of gene expression and physiological responses to better understand the physiological function of the genes. Specifically, the expression of *LhcSR* genes under different stress conditions (HL, LL, UV-B radiation and high salinity) was intensively studied by quantitative real-time PCR (qRT-PCR). In parallel, the effects of those conditions on photophysiological parameters were examined by Dual-PAM.

Materials and methods

Plant materials and growth conditions

The Antarctic ice alga, *Chlamydomonas* sp. ICE-L (Liu et al. 2006), was isolated from floating ice near the

Zhongshan Research Station of Antarctica (69.8°S, 77.8°E) during China's 18th Antarctic Expedition (2001–2002). Algal cells were cultured at a light density of 40 μmol photons m^{-2} s^{-1} under a 12-h/12-h light/dark photoperiod in Provasoli seawater medium (Provasoli 1968). This cultivation was conducted in 500-mL Erlenmeyer flasks containing 300 mL of medium at 6 ± 1°C. All glassware and media used in the experiments were previously sterilized by autoclaving. The salinity of the seawater was 31‰. Cells in the exponential phase were used for the experiments.

RNA extraction and cDNA synthesis

Total RNA was extracted using TRIzol Reagent (Invitrogen, USA) and dissolved in RNase-free dH$_2$O. The integrity of the total RNA was verified by running samples on 1.0% formaldehyde denaturing agarose gels. The concentration of total RNA was determined by measuring the UV absorbance at 260 nm using a Thermo Scientific NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and the RNA purity was checked by determining the $A260/A280$ ratio. The cDNA used for PCR and qRT-PCR was synthesized using M-MLV reverse transcriptase (Promega Biotech Co., Madison, WI, USA) and oligo d(T)$_{18}$ (TaKaRa Biotech Co., Dalian, China).

Cloning of *LhcSR* fragments

Primers for partial cDNA of *LhcSR1* and *LhcSR2* (Table 1) were designed from the nucleotide conserved regions of *LhcSR* from *C. reinhardtii*. *LhcSR* gene-related cDNA fragments were amplified by PCR consisting of 38 cycles of 94°C for 1.0 min, 58°C for 30 s and 72°C for 1.0 min, after which the PCR products were electrophoresed in 1% agarose gel and then visualized. The interesting fragments were excised and purified using a Tiangen gel purification kit (Tiangen Biotech Co., Beijing, China). The purified PCR products were cloned into the pMD18-T vector (TaKaRa Biotech Co., Dalian, China), after which they were transformed into Top10 *Escherichia coli* cells. Five randomly selected clones were identified as positive clones using LB culture with the addition of Amp (100 μg/μL) and then sequenced at least twice (GenScript, Nanjing, China).

Rapid amplification of cDNA ends

Based on the partial sequences of the two genes, the 3′ and 5′ ends of the cDNA were obtained by the RACE approach using gene-specific primers and adapter primers. The reverse transcription reactions were conducted using total mRNA, 5′-RACE CDS Primer A, 3′-RACE CDS Primer A and SMARTScript Reverse Transcriptase (Clontech Biotech Co., Mountain View, CA, USA). The RACE reactions were conducted using cDNA, the GSP1 and GSP2 primers, and a SMART^{TM} RACE cDNA Amplification Kit (Clontech Biotech Co., Mountain View, CA, USA) according to the manufacturer's instructions. The primers used in the experiment are shown in Table 1. The 5′- and 3′-RACE products were purified from an agarose gel and then ligated into the T/A cloning vector pMD18-T (TaKaRa Biotech Co., Dalian, China), after which they were transformed into competent *E. coli* Top10 cells. Each sample was sequenced

Table 1 Primers used in this study

Primers	Sequence (5′–3′)
Primers for partial cDNA	
LhcSR1-F	CCATCTACCACTTCCAGCAG
LhcSR1-R	CAGGATAACCTCCTTCTCGAA
LhcSR2-F	CCCGCCATCTATCACTTCCA
LhcSR2-R	TCACGCTCAATGTCGTCCAG
Primers for RACE	
LhcSR1-GSP1	CAATGAGCAGAGGCTCCCAGAATCCC
LhcSR1-GSP2	ACAACCTGAAGGAGGAGTATGAGATGGGCA
LhcSR2-GSP1	CCGTCTCGCCATGATTGCCATCGCTG
LhcSR2-GSP2	TCGGCCAGACCAATGCAAATGAGCA
Primers for qRT-PCR	
LhcSR2-qF	CTGAAGGTCATGCAGACTAA
LhcSR2-qR	TCACAACCTCATCAGCAGCT
LhcSR1-qF	TCTGGGAGCCTCTGCTCATT
LhcSR1-qR	GGTTGCCCATCTCATACTCCTC
18S-F	ATGGAATAACACGATAGGACTCTGG
18S-R	ACCTCTGACAATGAAATACGAATGC

at least twice (GenScript, Nanjing, China). The full-length cDNA of the two genes was then merged with partial cDNA nucleotide fragments and RACE-PCR fragments, after which the sequences of the open reading frame of the two genes were checked by PCR.

Bioinformatics analysis

The sequences were examined using the BLAST program available at the National Center for Biotechnology Information (NCBI) Web site (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The deduced amino acid sequence was analyzed with the Six Frame Translation of Sequence system (http://searchlauncher.bcm.tmc.edu/seq-util/Options/sixframe.html). The theoretical molecular weight (Mw) and isoelectric point (pI) of the protein were computed using the ExPASy Compute pI/Mw tool (http://www.expasy.org/tools/pi_tool.html). The domains of the sequences were then predicted in Pfam HMM (http://pfam.sanger.ac.uk/search), after which the membrane-spanning helix transmembrane structure was predicted by TMpred (http://www.ch.embnet.org/software/TMPRED_form.html) and NPS (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_nn.html). Multiple sequence alignments were generated using CLUSTAL X (Thompson et al. 1997) and subsequently analyzed using Editor Program v7.0.5 (Hall 1999). A phylogenetic tree was constructed using the neighbor-joining algorithm of the MEGA 4.0 program (Tamura et al. 2007). The full-length cDNA sequences of the genes were merged according to the RACE-PCR results using DNAStar 7.1 (DNASTAR Inc., USA).

Chlorophyll fluorescence measurements

To measure the chlorophyll fluorescence, a fresh 2-mL sample was placed in a 5-mm quartz cuvette. The pulse amplitude modulated fluorescence was then recorded at room temperature using a dual-wavelength pulse amplitude modulated fluorescence monitoring system (Dual-PAM, Heinz Walz, Germany). Prior to measurement of the fluorescence, samples cultivated under normal conditions were dark adapted for 15 min (done in triplicate). A pulse of saturating light (10000 μmol photon m^{-2} s^{-1} for 300 ms) was then applied to determine the maximum fluorescence (F_m). Once steady state fluorescence was achieved, saturating pulses were applied every 30 s to measure the F_m under actinic light (F_m'). Samples cultured under the induced conditions were analyzed directly in the Dual-PAM fluorometer. The energy dissipation that was not used for PSII photochemistry was determined based on the fluorescence quenching effect using the ratio NPQ = $(F_m - F_m')/F_m'$ (Bilger and Björkman 1990). Variable fluorescence (F_v) was

calculated as $F_m - F_o$, and the maximum PSII photochemical efficiency was calculated as $F_v/F_m = (F_m - F_o)/F_m$ (Krause and Weis 1991). All experiments were conducted in triplicate.

Stress treatments

In the HL stress treatment, algae were exposed to 400 μmol photons m^{-2} s^{-1} for 0.5, 1, 2, 3, 6 and 9 h to investigate the effects of HL exposure on mRNA expression of *LhcSR1* and *LhcSR2*. In the LL treatment, algae were exposed to 1 μmol photons m^{-2} s^{-1} for 0.5, 1, 2, 3, 6 and 9 h. In the UV-B radiation treatment, a suspension of algal cells was placed under two UV-B lamps (Beijing Normal University, Beijing, wavelength 290–320 nm, 8 W) and subjected to 60 μw cm^{-2} irradiance for 0.5, 1, 2, 4 and 6 h to investigate the mRNA expression of *LhcSR1* and *LhcSR2*. In high salinity treatments, additional NaCl was added to Provasoli seawater medium to give a final salinity of 93‰. Algal cells were then kept in culture medium containing 93‰ NaCl for 1, 2, 3, 6, 12, and 24 h to investigate the effects of salt stress on mRNA level. The expression of the two genes under different stress conditions was measured by qRT-PCR.

Analysis of gene expression by quantitative real-time PCR

qRT-PCR was conducted using an ABI StepOne Plus Real-time PCR system (Applied Biosysytems, USA) in a reaction mixture with a total volume of 20 μl that contained 10 μl of 2× SYBR Premix Ex Taq^{TM} II (TaKaRa Biotech Co., Dalian, China), 0.5 μl (10 μM) of each primer, 2.0 μl of the diluted cDNA mix and 7 μl of dH$_2$O. A product of approximately 120 bp was amplified using the *LhcSR1* and *LhcSR2* primers (Table 1) and sequenced to verify the PCR specificity. The 18S primers (Table 1) were used to amplify a 103-bp fragment as an internal control to verify the successful reverse transcription and calibrate the cDNA template. The thermal profile for real-time PCR was 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, 55°C for 10 s and 72°C for 30 s. Dissociation curve analysis of the amplification products was conducted at the end of each round of PCR to confirm that only one specific PCR product had been amplified and detected. Triplicate qRT-PCRs were conducted for each sample. After the PCR program was conducted, the data were analyzed using the comparative Ct ($2^{-\Delta\Delta C_T}$) method (Livak and Schmittgen 2001). To maintain consistency, the baseline was set automatically by the software. The mean ± SD was calculated for each experiment and analyzed using the SPSS 17.0 data processing system software.

Results

Cloning and sequencing of *LhcSR1 and LhcSR2* genes

LhcSR1 and *LhcSR2* cDNA fragments were amplified from the total cDNA using primers for the partial cDNA (Table 1). Agarose gel electrophoresis revealed that the amplified fragments were about 500 bp. The amplified fragments were cloned and sequenced, after which their nucleotide sequences were confirmed to correspond to the *LhcSR* genes of *C. reinhardtii*. The 5′ and 3′ fragments of two full-length cDNA samples were obtained through 5′-RACE and 3′-RACE, respectively. The full length of the cDNA was merged according to the amplified fragments and the RACE-PCR results using DNAStar 7.1. The results revealed the presence of an open reading frame and 5′ and 3′-untranslated regions encoding chlorophyll *a*/*b* binding proteins. The theoretical Mw and p*I* of the LhcSR1 protein were 28.1 kDa and 4.52, respectively. The LhcSR2 protein had a deduced Mw of 28.6 kDa and a p*I* of 4.83. The sequences of LhcSR1 and LhcSR2 were submitted to GenBank and assigned accession numbers HQ639411 and HQ286903, respectively.

Bioinformatic analysis

The deduced amino acid sequences were examined for homology with other known sequences using the protein BLAST program available at the NCBI Web site. The amino acid sequence of LhcSR1 was 62%, homologous with LhcSR1 of *C. reinhardtii*, while LhcSR2 shared 64% homology with LhcSR2/3 of *C. reinhardtii*. Pfam HMM was used to predict the domains, and the results showed that amino acids 5–156 of LhcSR1 comprised chlorophyll *a–b* binding protein (Bit score 121.9), while in LhcSR2, amino acids 3–156 comprised the chlorophyll *a–b* binding protein (Bit score 130). The TMpred program, which was used to predict the membrane-spanning regions and their orientation, revealed that there were three possible transmembrane helices in the two genes (Fig. 1), among which the first and third helices of the LhcSR proteins included the well-conserved generic LHC motif. The generic LHC motif contains three invariantly charged amino acids, glutamic acid (Glu) and arginine (Arg), as well as glycine (Gly) residues (Fig. 1). These results indicated that the two genes belong to the LHC protein superfamily.

The similar LhcSR protein sequences from other algae were retrieved from the NCBI Web site, and a phylogenetic tree was constructed based on their alignment. The unrooted phylogenetic tree (Fig. 2) showed two clades, designated A and B. Clade A consisted of green algae in which the LhcSR protein was chlorophyll *a*/*b* protein. Clade B was composed of diatom algae in which the protein was chlorophyll *a*/*c*. In the diatom algae, the chlorophyll *a*/*c* binding proteins were homologous to those of green algae and higher plants. These results indicate that those genes existed before the divergence of green algae and diatoms, after which they evolved in different species with gene duplication.

Effects of light intensity on chlorophyll fluorescence and genes' expression

To determine if the expression of *LhcSR* genes was dependent on light intensity, the samples were subject to HL (from 40 to 400 μmol photons m^{-2} s^{-1}) and LL (from 40 to 1 μmol photons m^{-2} s^{-1}) exposure. The maximum PSII photochemical efficiency (F_v/F_m) and NPQ were measured to determine if HL and LL could induce stress response. The expression of *LhcSR* under 40 μmol photons m^{-2} s^{-1} was set to 1 and the accumulation was observed at each time slot.

As shown in Fig. 3a, F_v/F_m was reduced from 0.52 to 0.32 after 9 h of HL exposure, while NPQ increased when compared with the normal cultured condition, peaking after 6 h of HL. The lower F_v/F_m indicated stress conditions, and the higher NPQ showed energy dissipation. The expression of *LhcSR1* and *LhcSR2* increased continuously during the first 3 h of HL, and peaked at levels 5.1- and 3.1-fold higher than the control at 3 h (Fig. 3b). Three hours later, the transcript levels of the two genes began to decrease, but they were still higher than those observed under normal light intensity. These findings indicated that all investigated genes were HL inducible, but that the response varied.

For the LL experiment, F_v/F_m changed only slightly under LL condition (Fig. 4a), which indicated that *Chlamydomonas* sp. ICE-L was not obviously stressed. Accordingly, NPQ showed a small change after 0.5 h and then decreased to 0 after 2 h (Fig. 4a). As shown in Fig. 4b, expression of *LhcSR1* was down-regulated with time, while *LhcSR2* was up-regulated during the first 0.5 h and then decreased.

Taken together, these results indicated that the mRNAs encoding LhcSR1 and LhcSR2 were differentially regulated in response to light intensity. Moreover, *LhcSR1* was found to be more sensitive to high light than *LhcSR2*, indicating that it may play a primary role in protection of the algal cells from potential photodamage under excess light conditions. Under LL conditions, *LhcSR2* might be necessary to collect photons as efficiently as possible for photosynthesis, which is as expected for a typical light-harvesting gene.

Chlorophyll fluorescence and genes' expression levels in response to UV-B radiation

To investigate the effects of UV-B radiation on expression levels of the two genes, ice algae *Chlamydomonas* sp. ICE-L in exponential growth were subjected to UV-B radiation
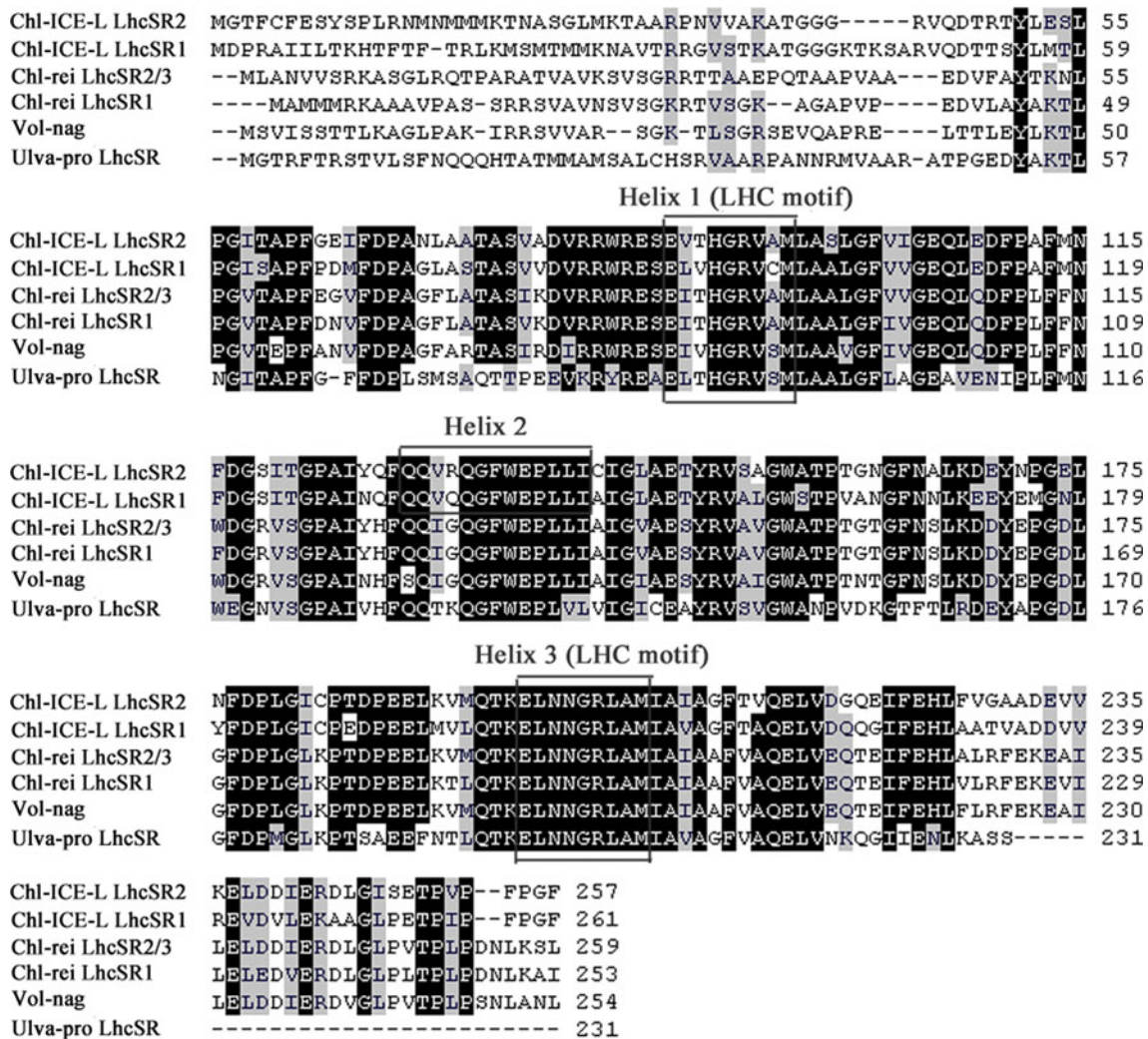
Fig. 1 Alignment and comparison of the deduced amino acid sequences of LhcSR with other species. There were three possible trans-membrane helices in the two genes, among which Helix1 and Helix3 included the well-conserved generic LHC motif. Ch-ICE-L lhcSR2 (*Chlamydomonas* sp. ICE-L, HQ286903), Ch-ICE-L lhcSR1 (*Chlamydomonas* sp. ICE-L, HQ639411), Chl-rei lhcSR2/3 (*C. reinhardtii*, XP_001696064), Chl-rei lhcSR1 (*Chlamydomonas reinhardtii*, XP_001696125), Volnag (*Volvox carteri f. nagariensis*, XP_002948670) and Ulva-pro lhcSR (*Ulva prolifera*, HQ622096.1). *Black bars* show identical amino acid residues. Deletions are indicated by *dashes*

for 0–6 h. F_v/F_m rapidly decreased to 0.15 after 0.5 h, where it remained for the rest of the UV-B treatment (Fig. 5a). NPQ was induced under UV-B stress, and reached its maximum value at 4 h. These parameters showed that algal cells were under stress condition. The expression of *LhcSR1* and *LhcSR2* at normal light intensity (40 μmol photons m^{-2} s^{-1}) without UV-B radiation was set to 1. *LhcSR1* and *LhcSR2* transcripts accumulated almost linearly under prolonged UV-B stress during the first 4 h, and peaked at 4 h by 6.4 and 6.2-fold, respectively, compared with the control (Fig. 5b). Then the transcript levels of the two genes began to decrease, but they were still higher than those observed under normal light intensity.

Effects of high salinity on chlorophyll fluorescence and expression of the *LhcSR* genes

To investigate the expression levels of the two genes in the high salinity treatment, *Chlamydomonas* sp. ICE-L was kept in culture medium containing 93‰ NaCl for different lengths of time (1, 2, 3, 6, 12, and 24 h). The expression of *LhcSR1* and *LhcSR2* was set to 1.

Under HS conditions (Fig. 6a), F_v/F_m reached its lowest point from 1 to 3 h, while the NPQ increased, after which they returned to their normal levels slowly. These results demonstrated that short-term HS conditions were stress condition for *Chlamydomonas* sp. ICE-L, but the algal cells can accommodate the longer periods of HS stress.

Fig. 2 Phylogenetic analysis of LhcSR (LI818)-like proteins. A neighbor-joining (NJ) tree constructed based on analysis of the LHCSR (LI818)-like protein in *Chlamydomonas* sp. ICE-L and sequences downloaded from GenBank
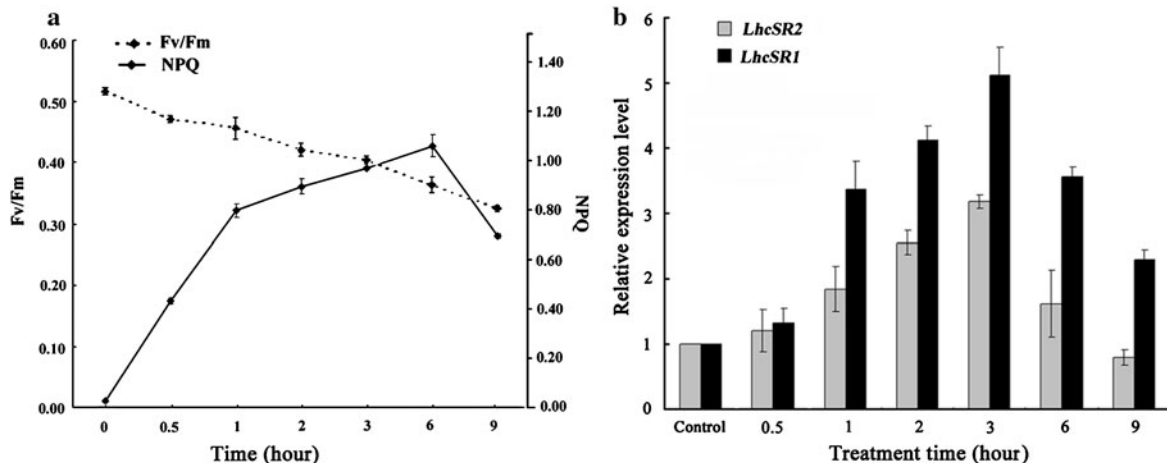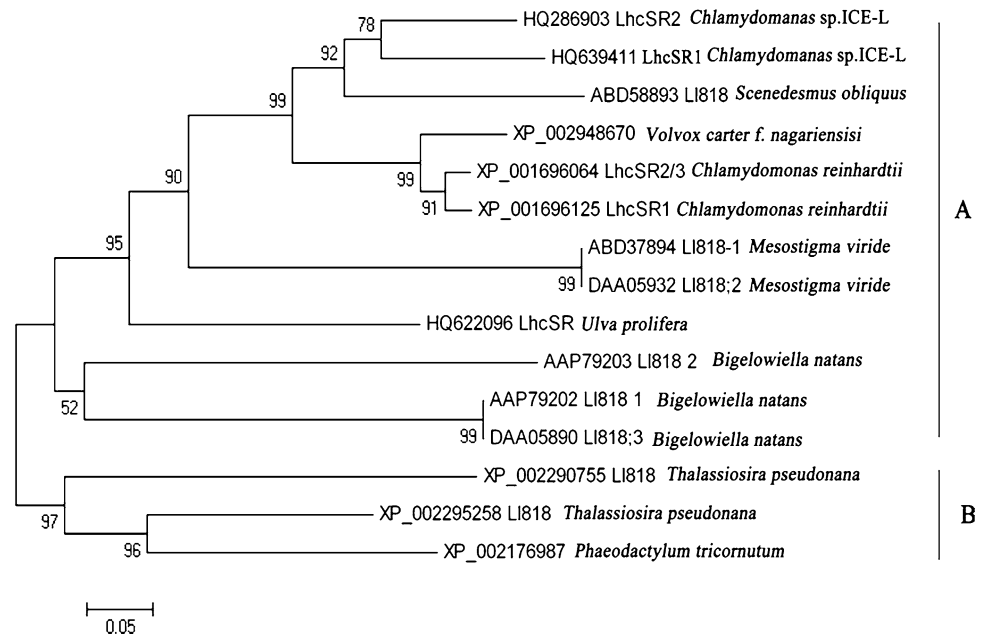

Fig. 3 a Maximum PSII photochemical efficiency (F_v/F_m) and NPQ for *Chlamydomonas* sp. ICE-L under HL condition; **b** Relative mRNA expression of *LhcSR1* and *LhcSR2* in algal cells in response to HL for different times. *Standard error bars* are shown

Figure 6b showed that there was an abrupt increase in the two genes during the first 2 h of HS stress, followed by a continuous decrease. The expression of *LhcSR1* and *LhcSR2* reached the highest levels at 2 h of 15.68- and 12.72-fold compared to those of the control, respectively. Overall, the data presented here suggest that these genes play a photoprotective role during HS stress.

Discussion

As light intensities increase and stress conditions are induced, the kinetic imbalance between the rates of photon excitation and thermally activated electron transport causes saturation of the rate of photosynthesis (Yamano et al.

2008). Photosynthesis is then incapable of using all of the energy absorbed by the LHCs. Plants, green algae and diatoms grown under different light intensities and stress conditions are able to adjust the size of their light-harvesting antenna through gene expression and/or protein degradation to balance light absorption and utilization (Walters and Horton 1994; Escoubas et al. 1995; Lindahl et al. 1995; Maxwell et al. 1995; Oeltjen et al. 2004). The NPQ mechanism has been found to have the fastest response to excess light in plants under stress conditions. In this way, the qE mechanism, which is the most important constituent of NPQ, provides efficient photoprotection. In vascular plants, the PSII polypeptide, PSBS, is essential for efficient qE (Li et al. 2000). Additionally, Peers et al. (2009) found that in *C. reinhardtii*, effective qE is
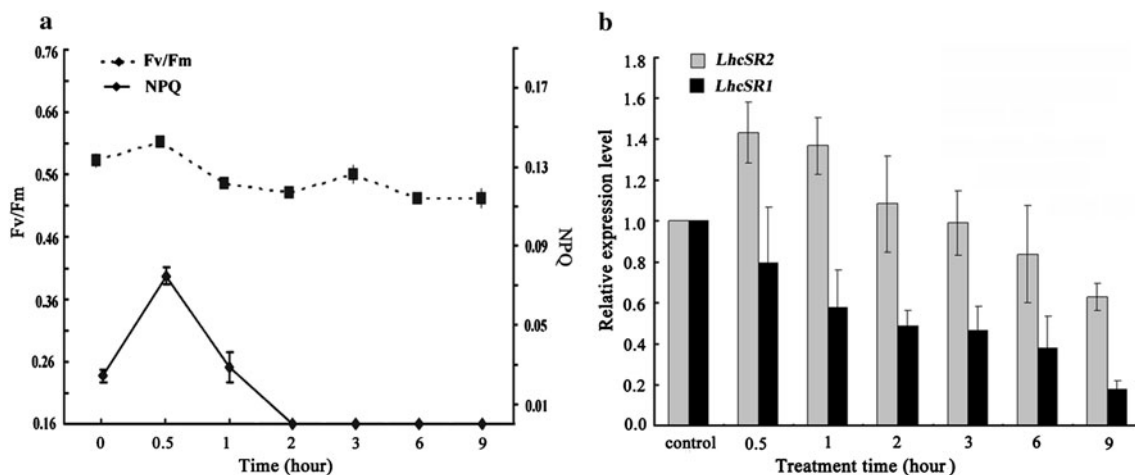
Fig. 4 Chlorophyll fluorescence (**a**) and expression levels of the *LhcSR* genes (**b**) in *Chlamydomonas* sp. ICE-L exposed to LL (1 μmol photons m^{-2} s^{-1}) for different times. *Standard error bars* are shown
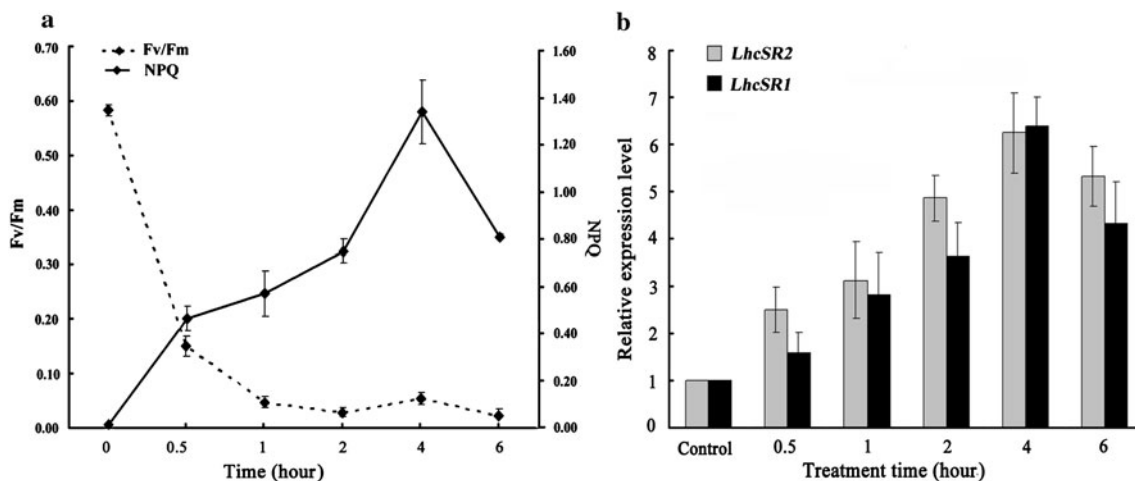

Fig. 5 Effects of UV-B radiation on chlorophyll fluorescence (**a**) and expression levels of *LhcSR1* and *LhcSR2* in algal cells (**b**). *Standard error bars* are shown
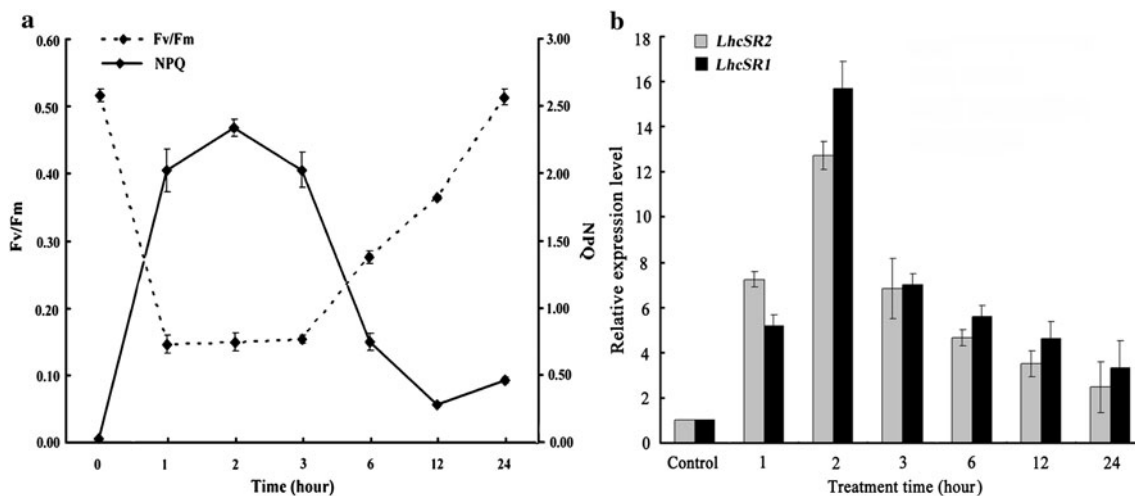

Fig. 6 Chlorophyll fluorescence (**a**) and expression of the *LhcSR* genes (**b**) in *Chlamydomonas* sp. ICE-L exposed to high salinity condition for different times. *Standard error bars* are shown

dependent on LhcSR3. However, there is currently no evidence that PSBS participates in the establishment of qE in *C. reinhardtii* in addition to LhcSR3, because no protein expression of PSBS in algae has been reported (Terashima et al. 2011). In *C. reinhardtii*, *LhcSR* is required for survival in a dynamic light environment and regulated over a short period of time (Peers et al. 2009). In *T. pseudonana* (Zhu and Green 2010), the expression of *Lhc*SR homologs (*Lhcx1*, *Lhcx4*, *Lhcx5*, *Lhcx6*, *lhcf2*, *lhcf4* and *lhcf5*) was studied under LL and HL conditions. The results showed that the transcript levels of *Lhcx1*, *Lhcx4* and *Lhcx6* increased dramatically after transfer to HL and peaked at 15 min. Furthermore, *lhcf2* had higher expression under LL conditions and was expected to be a typical light-harvesting gene (Zhu and Green 2010). These results indicated that *LhcSR* (*LI818*) homologs accumulated transiently under HL stress via a short-term photoprotective mechanism.

Chlamydomonas sp. ICE-L is a primary green alga found in the Antarctic region, and its responsive mechanisms of adaptation to stress provide an important basis for study of the Antarctic environment and species. The Antarctic marine ecosystem is one of the largest ecosystems on earth, and photosynthetic microorganisms dominate the biomass and metabolic activity in extreme environments (Morgan-Kiss et al. 2006). In its natural environment, *Chlamydomonas* sp. ICE-L generally grows in LL, low UV-B radiation, and seawater with a salt concentration of about 30‰. Liu et al. (2010) found that the CiHsp70 mRNA expression levels of *Chlamydomonas* sp. ICE-L increased in response to UV-B radiation and high salinity. In the present study, light intensity, UV-B radiation and high salinity were found to be stress conditions that could affect the physiology and metabolism of algae in polar environments. In this study, we found that the expression of *LhcSR1* and *LhcSR2* were both up-regulated under HL, UV-B radiation and high salinity stress conditions, which enable a better understanding of the physiological function of *Chlamydomonas* sp. ICE-L under stress conditions.

In the present study, the expressions of *LhcSR1* and *LhcSR2* did not obviously increase during the first 0.5 h of HL. However, they increased rapidly from 0.5 h and peaked at 3 h. These findings demonstrated that both *LhcSR1* and *LhcSR2* were sensitive to light intensity, but that their response time was longer than that of *C. reinhardtii* and *T. pseudonana*. This longer response to HL might relate to the unique physiology of polar environments. When the cells were shifted from normal light conditions to LL, the expression of *LhcSR1* decreased gradually, while *LhcSR2* was up-regulated during the first 0.5 h and then decreased. As shown in Fig. 4a, there was no thermal energy dissipation based on the absence of NPQ after 2 h, and the little changes of F_v/F_m showed that the cells were not under stress treatment. These findings

indicated that *LhcSR2* might be necessary to collect photons as efficiently as possible under LL conditions. UV-B and high salt stress induced physiological changes, suggesting that photosynthesis was incapable of using all of the energy absorbed by the LHCs and that the NPQ mechanism was the response to excess light under UV-B and high salt stress. The up-regulation of *LhcSR1* and *LhcSR2*, which was strongly induced by UV-B and high salt stress, was accompanied by a high level of NPQ. These findings indicated that *LhcSR1* and *LhcSR2* might play a primary role in reducing the excited state of excess photons to avoid photodamage. Under HS condition, short-term HS was a stress condition for *Chlamydomonas* sp. ICE-L, suggesting that algal cells can accommodate the longer periods of HS stress, which would occur in Antarctic winter. These findings indicated that both *LhcSR1* and *LhcSR2* play roles in protecting algal cells from potential photo-oxidative damage to the photosynthetic machinery. Under stress, the high level of NPQ is accompanied by up-regulated expression of *LhcSR* genes, suggesting that *LhcSR* plays a role in thermal energy dissipation (NPQ) or that it provides increased stability to the thylakoid membrane assembly under stress conditions. The excess absorption of light energy by photosynthetic pigments has led to the evolution of protective mechanisms (Peers et al. 2009). As a result, the *LhcSR* protective mechanisms of *Chlamydomonas sp*. ICE-L might be related to the polar environment.

Light signals and CO_2 are indispensable for photosynthesis, and it has been proposed that they are strongly associated with each other. Yamano et al. (2008) revealed that induction of the carbon-concentrating mechanism (CCM), which *Chlamydomonas* requires to allow the uptake of inorganic carbon (Ci) to acclimate to CO_2-limiting conditions, is not solely dependent on absolute environmental Ci concentrations, but is also affected by light intensity. In *C. reinhardtii*, *LhcSR1*, which was found to be a CCM-associated gene that is inducible under light stress, is highly up-regulated under low CO_2 (Yamano et al. 2008) and HL conditions, suggesting that the LhcSR1 protein plays a role in protecting chlorophyll proteins from excitation pressure under these conditions. In addition, *LhcSR1* is also induced under sulfur starvation (Zhang et al. 2004) and phosphorus deprivation (Moseley et al. 2006), and *LhcSR3* is strongly induced by HL (Yamano et al. 2008) and iron deficiency (Naumann et al. 2007). Under stress conditions, LHCSR genes and proteins were strongly induced, and they de-excited excess photons to prevent photodamage and photo-oxidization (Terashima et al. 2011). In the present study, the expression of *LhcSR1* and *LhcSR2* accumulated under HL, LL, UV-B radiation and high salinity, indicating that the two genes participated in a photoprotective effect under those stress conditions. These

findings also showed that aquatic and terrestrial plants in Antarctica have become well adapted to solar UV-B through the development of UV screens (Rozema et al. 2002). The up-regulation of *LhcSR* under different stress conditions in *Chlamydomonas* sp. ICE-L might form a long-term photoprotective mechanism to adapt to the seasonal light intensity of the Antarctic Ocean.

In higher plant photosynthesis, the PSBS protein is a critical component in regulation of NPQ that can remove excess absorbed light energy harmlessly as heat (Sami et al. 2010). However, the green alga *C. reinhardtii* shows different requirements for NPQ activation, in that it needs LhcSR for activation of heat dissipation instead of PSBS (Peers et al. 2009). Many studies have recently shown that *LhcSR* is up-regulated and plays a role in photoprotection under HL and other stress conditions (Peers et al. 2009; Zhu and Green 2010). In the present study, the expression of *LhcSR* can be used to analyze the photoprotective mechanism of Antarctic microalgae under stress conditions. Although *C. reinhardtii* and *Chlamydomonas* sp. ICE-L belong to the same genus, the response mechanism of *LhcSR* to HL exposure differed, which might reflect the special physiology of organisms native to polar environments. The long-term regulation of *LhcSR* in *Chlamydomonas* sp. ICE-L might be relevant to the adaptation of the Antarctic Ocean. However, further studies are needed to confirm whether *LhcSR* expression is related to NPQ in the short-term response to changing light intensity. Future experiments should also include an investigation of protein expression and biophysical and biochemical measurements relevant to photosynthesis.

Conclusions

In this study, physiological parameters of cells subjected to different conditions (HL, LL, UV-B radiation and high salinity) were examined by Dual-PAM. A lower F_v/F_m (meantime maximum PSII photochemical efficiency) indicated that cells had undergone a stress response under these conditions. HL induced the up-regulation of *LhcSR1* and *LhcSR2*, and these two genes were shown to be inducible, with *LhcSR1* being more sensitive to light stress than *LhcSR2*. Under LL, the *LhcSR2* gene was up-regulated, which is to be expected for a typical light-harvesting gene. Similarly, the expression of *LhcSR1* and *LhcSR2* accumulated under UV-B radiation and high salinity, indicating that the two genes participated in a photoprotective mechanism to protect the cell against excitation pressure. Under stress, the high level of NPQ was accompanied by up-regulated *LhcSR* gene expression, suggesting that *LhcSR* played a role in thermal energy dissipation (NPQ). The results presented here demonstrated that these genes might play a primary role in protecting algal cells against potential photodamage to the photosynthetic machinery. In Antarctic marine ecosystems, *Chlamydomonas* sp. ICE-L was acclimated to varying environmental factors that control genetic and physiological responses, especially UV-B radiation and high salinity stress. In the present study, we found that the *LhcSR* genes of *Chlamydomonas* sp. ICE-L could be induced and up-regulated by HL, as well as UV-B radiation and high salinity stress. These findings indicate that ICE-L might be involved in adaptation to these stress conditions in Antarctic marine ecosystems.

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