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Identification and expression analysis of four light harvesting-like (*Lhc*) genes associated with light and desiccation stress in *Ulva linza*

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ABSTRACT

The marine alga *Ulva linza* inhabits the intertidal zone, where it is strongly influenced by different kinds of physical stress, such as high light and desiccation. To cope with these stress conditions, *U. linza* has evolved stress tolerance strategies to protect against high light-induced photodamage. In the present work, we identified four light harvesting complex (Lhc)-like genes (*Elip*L1, *Elip*L2, *Cbrx* and *OHP*), which encode proteins that are relatives of light-harvesting complex proteins in *U. linza*. The mRNA levels of the four genes increased and reached maximum within 3 h under high light, and then rapidly returned to a low level. By contrast, these four genes displayed their highest expression levels at 6 h under desiccation stress. Up-regulation of *Cbrx* was more significant than the other three genes under both conditions. When compared with the mRNA expression data, the protein levels were not consistent, showing a slight delay under both conditions. These results suggested putative photoprotection functions for *Elip*L1, *Elip*L2, *Cbrx* and *OHP* in *U. linza* under both high light and desiccation stresses.

1. Introduction

Light is essential for photosynthesis, and oxygenic photosynthesis relies on energy from sunlight to catalyze the reduction of CO_2 to carbohydrates. However, in the natural environment, plants often experience stress conditions that disturb the balance between energy consumption and utilization (Takahashi and Murata, 2008; Li et al., 2009). Plants have to deal with a fluctuating light environment and regulation of the photosynthetic apparatus is vital for their survival. The large multigenic family of nuclear-encoded chloroplast proteins called the light harvesting complex (LHC) is involved in light harvesting and in photoprotection (Floris et al., 2013; Sturm et al., 2013). Across all recently studied bacterial and eukaryotic photosynthetic organisms, the extended LHC protein superfamily comprises the LHC, LHC-like and Photosystem II 22 kDa protein (PSBS) families (Sturm et al., 2013). The LHC-like protein family includes early light-induced proteins (ELIPs), stress-enhanced proteins (SEPs), one-helix proteins (OHPs), and carotene biosynthesis-related proteins (CBRs) (Dittami et al., 2010; Neilson and Durnford, 2010; Heddad et al., 2012).

Members of the LHC-like families are mainly involved in photoprotection and photoacclimation. Early light-induced proteins (ELIPs) are also induced in mature green plants exposed to variety of physiological stresses. The gene's expression of *Elips* culminates during exposure to high light stress conditions (Meyer and Kloppstech, 1984; Kimura et al., 2001). In addition to light stress, nutrient deprivation or dehydrative processes, such as desiccation (Gechev et al., 2012), cold stress and salt stress (Jin et al., 2010) also lead to induction of *Elip* genes. Carotene biosynthesis-related proteins (CBRs) of *Dunaliella* are overexpressed during light stress (Hsu and Lee, 2012). The CBR is closely associated with a minor LHCII (Chen et al., 2008) and functions as a zeaxanthin-binding protein (Levy et al., 1993). The *OHP*s genes of the LHC-like gene family in various algal species and cyanobacteria are encoded by the chloroplast, cyanelle or nucleoid genomes (Adamska, 2004). Two types of OHPs have been characterized: the OHP1/HLIP-type present in cyanophages, cyanobacteria and photosynthetic eukaryotes, and the OHP2-type restricted to eukaryotic organisms (Neilson and Durnford, 2010; Engelken et al., 2010). One-helix proteins (OHPs) can be induced by stress condition treatments, and OHPs accumulate in response to high light in *Arabidopsis thaliana*, suggesting their function of photoprotection (Heddad et al., 2012).

In recent years, green tides caused by the excessive growth and drifts of *Ulva* have occurred frequently in the South Yellow Sea of China, and its biomass has caused ecological and social problems (Ye et al., 2008,

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2011). The marine alga *Ulva linza*, as a member of diminutive green tides (Liu et al., 2010), rapidly propagates during spring along the coastline of Qingdao, Shandong Province (Yellow Sea, China). Most *Ulva* species have strong tolerances to temperature, salinity, pH and high light (Bolam et al., 2000; Dong et al., 2012). It is thought that *U. linza* has a series of photoprotection mechanisms to withstand high and variable illumination in the intertidal zone habitats. The genes' expressions of *Psb*S and *Lhc*SR in *U. linza* were positively regulated under stress conditions. Both LHCSR and PSBS-dependent non-photochemical quenching (NPQ) may be important strategies for adapting to the environment and they play an important role in the photoprotective mechanism (Dong et al., 2012; Zhang et al., 2013). The expressions of putative early light-induced genes have also been analyzed under different stress conditions in *U. linza*. The results suggested that *Elip*L1 and *Elip*L2 participate in photoprotection in *U. linza* under high light, low temperature and low osmotic stress conditions (Zhang et al., 2013).

In this study, two new LHC-like genes were identified in transcriptome databases of *U. linza*, and designated them as *Cbrx* and *OHP*. To understand the putative functions of *Cbrx* and *OHP*, together with *Elip*L1 and *Elip*L2, their mRNA and protein levels under high light and desiccation stress conditions were analyzed.

2. Materials and methods

2.1. Sampling and culture conditions

Samples of *U. linza* were collected in May of 2013 from the intertidal zone (35°35'N, 119°30'E) of Zhanqiao Wharf, Qingdao, China. In the laboratory, the intact samples were washed several times with sterile seawater, sterilized with 1% sodium hypochlorite for 2 min, and then rinsed with autoclaved seawater. The sterilized material was then placed into an aquarium containing enriched seawater (500 μM $NaNO_3$ and 50 μM NaH_2PO_4), aerated and maintained at 13 °C under a 12:12 h light–dark photoperiod with 120 μmol photons m^{-2} s^{-1} provided by cool-white fluorescent tubes. Samples for real-time PCR were treated with high light and desiccation. For desiccation stress, the algal samples, removed from the sea water and hanged on the glass rod in the incubator, were cultured at 13 °C and 120 μmol photons m^{-2} s^{-1} for 1, 2, 3, 6 and 9 h, separately. For high light stress, the material was submerged and induced by exposure to 2000 μmol photons m^{-2} s^{-1} for different durations (1, 3, 6, 9 and 12 h).

2.2. Fluorescence measurements

Chlorophyll fluorescence was measured with a dual-wavelength pulse amplitude-modulated fluorescence monitoring system (Dual-PAM, Heinz Walz, Germany). Prior to measurement, samples cultivated under normal conditions were dark adapted for 20 min (done in triplicate). A pulse of saturating light (10,000 μmol photon m^{-2} s^{-1} for 300 ms) was then applied to determine the maximum fluorescence (Fm). Once steady state fluorescence was achieved, saturating pulses were applied every 30 s to measure the Fm under actinic light (Fm′). The energy dissipation that was determined based on the fluorescence quenching effect using the ratio NPQ = (Fm − Fm′) / Fm′ (Bilger and Björkman, 1990) and NPQ is a direct indicator of the stress response via its function in photoprotective dissipation of excess absorbed light energy as heat. Variable fluorescence (Fv) was calculated as Fm − F_0, and the maximum PSII photochemical efficiency was calculated as Fv/Fm = (Fm − F_0) / Fm (Krause and Weis, 1991).

The maximum PSII photochemical efficiency (Fv/Fm) and NPQ were measured to determine if high light and desiccation could induce stress response. For desiccation stress, plants were measured with 120 μmol of photons m^{-2} s^{-1} of actinic light. For high light stress, the samples were measured with 2000 μmol photons m^{-2} s^{-1} of actinic light.

2.3. Genes and sequence analysis

The full-length cDNAs of the four genes were deduced from the transcriptome of *U. linza* (Zhang et al., 2012a, 2012b). The sequences were examined for identity with other known sequences using the BLASX program available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast). The four genes were named as *Elip*L1, *Elip*L2, *Cbrx* and *OHP*. The deduced amino acid sequences were analyzed using the Expert Protein Analysis System (http://www.expasy.org/). Pfam HMM (http://pfam.sanger.ac.uk/search) was used to predict protein domains, and HMMTOP (http://www.enzim.hu/hmmtop/html/submit.html) was used to predict membrane-spanning helices. ClustalX (Thompson et al., 1997) generated multiple sequence alignments, which were analyzed using the program BioEdit (Thompson et al., 1997; Chenna et al., 2003). The MEGA 5.0 program (Tamura et al., 2007) constructed the phylogenetic tree using the neighbor-joining algorithm. A total of 1000 bootstrap replicates were performed.

2.4. RNA extraction and reverse transcription

After the pretreatment, more than three samples were crushed together and mixed. Samples were frozen in liquid nitrogen and then ground into a fine powder using a mortar and pestle. Total RNA was subsequently extracted using the Trizol Reagent according to the manufacturer's instructions, after which it was dissolved in diethylpyrocarbonate (DEPC)-treated water. In this protocol, DNA was digested with DNaseI. The cDNA used for real-time PCR was synthesized from total RNA using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega Biotech Co., Madison, WI, USA) and oligo d(T)18 (TaKaRa Biotech Co., Dalian, China).

2.5. Expression of ElipL1, ElipL2, Cbrx and OHP under different stress conditions

Primers for real-time PCR of *Elip*L1, *Elip*L2, *Cbrx* and *OHP* (Table 1) were designed from the conserved regions of EliP, CbrX and OHP from *U. linza*. The 18S rDNA 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 real-time quantitative PCR reactions were performed with the ABI Step One Plus Real-Time PCR System (Applied Biosystems, Bedford, MA, USA) using SYBR Green fluorescence (TaKaRa Biotech Co., Dalian, China), according to the manufacturer's instructions. 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 performed at the end of each PCR reaction to confirm that only one specific PCR product was amplified and detected. Triplicate qPCRs were performed for each sample. The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used to analyze the quantitative real-time PCR data.

Table 1
Gene primers used in real-time PCR.

Genes	Oligo nucleotide sequence 5′–3′
18S	Forward: TGCCTAGTAAGCGCGAGTCA
	Reverse: AAACGATGGGCAGGGAAAC
*Elip*L1	Forward: AGCGCAAAGCGGAGAGACT
	Reverse: AATCACAATGGTGGCCACAAG
ElipL2	Forward: TCATCTTTGCGGCTGTGTCT
	Reverse: TGCCTGGGAGTTGCTGTTG
Cbrx	Forward: CGGGAAGAGCACACAACACAT
	Reverse: TCAGGCTCAACGTATCTACAAAGC
OHP	Forward: GACACCAGCGAACGAGAT
	Reverse: ACCCCTGTTGCATATTCTGT