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Circadian and irradiance effects on expression of antenna protein genes and pigment contents in dinoflagellate *Prorocentrum donghaiense* (Dinophycae)

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ABSTRACT

PCP and acpPC are the two major antennae proteins that bind pigments in peridinin-containing dinoflagellates. The relationship between antennae proteins and cellular pigments at molecular level is still poorly understood. Here we identified and characterized the two antennae protein genes in dinoflagellate *Prorocentrum donghaiense* under different light conditions. The mature PCP protein was 32 kDa, while acpPC was a polyprotein each of 19 kDa. Both genes showed higher expression under low light than under high light, suggesting their possible role in a low light adaptation mechanism. The two genes showed differential diel expression rhythm, with PCP being more highly expressed in the dark than in the light period and acpPC the other way around. HPLC analysis of cellular pigments indicated a diel change of chlorophyll *c2*, but invariability of other pigments. A stable peridinin: chlorophyll *a* pigment ratio was detected under different light intensities and over the diel cycle, although the diadinoxanthin: chlorophyll *a* ratio increased significantly with light intensity. The results suggest that 1) PCP and acpPC genes are functionally distinct, 2) PCP and acpPC can function under low light as an adaptive mechanism in *P. donghaiense*, 3). the ratios of diadinoxanthin:chlorophyll *a* and peridinin: chlorophyll *a* can potentially be used as an indicator of algal photophysiological status and a pigment signature respectively under different light conditions in *P. donghaiense*.

1. Introduction

Dinoflagellates and other phytoplankton live in a variable light environment and during photoacclimation may modulate their pigment abundance and distribution. Cellular concentrations of photosynthetic pigments are expected to correlate with the expression of individual chlorophyll-binding proteins (Iglesias-Prieto and Trench, 1997). Changes in pigments under variable light intensities experienced by dinoflagellates and other phytoplankton are usually associated with antenna remodeling (Peers et al., 2009). Each phytoplankton species has a specific distribution of pigment in its pigment-protein complexes and the distribution is rearranged during photoacclimation (Iglesias-Prieto and Trench, 1997). For example, high light can induce loss of photosynthetic pigments and lead to photobleaching and photoinhibition, as documented in *Symbiodinium* (Takahashi et al., 2008), a genus of dinoflagellates mostly endosymbiotic with corals and other marine invertebrates. The loss of photosynthetic pigments through the loss of antenna protein, particularly acpPC is believed to be responsible for coral bleaching (Takahashi et al., 2008). Thus, understanding the responses of the two antennae proteins to the variable ambient light environment will provide valuable information regarding the functional significance of changes in cellular pigment concentration (Iglesias-Prieto and Trench, 1997).

PCP (peridinin- chlorophyll *a*-binding protein) and acpPC (chlorophyll *a*-chlorophyll *c2*-peridinin-protein) are two major light harvesting components in peridinin-containing (i.e. "typical") dinoflagellates (Michael and Miller, 1998; Takahashi et al., 2008), which form two antennas with associated pigments in the photosynthesis system (Iglesias-Prieto and Trench, 1997; Takahashi et al., 2008). PCP is a water-soluble protein, which only occurs in light harvesting complex in dinoflagellates, usually located in

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the thylakoid membrane (Nassoury et al., 2001) to bind its luminal side and can exist in a short (of 15 kDa weight) or a long form (of 30–35 kDa weight) (Reichman and Vize, 2014). The long form is believed to have evolved from the short form via gene duplication (Hofmann et al., 1996). In *Glenodinium* sp., PCP synthesis is light regulated at the transcriptional level (Roman et al., 1988). In *Lingulodinium polyedrum*, PCP was reported to be regulated at the translational level by circadian signals (Nassoury et al., 2001). The photosynthetic capacity in *L. polyedrum* is maximal in the middle of the day and minimal in the middle of the night (Hollnagel et al., 2002), while the protein abundance is higher in the period from late of the day to the end of the night (Nassoury et al., 2001). In *Prorocentrum donghaiense*, this protein has also been reported to decrease under nitrogen limitation (Zhang et al., 2015). In contrast to PCP's water-soluble nature, acpPC usually is embedded in thylakoid membranes in dinoflagellates with functions similar to chlorophyll *a*/*b*-binding proteins in higher plants (Hiller et al., 1995). The gene usually encodes a polyprotein that can form as many as 10 different tandem repeated mature polypeptides, with ~19 kDa each (Hiller et al., 1995). In *Symbiodinium* spp., it was reported that acpPC was regulated by heat stress at the translational level (Takahashi et al., 2008) and the majority of the photosynthetic pigments were associated with this protein (Iglesias-Prieto and Trench, 1997). In *Amphidinium carterae*, acpPC gene expression was shown to be regulated by light intensity (Michael and Miller, 1998). So far, the relationship between photosynthetic pigments and these antenna proteins in dinoflagellates has been understudied.

P. donghaiense is a typical red tide dinoflagellate species, forming large scale blooms in East China Sea every year (Lu et al., 2005). Previous studies showed that the high turbidity and low light condition were conducive to *P. donghaiense* bloom formation (Sun et al., 2008). Pigments in this species have been used as a biological indicator of cell physiological status (Hou et al., 2007). It is well recognized that the dynamic process of the red tide event formed by *P. donghaiense* can be monitored by satellite based on the specific cellular pigment in this species (Lei et al., 2011). In this study, in order to further understand the functional significance of changes in cellular pigment concentration at the molecular level, the relationship between cellular pigments and gene expression of pigment-binding protein genes at different circadian times and under different light intensities was investigated. HPLC analysis was used to measure cellular contents of pigments over a diel cycle and under light intensities. PCP and acpPC genes were isolated, and their expression profiles in the diel cycle and under different growth light intensities were characterized.

2. Method

2.1. Algal culture and sample collection for gene isolation

P. donghaiense culture was grown in 1-L seawater medium (without silicate) and was treated with a cocktail of antibiotics including ampicillin (200 mg/L), kanamycin (100 mg/L) and streptomycin (100 mg/L) to minimize bacterial presence (Lin et al., 2015). The culture was kept at 20 ± 1 °C under a 14:10 h light dark cycle (LD) at a photon flux of 100 μmol photons m^{-2} s^{-1}. Cell concentration was monitored using a Sedgwick-Rafter counting chamber under a microscope. Exponential phase cells (~10^7 cells per sample) were harvested by centrifugation at 3000×*g* under 20 °C for 10 min. For RNA isolation, the cell pellet was resuspended in 1 mL TRIzol Reagent (Invitrogen, Carlsbad, CA), mixed thoroughly by vortex and stored at −80 °C for subsequent RNA extraction.

2.2. Diel sample collection for pigment and gene expression analyses

A master culture was first synchronized as previously reported (Shi et al., 2013). The synchronized culture was then transferred into 7.5-L L1 medium in triplicate. The culture condition was the same as described above. When the cultures entered the early exponential phase (3 days after transfer), a sample was taken from each of the triplicate cultures every 2 h for a 24 h period. At each time point, 25 mL and 300 mL samples were harvested for pigments analysis and RNA extraction, respectively. Pigment samples were filtered onto Whatman GF/F membrane of 25 mm diameter with 0.7-μm pore size under a gentle vacuum (<150 mmHg). The membrane was folded and wrapped with aluminum foil and frozen in liquid nitrogen. The frozen sample was stored at −80 °C for subsequent pigment extraction. The RNA samples were collected using centrifugation. The cell pellets were suspended in 1 mL TRIzol Reagent, mixed thoroughly, and stored at −80 °C until RNA extraction.

2.3. Light manipulation to study responses of PCP expression, pigment content, and photochemical efficiency in P. donghaiense

The synchronized culture was transferred into 1-L L1 medium in 9 bottles under 100 μmol photons m^{-2} s^{-1} to be set up in triplicate for each of the three light conditions used in the experiment. When the culture entered into exponential phase, each set of triplicate cultures was transferred to a different light density, 20 μmol photons m^{-2} s^{-1}, 100 μmol photons m^{-2} s^{-1} and 600 μmol photons m^{-2} s^{-1}, respectively. All the experiment was conducted under a 14:10 h LD light cycle. The light density experiment was started at middle light phase. Samples were collected in 0 h, 24 h and 48 h after transfer to respective light densities. Cell concentration was monitored as described above every day. Growth rate was calculated by [ln(N2)–ln(N1)]/(t2–t1), where N2 and N1 are cell concentrations at time t2 and t1, respectively. After 24 and 48 h treatment, 25 mL and 300 mL samples were harvested at the same time point of the day for pigments analysis and RNA extraction as described above, respectively in each time point. The maximum quantum efficiency of PSII photochemistry Fv/Fm = (Fm–Fo)/Fm was measured using FIRe fluorometer system (Satlantic, Halifax, NS, Canada) as described previously (Cui et al., 2017).

2.4. Pigment analysis

The frozen filter was treated with freeze drier under −80 °C for 12 h. The dried filter was soaked in 2 mL N, N-dimethylformamide and extracted in a freezer (−20 °C) for 2 h (Furuya et al., 1998). The extractions were then filtered using Whatman GF/F filters of 13 mm diameter with 0.7 um pore size (Swinnex Filter Holder) to remove cell debris and then mixed with the same volume of ammonium acetate solution (1 M). An aliquot of the extracted mixture was partially injected into an Agilent HPLC system equipped with a 3.5 μm Eclipse XDB C8 column (100 × 4.6 mm; Agilent Technologies). The HPLC equipment was consisted of a Shimadzu LC-20A pump with a low-pressure gradient unit FCV-20AL, an on-line degasser DGU-3A, and a photodiode array UV–vis detector SPD-M20AV with wavelength resolution in 1.2 nm size. The mobile phase were A (methanol: 1 M ammonium acetate, 80:20) and B (methanol). The LC gradients were (min, solvent A%, solvent B%): (0, 100, 0), (16, 45, 55), (27, 0, 100), (32, 0, 100), (40, 100, 0) and the flow rate was maintained at 1 mL min^{-1}. The specific peak of each pigment was identified based on their retention time and absorption spectrogram compared with those of pure standards purchased from Danish Hydraulic Institute (DHI) Water and