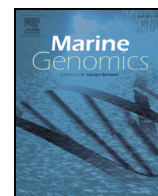




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Marine Genomics



The upstream regulatory sequence of the light harvesting complex *Lhc2* gene of the marine diatom *Phaeodactylum tricornutum* enhances transcription in an orientation- and distance-independent fashion

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ABSTRACT

Diatoms are a key phytoplankton group in the contemporary ocean, showing extraordinary adaptation capacities to rapidly changing environments. The recent availability of whole genome sequences from representative species has revealed distinct features in their genomes, like novel combinations of genes encoding distinct metabolisms and a significant number of diatom-specific genes. However, the regulatory mechanisms driving diatom gene expression are still largely uncharacterized. Considering the wide variety of fields of study orbiting diatoms, ranging from ecology, evolutionary biology to biotechnology, it is thus essential to increase our understanding of fundamental gene regulatory processes such as transcriptional regulation. To this aim, we explored the functional properties of the 5'-flanking region of the *Phaeodactylum tricornutum Lhc2* gene, encoding a member of the Light Harvesting Complex superfamily and we showed that this region enhances transcription of a GUS reporter gene in an orientation- and distance-independent fashion. This represents the first example of a cis-regulatory sequence with enhancer-like features discovered in diatoms and it is instrumental for the generation of novel genetic tools and diatom exploitation in different areas of study.

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1. Introduction

Marine diatoms are unicellular algae of great interest in a wide variety of fields of study ranging from ecology, evolutionary biology to biotechnology. Contributing to at least 20% of the global CO₂ assimilation and to the biogeochemical cycling of important nutrients, such as carbon, nitrogen and silicon (Smetacek, 1999), diatoms play fundamental roles in the ocean and are at the base of the marine food chain. Because of their origin from secondary endosymbiosis, diatoms occupy a peculiar position in the eukaryotic tree of life, they are distant from animals and plants and show peculiar metabolic features (Armburst, 2009). Moreover, these organisms have been recently recognized as an important source of biofuel, food products and pharmaceutically active substances (Bozarth et al., 2009; Levitan et al., 2014). For all these reasons, significant efforts have been devoted during the past years to

the development of molecular based techniques and genome-enabled resources in diatoms. In particular, genetic transformation has been set up for several diatom species (Dunahay et al., 1995; Apt et al., 1996; Falciatore et al., 1999; Fischer et al., 1999; Poulsen and Kröger, 2005; Poulsen et al., 2006; Miyagawa-Yamaguchi et al., 2011; Muto et al., 2013; Miyahara et al., 2013; Ifuku et al., 2015; Karas et al., 2015; Sabatino et al., 2015), and novel tools to modulate gene expression, like overexpression and gene silencing, have been developed for the molecular model species *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* (Siaut et al., 2007; De Riso et al., 2009; Bertrand et al., 2012). Targeted mutagenesis induced by both meganucleases and TALE nucleases has also been shown in *P. tricornutum* (Daboussi et al., 2014; Weyman et al., 2014). Moreover, several genomes from representative species have already been sequenced (Armburst et al., 2004; Bowler et al., 2008; Lommer et al., 2012; Tanaka et al., 2015; <http://genome.jgi.doe.gov/bacillariophyta/bacillariophyta.info.html>), and many others will be available in the close future. Finally, a growing flux of genome-based information is also arising from the Marine Microbial Eukaryote Transcriptome Sequencing Project (<http://marinemicroeukaryotes.org/>) and from metagenomic data (Keeling et al., 2014; Toseland et al., 2014). On one hand, these resources are providing an unprecedented opportunity to explore diatom biology;

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