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Physiological and Molecular Evidence that Environmental Changes Elicit Morphological Interconversion in the Model Diatom *Phaeodactylum tricornutum*

Alessandra De Martino[a,1], Ana Bartual[b], Anusuya Willis[a,2], Agnes Meichenin[a], Beatriz Villazán[b], Uma Maheswari[a,3], and Chris Bowler[a,4]

[a]Institut de Biologie de l'Ecole Normale Supérieure (IBENS), CNRS UMR8197 INSERM U1024, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05, France
[b]Dpto. Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Avda República Saharaui s/n. 11510, Puerto Real, Cádiz, Spain

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Over the last decades *Phaeodactylum tricornutum* has become a model to study diatom biology at the molecular level. Cells have the peculiarity to be pleiomorphic and it is thought that this character is triggered by culture conditions, although few quantitative studies have been performed and nothing is known at the molecular level. Our aim was to quantify the effect of growth conditions on cell morphology of different *P. tricornutum* strains by quantitative microscopy, cellular imaging, and non-targeted transcriptomics. We show that morphotype changes can be regulated by changing culture conditions, depending on the strain, and show a common trend of increased oval cell abundance as a response to stress. Examination of expressed sequence tags (ESTs) from triradiate cells infers the importance of osmoregulation in the maintenance of this morphotype, whereas ESTs derived from oval cells grown in hyposaline and low temperature conditions show a predominance of genes encoding typical components of stress pathways, especially in signaling, cell homeostasis and lipid metabolism. This work contributes to better understand the importance of the unique capability of morphotype conversion in *P. tricornutum* and its relevance in acclimation to changing environmental conditions.

Key words: Diatoms; life cycle; morphotypes; phenotypic plasticity; stress acclimation; time-lapse microscopy.

Introduction

Diatoms are important contributors to the carbon cycle, generating approximately 20% of the organic carbon that is produced each year by photosynthesis. *Phaeodactylum tricornutum* has been used as a model over several decades to explore diatom physiology, and more recently molecular tools have been developed for reverse genetics and cell biology (De Martino et al. 2009; De Riso et al. 2009;

[1]Present address: Algenics™, Pole BioOuest, rue du Moulin de la Rousselière, 44800 St Herblain, France.
[2]Present address: School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA
[3]Present address: EMBL—European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, United Kingdom
[4]Corresponding author; fax +33 1 44 32 39 35
e-mail cbowler@biologie.ens.fr (C. Bowler).

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Falciatore et al. 1999; Siaut et al. 2007). The *P. tricornutum* genome has been recently sequenced (Bowler et al. 2008) and more than 130 000 Expressed Sequenced Tags (ESTs) derived from cells grown in 16 different conditions have been generated (Maheswari et al. 2009, 2010). These resources open up new possibilities to assess gene function in diatoms, in order to understand more about their biology and their ecological roles in the oceans and other aquatic environments.

We have recently investigated the inferred genetic and phenotypic characteristics of ten different strains of *P. tricornutum* collected from different locations worldwide, and that show morphological and physiological variability (De Martino et al. 2007). In nature, this diatom is not highly abundant but it appears to be a cosmopolitan, coastal species found principally in unstable environments such as estuaries and rock pools (De Martino et al. 2007). In these environments, salinity and temperature can rapidly change as a consequence of tidal effects, solar irradiation and human activities (Howland et al. 2000). The ability to acclimate to such fluctuating environmental conditions could be related to the pleiomorphic character of *Phaeodactylum* cells because the organism can typically be found as one of three morphotypes: fusiform, oval, or triradiate. A fourth 'round' morphotype was also proposed more recently, as a possible resting-cell (De Martino et al. 2007). The unique morphological plasticity of this diatom is possible because its organic cell frustule is only facultatively silicified (Borowitzka and Volcani 1978; De Martino et al. 2007). In fact, the oval cell is the only morphotype that forms a single silica frustule, embedded in an organic casing, which contains a raphe and allows the gliding motility typical of raphid diatoms (Borowitzka and Volcani 1978; Tesson et al. 2009; Wetherbee et al. 1998).

The different forms of *P. tricornutum* were initially described by Wilson (1946), and electron micrographs and ultrastructure of each morphotype have been described previously to some extent (Borowitzka and Volcani 1978; Lewin et al. 1958; Tesson et al. 2009). Transformation from one morphotype into another was shown to occur in laboratory cultures (Borowitzka and Volcani 1978; Lewin et al. 1958; Wilson 1946), and modalities of morphotype transformation, passing through intermediate shapes, have been proposed based on microscopic observations (Borowitzka and Volcani 1978; Gutenbrunner et al. 1994; Lewin et al. 1958; Tesson et al. 2009; Wilson 1946). Transformation of cells into the oval morphotype generally occurs in non-shaken cultures, on solid media, and in stationary phase cultures (Cooksey and Cooksey 1974; Gutenbrunner et al. 1994). It has been postulated that shape changes in *P. tricornutum* may represent a response to changes in environmental conditions (Borowitzka and Volcani 1978; De Martino et al. 2007; Wilson 1946), and differential fitness of morphotypes have been proposed (Bartual et al. 2008). Notwithstanding, quantitative studies are lacking, and it has been notoriously difficult to maintain triradiate cells in culture (Lewin et al. 1958).

Our aim here was to reinvestigate the pleiomorphic character of *P. tricornutum* in response to different culture conditions using quantitative and molecular approaches. Our studies have been further potentiated with respect to previous work by the use of different *P. tricornutum* strains that display different morphotypes and have differential sensitivities to temperature and salinity (De Martino et al. 2007), two parameters that vary considerably in the estuaries and rock pools where this diatom has been found.

On the basis of a previous detailed description of different strains (De Martino et al. 2007) we selected the most representative to study responses to changing culture conditions. First, to study cold stress we selected a 'tropical strain' (Pt9) that displays a majority fusiform morphotype at 25-28 °C in 100% seawater (denoted here $Pt9_{F28\,^{\circ}C,100sw}$) but a majority oval morphotype at 19 °C ($Pt9_{O28\,^{\circ}C,100sw}$) (De Martino et al. 2007). Second, an 'oval strain' (Pt3) characterised by a majority of oval cells at 19 °C in 100% seawater ($Pt3_{O19\,^{\circ}C,100sw}$) (De Martino et al. 2007) was used to study the effects of both hyposalinity and cold stress on the oval morphotype. Third, a strain that displays a majority triradiate morphotype at 19 °C in 100% seawater ($Pt8_{T19\,C,100sw}$) was used to study cold and hyposalinity stresses on the triradiate morphotype. Finally, we chose the genomic strain Pt1 8.6_F, used previously for whole genome sequencing (Bowler et al. 2008; De Martino et al. 2007), as a reference and representative of the fusiform morphotype. These cultures display primarily fusiform cells at 19 °C in 100% seawater (De Martino et al. 2007), and were used as a comparison for the temperature and hyposalinity stress experiments.

In the current report, quantitative studies of morphotype abundance during acclimation processes to cold temperature and hyposalinity have been explored, and a time-lapse imaging approach has been developed to study the dynamics of morphotype division and conversion. In addition, we have generated EST libraries to assess differential gene expression in triradiate and oval cells, particularly in low salinity and cold stress conditions. This work contributes to our knowledge of differential gene