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Characterization of a Modular, Cell-Surface Protein and Identification of a New Gene Family in the Diatom *Thalassiosira pseudonana*

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We report the characterization of a cell-surface protein isolated from copper-stressed cells of the centric diatom *Thalassiosira pseudonana* Hasle and Heimdal (CCMP 1335). This protein has an apparent molecular weight of 100 kDa and is highly acidic. The 100 kDa protein (p100) sequence is comprised almost entirely of a novel domain termed TpRCR for *T. pseudonana* repetitive cysteine-rich domain, that is repeated 8 times and that contains conserved aromatic, acidic, and potential metal-binding amino acids. The analysis of the *T. pseudonana* genome suggests that p100 belongs to a large family of modular proteins that consist of a variable number of TpRCR domain repeats. Based on cell surface biotinylation and antibody data, p100 appears to migrate more rapidly with SDS—PAGE when extracted from cells exposed to high levels of copper; however, the discovery of a large family of TpRCR domain-containing proteins leaves open the possibility that the antibody may be cross-reacting with members of this protein family that are responding differently to copper. The response of the gene encoding p100 at the mRNA level during synchronized progression through the normal cell cycle is similar to previously characterized genes in *T. pseudonana* encoding cell wall proteins called silaffins.

Key words: Bacillariophyta; cell cycle; cell-surface; copper; diatom; stress.

Introduction

Diatoms are found throughout the world in both freshwater and marine environments, and are dominant members of coastal ecosystems. These unicellular, eukaryotic microorganisms are typically photosynthetic (Round et al. 1990) and are important contributors to global carbon fixation (Nelson et al. 1995; Werner 1977). A notable feature of diatoms is that they utilize silicic acid from the environment to create shells made of silica (reviewed in Davis and Hildebrand 2007) and are dominant contributors to biosilicification (Tréguer et al. 1995). The silica shell, termed frustule, is comprised of silicified components that collectively encase the cell. The two halves of the

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Abbreviations: ASW, artificial sea water; RACE, rapid amplification of cDNA ends; SBH, succinimidyl 6-(biotinamido) hexanoate; TpRCR domain, *T. pseudonana* repetitive cysteine rich domain

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frustule fit together in a Petri dish-like manner, and each half consists of a valve and silicified strips, called girdle bands, that hold the two halves together. Frustule morphology is species-specific and genetically encoded, being reproduced from generation to generation with fidelity, but it is highly variable among the estimated tens of thousands of diatom species. Diatoms can be classified into general morphological groups that display either bilateral or radial symmetry and are referred to as pennate or centric diatoms, respectively, although this morphological distinction is thought to mask evolutionary complexity (Medlin and Kaczmarska 2004).

Since silica cell wall formation is a major characteristic of diatoms, the protein composition of the cell wall is obviously of great interest. *Thalassiosira pseudonana* (Armbrust et al. 2004) and subsequently *Phaeodactylum tricornutum* are the first two diatoms to have their genomes sequenced and made publicly available (www.jgi.doe.gov/) facilitating investigations into the cell wall protein complement of diatoms and the genes that encode these proteins. Three families of cell wall proteins, frustulins (Kröger et al. 1994, 1996), pleuralins (Kröger et al. 1997; Kröger and Wetherbee 2000), and silaffins (Kröger et al. 1999), were originally identified in the pennate diatom *Cylindrotheca fusiformis*. However, silaffins have recently been characterized from *T. pseudonana* (Poulsen and Kröger 2004) and frustulins have been identified in the *T. pseudonana* (Armbrust et al. 2004) and *P. tricornutum* genomes. Recently, mRNA levels corresponding to two of the silaffin genes, *SIL1* and *SIL3*, in *T. pseudonana* during synchronized cell cycle progression were examined; silaffin mRNA levels revealed peaks of induction during girdle band and valve synthesis resulting in their characterization as marker genes for cell wall synthesis and against which mRNA levels of other genes suspected of being involved in cell wall synthesis could be compared (Frigeri et al. 2006).

The cell-surface protein complement of diatoms changes with exposure to environmental stress (Davis et al. 2005). A survey of copper-induced changes in the cell-surface protein complement of *T. pseudonana* was performed. Cell-surface proteins of copper-exposed and control cells were biotinylated, extracted, and compared through western blot analysis. Three proteins were different from those observed in control cells. These proteins had apparent molecular weights of 100, 130, and 150 kDa by SDS-PAGE and are referred to as p100, p130, and p150, respectively. The characterization of p150 has been reported (Davis et al. 2005). The p150 is a girdle band protein that is also more highly expressed during silica depletion and iron depletion in addition to copper excess. Here we report the characterization of p100.

Results

Copper Concentration and Cell Growth

We examined the response of *T. pseudonana* cells to elevated levels of Cu. Based solely on inorganic speciation, the free copper concentration was estimated to be $pCu = 7.82$ in amended cultures, however this number likely represents an overestimate (See Davis et al. 2005). In a representative experiment, Cu was added to a culture of *T. pseudonana* at a cell density of $\sim 1.8 \times 10^5$ cells mL^{-1}, and this density did not significantly change after a 24 h incubation (1.4×10^5 cells mL^{-1}) indicating that cell division had arrested. In contrast, cell division continued in the control culture (3.3×10^5 cells mL^{-1}) during a 24 h incubation.

Identification and Purification of Copper-Induced Cell-Surface Proteins

Using SBH to biotinylate cell-surface proteins, a protein with an apparent molecular weight of 100 kDa, referred to as p100 for convenience, was uniquely identified in copper exposed *T. pseudonana* cells. This protein, which was followed by SBH-labeling, was greatly enriched in SDS-extracted fractions, which contained cell wall material, and was solubilized by 8 M urea (Fig. 1A). This protein does not represent a general stress response as it was not induced by phosphate or nitrate limitation or by exposure to other metals such as cadmium or zinc (Davis et al. 2005). p100 was consistently smaller than a protein of similar size present in control conditions (Fig. 1A). Based on SBH labeling it could not be determined if p100 represented a uniquely expressed protein, or the same protein displaying increased electrophoretic mobility during SDS—PAGE. p100 consistently displayed faint staining with periodic acid-Schiff (data not shown) suggesting the presence of covalently attached carbohydrate residues.

Polyclonal antibodies were originally made in chickens against the native p100 extracted from *T. pseudonana*. This antibody was not specific,