

Functional characterization of the alpha glucosidase II (GSII) in the green microalga *Chlamydomonas reinhardtii*

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The *N*-glycosylation pathway of proteins is one of the most important co- and post-translational modification of proteins in Eukaryotes¹. Glycoproteins are produced firstly in the endoplasmic reticulum (ER) where molecular actors (such as glucosidases I and II, lectins) are responsible for the proper folding and 3D conformation of proteins before their transfer into the Golgi apparatus². In a context of bioproduction of therapeutic recombinant glycoproteins, new cheaper expression systems emerged, such as microalgae^{3,4}. The growing interest of using *C. reinhardtii*, a green microalga, as an emerging cell factory for the industrial production of recombinant glycoproteins require overall an in-depth understanding and analysis of protein *N*-glycosylation in this organism. Despite a relatively well-investigated *N*-glycosylation pathway in *C. reinhardtii*^{5,6,7,8,9}, many glyco-enzymes involved in the *N*-glycosylation pathway in the ER are still not characterized. In this work, functional characterization of the putative alpha-glucosidase II (GSII) was conducted using combinatory approaches on *C. reinhardtii* insertional mutant cells in order to evaluate the impact of the *GSII* gene deletion compared to the WT cells and to give new insights on the ER *N*-glycosylation pathway in this microalga. Accumulation of free oligosaccharides (fOSs) and *N*-glycans such as Glc₂Man₅GlcNAc₂ are observed in the GSII mutant cells. Moreover, an over-expression of both Cal and PDI genes, respectively involved in the quality control of misfolded proteins and UPR pathway, was observed at 6 days of culture growth in addition to an accumulation of reactive oxygen species (ROS), suggesting a physiological stress in the microalgae mutant cells.

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