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Understanding the glycosylation pathway and increasing the production yield of therapeutic proteins in plant and microalgae cells

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The *N*-glycosylation pathways of glycoproteins are crucial co- and post-translational modifications in eukaryotic cells, impacting the physiochemical properties and biological functions of proteins (Toustou et al. 2022). Glycoproteins are initially synthesized in the endoplasmic reticulum (ER), where chaperones like calreticulin (CRT) and calnexin (CNX) are responsible for proper folding and 3D conformation before transit to the Golgi apparatus (Aebi 2013). In a context of production of therapeutic glycoproteins also called biologics, new expression systems emerged such as microalgae and plant cells to offer an alternative to the current Chinese Hamster Ovary cells (Herman et al. 2021). This PhD project has two objectives: i) the functional characterization of the CRT and CNX in microalgae and plant cells to give new insights on the ER *N*-glycosylation pathway and ii) the overexpression of CRT and CNX in both microalgae and plant cells in order to enhance the production yield of recombinant glycoproteins. This research involves working with insertional mutants lacking functional CRT and/or CNX generated using CRISPR/Cas9 technology in both models. Analysis of mutant cells include molecular characterization like gene expression using RT-qPCR and phenotypical characterization such as cell motility evaluation, growth kinetics, starch and chlorophyll content as well as structural analysis of *N*-glycoproteins using mass spectrometry. Additionally, the overexpression of CRT and CNX is conducted in this project on strains producing a glycosylated fluorescent protein. The objective is to evaluate the impact of this overexpression on the yield and quality of the recombinant glycoprotein.

Aebi, Markus. 2013. « N-Linked Protein Glycosylation in the ER ». *Biochimica Et Biophysica Acta* 1833 (11): 2430-37. <https://doi.org/10.1016/j.bbamcr.2013.04.001>.

Herman, Xavier, Johann Far, Adeline Courtoy, Laurent Bouhon, Loïc Quinton, Edwin De Pauw, François Chaumont, et Catherine Navarre. 2021. « Inactivation of N-Acetylglucosaminyltransferase I and α 1,3-Fucosyltransferase Genes in *Nicotiana tabacum* BY-2 Cells Results in Glycoproteins With Highly Homogeneous, High-Mannose N-Glycans ». *Frontiers in Plant Science* 12. <https://www.frontiersin.org/articles/10.3389/fpls.2021.634023>.

Toustou, Charlotte, Marie-Laure Walet-Balieu, Marie-Christine Kiefer-Meyer, Marine Houdou, Patrice Lerouge, François Foulquier, et Muriel Bardor. 2022. « Towards Understanding the Extensive Diversity of Protein *N* -glycan Structures in Eukaryotes ». *Biological Reviews* 97 (2): 732-48. <https://doi.org/10.1111/brv.12820>.

Key Words: *N*-glycosylation; calreticulin; calnexin; insertional mutants; Crispr/Cas9; endoplasmic reticulum, *C. reinhardtii*, *N. tabacum*