Towards Humanizing the N-glycosylation of Antibodies Produced in Phaeodactylum tricornutum through PtTMEM165

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Résumé

Microalgae are emerging as promising cell factories for producing complex recombinant proteins, such as monoclonal antibodies. However, optimizing the effector functions of antibodies produced by these microalgae for therapeutic applications underscores the need for optimizing the N-glycosylation pathway. In this context, the present study focuses on investigating N-glycosylation in Phaeodactylum tricornutum to produce antibodies with a human-like glycosylation pattern.

Recent insights into mammalian cells reveal that the biosynthesis of complex-type N-glycans is regulated by manganese ion homeostasis, with the involvement of the transmembrane protein TMEM165. It has been demonstrated that defects in this protein led to Golgi glycosylation abnormalities, notably hypogalactosylation and hyposialylation.

Recently, we identified an ortholog of TMEM165 named PtTMEM165 in P. tricornutum, strongly suggesting its potential role in regulating Golgi N-glycosylation. To assess PtTMEM165's function and role, we conducted heterologous complementation in HEK KO TMEM165 deficient cell line, generated TMEM165-deficient P. tricornutum lines using CRISPR-Cas9, and overexpressed PtTMEM165 in wild-type P. tricornutum cells. Such experiments allowed us to demonstrate 1-that PtTMEM165 is able to restore a normal glycosylation profile in HEK cells and 2-the overexpression of PtTMEM165 facilitated the synthesis of N-glycans containing N-acetylglucosamine residues at the terminal position. Additionally, subcellular localization studies in HEK cells revealed that PtTMEM165 predominantly localizes to the endoplasmic reticulum. The phenotypic alterations of P. tricornutum cells lacking TMEM165 activity are under studied.

In conclusion, this study advances our understanding of PtTMEM165's role in P. tricornutum N-glycosylation and highlights its potential for engineering humanized glycosylation pathways to produce antibodies.

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Mots-Clés: Phaeodactylum tricornutum, N.glycosylation, PtTMEM165, Humanization, N.acetylglucosamine, Antibodies