Glycoengineering of Yeast Cells for Humanized *N*-glycans

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Due to its capacity to glycosylate proteins, yeasts have been considered for the production of glycoproteins derived from higher eukaryotes. However, yeast *N*-glycosylation pathway is only partially homologous to the pathway in human cells. Here we report the remodeling of glycosylation pathway for biosynthesis of humanized *N*-glycans in the methylotrophic yeast *Hansenula polymorpham*, which is rapidly gaining favor as a promising host for the production of recombinant proteins (1). Especially, *H. polymorpha* shows some advantages over the traditional yeast *Saccharomyces cerevisiae* in the production of recombinant glycoproteins for human therapeutic use. Most *N*-linked oligosaccharide species attached to *H. polymorpha*-derived glycoproteins have core-sized structures ($Man_{8-12}GlcNAc_2$) without highly immunogenic terminal α-1,3-linked mannose residues. Moreover, the outer chains of *H. polymorpha N*-linked glycans were shown to be extended mostly in 1,2-mannose linkages and to have very short 1,6 extensions, mainly composed of single α-1,6-linked mannose (2). As a first step toward humanizing *H. polymorpha N*-glycosylation pathway, the *H. polymorpha och2* mutant strain having a defect in the outer chain initiation on the core oligosaccharide $Man_8GlcNAc_2$ was developed and further engineered with the targeted expression of *Aspergillus saitoi* α-1,2-mannosidase in the ER. The engineered *H. polymorpha och2* strain was shown to produce the human high mannose-type $Man_5GlcNAc_2$ oligosaccharide as a major *N*-glycan (3). As an alternative approach, the remodeling of core oligosaccharide assembly pathway was carried out with additional deletion of theH. polymorpha ALG3 gene (HpALG3), encoding Dol-P-Man:-$Man_5GlcNAc_2$-PP-Dol mannosyltransferase. The engineered double deletion (alg3och2) mutant strain expressing *A. saitoi* α-1,2-mannosidase generated mainly the trimannosyl-core form glycan ($Man_3GlcNAc_2$), which is an intermediate for further maturation to hu-

man-like complex *N*-glycans. These results demonstrate the potential of *H. polymorpha* to be developed as a host for the production of therapeutic glycoproteins with homogeneous complex *N*-glycan structures.

References

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