Production of humanized IgG in *Saccharomyces cerevisiae*

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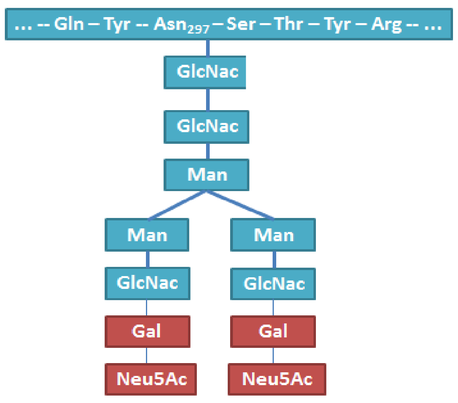
Introduction

The N-glycosylation of antibodies for their use as drugs

Glycosylation of antibodies is something essential to avoid the development of an **immune response**. Moreover, it is expected that more than a 30% of the licensed drugs during the next decade will be antibodies, so the reduction of their production costs is crucial in a competitive market.

IgG are N-glycosylated at the position Asn_{297}. The oligosaccharide always contains, at least, the blue heptasaccharide, but further modifications could take place to change the antibody activity. **Terminal sialic** acids have shown increased **ADCC** (antibody dependent cellular cytotoxicity) and, for this reason, they are an interesting option for the synthesis of drugs against **autoimmune diseases and inflammatory disorders**.



Current antibodies production methods

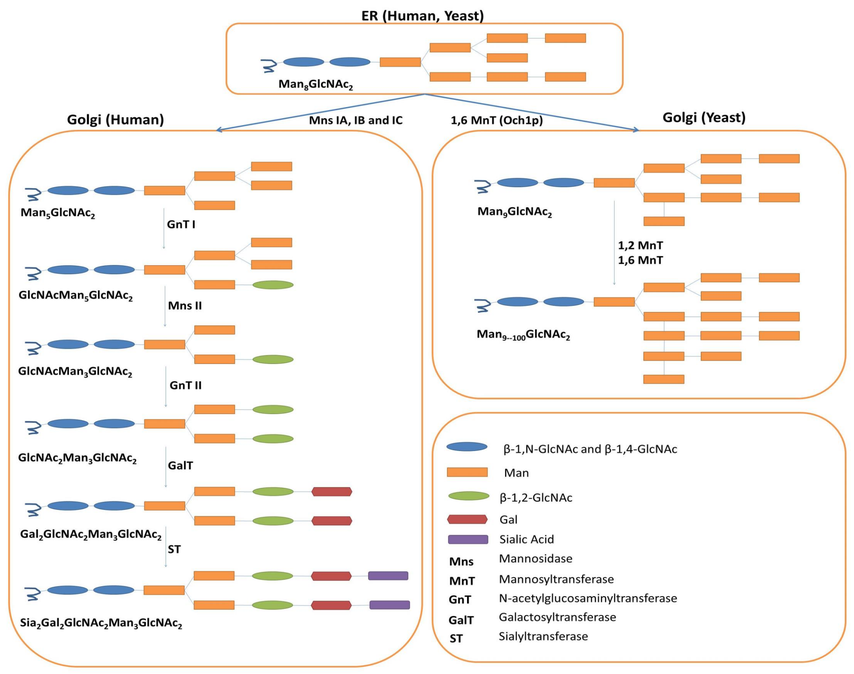
1. **Engineered CHO cells**: They have incorporated the genes of the human N-acetylglucosaminyltransferase-III and alpha-2,6-sialyltransferase-I .
2. ***Picchia pastoris***: Glycofi (Lebanon, USA) obtained a humanized strain of *P. pastoris* which produced glycoproteins with the N-glycosylation of humans using synthetic biology tools.

Goals

1. To obtain a recombinant strain of *S. cerevisiae* that produces humanized N-glycosylated proteins.
2. To compare the results between the strain obtained by directed genetic engineering and the strain obtained by synthetic biology.
3. To discuss which improvements could be made to obtain a better humanized strain of *S. cerevisiae*.

Directed genetic engineering

Endogenous N-glycosyaltion pathway in humans and yeasts

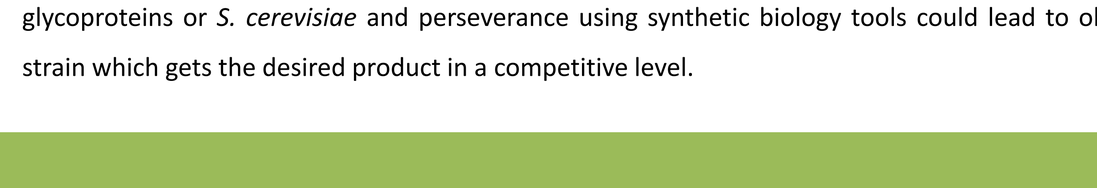


Genetic engineering

An strain producing glycoproteins with $\mathbf{Man_5GluNAc_2}$ was achieved. The process consisted of two steps:

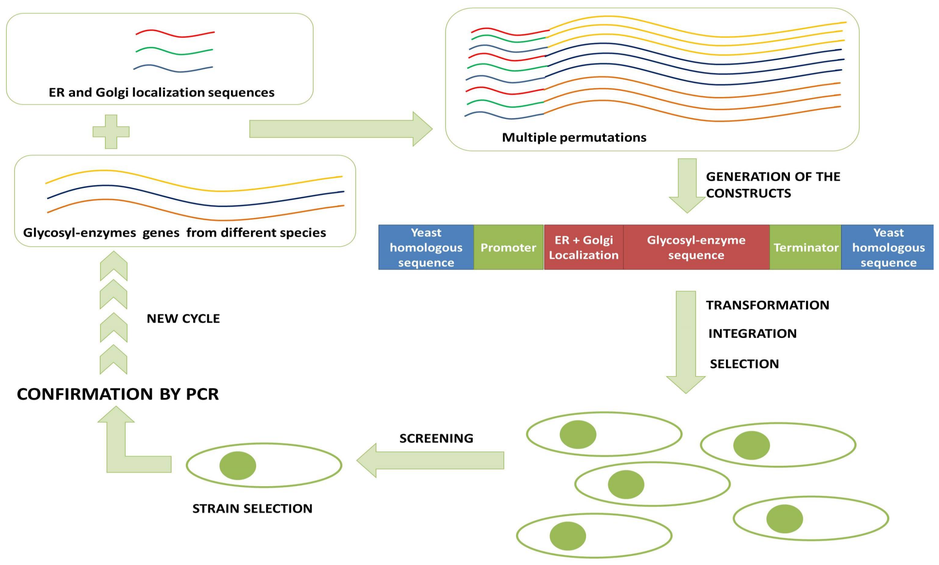
1. Those genes of the yeast implied in the hyper-mannosylation of the glycoprotein in the Golgi were deleted (***ΔOCH1, ΔMNN1***). As a result, the yeast could not modify $\mathbf{Man_8GluNAc_2}$.
2. The gene **α-1,2-mannosidase** of *Aspergillus saitoi* was added with a plasmid. This enzyme hydrolyzes the mannoses of $Man_8GluNAc_2$. Its sequence was fused to **HDEL** sequence for the retention of the enzyme into the endoplasmic reticulum (**ER**).

Result: Heterogeneous product and growth deficiencies.



Synthetic biology

Glycode SAS (Uzerche, France) developed a recombinant strain of *S. cerevisiae* which could produce the oligosaccharide of interest with more than a **75% of homogeneity**. It consisted in using **integrative vectors** which were specifically integrated into **auxotrophic cassettes** of the genome of *S. cerevisiae.* It was a step by step optimization process which was based on a **high throughput screening** of the results obtained with multiple combinations of ER and Golgi localization sequences and different glycosyl-enzymes genes. The screening consisted of two steps: Production of an heterologous protein in a microfermenter and MALDI-TOF analysis.



Each strain had a different name and it was obtained after the performance of one cycle as it is shown in the figure above.

Strain's name	Gene	Source of the gene	Promoter	Product
Amélie	α-1,2-mannosidase I	C. elegans	pGAP	$Man_5GlcNAc_2$
Arielle	N-acetylglucosaminyltransferase I	Human	pGAP	$GlcNacMan_5GlcNAc_2$
Anaïs	Mannosidase II	Mice	PGK	$GlcNacMan_3GlcNAc_2$
Alice	N-acetylglucosaminyltransferase II	Human	PMAI	$GlcNac_2Man_3GlcNAc_2$
Athena	B-1,4-galactosyltransferase I	Human	CaMV	$Gal_2GlcNac_2Man_3GlcNAc_2$
Aeron	α-2,3-sialiltransferase	Human	SV40	$Sia_2Gal_2GlcNAc_2Man_3GlcNAc_2$

Conclusions

1. The strain obtained using directed genetic engineering achieved just the first steps of a modified N-glycosylation pathway. Moreover, the product was heterogeneous and the growth of the recombinant strain was diminished.
2. The recombinant strain obtained using the tools of synthetic biology achieved the desired product with higher homogeneity (more than a 75%). However, the titers of humanized IgG achieved with this cell culture have not been reported and Glycode SAS is no longer active.
3. Research has focused on improving the mainstream methods for the production of humanized antibodies (improved CHO cells).
4. The knowledge of other N-glycosylation pathways and the implications of the modification of the endogenous glycoproteins or *S. cerevisiae* and perseverance using synthetic biology tools could lead to obtaining a recombinant strain which gets the desired product in a competitive level.

References

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