# USE OF SYNTHETIC BIOLOGY TO HUMANIZE THE YEAST *PICHIA PASTORIS* APPLIED IN MONOCLONAL ANTIBODIES PRODUCTION

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## OBJECTIVES

- obtaining a humanized *Pichia pastoris* strain able to produce N-gycosilated proteins with proper folding, pharmacokinetic stability, and efficacy.
- application in the production of monoclonal antibodies.

## INTRODUCTION

Therapeutic proteins are the fastest-growing source of new therapies in the biopharmaceutical industry resulting in a demand for more efficient protein expression systems.

Yeast-based expression systems offer a variety of advantages: shorter fermentation times, cheaper operating costs, chemically defined media, no viral contamination, higher protein titers, and much shorter development times from gene to protein.

However, for the production of glycoproteins for human use, native highmannose yeast glycosylation is not suitable and therefore represents a major limitation for yeast based protein expression systems.

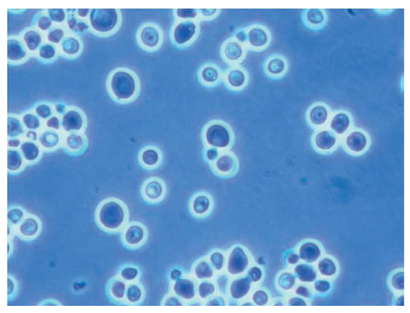

***Fig.1.*** *Pichia pastoris* yeast cells. [1]

## N-GLYCOSYLATION IN YEAST AND HUMAN

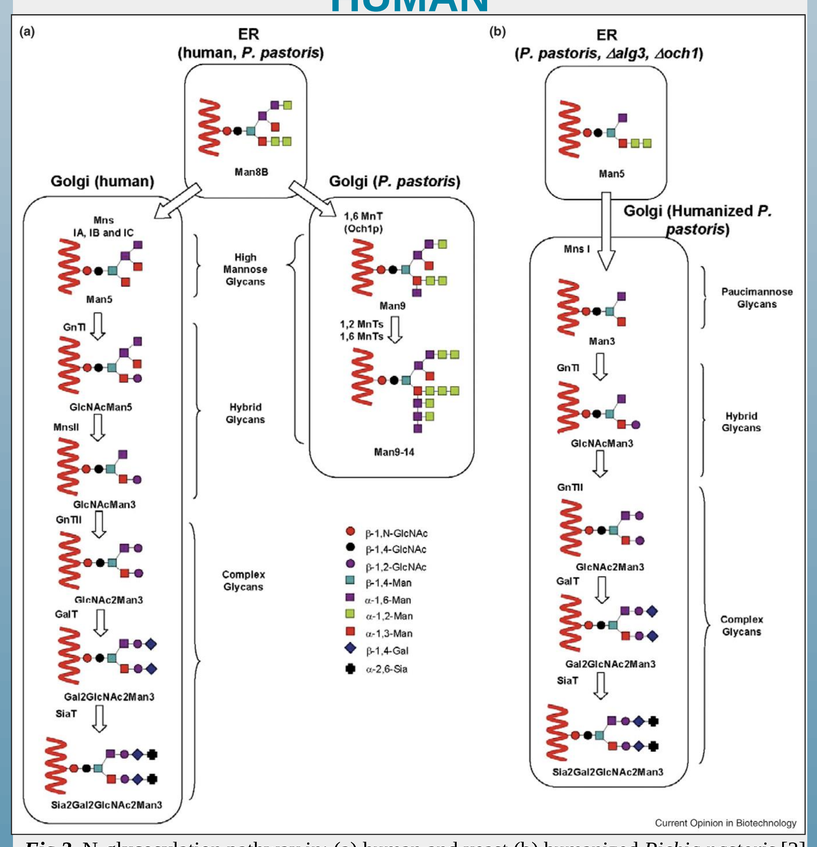


***Fig.2***. N-glycosylation pathway in: (a) human and yeast (b) humanized *Pichia pastoris* [2]

## HUMANIZING GLYCOSYLATION PATHWAY IN *PICHIA PASTORIS*

### EARLY APPROACHES

Creation of ***och1*** **knockout strain**

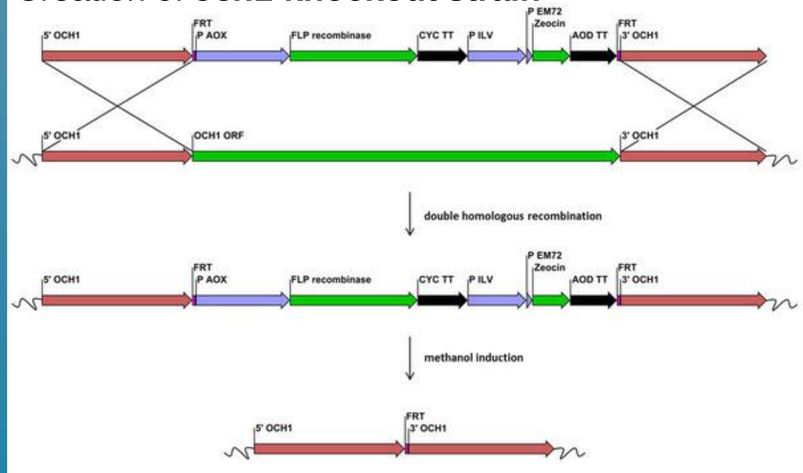


***Fig.3.*** Schematic workflow of the knockout of *OCH1* using a flipper cassette. [3]

## REBUILDING THE HUMAN SECRETORY PATHWAY

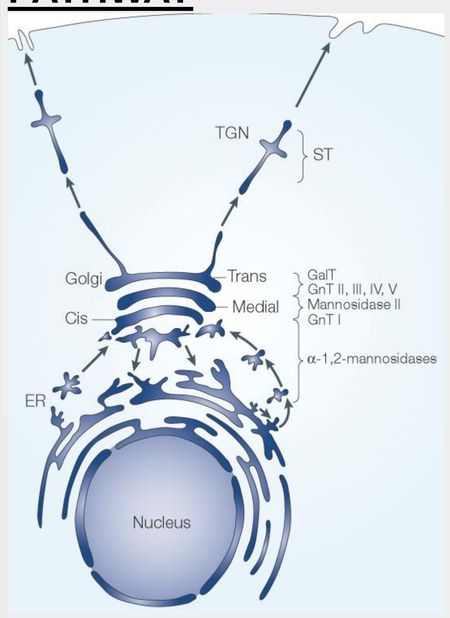


***Fig.4.*** A working model for the cellular distribution of glycosyltransferases throughout the secretion pathway. [4]

- Screening of a **combinatorial library** of α-1,2 mannosidases.
- Screening of a library of catalytic domains encoding N-acetyl-glucosaminyltransferase I (**GnTI**) fused to a library of yeast localization signals.
- Use of the combinatorial library approach to introduce **mannosidase II** and **GnTII**.

## GALACTOSYLATION

A galactosyl transferase should add one galactose sugar onto every branch. The mutant strain capable of producing complex human N-glycans was accomplished through deletion of PpALG3 and expression of GnT II and GalT.

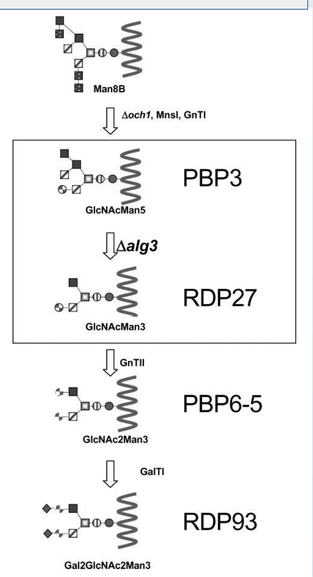


***Fig.5***. *P.pastoris* glycoengineering to produce complex human N-glycans. [5]

## SIALIC ACID TANSFER

The transfer of sialic acid onto terminal β-1,4 galactose sugars of complex glycoproteins requires a library with catalytic domains, a library with several N-terminal fragments for the location and a pool of CMP-sialic acid available (not available in yeast).

Therefore a pathway to produce CMP-sialic acid and a CMP-sialic acid transporter have to be introduced to the system.

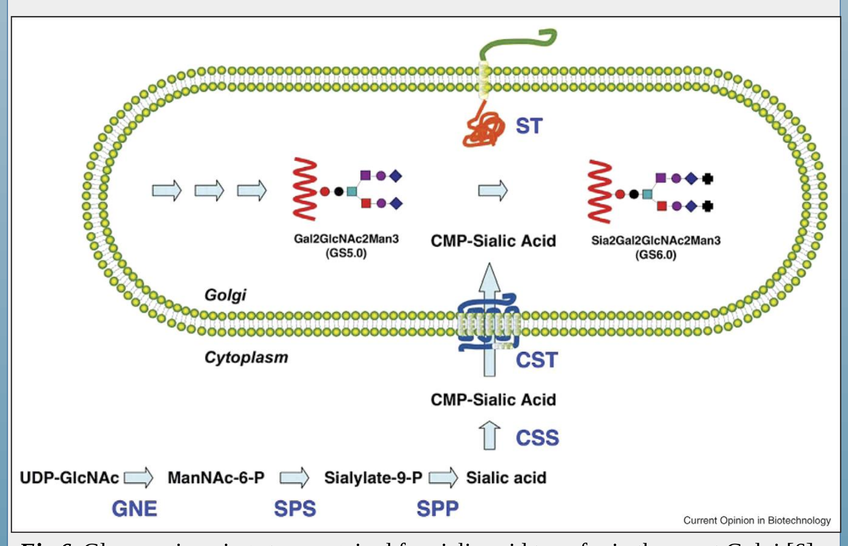


***Fig.6***. Glycoengineering steps required for sialic acid transfer in the yeast Golgi.[6]

## CONSTRUCTION OF EXPRESSION STRAINS

### Expression vectors

Expression of any foreign gene *in P. pastoris* requires three basic steps:

- the insertion of the gene into an expression vector.
- introduction of the expression vector into the *P. pastoris* genome.
- examination of potential expression strains for the foreign gene product.

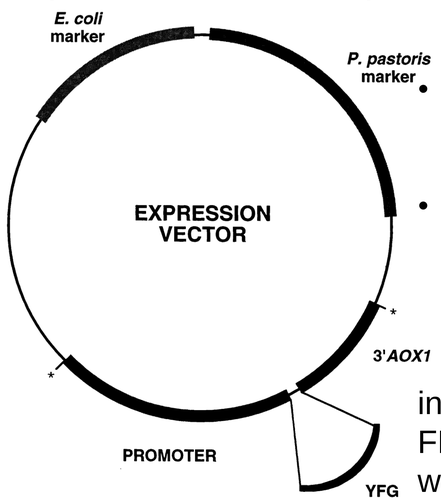


***Fig.7.*** General diagram of a *P. pastoris* expression vector. YFG (Your Favorite Gene) sites for cassette amplification. [7]

**Expression vectors** are integrated into the *P. pastoris* genome to maximize the stability of expression strains. This can be done in two ways:

- restricting the vector at a unique site in either the marker gene or the AOX1 promoter fragment and then to transform it.
- digesting certain *P. pastoris* expression vectors in such a way that the expression cassette and marker gene are released, flanked by 5' and 3' AOX1 sequences.

**Multicopy strains** can be approached:

- Vector with multiple head-to-tail copies of an expression cassette flanked by restriction sites with complementary termini.
- Expression vectors that contain the *P. pastoris* HIS4 and kanamycin resistance gene.
- Vector with resistance to the antibiotic zeocin.

**Selectable markers**

- biosynthetic markers: HIS4, ARG4, ADE1, URA3
- drug-resistance markers: $kan^R$ gene, Sh ble gene.

Combinations of these different markers are employed for efficient selection of true transformants.

## APPLICATION IN MONOCLONAL ANTIBODIES PRODUCTION

Antibodies often achieve therapeutic benefit through two binding events:

- the variable domain of the antibody binds a specific protein on a target cell.
- the effector cells bind to the constant region (Fc) of the antibody and destroy cells to which the antibody has bound.

**Construction of the antibody expression vector**

The plasmid pGLY2988 (containing IgG1 heavy and light chain under control of AOX1 promoter) was transformed into YGLY638 by electroporation to yield YGLY4140.

The host strain has been modified to secrete recombinant proteins with highly uniform N-linked glycans of the type Man5GlcNAc2.

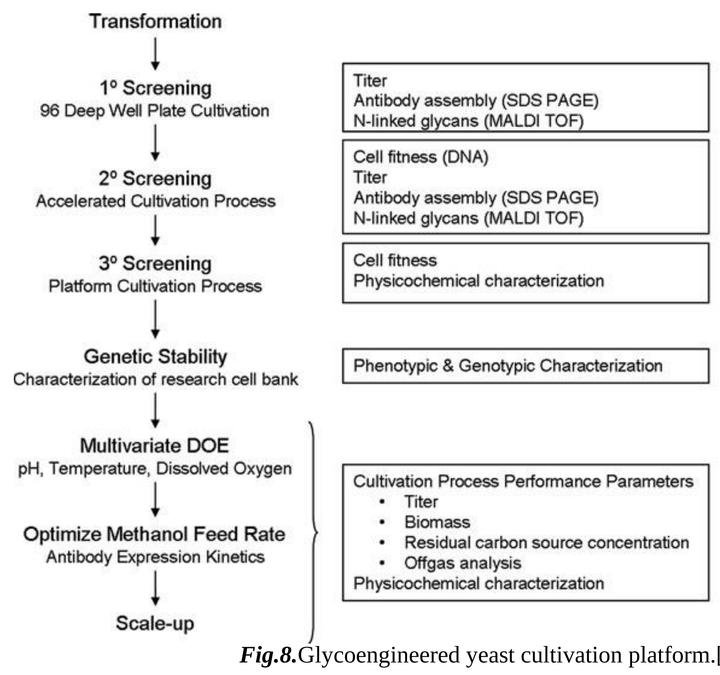


***Fig.8.***Glycoengineered yeast cultivation platform.[8]

## CONCLUSIONS

- Compared to CHO cells, the glycoengineered yeast produced antibody with similar motilities on SDS-PAGE, comparable size exclusion chromatograms and antigen binding affinities but with highly uniform N-linked glycans of the type Man5GlcNAc2.
- The production of monoclonal antibodies in *P. Pastoris* offers a robust and economically viable alternative to mammalian cell expression.

## REFERENCES

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