Glycoengineerization of *Pichia pastoris* for recombinant erythropoietin production

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INTRODUCTION

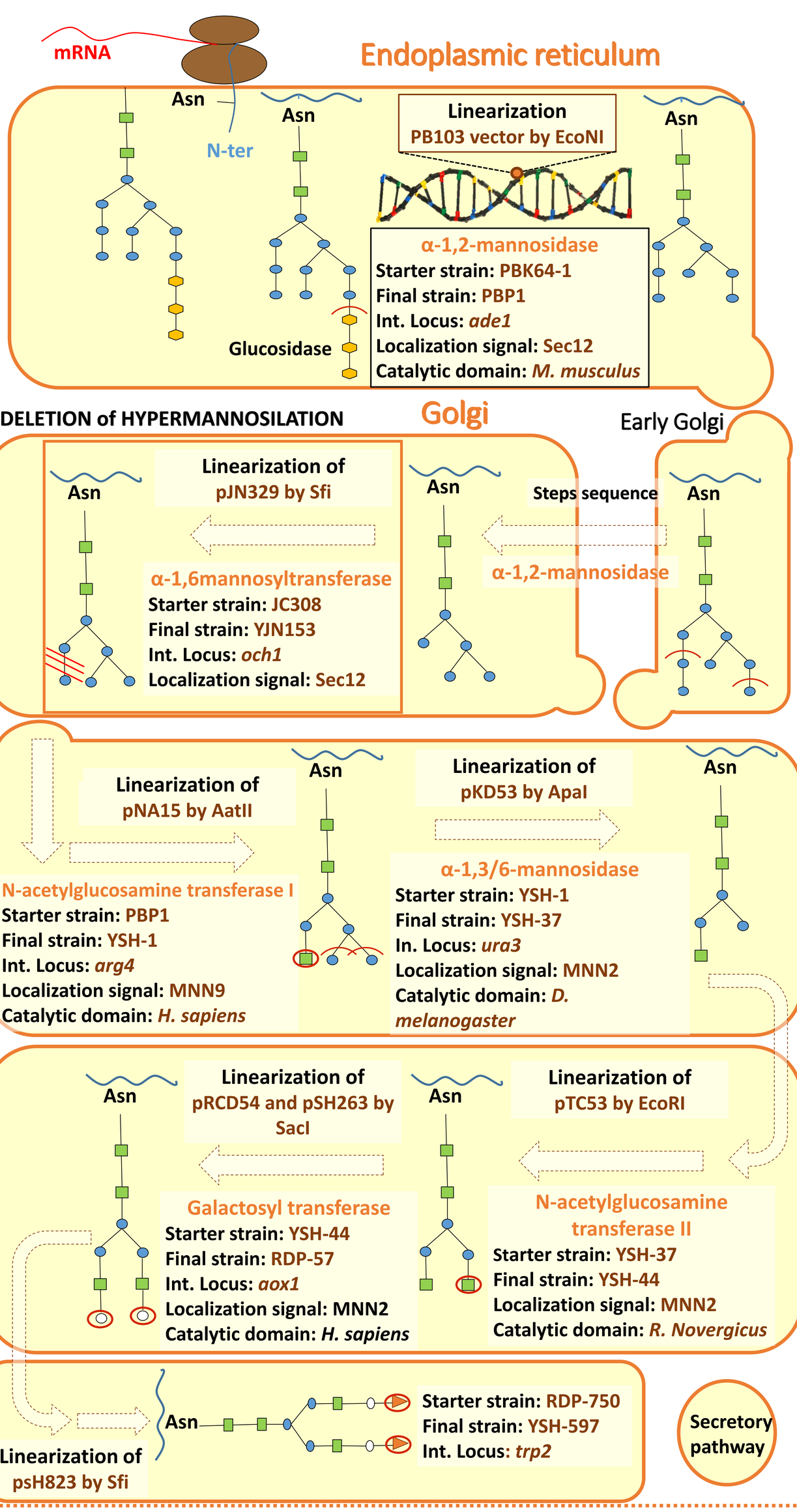
The recombinant production of glycosylated proteins is one of the main limitations of yeast expression system. Glycosylation, especially N-glycosylation is very important in multiple aspects like correct folding, half-life, inmunogenicity and therapeutic protein functionality.
Because of this, the next study is focused on N-glycosylation type reproduced by Pichia pastoris. Pichia Pastoris incorporates less mannose-type glycans on the protein structure and has some advantatges compared with animal cell culture. One of them is its larger potential production scale.

OBJECTIVE

Reproduce the mammalian glycosylation pattern in a glycoengineered yeast. For this purpose, it is necessary to use a reporter protein with a terminal sialylated site.

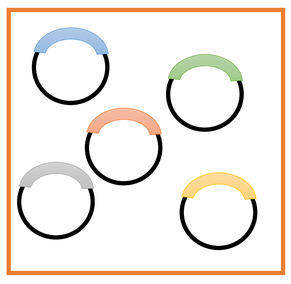
RESULTS

Engineered glycosylation pathway

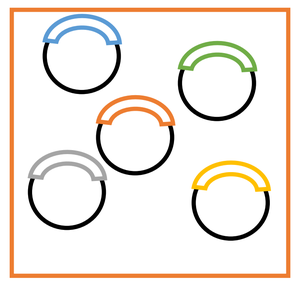


METHODOLOGY

Main technique: combinatory genetic libraries



Genetic library searching for yeast's transmembrane domain II

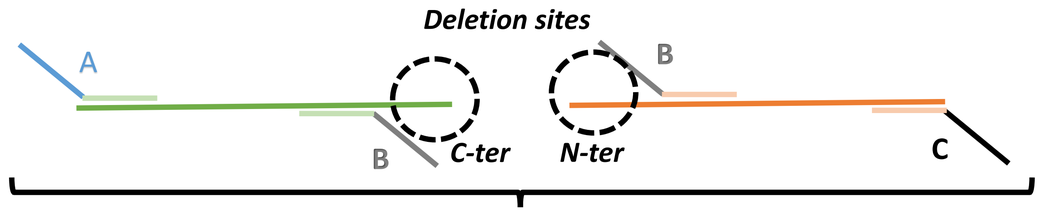


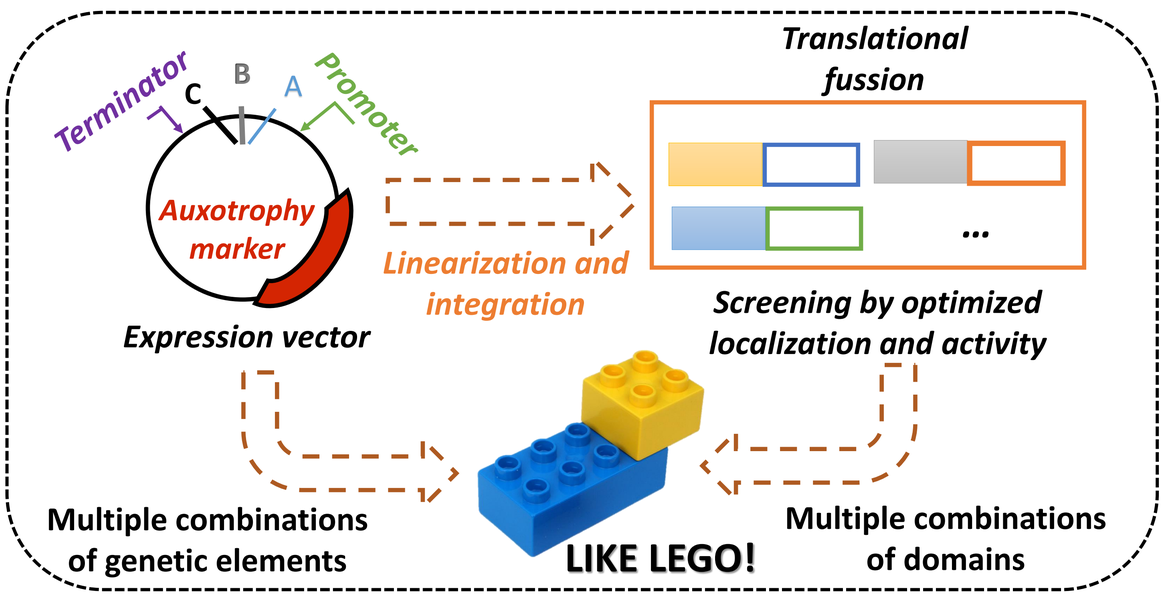
Genetic library searching for mammalian's catalytic domain

Primer design for bp deletion and restriction sites

PCR and digestion by A and B restriction enzymes

PCR and digestion by B and C restriction enzymes





Promoters and expression vectors

Promoter	Features	Expression vectors	Origin
GADPH	Constitutive promoter	pNA15, pKD53, pTC53, pRCD54	PCR-2.1-TOPO
AOX1	Methanol's inducible promoter	pJN329	pUC19 vector
PMA1	Constitutive promoter		

Mannose
N-acetylglucosamine
Galactose
Sialic acid

D. melanogaster: *Drosophila melanogaster*
R. novergicus: Rattus novergicus
H. sapiens: Homo sapiens
M. musculus: Mus musculus

DISCUSSION AND CONCLUSIONS

A glycoengineered strain of Pichia pastoris is obtained in this study by serial integrations of expression vectors in the genome. These vectors express chimeric proteins which make possible to obtain erythropoietin with the desired glycosylation pattern. Therefore, synthetic and metabolic engineering provide an efficient and easy method for this purpose.
The robust platform developed could give rise to a great large scale production system. Even so, industry level-news has nearly come to a standstill since 2006. More studies to resolve the bottlenecks of the system are needed.

References

1. Choi BK, Bobrowickz P, Davidson RC, Hamilton SR, Kung DH, Li H, et.al Use of combinatorial genètic libraries to humanize N-linked glycosylation in the yeast *Pichia pastoris*. *PNAS.* 2003; 100(9):5022-5027.
2. Hamilton SR, Davidson RC, Sethuraman N, Nett JH, Jiang Y , Rios S et.al Humanization of Yeast to produce complex terminally sialylated glycoproteins. *Science*. 2006; 313:1441-1443.