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Current Opinion in Biotechnology

Biopharmaceutical discovery and production in yeast

Michael A Meehl and Terrance A Stadheim

The selection of an expression platform for recombinant biopharmaceuticals is often centered upon suitable product titers and critical quality attributes, including post-translational modifications. Although notable differences between microbial, yeast, plant, and mammalian host systems exist, recent advances have greatly mitigated any inherent liabilities of yeasts. Yeast expression platforms are important to both the supply of marketed biopharmaceuticals and the pipelines of novel therapeutics. In this review, recent advances in yeast-based expression of biopharmaceuticals will be discussed. The advantages of using glycoengineered yeast as a production host and in the discovery space will be illustrated. These advancements, in turn, are transforming yeast platforms from simple production systems to key technological assets in the discovery and selection of biopharmaceutical lead candidates.

Addresses
GlycoFi, Biologics Research, Merck & Co., Inc., 16 Cavendish Court, Lebanon, NH 03766, USA

Corresponding author: Stadheim, Terrance A (andy_stadheim@merck.com)

Current Opinion in Biotechnology 2014, **30**:120–127

This review comes from a themed issue on **Pharmaceutical biotechnology**

Edited by **Beth Junker** and **Jamey D Young**

http://dx.doi.org/10.1016/j.copbio.2014.06.007

Introduction

The dawn of recombinant DNA techniques ushered in the modernization of protein-based pharmaceuticals, known as biopharmaceuticals or biologics. In the early 1980s, recombinant human insulin was marketed and provided Type I diabetics with a more homogenous and reliable medicine that no longer required sourcing from animal pancreas. The development of recombinant insulin helped pave the way for the marketing approval of *Escherichia coli* and *Saccharomyces cerevisiae* derived products. Three decades later, the current quality of recombinant glycoprotein expression in yeast rivals that of traditional mammalian cell culture systems. Such engineering and process development efforts have occurred in different systems with varying degrees of success. Recent advancements in yeast engineering have provided yeast-based platforms with more than just the ability to manufacture aglycosylated peptides or proteins with low complexity. Considerable effort has focused on strain improvements to develop glycoengineered yeast systems for the production of recombinant glycoproteins with desired physiochemical properties and efficacy. Innovations in yeast genetics and protein secretion will also be discussed that enable yeast to be a state-of-the-art tool for biopharmaceutical research and development.

Yeast production systems and derived products

Early biopharmaceuticals originated from animal and human sources. However, the majority of newly marketed products are derived from recombinant expression systems. Yeast, along with bacterial (*i.e. E. coli*) and mammalian hosts (*i.e.* Chinese Hamster Ovary) represent the most frequently used expression systems for biopharmaceuticals. In the early 1980s, ZymoGenetics industrialized *S. cerevisiae* as a host for recombinant human insulin leading to the marketing of Novolin® with Novo Nordisk in 1987. Today, the *S. cerevisiae* platform currently delivers half of the world supply of insulin. With the recent interest in biosimilar products, *Pichia pastoris* has been used to produce recombinant human insulin with titers of 3 g/L [1,2]. In fact, Biocon is the world's fourth largest supplier of insulin products and utilizes *P. pastoris* [URL: http://www.biocon.com]. The example of recombinant insulin demonstrates the capabilities of yeast-based biopharmaceutical expression and production. In recent years, many new biopharmaceutical candidates were produced in *S. cerevisiae*, *P. pastoris*, and *Hansenula polymorpha* platforms [3–5,6•,7].

The most recent FDA approval of a yeast-expressed biopharmaceutical was in 2012 for ocriplasmin, sold as Jetrea® by ThromboGenics [8]. Ocriplasmin is an aglycosylated protease expressed in *P. pastoris* approved for the treatment of symptomatic vitreomacular adhesion [9]. Other notable yeast-based biopharmaceuticals with marketing approval include: a Hepatitis B subunit vaccine using Hepatitis B surface antigen (*S. cerevisiae*, *P. pastoris*, and *H. polymorpha*); recombinant human granulocyte macrophage-colony stimulating factor, Leukine®(sargramostim, *S. cerevisiae*, 1991); recombinant human platelet derived growth factor, Regranex® (becaplermin, *S. cerevisiae*, 1997); human papillomavirus subunit vaccine, Gardasil® (*S. cerevisiae*, 2006); and a kallikrein inhibitor, Kalbitor® (ecallantide, *P. pastoris*, 2009).

Numerous yeast-based biopharmaceuticals are in clinical development. Ablynx reports at least two clinical programs focused on nanobody-based therapeutics

expressed in *P. pastoris* [URL: http://www.ablynx.com]. Alder Biopharmaceuticals reports two clinical antibody leads targeting interleukin-6 (IL-6), named ALD518 (BMS-945429), and calcitonin gene-related peptide (CGRP), named ALD403, in their pipeline using the *P. pastoris* expression system [URL: http://alderbio.com]. In the case of ALD518 (BMS-945429), the humanized antibody was engineered to lack Fc glycosylation. However, serum half-life is preserved and is comparable to other marketed glycosylated antibodies [10,11]. This latter example of full-length antibody expression in *P. pastoris* signifies a shift towards the production of more complex biopharmaceuticals. Indeed, there are reports of the production in *P. pastoris* of biopharmaceuticals with human-like glycosylation patterns, including antibodies and therapeutic proteins [12–14,15••,16–18,19••,20–22].

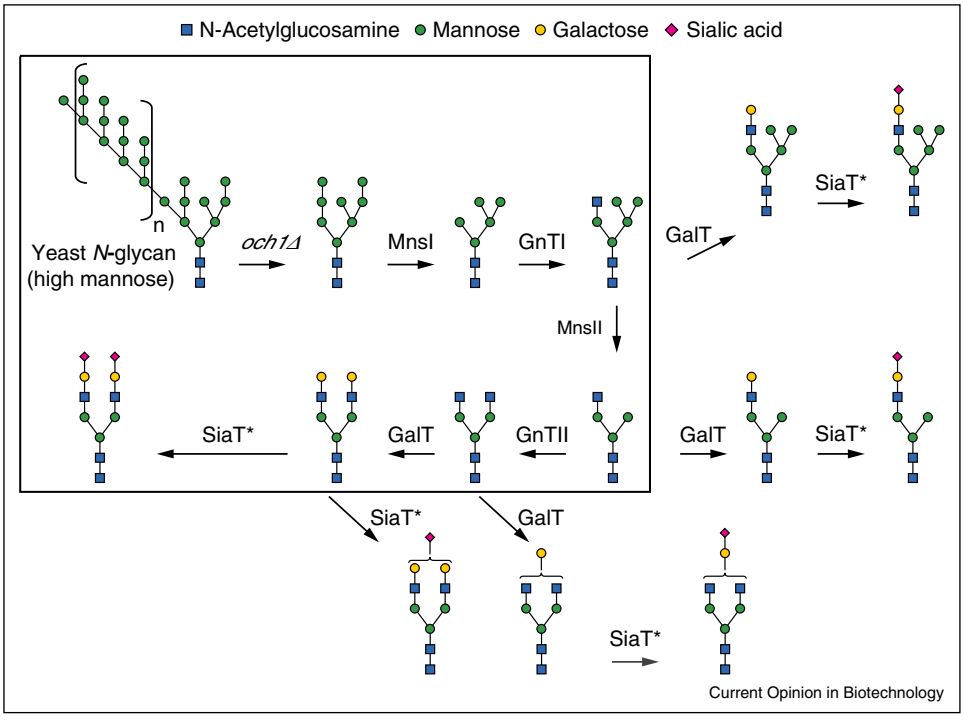
Strain engineering and process improvements

N-glycan modifications

Many biopharmaceuticals require proper post-translational modifications, including glycosylation, for optimal pharmacokinetic (PK) and pharmacodynamic (PD) properties [23,24]. Recombinant biopharmaceuticals with yeast-derived high mannose *N*-glycans are often not suitable for clinical use. Proteins with yeast-type glycosylation, when delivered *in vivo*, interact with human C-type lectins of the innate immune system resulting in altered pharmacokinetic properties, activation of complement, enhanced anti-drug–antibody formation, or sequestration by circulating anti-glycan antibodies [20,25–27,28••]. Furthermore, mannose glycans have been shown to alter the efficacy of recombinant vaccines [29,30]. Many therapeutic glycoprotein candidates require a particular human-like glycoform for optimal efficacy, such as sialic acid for half-life extension [18,31], lack of fucose on antibodies for improved cytotoxic properties [32], and paucimannose for selective targeting to macrophages [33]. While *in vitro* glycan modifications using processing enzymes are possible, the most robust and efficient methods to produce particular glycosylation profiles are those that can be synthesized *in vivo*, such as using glycoengineered yeast.

N-glycan humanization centers on the conversion of an extant fungal high mannose oligosaccharide to a complex oligosaccharide that can be further optimized to contain specific terminal sugars (*i.e.* *N*-acetylglucosamine (GlcNAc), galactose and sialic acid) (see Figure 1). Over the past several years, this feat of genetic engineering

Figure 1



Yeast *N*-glycan engineering. The *N*-glycosylation pathway of glycoengineered *Pichia pastoris* was previously reviewed [34]. Glycoproteins harboring predetermined glycoforms [78] are obtained depending on the glycoengineered yeast host used, each of which contains a unique set of gene deletions and glycosylation enzymes, as indicated by arrows. The main glycosylation pathway to obtain mammalian biantennary glycans is shown in the upper left rectangle. As indicated by (*), sialic acid linkages may be exclusively α-2,6 or α-2,3 depending on the chosen sialyltransferase. Other yeast modifications (*e.g.* beta-linked mannose, mannosylphosphate) are not depicted in the figure.