Researchers report full humanization of therapeutic proteins from yeast

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Researchers at Dartmouth's Thayer School of Engineering, Dartmouth Medical School, and the biotechnology firm GlycoFi, Inc., report a significant advance in the production of therapeutic proteins. Reported in the Sept. 8 issue of the journal *Science*, the Dartmouth/GlycoFi team announced the complete humanization of the glycosylation pathway in the yeast Pichia Pastoris.

"We've successfully completed one of the most complex cellular engineering endeavors undertaken to date," said Tillman Gerngross, chief scientific officer of GlycoFi and professor of engineering at Dartmouth.

Protein-based therapies represent more than half of all the drugs currently in development, and they have to be manufactured by living cells, which are genetically engineered to produce a given protein of interest. However, most of these proteins require the attachment of sugar structures, a process known as glycosylation, to attain full biological function. To date, this has required the expression of such proteins in mammalian cells that have the ability to attach human-like sugar structures.

This new finding replicates all the steps of human glycosylation within a yeast cell, eliminating the need for mammalian cells. Plus, report the researchers, the technology offers numerous advantages over the conventional use of mammalian cell cultures, namely reduced risk of contamination by pathogens and infectious agents along with improved

drug performance and manufacturing efficiency.

"Humanizing glycosylation in yeast was a tour de force of genetic engineering, requiring the knockout of four yeast genes and the introduction of over 14 heterologous genes," said Stephen Hamilton, the lead author on the study and a senior scientist at GlycoFi.

The study details the genetic engineering of the yeast Pichia pastoris to secrete human glycoproteins with fully complex, terminally sialyated N-glycans. The researchers demonstrated the effectiveness of this approach when the glycoengineered yeast strain was used to produce functional erythropoietin, a protein widely used in the treatment of anemia, and considered to be the most successful biotech drug to date.

Gerngross noted that the GlycoFi/Dartmouth research team previously demonstrated the importance of glycosylation structures on other commercially relevant therapeutic proteins such as antibodies (published in *Nature Biotechnology* earlier this year). Like with most glycoproteins the researchers were able to show that, by controlling glycosylation, they could significantly improve an antibodies ability to kill cancer cells.

"By engineering yeast to perform the final and most complex step of human glycosylation, we will be able to conduct far more extensive structure-function investigations on a much wider range of therapeutic protein targets," Gerngross said.

Source: Dartmouth College