

ENHANCEMENT BY REDUCTION - PUSHING THE N-GLYCOSYLATION CAPACITY OF CHO CELLS BY CLEANING UP THE GOLGI

Nina Bydlinski, Department of Biotechnology, BOKU University of Natural Resources and Life Sciences, Austria
nina.bydlinski@boku.ac.at

Daniel Maresch, Department of Biochemistry, BOKU University, Austria

Richard Strasser, Department of Applied Genetics and Cell Biology, BOKU University, Austria

Nicole Borth, Department of Biotechnology, BOKU University, Austria

Key Words: CHO cells, N-glycosylation, multi-gene knock-out

CHO cells have gained their position as the most commonly used production system for complex biological therapeutics for a variety of reasons, including their ability to produce human like N-glycosylation patterns: correct N-glycan structures ensure that a product performs adequately in terms of efficacy and without the risk of eliciting immunogenic reactions.

Nevertheless, N-glycosylation in CHO cells has posed several challenges. Many cell-engineering approaches have tackled the problem of low levels of sialylation and the lack of α -2,6-linked sialic acid by introducing additional sialyltransferases as well as boosting the sialic acid pathway and the transport of CMP-NA precursors into the Golgi. Various reports show that the process of N-glycan maturation can run into limitations when the production load is high, a problem of increasing relevance as the boundaries of productivity of CHO cells are being pushed further and further. In this regard, the link between high productivity and reduced sialylation and galactosylation as well as the occurrence of high-mannose structures has been established. This observation can be partially explained by lack of sugar-precursors due to the depletion of nutrients towards the end of a bioprocess, but it has also been proposed that there is a limited capacity of the Golgi for N-glycan processing. With more glycoprotein traversing through the secretory pathway, the abundance of glycosyltransferases in the Golgi membrane might not be sufficient to act upon all N-glycan molecules.

Our strategy is based on the knock-out of multiple galactosyltransferases and sialyltransferases that have no or only a minor role in N-glycosylation of recombinant proteins to generate free space in the Golgi membrane, which can then be re-populated with the most effective isoenzymes to ensure high levels of glycan maturation even at high production rates. For sialylation, ST3GAL4 was previously identified as the key player. Out of the four galactosyltransferases involved in N-glycosylation, B4GALT1 has been proposed to be the dominant isoform, but published results vary concerning the contributions of the other isoenzymes. Therefore, we studied the activity of each of these four galactosyltransferases individually by removing the respective other three isoenzymes using CRISPR and a paired sgRNA deletion strategy. Three different glycoproteins (Epo-Fc, IFNG and a heavily glycosylated Fc fusion protein) were produced transiently and analysed by mass spectrometry for site specific N-glycans. The results clearly show that B4GALT1 alone is sufficient for high levels of galactosylation of all model proteins. B4GALT2 and B4GALT3 contribute to different extents but only yield low levels of galactosylation when acting alone, with slightly protein and site-dependent effects.

To enhance N-glycosylation ST3GAL4 and B4GALT1 will be overexpressed in a cell line with a thus “cleaned-up” Golgi, referring to the lack of the other isoenzymes. The superiority of this system will be validated with a transient expression system based on mRNA transfection to obtain high productivity mimicking the high production load of industrial cell lines.