Rewiring the synthesis of protein-linked glycosylation in eukaryotic biopharmaceutical expression hosts.

Nico Callewaert.

Medical Biotechnology Center, VIB and UGhent, Ghent, Belgium.
Correspondence: nico.callewaert@vib-ugent.be.

Glycosylation is the enzymatically catalysed modification of biomolecules with carbohydrate structures. For secreted and membrane-integral proteins, it is the most common post-translational modification. Glycan structures characterize the molecular environment immediately outside of all cell types and hence have critical functions in interactions of any cell with its environment (cell-cell, cell-pathogen, cell-molecule). The field of glycobiotechnology is concerned with understanding and re-engineering of these glycosylation-dominated interactions. In particular, the understanding of the synthetic pathways and functions for eukaryotic N- and O-glycosylation, gained over the past few decades, has enabled the rewiring of these pathways for the benefit of pharmaceutical applications. Based on the conservation of the core pathways between eukaryotes, it has been possible to transfer the efficient synthesis of particular human-specific glycan structures to other eukaryotes such as yeasts and plants. This is enabling the cost-effective production of biopharmaceutical proteins with glycosylation patterns customized to particular therapeutic functionality (e.g. targeting to particular glycan receptors, or customized for particular pharmacokinetic behaviour). I will illustrate our work with regard to the production of human IgG-like glycosylation patterns in yeast[1], and the production of mannose-6-phosphate modified lysosomal enzymes for the treatment of human inherited lysosomal storage diseases[2]. Whereas these earlier synthetic biology endeavours were geared towards efficiently synthesising proteins with complex mammalian glycan structures in other eukaryotes, more recently we have generated mammalian cells and plants in which glycosylation complexity has been reduced to the bare minimum, while still being compatible with eukaryotic cell life and protein productivity. This 'GlycoDelete' technology[3,4] opens up many new structural biology and biopharmaceutical applications that are currently being explored in our laboratory.

1. Jacobs, P. P., Geysens, S., Vervecken, W., Contreras, R. & Callewaert, N. Engineering complex-type N-glycosylation in Pichia pastoris using GlycoSwitch technology. *Nat. Protoc.* **4,** 58–70 (2009).
2. Tiels, P. *et al.* A bacterial glycosidase enables mannose-6-phosphate modification and improved cellular uptake of yeast-produced recombinant human lysosomal enzymes. *Nat. Biotechnol.* **30,** 1225–1231 (2012).
3. Meuris, L. *et al.* GlycoDelete engineering of mammalian cells simplifies N-glycosylation of recombinant proteins. *Nat. Biotechnol.* **32,** 485–489 (2014).
4. Piron, R., Santens, F., De Paepe, A., Depicker, A. & Callewaert, N. Using GlycoDelete to produce proteins lacking plant-specific N-glycan modification in seeds. *Nat. Biotechnol.* **33,** 1135–1137 (2015).