Beyond the ribosome: engineering of post-translational processes in the eukaryotic secretory system

Nico Callewaert
VIB-UGent Center for Medical Biotechnology Center, Ghent, Belgium

Email: nico.callewaert@ugent.vib.be

Decades of research on protein biogenesis have brought detailed insight on the processes at work during transcription, translation and co/post-translational processes such as translocation across or insertion in membranes, protein folding and modifications such as glycosylation. Nevertheless, having a lot of knowledge on the parts of a biological system as complex as this does not necessarily mean that we have a sufficiently integrative view nor the tools to fully control it. This rapidly becomes evident to anyone who has ever tried to recombinantly produce a new protein of a certain degree of structural complexity. Apart from a majority of fundamental life sciences research, also pharmaceutical development heavily relies on our ability to produce often complex drug target proteins and protein drugs. However, many projects still get stalled because of intractable problems with protein expression. In our lab, we are developing ways and means to better understand and overcome such problems, in particular for the eukaryotic secretory system.
In particular, the understanding of the synthetic pathways for eukaryotic N- and O-glycosylation has enabled the rewiring of these pathways for the benefit of structural biological and 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. 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]. More recently we have generated mammalian cells, plants and yeast 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 applications that are currently being explored.

To gain a better understanding of the principles that underpin productive passage of proteins through the eukaryotic secretory system, we have recently developed a new methodology that generates proteome-wide data of protein domain 'secretability'. This now allows for the effect of particular molecular or environmental perturbations in the secretory system to be studied not on a few model proteins, but on tens of thousands of protein fragments simultaneously. Combined with increasing computational power, future work based on this may enable the construction of more predictive models of protein secretability.

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).