Glycoprotein Synthesis: Quality Matters

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Glycosylation is the process by which sugars are added to biological molecules such as proteins. It regulates a myriad of biochemical processes, such as protein folding, adhesion, targeting and recognition events. Conditions resulting from in-born defects of glycosylation manifest with wide-ranging pathologies impacting nearly every organ system and often result in severe and multi-system disease. These disorders are challenging to diagnose and difficult to map onto the clinical presentations, as a single defect in a sugar processing pathway may result in complex and varied downstream effects[1].

Glycosylation and protein folding

The addition of sugar chains to newly synthesised proteins in the endoplasmic reticulum (ER) plays an important role in controlling the correct folding of proteins (**Figure 1**).

- Proteins enter the ER and carbohydrate chains are transferred to the surface of the protein.
- Correctly folded proteins are exported to the Golgi body
- Misfolded proteins are exported to the cytosol. In the cytosol the enzyme **PNGase** removes the sugar chain and the protein is degraded by the **proteasome**

Loss of PNGase activity results in a build up of misfolded proteins which aggregate in the cell[1,2].

NGLY1 deficiency is a rare disease caused by a mutation in the gene that encodes PNGase. It results in a loss of action of the enzyme. Many patients with this disorder are wrongly diagnosed initially with Rett syndrome or mitochondrial disorders (source: Grace Wilsey Foundation).

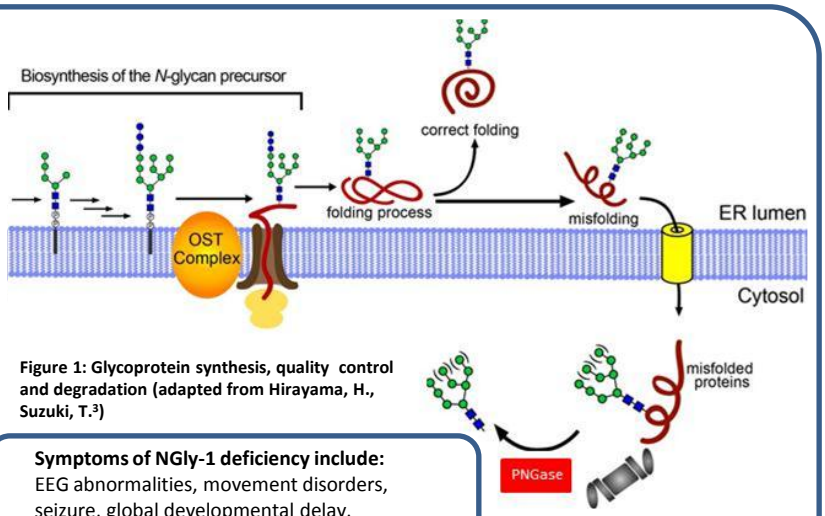

Figure 1: Glycoprotein synthesis, quality control and degradation (adapted from Hirayama, H., Suzuki, T.[3])

Symptoms of NGly-1 deficiency include:
EEG abnormalities, movement disorders, seizure, global developmental delay, hyptonia, liver disease, alacramia

PNGase as a tool for sugar analysis

- Plasmid encoding PNGase is introduced into *E. coli* (DE3) and the protein expressed
- PNGase is extracted by shocking the *E. coli* with cold water, releasing the protein
- Protein is purified by affinity chromatography
- Activity of enzyme quantified by monitoring deglycoslyation of RNAseB

- PNGase can be used *in vitro* to cleave sugars from proteins so they can be analysed
- Enzyme activity assay can be used as an *in vitro* assay for PNGase inhibition

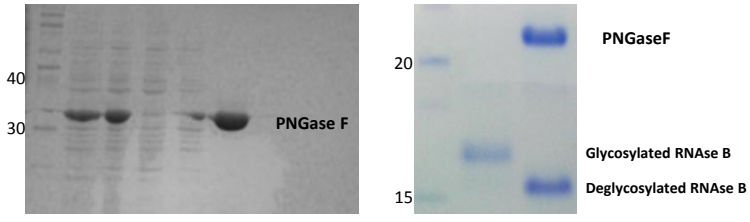

Figure 2: Purification of PNGaseF (left). Activity of PNGaseF *in vitro* (right). When RNAseB is incubated with PNGaseF, the enzyme cleaves the glycans from the protein surface causing a decrease in molecular weight

Effects of PNGase inhibition

- PNGase can be inhibited in vivo using siRNA knockdown or treatment with pharmacological inhibitors such as Z-VAD-fmk

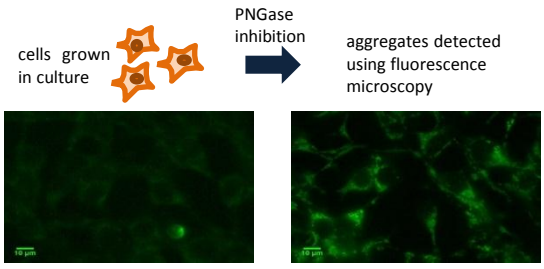

Figure 3: HEK293 cells stained with 5 µM ThT under normal (left) and stressed (right) conditions. Cells treated with 2 mM DTT to induce ER stress

Future work

Characterise the nature of the protein aggregates and their effect on cellular function
Characterise the impact of PNGaseF inhibition on ER stress and protein degradation pathways

Acknowledgments
Prof. Frances Ashcroft (Department of Physiology, Anatomy and Genetics, University of Oxford)
Dr. Konstantinos Lefkimmiatis (British Heart Foundation CRE, Cellular and Molecular Signalling Group, Department of Physiology, Anatomy and Genetics, University of Oxford)
Georgina Berridge (Nuffield Department of Medicine, University of Oxford)

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