GLYCOSYLTRANSFERASE COMPLEXES:

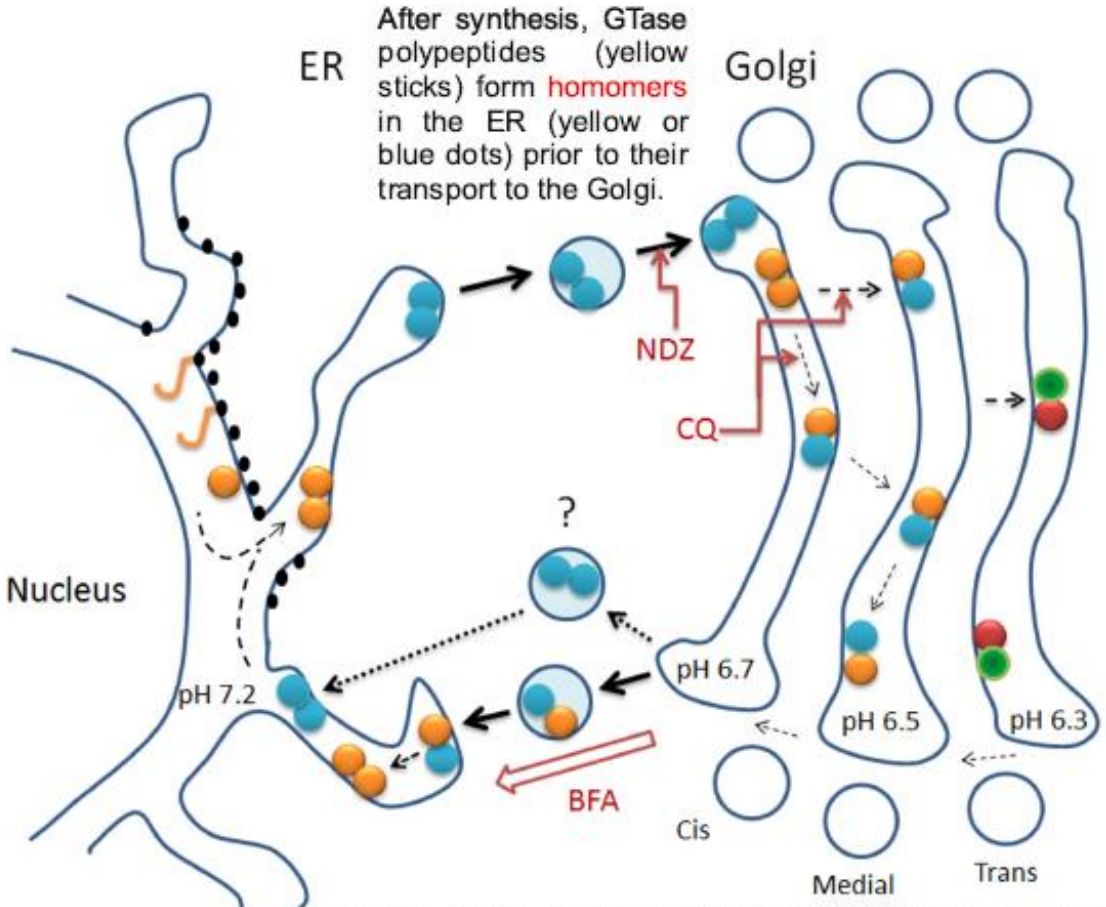
A NEW VIEW TO UNDERSTAND GLYCOSYLATION

Tuomo Glumoff, Antti Hassinen, Deborah Harrus, Fawzi Khoder Agha,
Elham Khosrowabadi, Imran Qadir, Minna Lempinen and Sakari Kellokumpu
Faculty of Biochemistry and Molecular Medicine, University of Oulu

BACKGROUND AND AIMS

Glycosylation is the most common modification of cellular proteins and lipids, and a major source of biological diversity between cells, tissues and organisms. Glycosylation products – i.e. glycans - carry structural information needed for many crucial cellular functions that range from regulation of gene expression to protein folding and trafficking, cell motility and signaling, and immunological defense. The importance of correct glycosylation for human health is highlighted by the presence of aberrant glycans in many diseases - including cancers. The Golgi apparatus is the main site of glycosylation in eukaryotes: it synthesizes O-glycans, proteoglycans and glycolipids, and processes high mannose type N-glycans made in the ER to complex N-glycans. It has been estimated that 4000 to 8000 distinct glycan chains carry important structural information that is relevant for cellular functions. To faithfully synthesize the needed variety of glycans without a template sequence of sugars requires careful sequential action of dozens of functionally distinct, competing, and co-existing glycosyltransferases (GTases) and glycosidases is needed. Our aim is to combine in vivo, in vitro, and in silico methods to explore how GTases function as enzymes and how they are regulated in order to complete this difficult task.

This figure summarizes our key idea of GTase function: they form complexes - both homo- and heteromeric complexes in live cells. Heteromers typically form between successively acting GTases and catalyze additions/deletions of relevant sugar residues to/from the growing glycan chain during processing in the Golgi. Moreover, heteromeric complexes appear to be catalytically more important, while homomers seem to have a regulatory function – given that the complexes undergo dynamic transitions between homo- and heteromers upon their recycling within the secretory compartments.



In the Golgi, acidic luminal microenvironment favours the formation of medial-Golgi (yellow-blue dots) and trans-Golgi (red-green dots) heteromers at the expense of homomers.

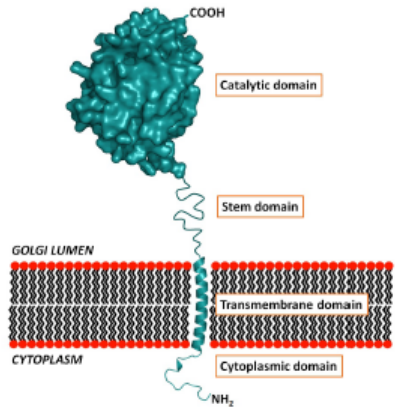
ACADEMY OF FINLAND

CIMO

GLYCO BIOLOGY in OULU

STRUCTURAL ASPECTS OF GLYCOSYLTRANSFERASE COMPLEXES

Most GTases are type II membrane proteins with the C-terminal globular catalytic domain (ca. 300 aa) on the luminal side. N-terminus, membrane-spanning α-helix and the stem region comprise about 100 residues. Altogether, ca. 250 GTases are known (http://www.CAZy.org).

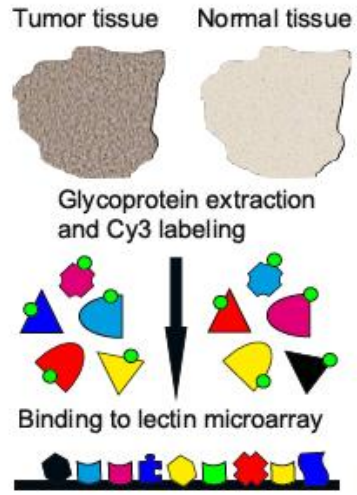


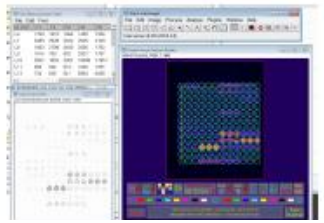
ST6-GalT1 heteromer constructed by molecular docking. Variant proteins are designed based on suggested surface contacts, and the relevance confirmed in vivo by FRET. We are also expressing and producing the proteins for crystallization. Based on FRET results there are both pH-sensitive and pH-insensitive GTase complexes.

Details of GTase complex formation is studied by molecular docking and x-ray crystallography. Critical interaction surface residues in the catalytic domains are mutated. We are also studying the possible role of the stem region in complex formation by constructing truncated variant GTases. Complex formation is verified in vivo in live cells by FRET analysis.

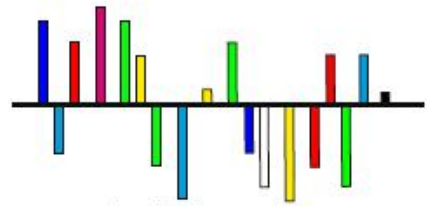
GLYCOME WIDE ANALYSIS OF ENZYME ACTIVITIES BY LECTIN MICROARRAYS

We have recently set up a lectin microarray platform to allow high-throughput glycan profiling of cell/tissue samples. Lectin microarray analyses are performed with cells expressing either wild type (interacting) or mutant (non-interacting) GTases to verify the functional significance of the complexes for glycan synthesis. Similar analyses with the wild type and mutant cells will thus enable detection of potential changes in the glycan fingerprints that are caused by the absence of the selected GTase complexes.





Scanning and data analysis



ANALYSIS OF GTase COMPLEXES IN VIVO BY FRET

We are currently using the Operetta High Content Imaging System (PerkinElmer) for fluorescence resonance energy transfer (FRET) measurements with mVen and mChe variants as the donor/acceptor FRET pair.

FRET microscopy measurements (Hassinen & Kellokumpu, 2014). GTase homomer formation was only inhibited by interacting heteromeric enzyme constructs in the Golgi.