Glycometabolic Biochemistry Laboratory (2023)

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(0) Research field
CPR Subcommittee**:** Biology
Keywords: glycoproteins, asparagine-linked glycans, metabolism, peptide:*N*-glycanase, Ngly1

(1) Long-term goal of laboratory and research background

Peptide:*N*-glycanase (PNGase) releases asparagine-linked (N-linked) glycans from glycoproteins/ glycopeptides. The cytoplasmic PNGases (NGLY1/Ngly1 in human/mouse or rat), ubiquitously found throughout eukaryotes, are now widely recognized as a component implicated in the ERAD (ER-associated degradation) process, which constitute one of the quality control machineries for newly synthesized misfolded glycoproteins exported out of the ER lumen. While the biosynthetic pathway for N-glycans has been clarified in detail, the catabolic pathway for the "free" *N*-glycans released by the cytoplasmic PNGase remains largely unknown. Although this "non-lysosomal" metabolic path for N-glycan may represent one of the very basic biological phenomena in eukaryotes, there are still many more enzymes/transporters that remains to be identified. We are currently trying to identify other players involved in this process and also taking a number of approaches to analyze the physiological importance of this non-lysosomal metabolic pathway.

(2) Current research activities (FY2023) and plan

NFE2L1 (NRF1) is a transcription factor playing a key role for counteracting various cellular stresses such as oxidative or proteostatic pstresses. It has also been well established that NRF1 can be activated through deglycosylation by NGLY1 (PNGase) – it is the only protein known to be activated by NGLY1 action. In fact, there are multiple processes involved for the activation of this molecule, i.e. N-glycosylation in the ER lumen followed by retrotranslocation into the cytosol; deglycosylation by NGLY1; limited proteolysis by DDI2; and translocation into the nucleus, creating various “molecular forms” with distinct size on SDS-PAGE. The precise mechanism, however, of how these forms are generated remains largely unknown. We have examined the N-glycosylation status of NRF1 and unexpectedly found that molecular size of NRF1 on SDS-PAGE hardly changed after PNGase digestion. In sharp contrast, Endo H-treatment resulted in expected size change. The abnormal behavior of PNGase-deglycosylated protein appeared to be due to the introduction of negative charge(s) by conversion of glycosylated Asn into Asp residues. We also demonstrate that NGLY1-mediated deglycosylation and DDI2-mediated proteolytic processing of NRF1 are not strictly ordered reaction. Our study will allow us to better understand the precise structures as well as biochemical properties of the various molecular forms of NRF1[1].

To accelerate NGLY1 deficiency research in Japan, finding Japanese NGLY1 deficiency patients has long been a high priority. It has been achieved by a group of Kyushu University (Profs. Shoichi Ohga and Yasunari Sakai), who published the clinical report of the very first Japanese NGLY1 deficiency patient [2]. While mutations in NGLY1 allele have been confirmed, we contributed to detecting the increased level of Asn-GlcNAc, a serum biomarker for NGLY1 deficiency.

Oligosaccharyltransferase (OST) normally transfer oligosaccharides onto proteins from dolichol-linked oligosaccharides (DLO), while it is also known that OST can hydrolyze DLO to form free N-glycans (FNGs), serving as an alternative pathway for forming FNGs in an NGLY1-independent fashion. Functional importance of the hydrolysis reaction, however, remains unknown. We showed that, in budding yeast, mutants for ubiquitin ligases involved in endoplasmic reticulum (ER)-associated degradation (ERAD) had the increased level of FNGs. Moreover, this hydrolysis is also upregulated when cells are treated with dithiothreitol, inducing folding defect in the lumen of ER. These results suggest that, in budding yeast, hydrolysis activity of OST is upregulated under conditions where misfold proteins are expected to be accumulated in the ER. Our results thus imply the possible function of FNGs formed by the OST as a molecular chaperone, helping protein folding in the lumen of the ER [3].

We have recently established the isolation/quantitation method for serum free oligosaccharides, while the precise mechanism of how they are formed remained unknown. We therefore analyzed how the free oligosaccharides are formed using rat primary hepatocytes. It has been demonstrated that various types of free oligosaccharides, i.e. sialyl/netral free N-glycans (FNGs), sialyl lactose/LacNAc-type glycans, whose structures are very similar to serum free oligosaccharides found in the same individual rat, are secreted from rat hepatocytes. Various inhibitor experiments also showed that OST is, at least in part, involved in the secretion of serum FNGs. Our results clearly indicate that liver is the source of various serum oligosaccharides,

secreting them into the serum [4].

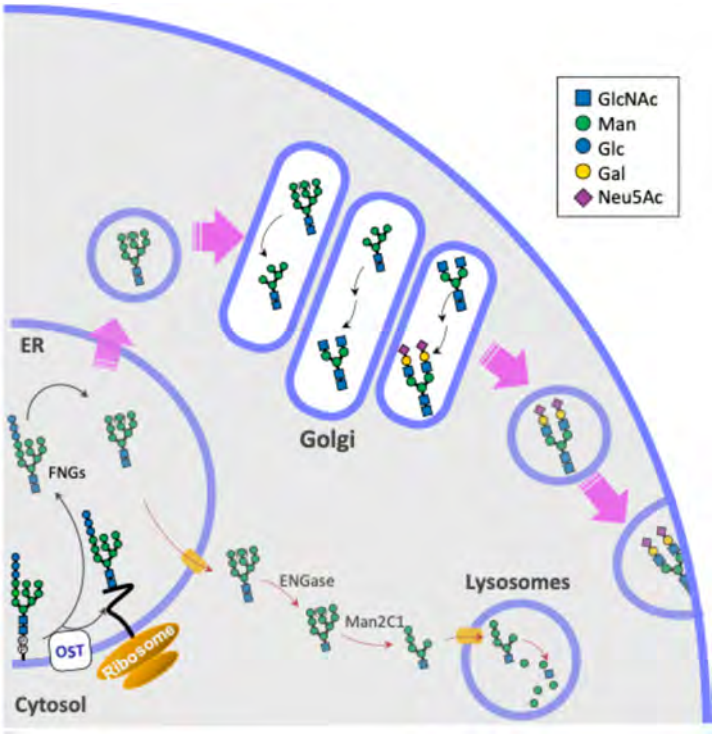


Figure: Model for the biosynthesis/secretion of serum free N-glycans (FNGs) [4]
Free oligosaccharides (FNGs) formed by OST are normally retrotranslocated into the cytosol by an ER transporter for oligosaccharides, and they are eventually degraded into the lysosomes (depicted in the lower part of the figure). On the other hand, in hepatocytes, at least a part of the luminal FNGs escaped the degradation pathway and secreted into the medium through secretory pathway (depicted in the upper part of the figure). During this process, structures of FNGs are remodeled into sialic acid-containing complex-type glycans.

This year Suzuki was awarded **IGO Hakomori Award** from the International Glycoconjugate Organization (IGO) for his long contribution on studies for NGLY1 and non-lysosomal glycan degradation process [5]. This award, one of the most prestigious international awards in the glycoscience field, was given by IGO every other year, to senior glycoscientists who have clearly advanced the field of glycobiology and show promise of continuing advancements. This was the third Japanese won this award, after Profs. Naoyuki Taniguchi (2001) and Taroh Kinoshita (2017).

In the future, we will continue to aim at clarifying the molecular mechanism for the catabolism of N-glycans and their precursors (dolichol-linked oligosaccharides). We will also aim at unveiling the species-specific glycan biosynthetic and degradation pathway, such as the molecular mechanism for free O-mannose glycan formation in budding yeast, to provide novel insights into the functional importance of glycans from the standpoint of “comparative glycobiology”. We will also carry out in-depth analysis of phenotypic consequences of *Ngly1*-KO mice, to clarify the pathophysiology of NGLY1 deficiency.

(3) Members (FY2023)

(Chief Scientist)
Tadashi Suzuki
(Senior Research Scientist)
Kenichi Moto
Masashi Ueki
Katsuhiko Kamada
(Research Scientist)
Hiroto Hirayama
Haruhiko Fujihira
(Technical Scientist)
Yuriko Tachida
(Special Postdoctoral Researcher)
Akinobu Honda
(Postdoctoral Researcher)
Shengtao Li
Stuart Emmerson
Ryosuke Koyama
(Technical Staff I)
Junichi Seino
Keiko Sato
(Assitant)
Yuko Suzuki
(Research Part Time Worker II)
Tsugiyo Matsuda

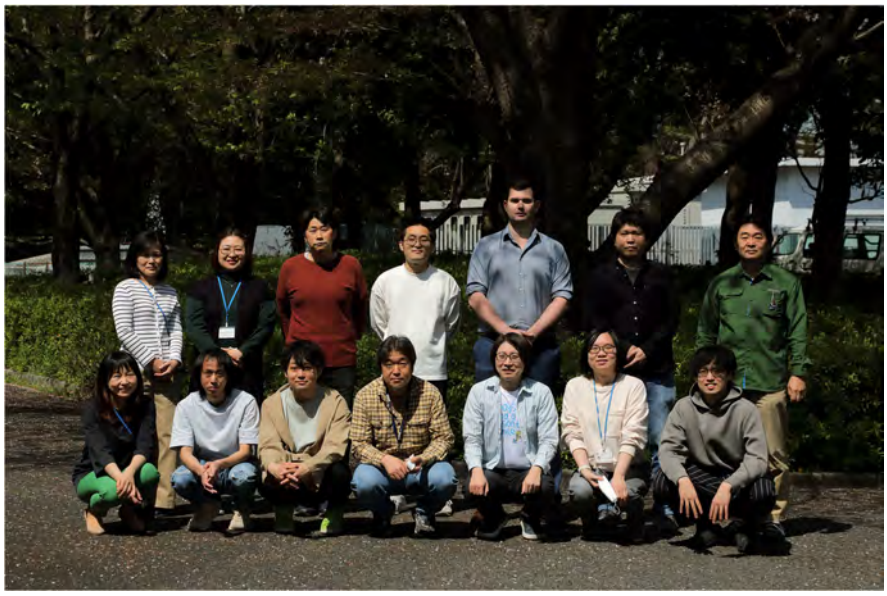
(4) Representative research achievements

(Lab members: double underline; T-CiRA/AMED-CREST members: single underline)

1. Y. Tachida#, H. Hirayama# and T. Suzuki* (2023) Amino acid editing of NFE2L1 by PNGase causes abnormal mobility on SDS-PAGE. ***Biochim. Biophys. Acta General Subjects*** **1867**, 130494. (#= equally contributed) (doi: 10.1016/j.bbagen.2023.130494)
2. Y. Sonoda, A. Fujita, M. Torio, T. Mukaino, A. Sakata, M. Matsukura, K. Yonemoto, K. Hatae, Y. Ichimiya, P. F. Chong, M. Ochiai, Y. Wada, M. Kadoya, N. Okamoto, Y. Murakami, T. Suzuki, N. Isobe, H. Shigeto, N. Matsumoto, Y. Sakai*, and S. Ohga (2024) Progressive myoclonic epilepsy as an expanding phenotype of NGLY1-associated congenital deglycosylation disorder: A case report and review of the literature. ***Eur. J. Med. Genet.*** **67**, 104895. (doi: 10.1016/j.ejmg.2023.104895)
3. S.-T. Li, H. Hirayama, C. Huang, T. Matsuda, R. Oka, T. Yamasaki, D. Kohda and T. Suzuki* (2024) 1 ***The FEBS Journal*** **291**, 884-896. (doi: 10.1111/febs.17011)
4. C. Huang, J. Seino, A. Honda, H. Fujihira, D. Wu, K. Okahara, S. Kitazume, S. Nakaya, K. Kitajima, C. Sato, and T. Suzuki* (2024) Rat hepatocytes secrete free oligosaccharides. ***J. Biol. Chem.*** **300**, 105712. (doi: 10.1016/j.jbc.2024.105712)
5. T. Suzuki NGLY1: the beauty of curiosity-driven science. The 26th International Symposium on Glycoconjugates (Glyco26) at Taipei (Taiwan) August 27th, 2023 (**IGO Hakomori Award Lecture**)

Supplementary

Group photo of RIKEN Glycometabolic Biochemistry Laboratory



Group photo of T-CiRA Ngly1 project Team

Laboratory Homepage

https://www.riken.jp/research/labs/chief/glycometab_biochem/index.html