Glycometabolic Biochemistry Laboratory（2020）
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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.

(1) Current research activities (FY2020) and plan (until Mar. 2025)

N-Glycosylated substrates are synthesized in the endoplasmic reticulum (ER), and their quality is closely monitored by various quality control systems in the ER. Proteins that undergo ERAD are often deglycosylated by Ngly1 during their proteasomal degradation in the cytosol. Consequently, the presence of “non-glycosylated” forms of such proteins after treatment with proteasome inhibitors is widely used as evidence for cytosolic deglycosylation by Ngly1. However, it was found that the accumulation of nonglycosylated RTAΔm, a model ERAD substrate, was still observed in mouse cells lacking cytosolic de-*N*-glycosylating enzymes, when treated with proteasome inhibitors. It was found that RTAΔm is normally partially *N*-glycosylated, while the nonglycosylated form is rapidly degraded by proteasomes in the cytosol. Our results suggest that the occurrence of 'nonglycosylated' ERAD substrates upon treatment with proteasome inhibitors is not necessarily a clue for cytosolic deglycosylation [1].

N-glycanase 1 (NGLY1) deficiency, an autosomal recessive disease caused by mutations in the *NGLY1* gene, is characterized by developmental delay, hypolacrima or alacrima, seizure, intellectual disability, movement disorders and other neurological phenotypes. Because of few animal models that recapitulate these clinical signatures, the mechanisms of the onset of the disease and its progression are poorly understood, and the development of therapies is hindered. In a collaboration with Takeda Pharmaceutical Co. (T-CiRA project), we successfully generated the systemic *Ngly1*-KO rats, which showed developmental delay, movement disorder, somatosensory impairment and scoliosis. These phenotypes in *Ngly1*-KO rats are consistent with symptoms in human patients. In accordance with the pivotal role played by NGLY1 in ERAD processes, loss of Ngly1 led to accumulation of cytoplasmic ubiquitinated proteins, a marker of misfolded proteins in the neurons of the central nervous system of *Ngly1*-KO rats. Histological analysis identified prominent pathological abnormalities, including necrotic lesions, mineralization, intra- and extracellular eosinophilic bodies, astrogliosis, microgliosis and significant loss of mature neurons in the specific brain regions of *Ngly1*-KO rats. Axonal degradation in the sciatic nerves was also observed, as in human subjects. *Ngly1*-KO rats, which mimic the symptoms of human patients, will be a useful animal model for preclinical testing of therapeutic options and understanding the detailed mechanisms of NGLY1 [2].

Ngly1-KO is embryonic lethal in C57BL/6 mice background, while additional deletion of the *Engase* gene, encoding another cytosolic deglycosylating enzyme (endo-β-*N*-acetylglucosaminidase; ENGase), partially rescued the lethality (Fujihira, *et al.* ***PLoS Genet.*** 2017). We hypothesized that, upon compromised Ngly1 activity, ENGase-mediated deglycosylation of misfolded glycoproteins may cause excess formation of *N*-GlcNAc proteins in the cytosol, leading to detrimental effects in the mice (*N*-GlcNAc hypothesis; **Figure**) and indeed, we have shown the formation of *N*-GlcNAc proteins using a model ERAD substrate (Huang, *et al.* ***PNAS*** 2015). Whether endogenous *N*-GlcNAc proteins are really formed in *Ngly1*-KO cells/animals or not remains unclarified. We therefore established a collaboration with Prof. Alma Burlingame/Dr. Jason C. Maynard (UCSF, USA), to aim at comprehensive identification of *O*- and *N*-GlcNAc proteins using cytosol prepared from wild type, *Ngly1*-KO, *Engase*-KO, and *Ngly1/Engase* double KO mouse embryonic fibroblasts. It was revealed that while there is no dramatic change in the level of *O*-GlcNAc proteins among cells examined, there was a vast increase of *N*-GlcNAc proteins in *Ngly1*-KO cells upon proteasome inhibition. Importantly, few *N*-GlcNAc proteins were observed in *Engase*-KO or *Ngly1/Engase* double-KO cells, clearly indicating that the cytosolic ENGase is responsible for the formation of *N*-GlcNAc proteins. The excess formation of *N*-GlcNAc

proteins may at least in part account for the pathogenesis of NGLY1-deficiency [3].

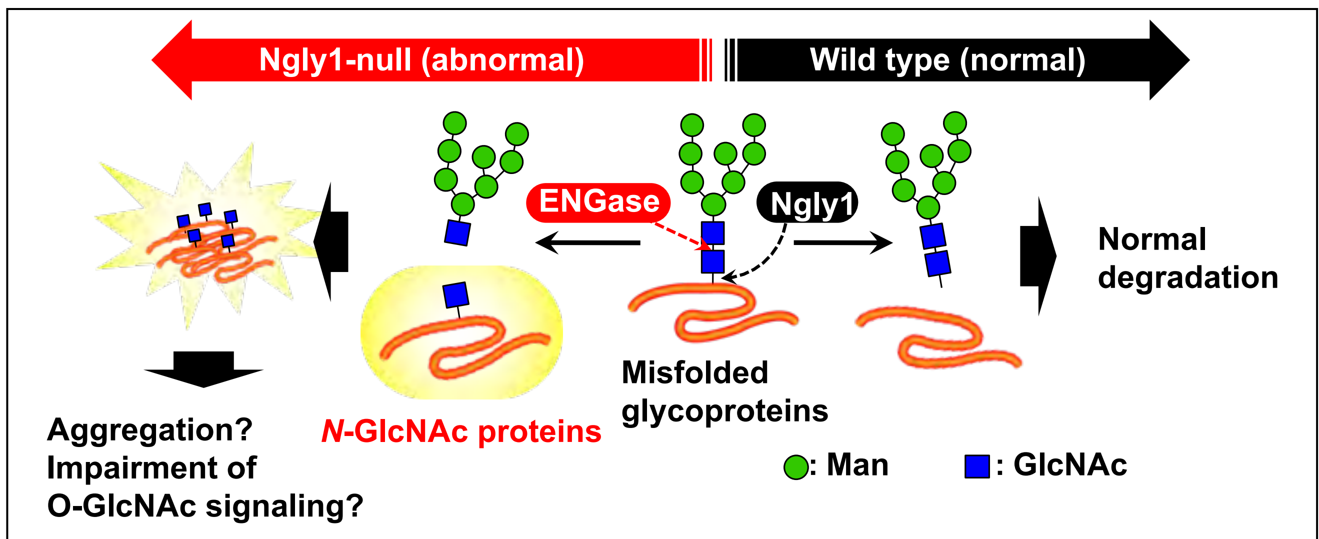

Figure *N*-GlcNAc hypothesis: Under normal conditions, deglycosylation of misfolded proteins are mainly catalyzed by Ngly1, and proteins were degraded by proteasomes. On the other hand, under conditions where Ngly1 activity was compromised, ENGase can stochastically act on misfolded glycoproteins, forming *N*-GlcNAc proteins. Excess formation of N-GlcNAc proteins may induce formation of toxic protein aggregates, or impair O-GlcNAc mediated signal transduction, leading to cytotoxicity, and this may at least in part explain the pathogenesis of NGLY1 deficiency.

Previously we reported that NGLY1 regulates *Drosophila* BMP signaling in a tissue-specific manner (Galeone et al., ***eLife*** 2017). As a collaborative effort with Prof. Hamed Jafar-Nejad (Baylor College of Medicine, USA), Dr. Antonio Galeone (Univ. of Milan, Italy), Prof. Markus Affolter (Univ. of Basel, Switzerland) and Dr. Cathleen M Lutz (The Jackson Laboratory, USA), we established the *Drosophila* Dpp and its mouse ortholog BMP4 as biologically relevant targets of NGLY1 and found, unexpectedly, that NGLY1-mediated deglycosylation of misfolded BMP4 was required for its retrotranslocation. Accumulation of misfolded BMP4 in the ER results in ER stress and prompts the ER recruitment of NGLY1. The ER-associated NGLY1 then deglycosylates misfolded BMP4 molecules to promote their retrotranslocation and proteasomal degradation, thereby allowing properly-folded BMP4 molecules to proceed through the secretory pathway. Our study redefines the role of NGLY1 during ERAD and suggests that impaired BMP4 signaling might underlie some of the NGLY1 deficiency patient phenotypes [4] (results obtained at T-CiRA project).

Translocation of soluble protein into the ER is regulated by the so-called signal peptide, an intrinsic peptide sequence for targeting the protein into the ER lumen, and accordingly, proteins without signal peptide has been considered not to be translocated into the ER lumen. Nevertheless, there are reports suggesting the occurrence of soluble proteins in the ER lumen while it lacks the recognizable signal peptide sequence. The molecular mechanism of the signal peptide-independent translocation in to the ER remained unclarified. We identified Ste24 as a factor to suppress the signal peptide-independent translocation into the ER. We also identified Rme1, a protein without a signal peptide, can be translocated into the ER in ste24Δ cells [5]. (Collaboration between Dr. Akira Hosomi (Shinshu University), Dr. Kazuko Iida (Tokyo Metropolitan Institute for Medical Sciences), and Prof. Hidekazu Iida (Tokyo Gakugei University)).

In the future, we will aim at clarifying the molecular mechanism for the catabolism of free *N*-glycans and its precursors (dolichol-linked oligosaccharides). We will also aim at unveiling the species-specific glycan biosynthetic and degradation pathway, to provide insight into the functional importance of glycans from the standpoint of “comparative glycobiology”. In addition, we will clarify the pathophysiology of Ngly1-KO mice and also contribute to develop the therapeutic means for NGLY1 deficiency through T-CiRA joint program.

(3) Members

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Fuka Onoue

(4) Representative research achievements

1. C. Huang and T. Suzuki* (2020) The occurrence of nonglycosylated forms of *N*-glycoprotein upon proteasome inhibition does not confirm cytosolic deglycosylation. ***FEBS Lett***. **594,** 1433-1442 (doi: 10.1002/1873-3468.13734.).
2. M. Asahina, R. Fujinawa, S. Nakamura, K. Yokoyama, R. Tozawa, and T. Suzuki* (2020) *Ngly1−/−* rats develop neurodegenerative phenotypes and pathological abnormalities in their peripheral and central nervous systems. ***Hum. Mol. Genet.*** **29,** 1635-1647 (doi: 10.1093/hmg/ddaa059.).
3. J. C. Maynard, H. Fujihira, G. E. Dolgonos, T. Suzuki* and A. L. Burlingame (2020) Cytosolic *N*-GlcNAc proteins are formed by the action of endo-β-*N*-acetylglucosaminidase. ***Biochem. Biophys. Res. Commun.*** **530,** 719-724 (doi: 10.1016/j.bbrc.2020.06.127.).
4. A. Galeone, J. M. Adams, S. Matsuda, M. F. Presa, A. Pandey, S. Y. Han, Y. Tachida, H. Hirayama, T. Vaccari, T. Suzuki, C. Lutz, M. Affolter, A. Zuberi, and H. Jafar-Nejad* (2020) Regulation of BMP4/Dpp retrotranslocation and signaling by deglycosylation. ***eLife*** **9,** e55596 (doi: 10.7554/eLife.55596.).
5. A. Hosomi, K. Iida, T. Cho, H. Iida, M. Kaneko, and T. Suzuki* (2020) The ER-associated protease Ste24 prevents N-terminal signal peptide-independent protein translocation into the ER in *Saccharomyces cerevisiae*. ***J. Biol. Chem.*** **295,** 10406-10419 (doi: 10.1074/jbc.RA120.012575.).

Supplementary

Group photo of RIKEN Glycometabolic Biochemistry Laboratory

Group photo of T-CiRA Ngly1 project

Laboratory Homepage

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