Glycometabolic Biochemistry Laboratory (2022)

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(0) Research field
CPR Subcommittee**:** Biology
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(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 (FY2022) and plan

NGLY1 is a molecule whose activity was first described in 1993, and its gene was identified in 2000, both by Suzuki and colleagues. While its physiological role and biological significance have been enigmatic for a long time, NGLY1 research has been greatly accelerated since the discovery of a human genetic disorder called NGLY1 deficiency, largely thanks to the devoting efforts by research foundations established by the patient's families. Upon request by the Japanese Biochemical Society, Suzuki and Yukiko Yoshida, Chief Scientist in Tokyo Metropolitan Institute of Protein Science, organized a special issue in ***J Biochem*** [1]. In this issue, Fujihira *et al.* summarized the NGLY1 research progress using model animals such as mice and rats, and Hirayama and Suzuki overviewed the current knowledge on the assay method for NGLY1 (PNGase) as well as the biomarker for NGLY1 deficiency [2, 3].

Precise determination of NGLY1 activities in patient-derived cells will be very critical for NGLY1 research. Currently, HPLC-based identification of reaction product using labeled glycopeptide is widely used for NGLY1 assay. This method however is often not accessible for non-experts, and therefore the development of a facile NGLY1 assay that can be used in clinical laboratory settings has been an urgent demand. Here Prof. Tsuyoshi Takahashi (Gunma University) and others developed a method to identify enzyme activity using a system called intein that autocatalytically splice protein through transpeptidation reactions. In this assay, it is designed that NGLY1-mediated deglycosylation leads to peptide splicing, and resultant NanoLuc luciferase activity is quantitated, thereby indirectly measuring NGLY1 activity. With this new method, activity of 0.2 nM PNGase derived from budding yeast was accurately detected. This method also was able to detect PNGase activity from crude extract of HeLa cells overexpressing NGLY1. Moreover, by fusing substrate glycopeptide with cell-penetrating nona-arginine (Arg) peptide, detection of cellular NGLY1 activity in NGLY1-overexpressing cells was achieved (Figure). This new probe can serve as a new modality to detect PNGase (NGLY1) activity without using HPLC [4].

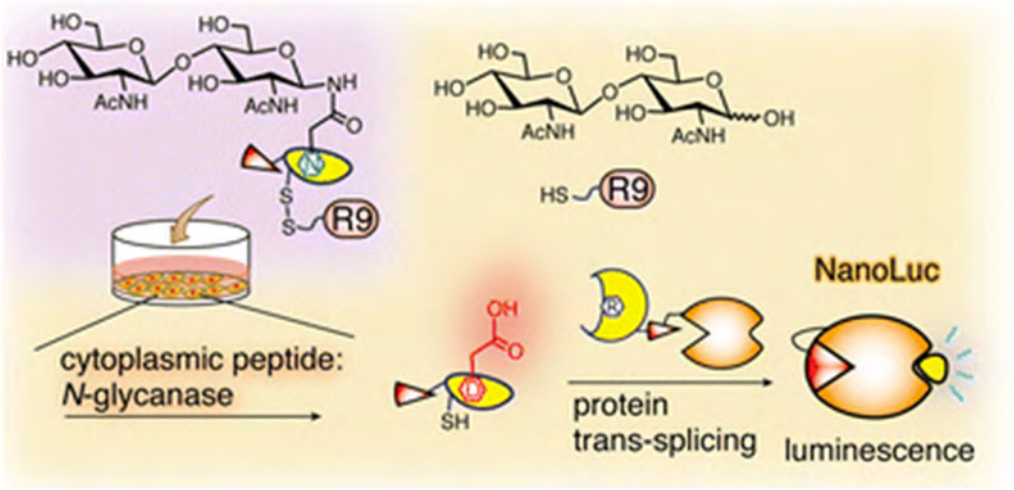

Figure: Outline of the method for cellular PNGase assay using the intein system [4]

Intein is a protein catalyzing “protein splicing”, and intein typically has two separate domains, and by meeting these two domains together, they ended up conjugating the two exteins (In this figure, NanoLuc luciferase is an extein, which was originally separated by two fragments). This reaction was called as PTS (protein transsplicing), and in this assay, GlcNAc2 is introduced in N-terminal intein, keeping it from binding to C-terminal intein. When PNGase act on the N-terminal intein, binding of the two inteins, followed by PTS reaction, was promoted, resulting in strong luminescence by NanoLuc, thereby indirectly measuring NGLY1 activity. In cellular assays, N-terminal intein is conjugated with nona-arginine (Arg9), promoting the cell penetration of the N-terminal intein, allowing the PTS reaction to occur inside cells.

N-linked glycans are attached to the protein in the endoplasmic reticulum (ER), and their structures were modified by enzymes in the ER and/or Golgi, and it has been well described that these glycan structures affect intra- or intercellular transport or localization of N-glycoproteins. Golgi is known to be fragmented during cell division, during which the secretory pathway has been impaired. It has been totally unknown as to how the glycosylation is regulated during Golgi fragmentation. As a collaborative effort with Prof. Yanzhang Wang (Michigan Univ), we clarified that Man1A1, one of the first enzymes glycoproteins will encounter when they reach Golgi, is phosphorylated by CDK1, resulting in compromised enzyme activity. This will change the glycan processing pathway, and it also reduces interaction between Man1A1 and MGAT1, another processing enzyme acting after Man1A1. These results collectively suggest that the glycosylation is tightly regulated during cell division [5].

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”. 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 program.

(3) Members (FY2022)

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Tadashi Suzuki
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Kenichi Moto
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Akinobu Honda
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Fuka Onoue
(Technical Staff I)
Reiko Fujinawa
Junichi Seino
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(Assistant)
Yuko Suzuki
(Research Part Time Worker II)
Ritsuko Oka
Tsugiyo Matsuda

(4) Representative research achievements

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

1. T. Suzuki***** and Y. Yoshida (2022) Ever-expanding NGLY1 biology. ***J. Biochem.*** **171**, 141-143 (doi: 10.1093/jb/mvab134)
2. H. Fujihira*, M. Asahina, and T. Suzuki (2022) Physiological importance of NGLY1, as revealed by rodent model analyses. ***J. Biochem.*** **171**, 161-167 (doi: 10.1093/jb/mvab101)
3. H. Hirayama* and T. Suzuki (2022) Assay for peptide:*N*-glycanase/NGLY1 and disease-specific biomarkers for diagnoses of NGLY1 deficiency. ***J. Biochem.*** **171**, 169-176 (doi: 10.1093/jb/mvab127)
4. T. Takahashi*, T. Uchibayashi, N. Ishii, I. Matsuo, Y. Yoshida and T. Suzuki (2022) Luminescence detection of peptide:*N*-glycanase activity using engineered split inteins. ***Chem. Commun.*** **58,** 13282-13285 (doi: 10.1039/D2CC04865E)
5. S. Huang#, Y. Haga#, J Li#, J. Zhang, H. K. Kweon, J. Seino, H. Hirayama, M. Fujita, K. W. Moremen, P. Andrews, T. Suzuki, and Y. Wang* (2022) Mitotic phosphorylation inactivates the Golgi mannosidase MAN1A1. ***Cell Rep.*** **41,** 111679 (doi: 10.1016/j.celrep.2022.111679) (#: equally contributed)

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