Catabolism of Glycans

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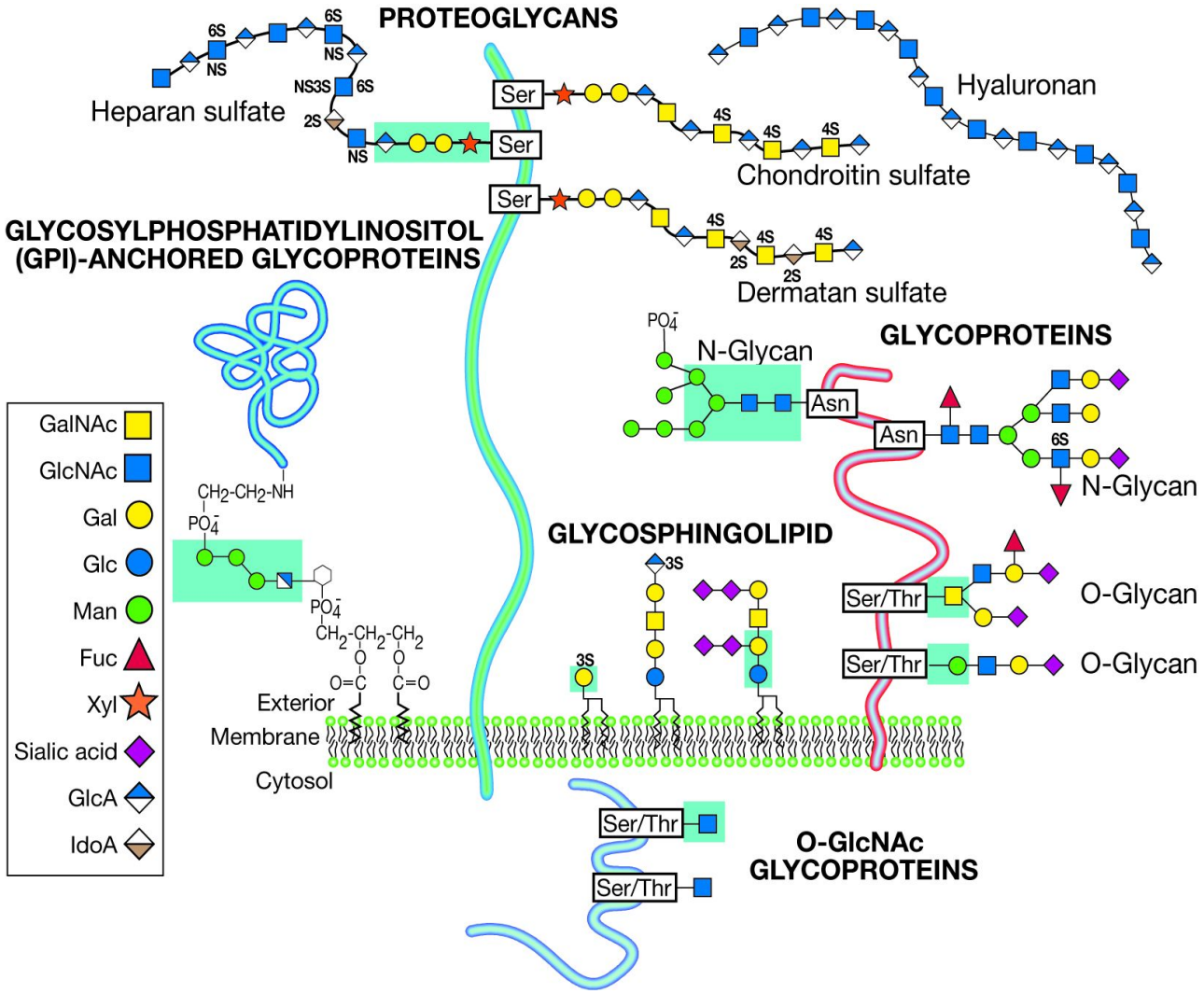
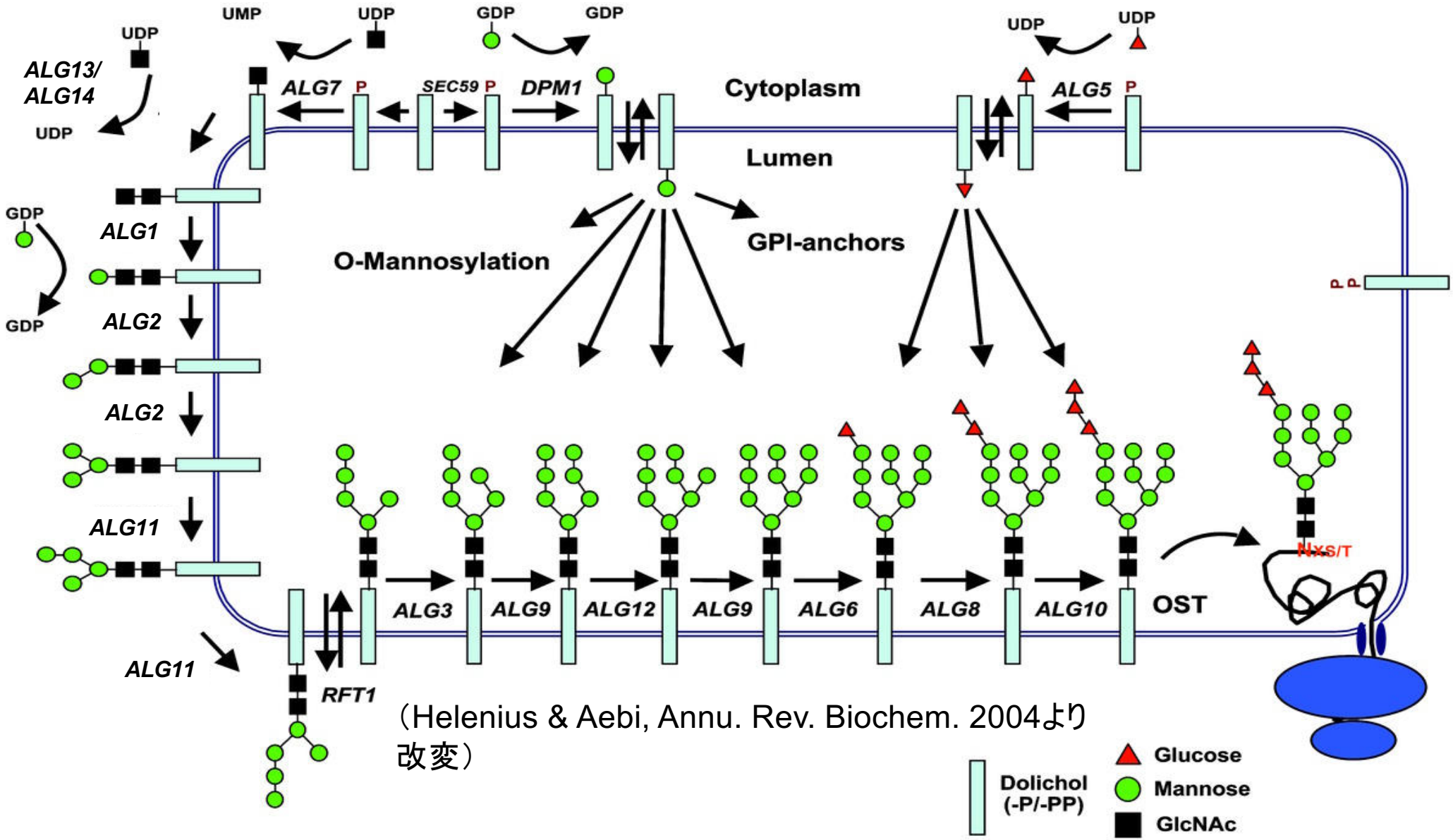
There are various types of glycans…



… but in this lecture we mainly deal with N-linked glycans.

Biosynthesis of *N*-glycans: in mammalian cells, almost all processes have been clarified.



（Helenius & Aebi, Annu. Rev. Biochem. 2004より改変）

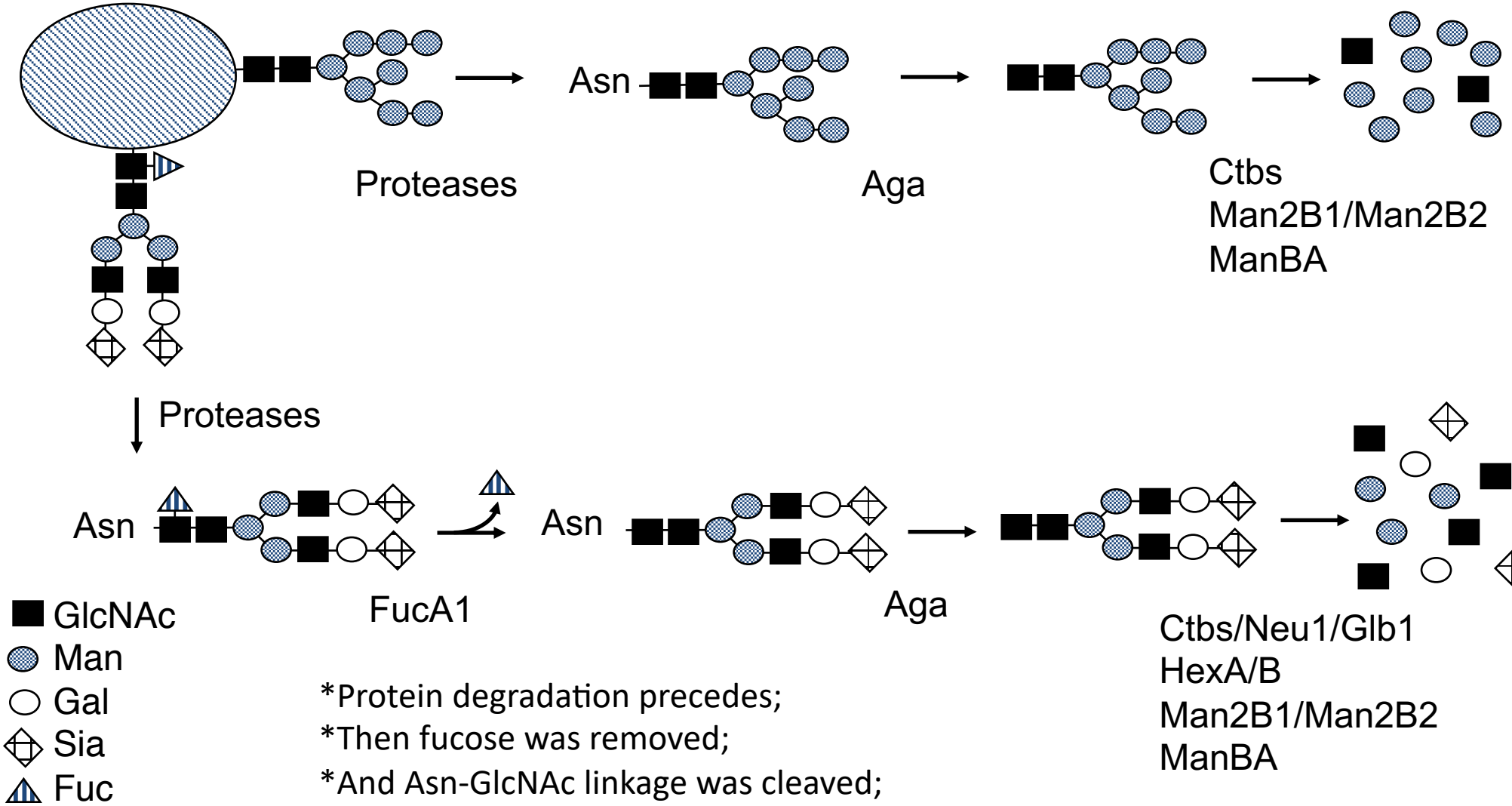
But when it comes to the catabolism of glycans…

*Our textbook knowledge is; “it occurs in the lysosomes”. Period.

*When there is a problem in the lysosomal catabolism of glycans, people will suffer from lysosomal storage diseases, which cause multisystemic problems

ex. Sialidosis, Galactosialidosis, etc.

Lysosomal catabolism of *N*-glycans (Suzuki, T., *Mol. Aspect Med.* 2016)

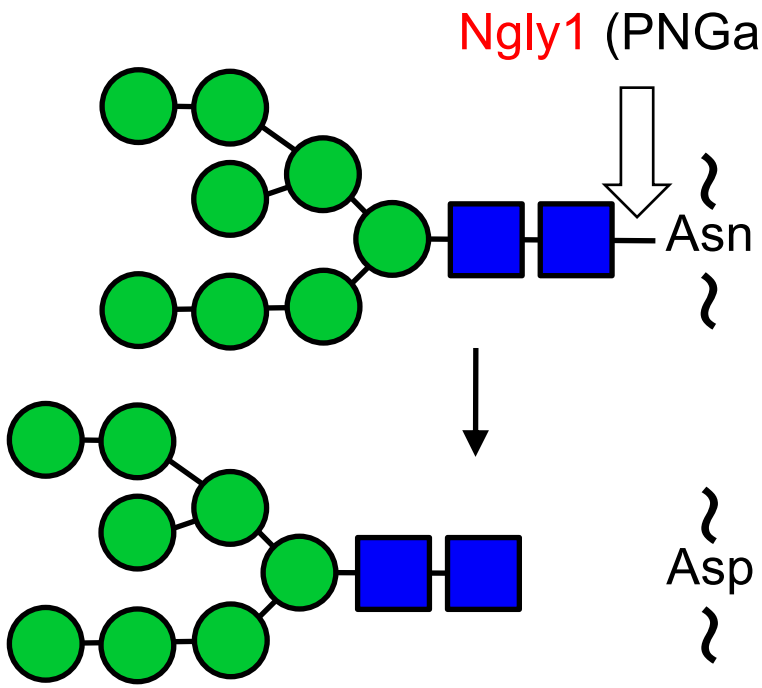


*Protein degradation precedes;
*Then fucose was removed;
*And Asn-GlcNAc linkage was cleaved;
*Which is followed by digestion by various glycosidases

Examples of lysosomal storage diseases which results in abnormal degradation of *N*-linked glycoproteins

Diseases	Defective Enzymes
Fucosidosis	α-Fucosidase（FucA1）
Sialidosis	Sialidase（Neu1）
α-Mannosidosis	α-Mannosidase（Man2B1）
β-Mannosidosis	β-Mannosidase（ManBA）
GM1-gangliosidosis	β-Galactosidase（Glb1）
Sandhoff disease	β-Hexosaminidase A/B（HexA/B）
Aspartylglucosaminuria	Aspartylglucosaminidase

Peptide:*N*-glycanse（PNGase；Ngly1); de-*N*-glycosylating enzymes



PNGase：Plant- or bacteria-derived enzymes have been widely used as a tool reagent to study structures/functions of *N*-glycans on glycoproteins.

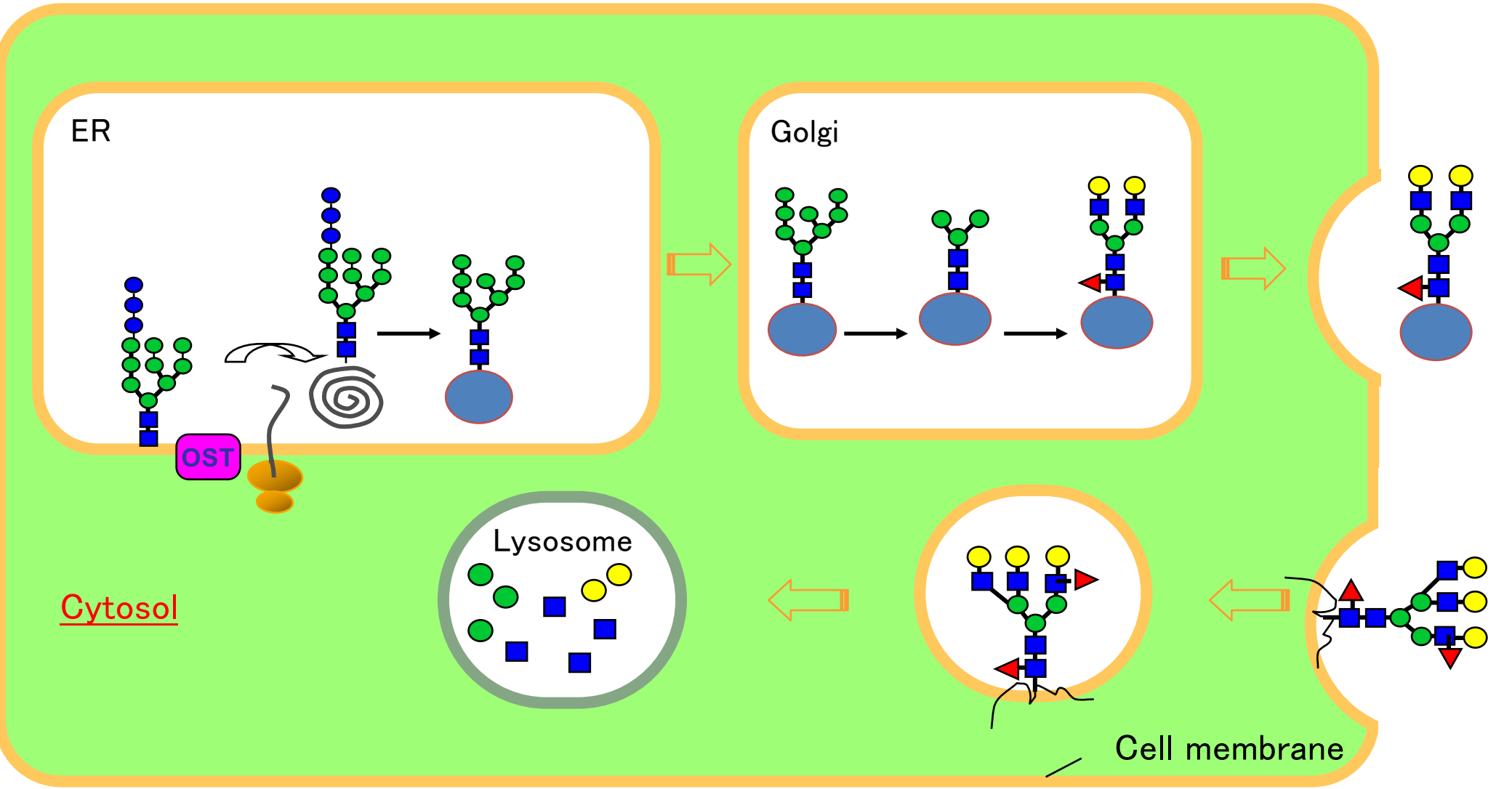
●：Mannose（Man）
■：*N*-acetylglucosamine（GlcNAc）

Prof. Inoue

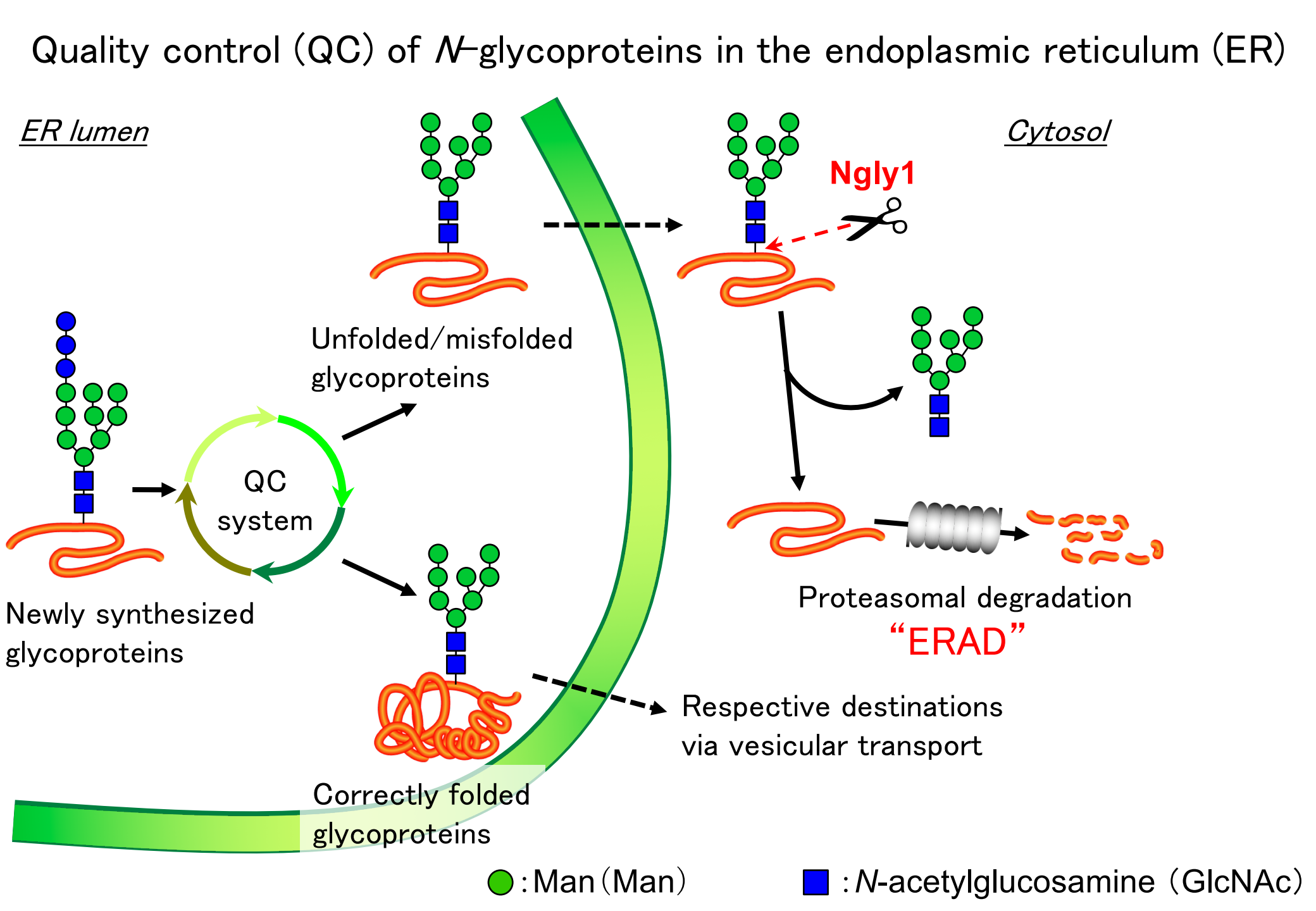
Prof. Lennarz

Discovery of cytosolic PNGase activity
(Suzuki, *et al.*, *BBRC* 1993; *JBC* 1994)
Identification of gene (*PNG1*) encoding the cytosolic PNGase
(Suzuki, *et al.*, *JCB* 2000)
-No obvious phenotypes for *png1* mutant
Ngly1 as a mammalian orthologue of *PNG1*
(Suzuki, *et al.*, *BBRC* 2003)

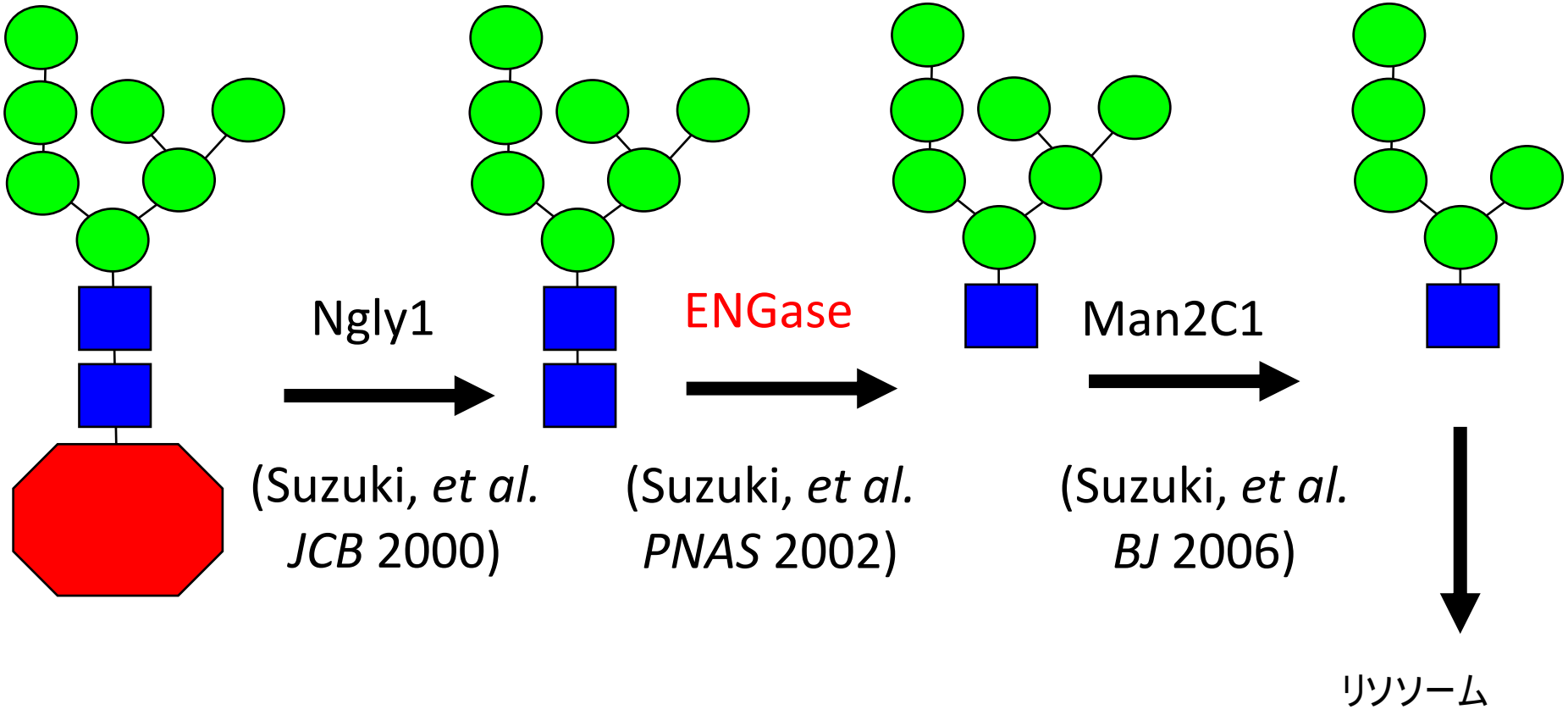
Birth and death of *N*–glycoproteins: Textbook knowledge



Cytosol and white part (where glycoproteins are supposed to be located) are segregated by lipid bilayer


Quality control (QC) of N-glycoproteins in the endoplasmic reticulum (ER)
ER lumen
Cytosol
Ngly1
Unfolded/misfolded glycoproteins
QC system
Newly synthesized glycoproteins
Proteasomal degradation
"ERAD"
Respective destinations via vesicular transport
Correctly folded glycoproteins
: Man (Man)
: N-acetylglucosamine (GlcNAc)

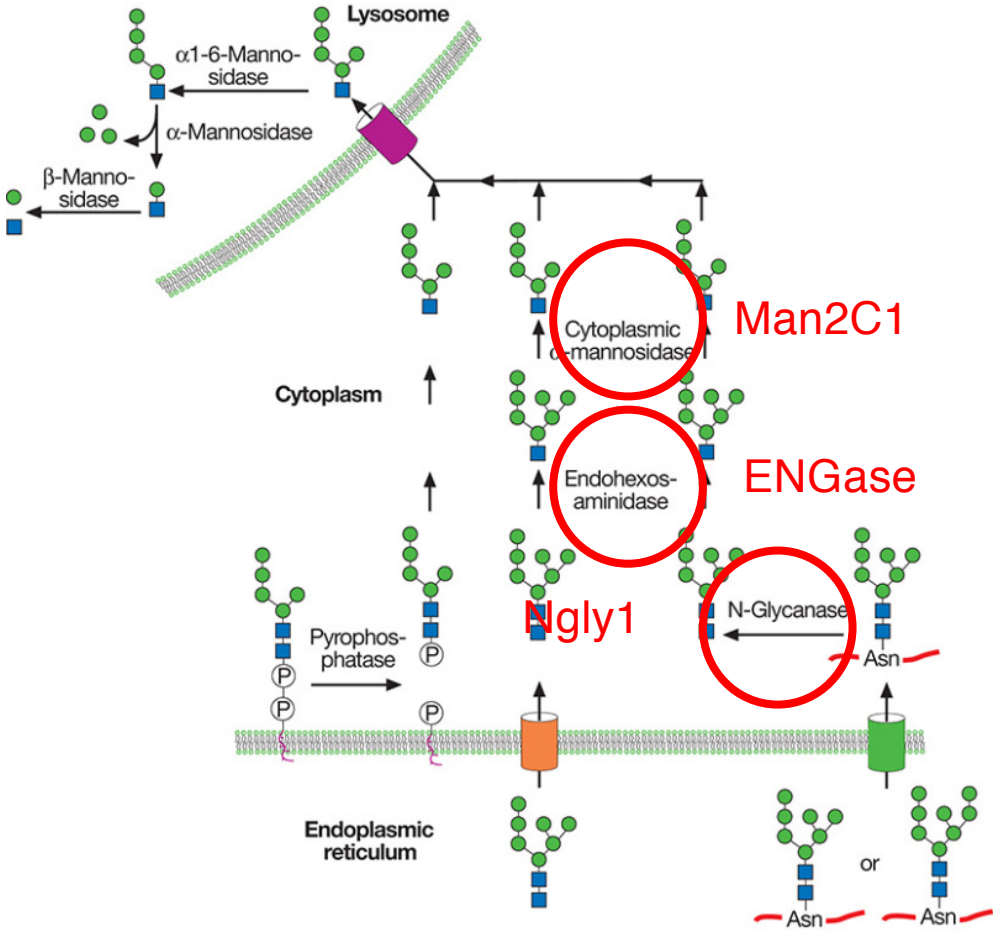
Novel catabolic pathway for glycans released by Ngly1 in the cytosol (“non-lysosomal glycan degradation”)



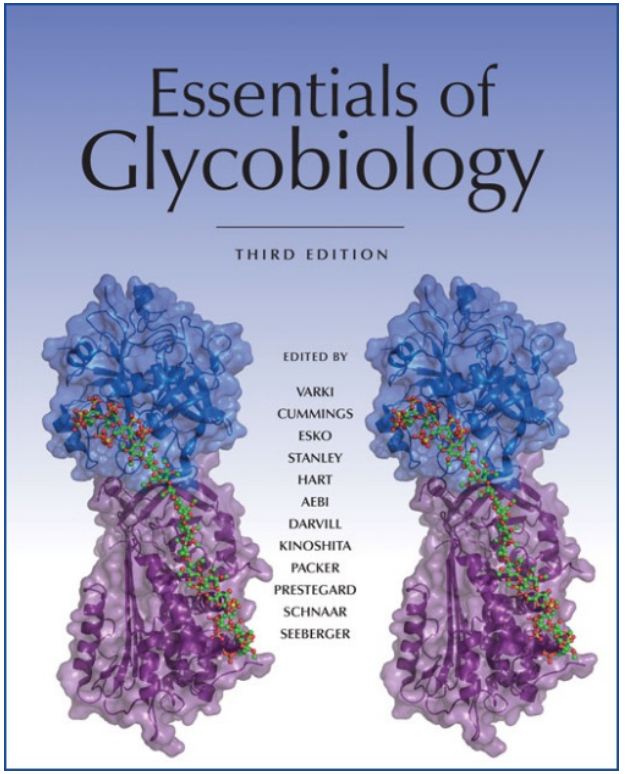
ENGase: endo-β-*N*-acetylglucosaminidase

-Believed to be involved in the downstream of Ngly1-mediated glycan catabolism

Non-lysosomal glycan catabolism-
Now appears in the textbook!



(Figure 39.3)



NGLY1-deficiency was discovered in 2012!

Ngly1-deficiency:
"Kids who don't cry"

(Grace Science Foundation Global NGLY1 Conference, 2017)

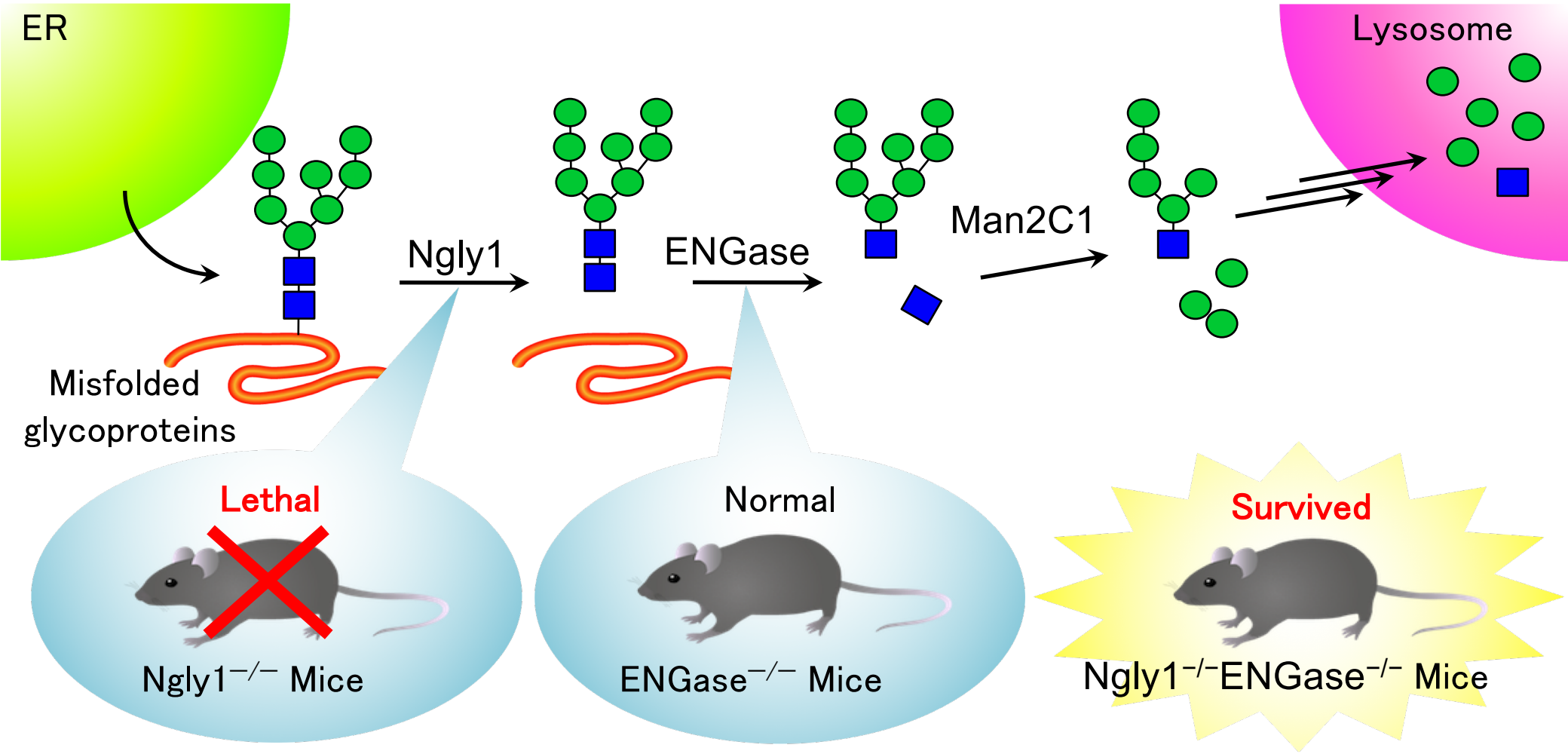
Major symptoms of NGLY1-deficiency:

Hypotonia
Movement Disorder
Epilepsy
Hypoalacrima
Scoliosis
Brain Atrophy/Abnormal EEG etc..

Wilsey Family

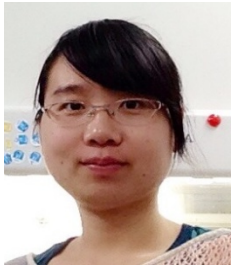
Might Family

Puzzling results on our Ngly1-KO mice（Serendipitous finding）: ENGase-KO partially rescue embryonic lethality of Ngly1-KO mice!



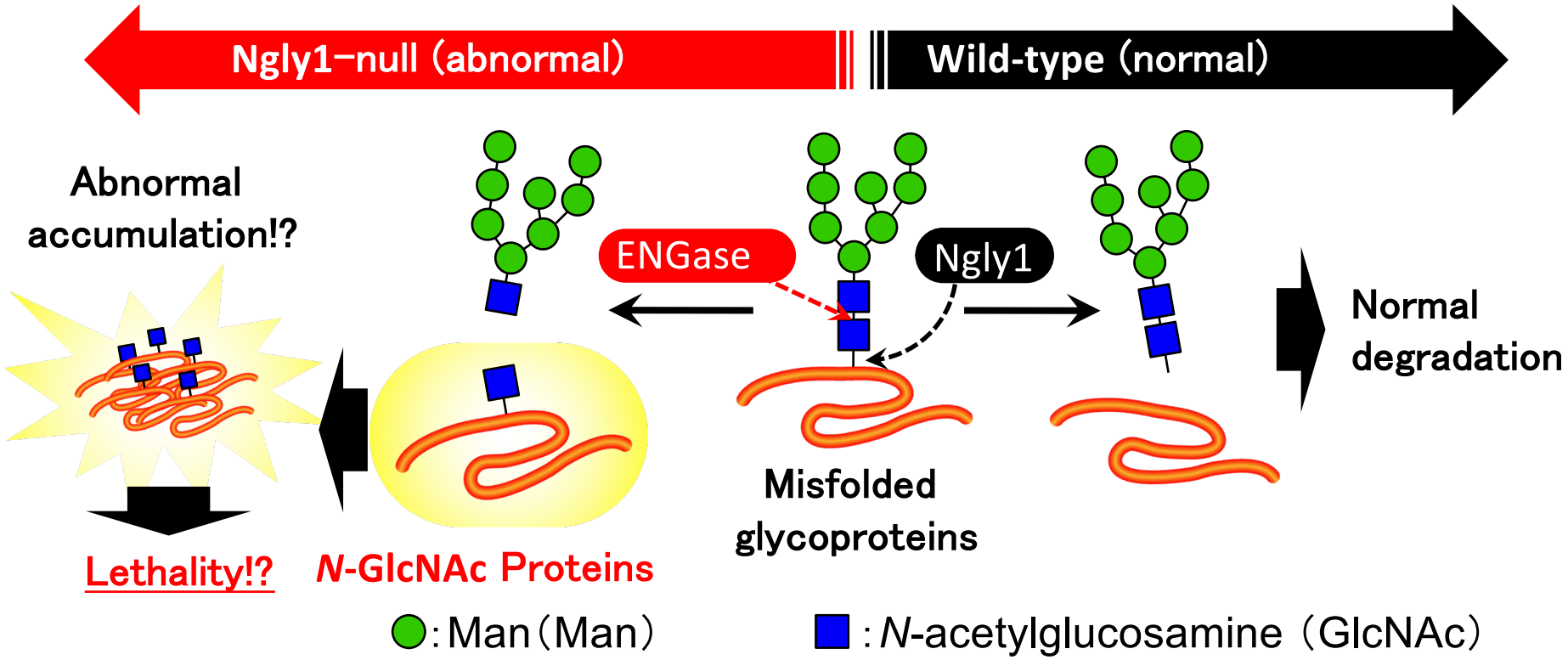
--- ENGase could be a possible therapeutic target for NGLY1-deficiency

Why on earth deletion of *Engase* can rescue the phenotypes of *Ngly1*-KO mice??



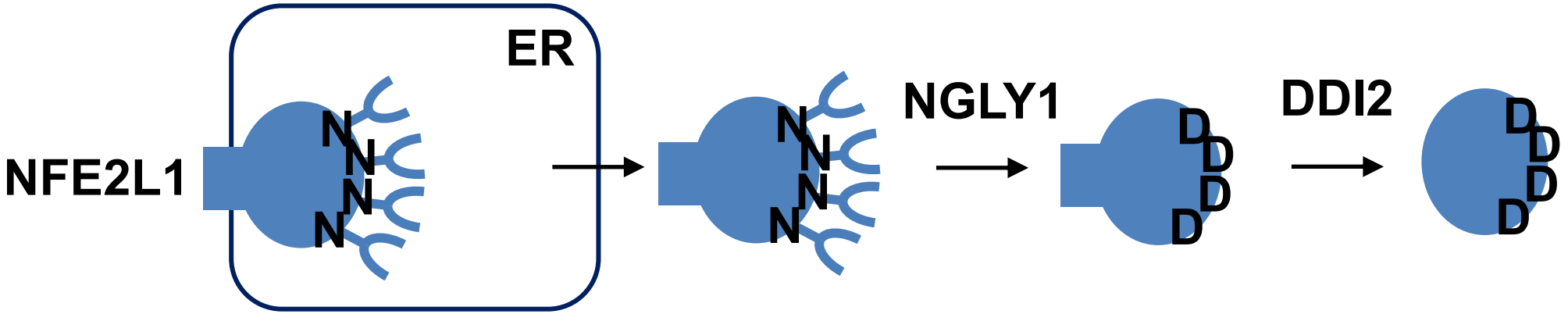
Dr. Huang

"*N*-GlcNAc hypothesis"–in the absence of Ngly1, ENGase could act on some of the misfolded glycoproteins, generating *N*-GlcNAc proteins potentially detrimental to cells/tissues



Huang, et al., (2015) Endo-beta-*N*-acetylglucosaminidase forms N-GlcNAc protein aggregates during ER-associated degradation in Ngly1-defective cells. ***Proc. Natl. Acad. Sci. USA***, 112, 1398-1403.

Question: Is “taking-care-of-junk” all Ngly1 does?



Modulation of activities for transcription factor(s) ??

(Lehrbach and Ruvkun, *eLife* 2016; Tomlin, *et al. ACS Cent. Sci.* 2017)

Possible functions of de-*N*-glycosylating enzymes (PNGase/ENGase) (Suzuki, et al., Glycoconj. J. 1995)

(a) Quality Control of Newly Synthesized *N*-glycosyl Glycoproteins

(b) Modulation of Receptor-ligand Interaction

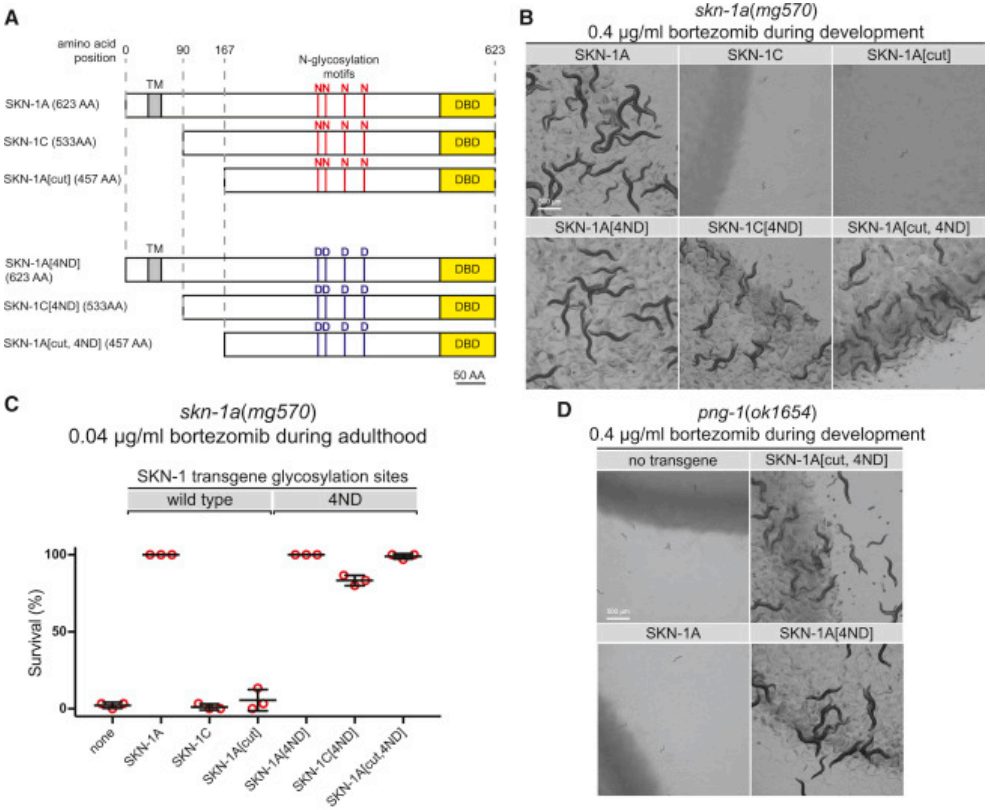
(c) Formation of Bioactive Molecules

(d) Regulation of Proteolytic Processing

(e) Generation of Structural Polymorphism

…Our ”fantasy” comes true after 20+ years!

NGLY1 is a protein-editing enzyme!!



Protein Sequence Editing of SKN-1A/Nrf1 by Peptide:N-Glycanase Controls Proteasome Gene Expression
Lehrbach et al. Cell 177, 737 (2019)

Gary Ruvkun
(Harvard Univ.)

Nic Lehrbach
(Mass. Gen. Hospital)

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 269, No. 26, Issue of July 1, pp. 17611–17618, 1994
Printed in U.S.A.

Purification and Enzymatic Properties of Peptide:*N*-Glycanase from C3H Mouse-derived L-929 Fibroblast Cells

POSSIBLE WIDESPREAD OCCURRENCE OF POST-TRANSLATIONAL REMODIFICATION OF PROTEINS BY *N*-DEGLYCOSYLATION*

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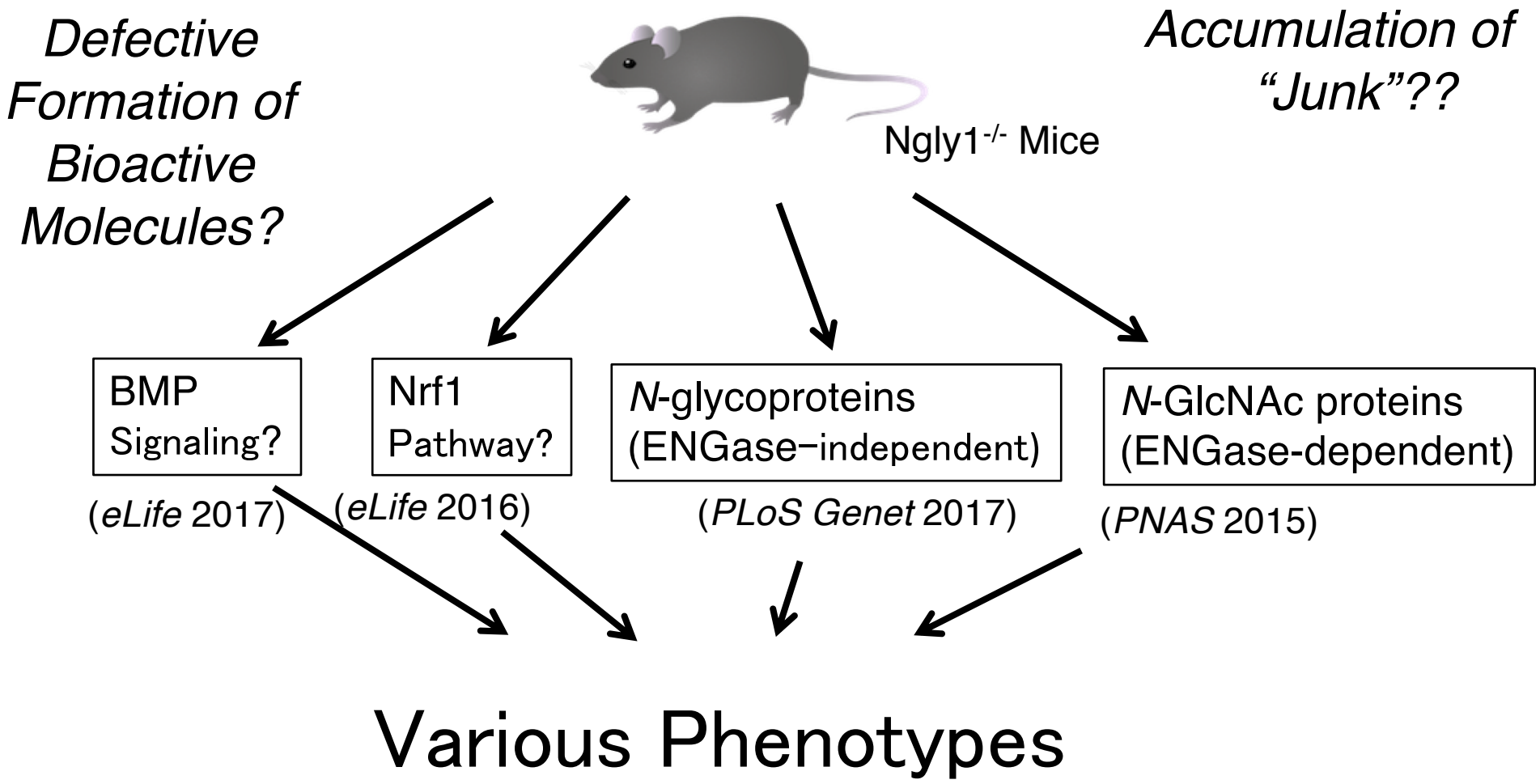
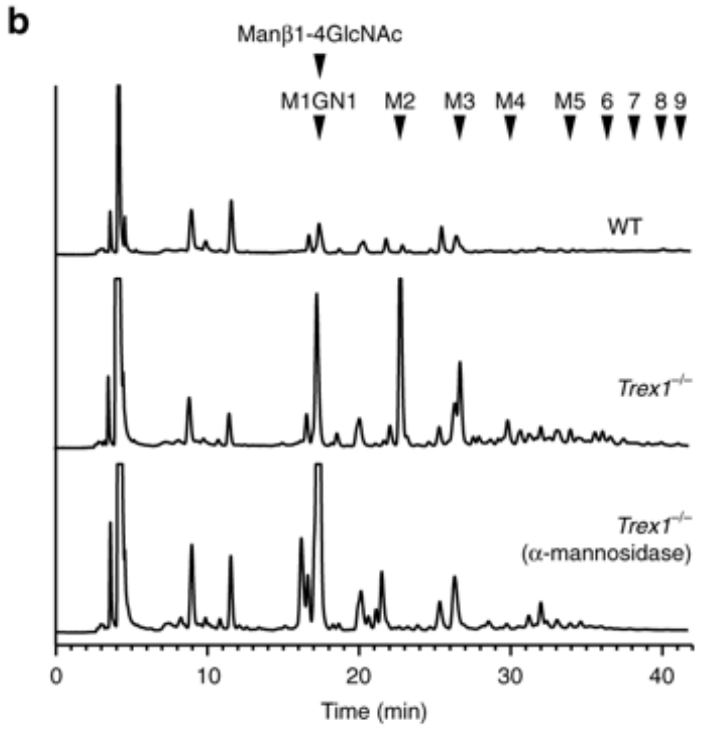
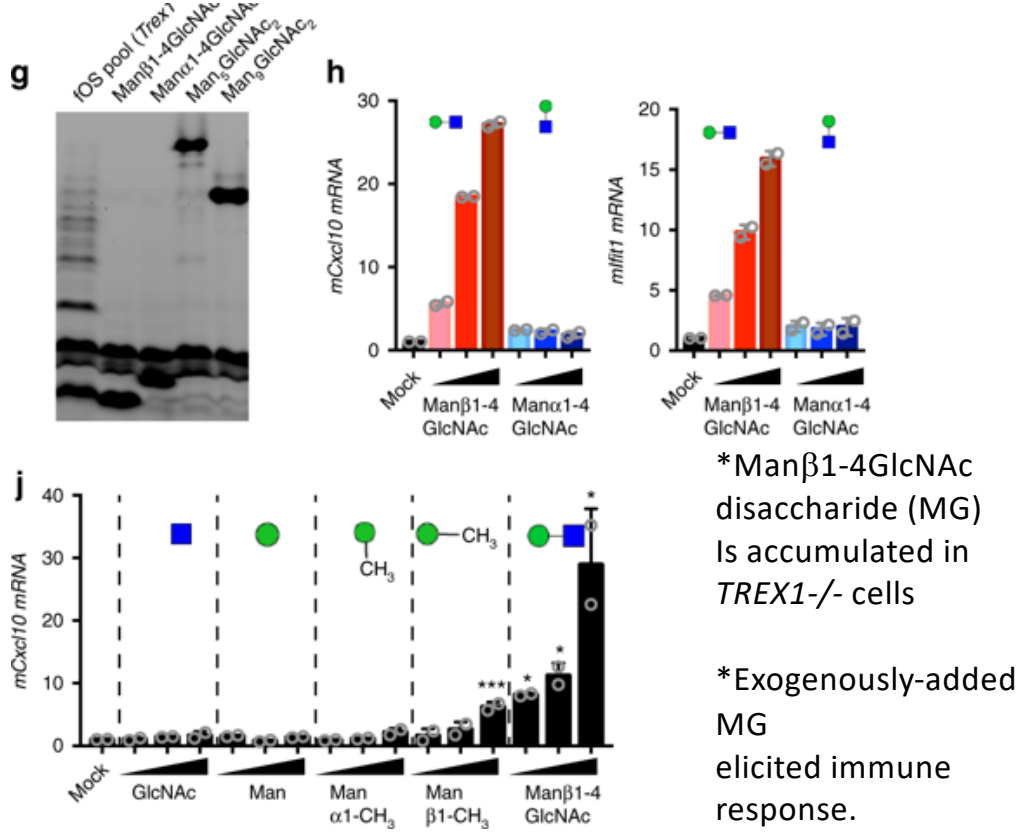
NGLY1-*deficiency* – Caused by Multiple Reasons?



Recent Topics : Degradation intermediate of glycans can elicit an innate immune response!
(Fermaintt, et al., Nature Communications 2019)

TREX1- known as DNase, but also modulates function of oligosaccharyltransferase (OST), and its defect leads to upregulate the formation of free glycans, leading to auto-immune disease.



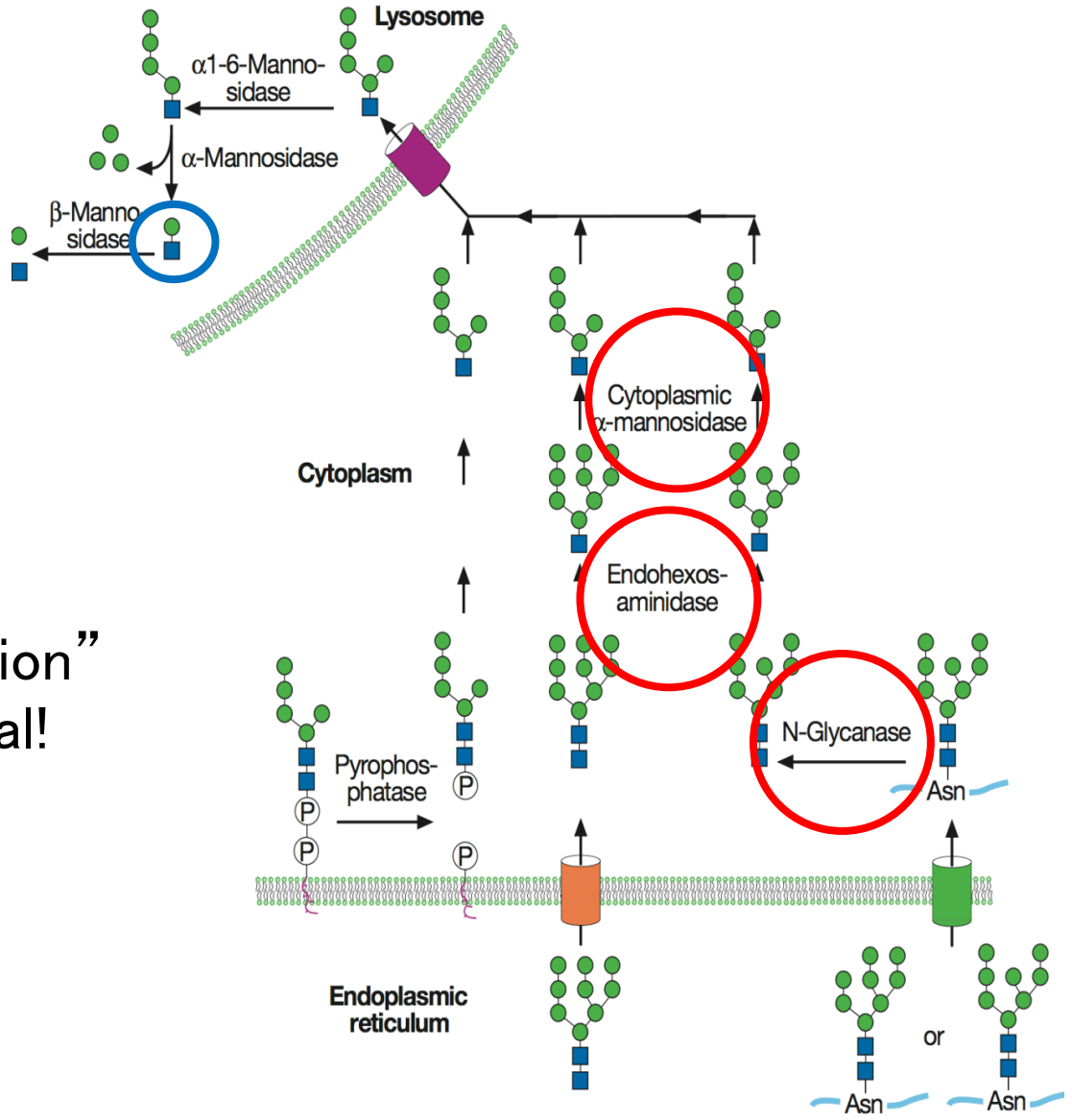


*Manβ1-4GlcNAc disaccharide (MG) Is accumulated in *TREX1-/-* cells

*Exogenously-added MG elicited immune response.

How is this immunoactive disaccharide（Manβ1-4GlcNAc) made?

"Non-lysosomal glycan degradation" may be critical!



Phenotypes of Ngly1-KO mice could be greatly influenced by their genetic background (Fujihira, et al., ***PLoS Genet.*** 2017)

Ms. Negishi-Masahara

Dr. Fujihira

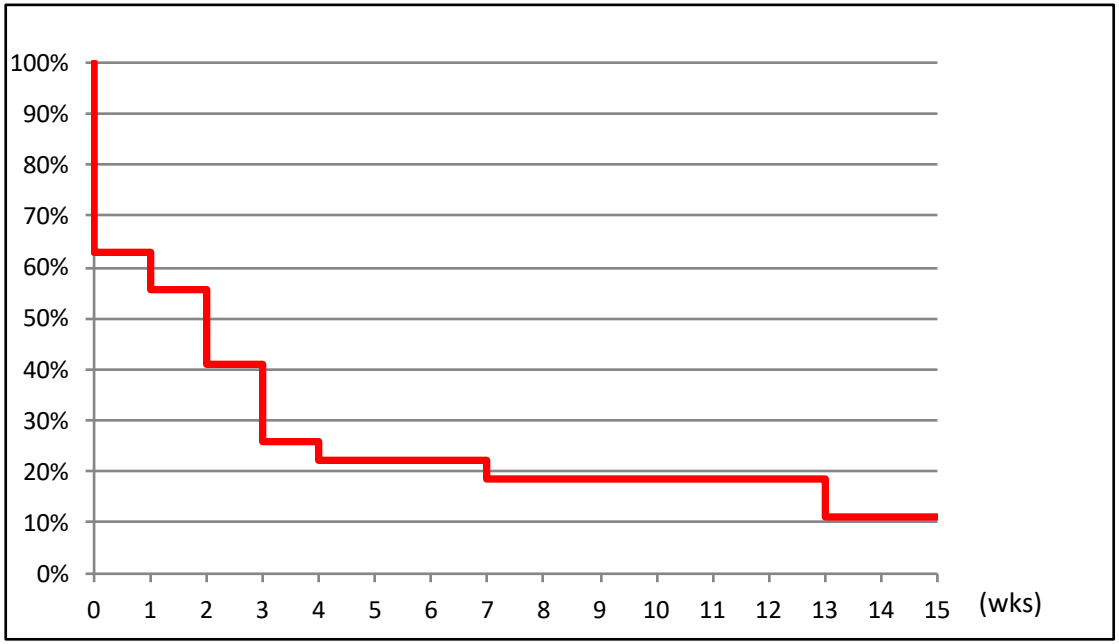
Ngly1-/- mice survive upon crosses with outbred ICR mice !

ICR x B6 (*Ngly1*-/+);
F2 (1 month old)

♂ *Ngly1*-KO

♂ *Ngly*1-WT

Survival Curve for
F2 *Ngly1*-KO mice

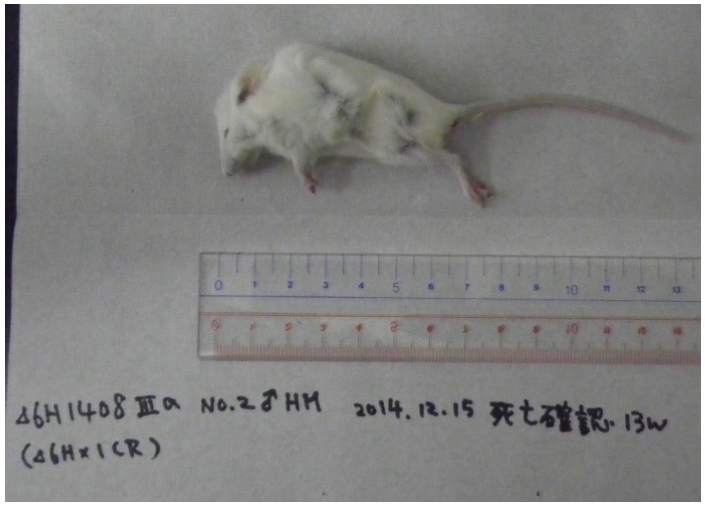


Much severer phenotypes than B6 *Ngly1-/- Engase-/-* KO mice

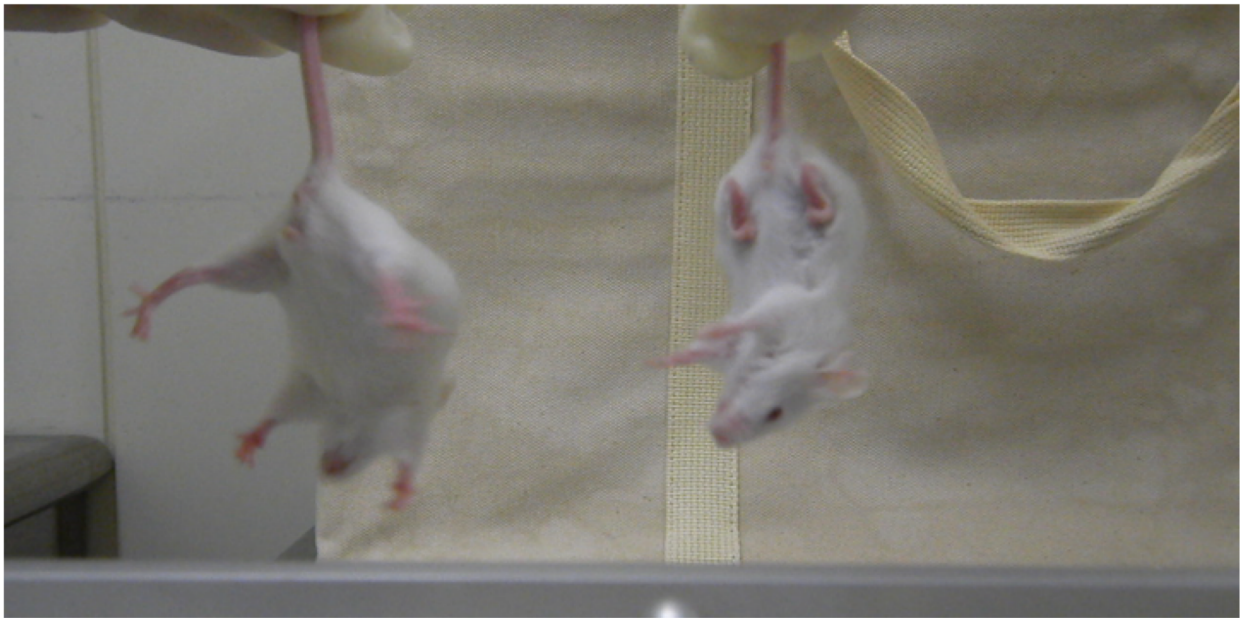
Fujihira, et al., *PLoS Genet.* 2017

Ngly1-/- mice from ICR x C57BL/6

4-wks old
♂WT 20.70g
♂KO 9.58g



Died in 13 wks (Bent spine)



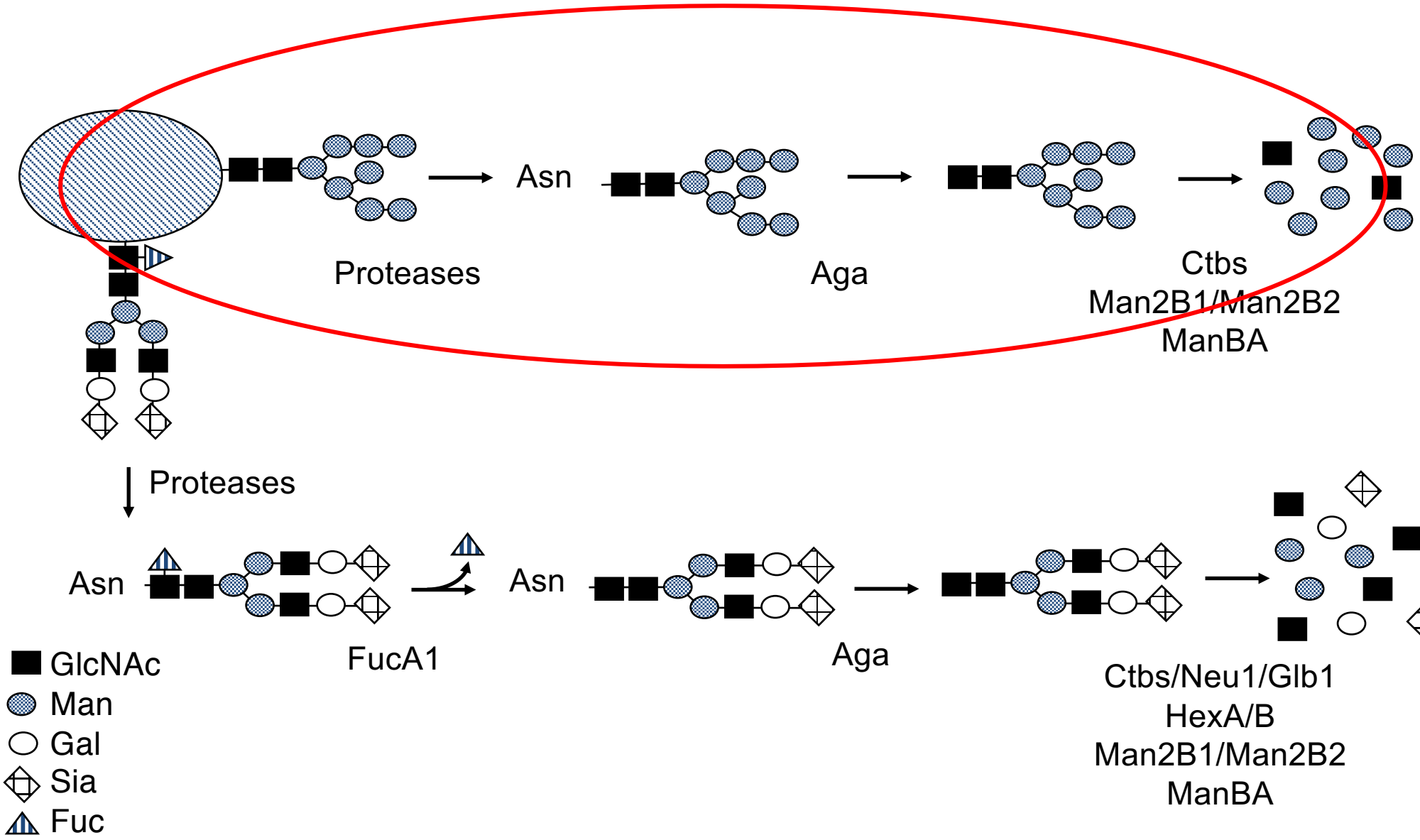
Hindlimb-clasping
(4-wks old; Ngly1-KO mice-right)

Take home message:
Possibility for drug development?

Considering the remarkable impact of the genetic background on phenotypes of *Ngly1-/-* mice,as well as the fact that genotype-phenotype relashionship was not so obvious for NGLY1-deficiency patients, development of effective drug to improve the various symptoms of this disease appears to be an achievable goal.

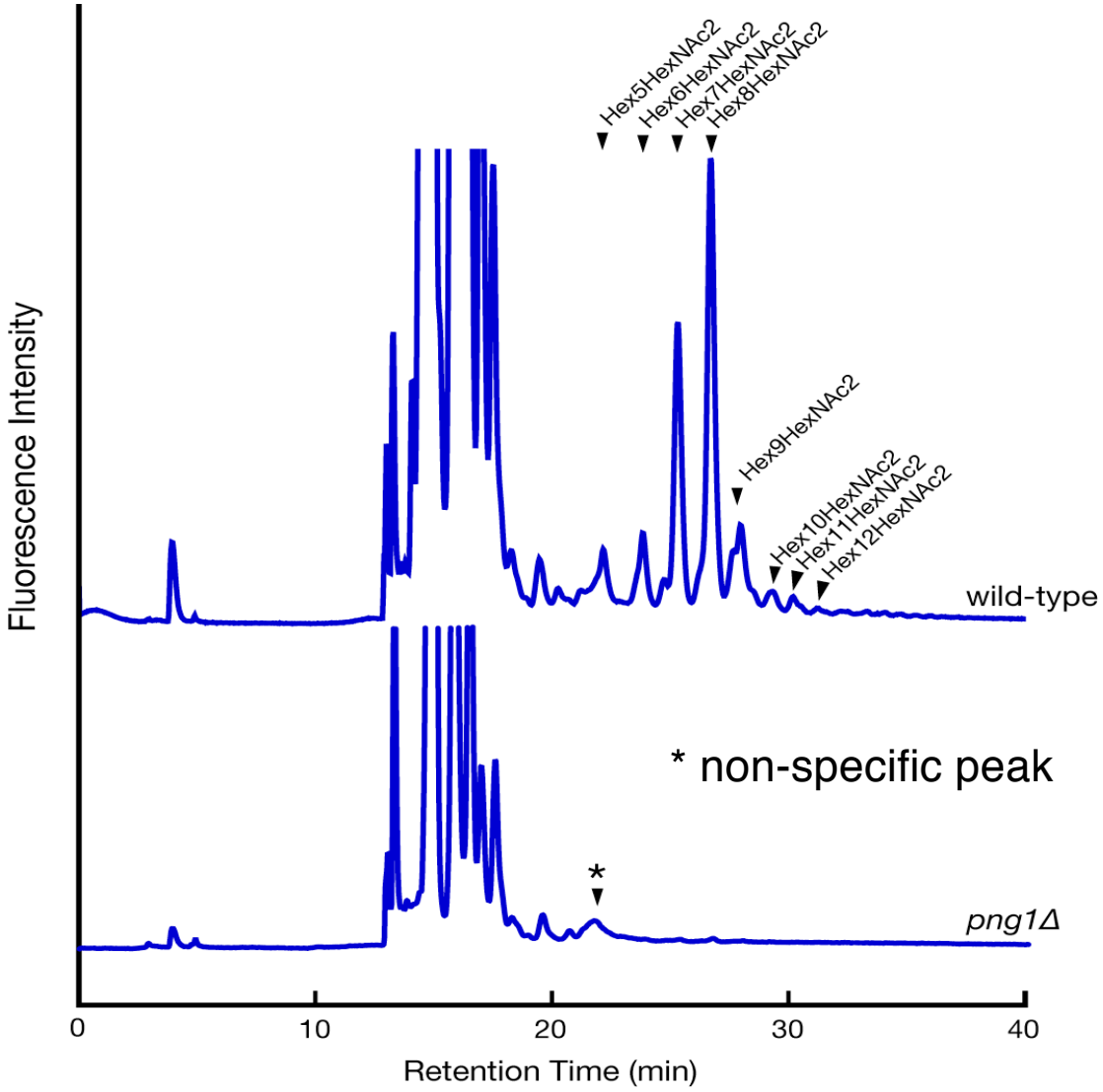
Ex. ENGase inhibitor

Appendix: *lysosomal degradation of N-glycans*: Cases in budding yeast (*S. cerevisiae*)



In budding yeast, almost all “free N-glycans” are formed by the action of the cytosolic PNGase

（they are released from misfolded glycoproteins during ERAD）



(Hirayama, et al. JBC, 2010)

Dr. Hirayama

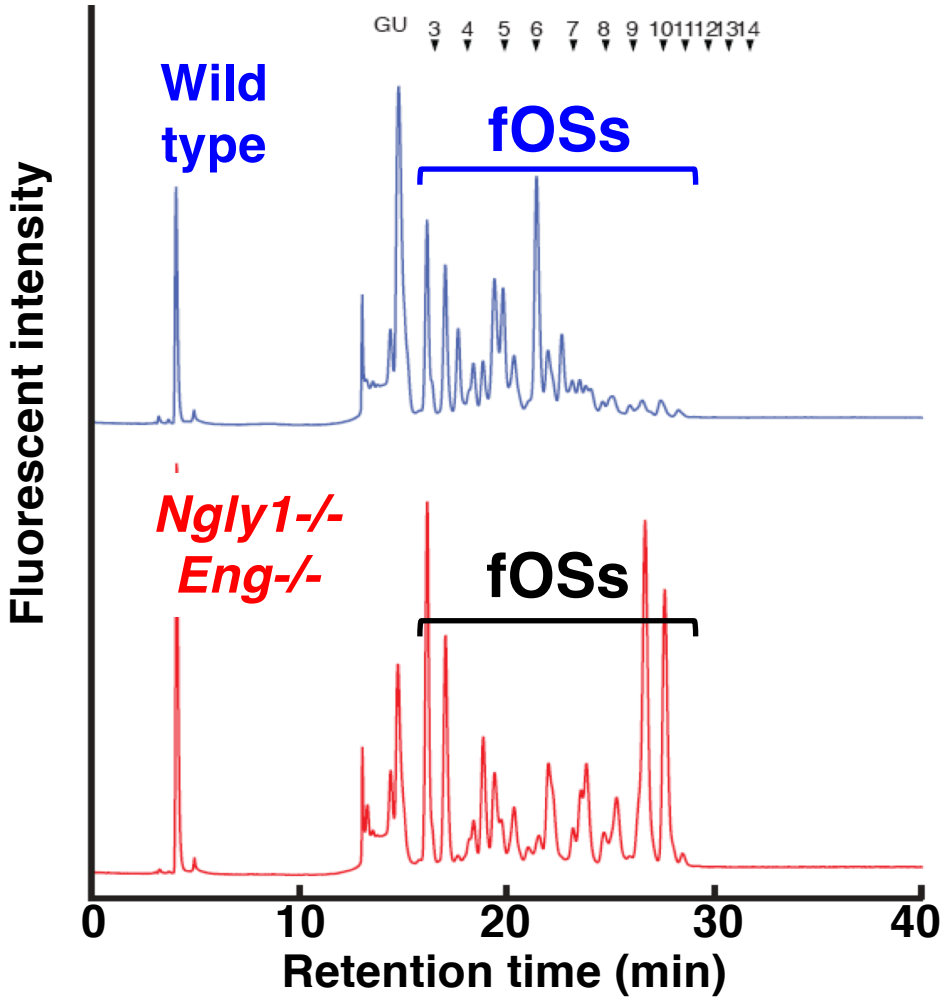
.. But in mammalian cells, almost all free N-glycans are generated in a PNGase-dependent fashion (hydrolytic activity of OST)

To our surprise…

There is NO data suggesting the importance of vacuoles (equivalent to lysosomes) in the degradation of *N*-glycans..

(Doubt the common sense!)

Mice embryonic fibroblasts



(Harada, et al. Glycobiology, 2015)

Glycan catabolisms can be drastically distinct among different organisms!

Dr. Harada

Summary

Our textbook knowledge is that glycan catabolism occurs in the lysosomes. (for your note, that does not apply for budding yeast, the best-characterized eukaryotic organisms).

Recently “non-lysosomal” glycan catabolism that occurs in the cytosol or ER has become also evident. This process is conserved from yeast to mammalian cells.

The cytosolic PNGase (NGLY1), which play a central role of the non-lysosomal glycan catabolism, is essential for normal development in human (which is not the case in yeast). The pathological mechanism for NGLY1-deficiency is, however, very complex.

Acknowledgement

RIKEN Team

T-CiRA Team

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grace science foundation

T-CiRA