Function and disease relevance of glycosyltransferase

Center for Highly Advanced Integration of
Nano and Life Sciences (G-CHAIN)

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Contents

1. Glycan (glycoconjugate) and glycosyl-transferase

2. Alzheimer’s disease and glycosyltransferase

3. Cancer and glycosyltransferase

Contents

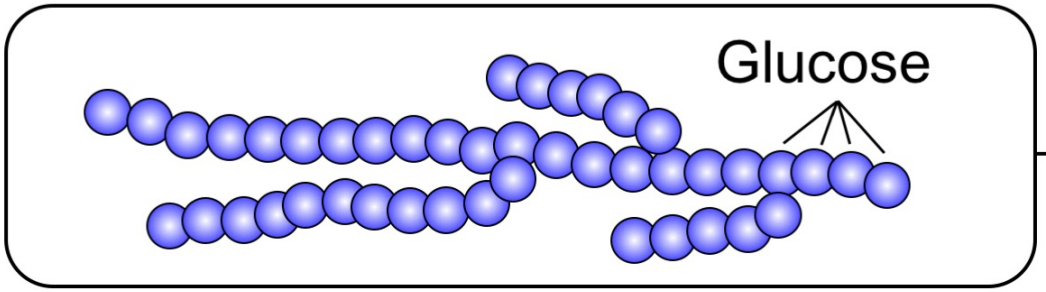
Basics of glycans

Glycan ＝ 「Sugar chain」

Basics of glycans

Homopolysaccharide: energy, structure

Starch, glycogen

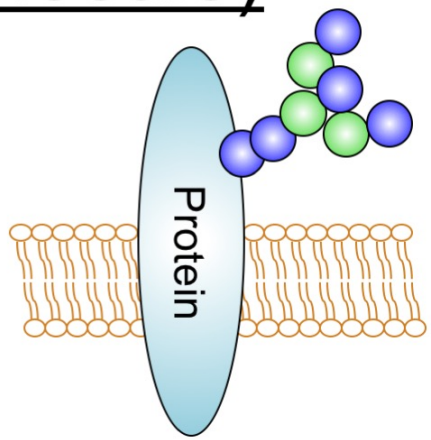


Energy

Cellulose, chitin ——— Fiber, structure

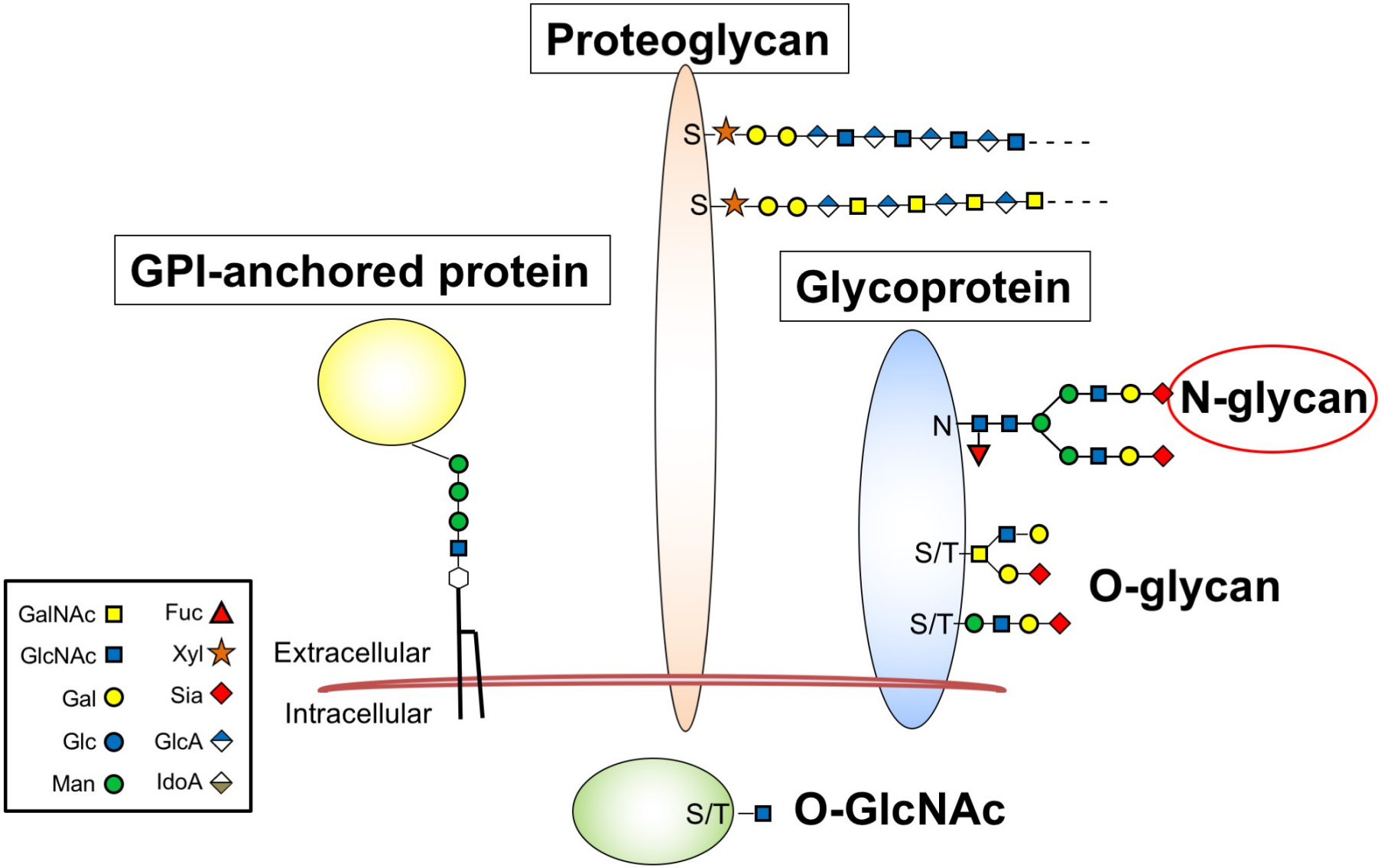
Glycoconjugate (sugar + another molecule)

- Glycoprotein: sugar + protein
- Glycolipid: sugar + ceramide



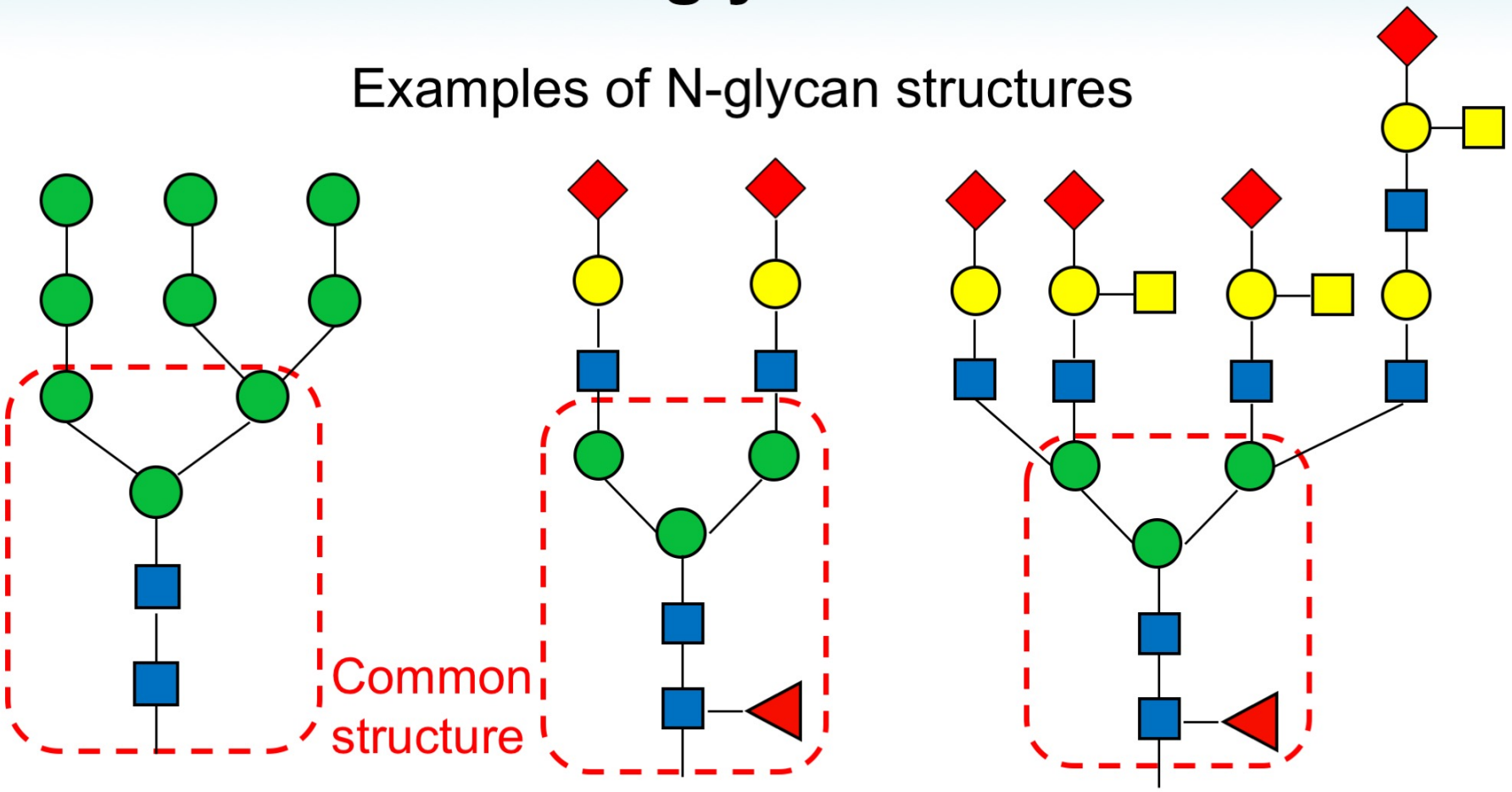
Mammalian glycoproteins

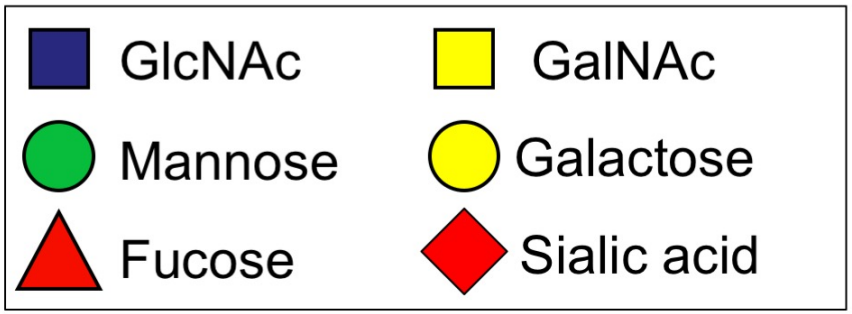
Over half of proteins are glycosylated in mammals



N-glycan

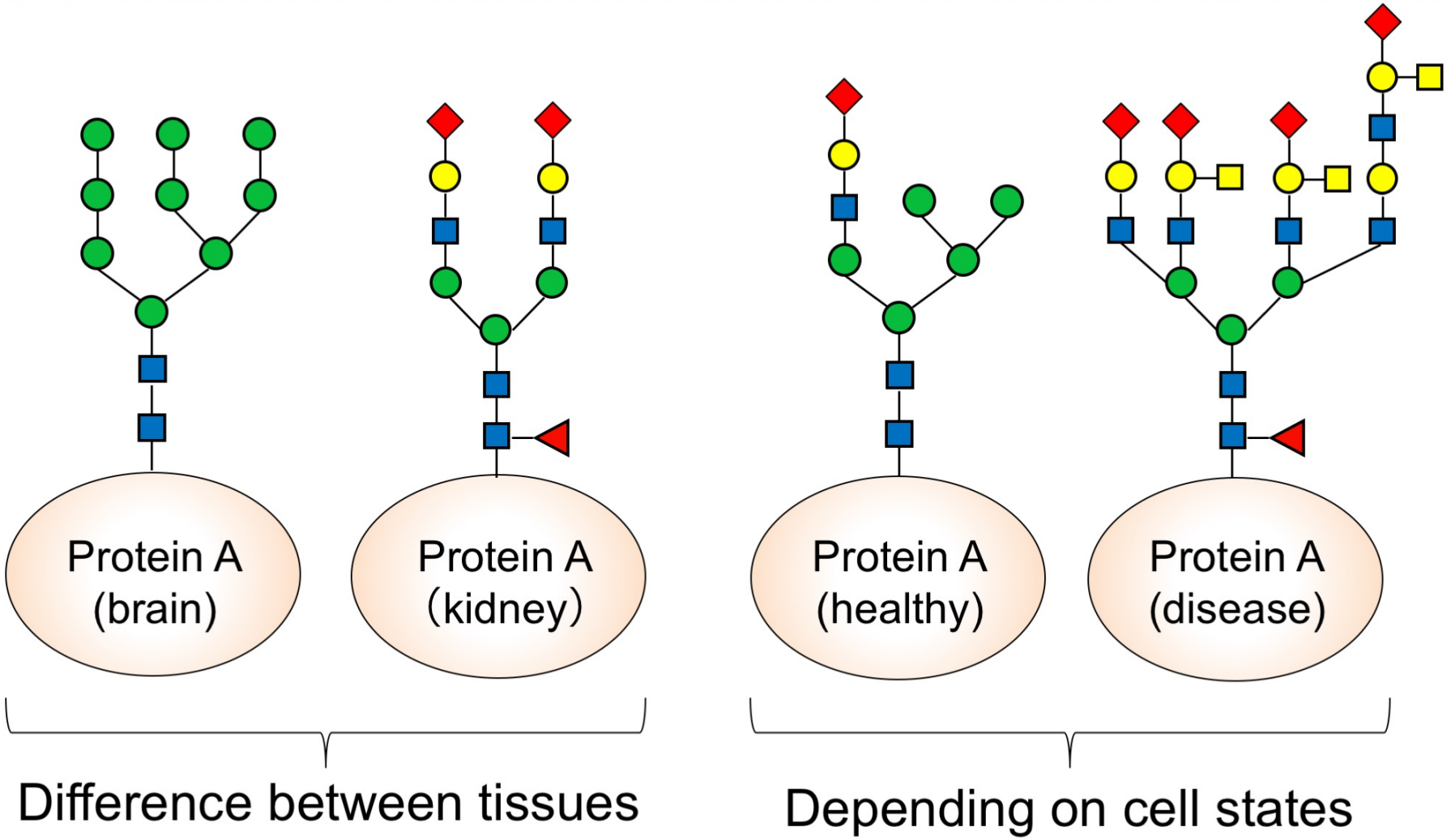
Examples of N-glycan structures





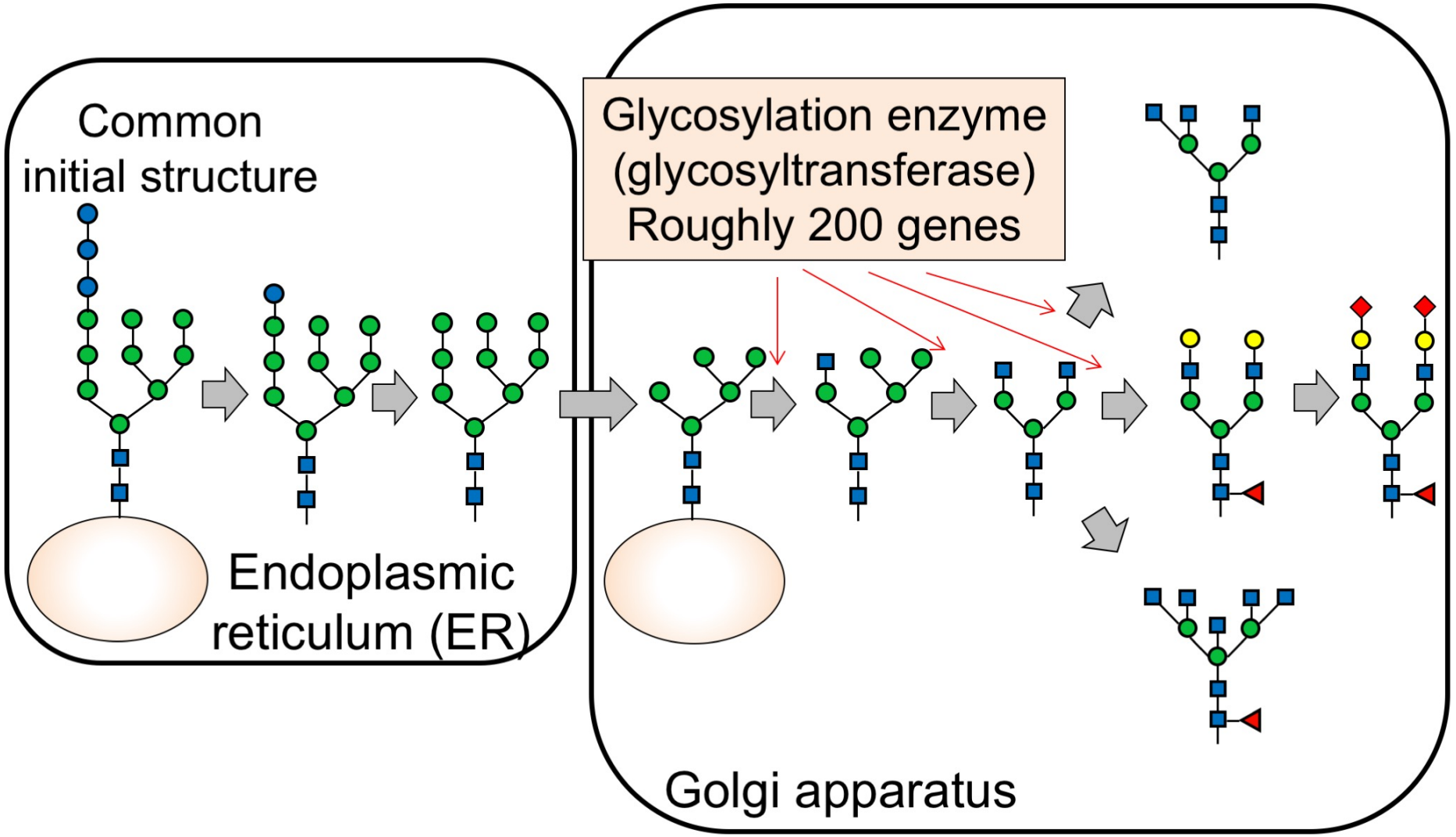
N-glycan structures
are very complex

A significance of glycan: diversity



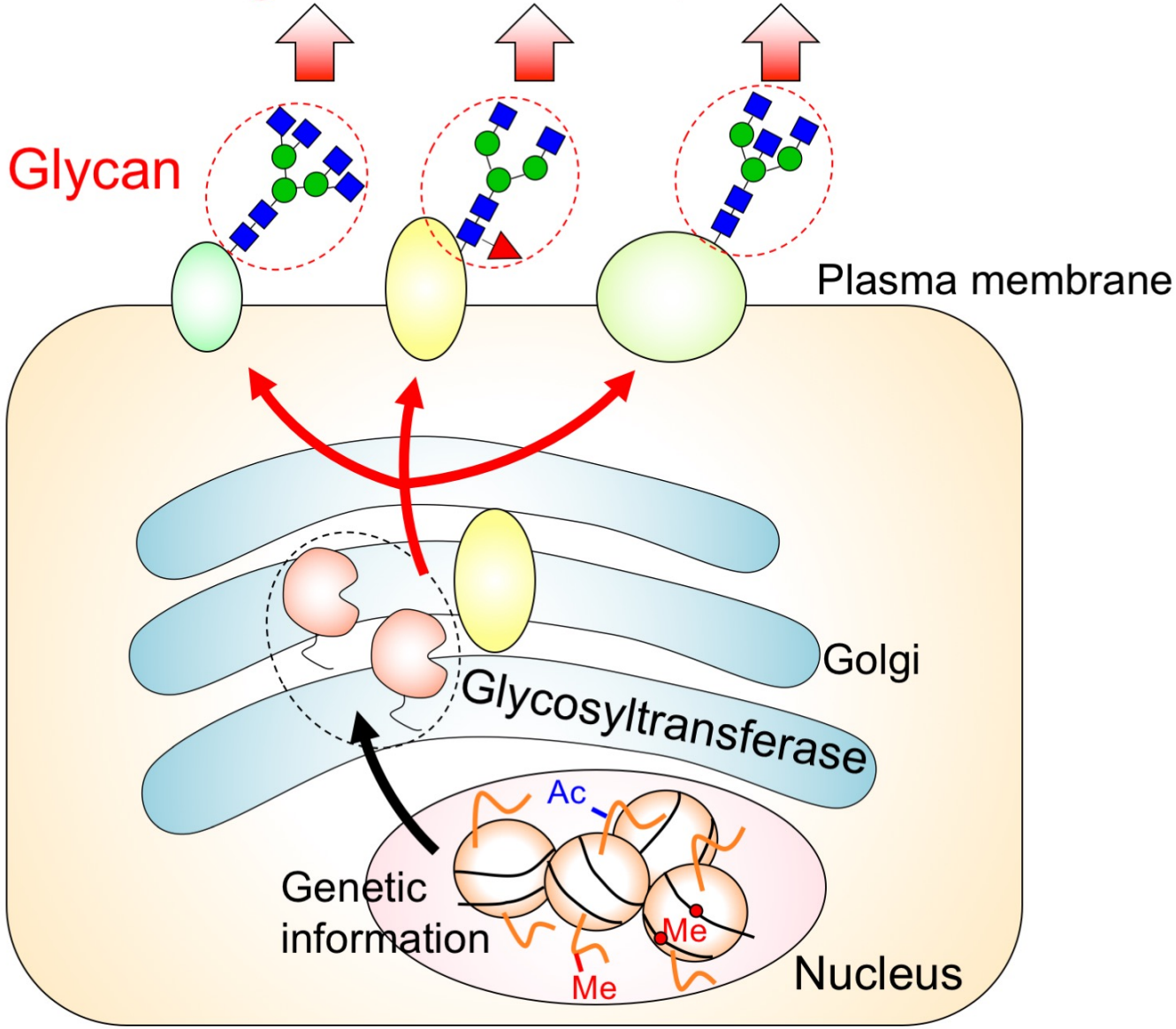
It gives dramatic structural diversity to a certain protein
(protein variations from the limited number of genes)

How is N-glycan biosynthesized?

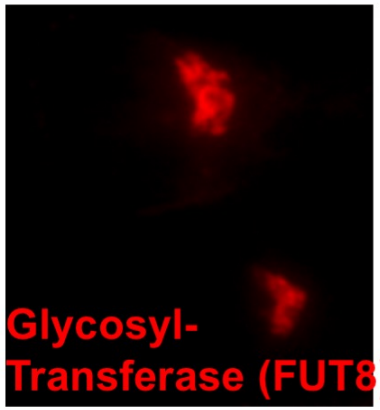


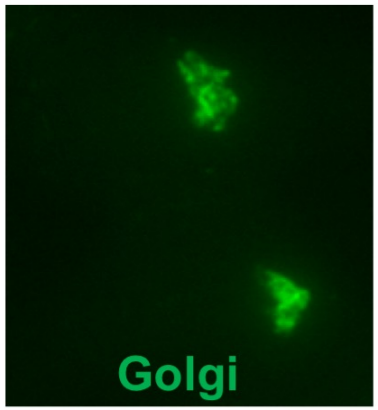
Glycan biosynthesis by glycosyltransferases

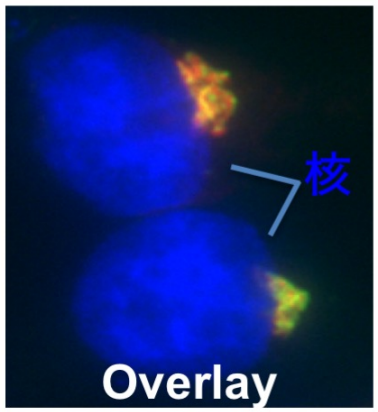
Biological functions, diseases



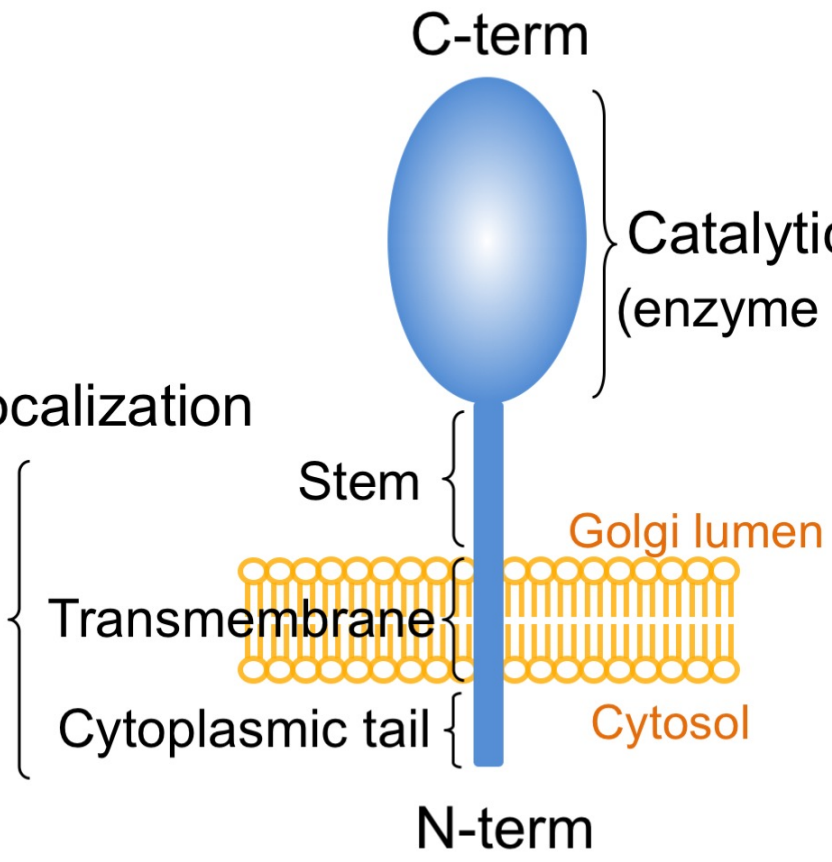
Typical domain structure of glycosyltransferase

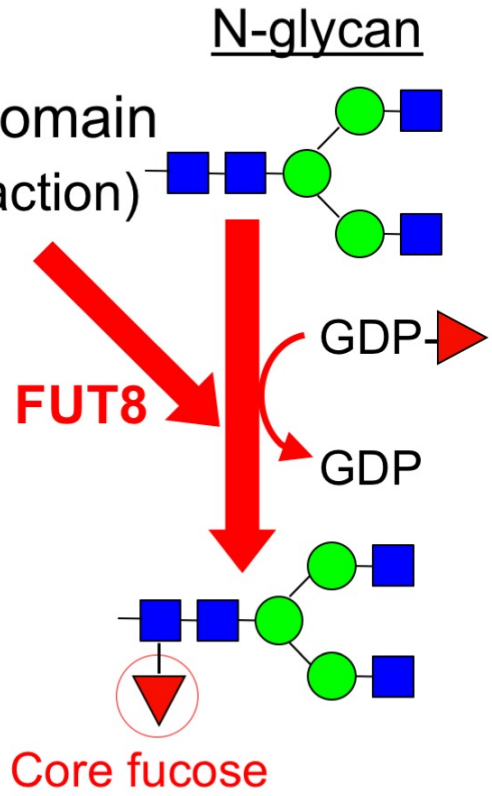






Localization

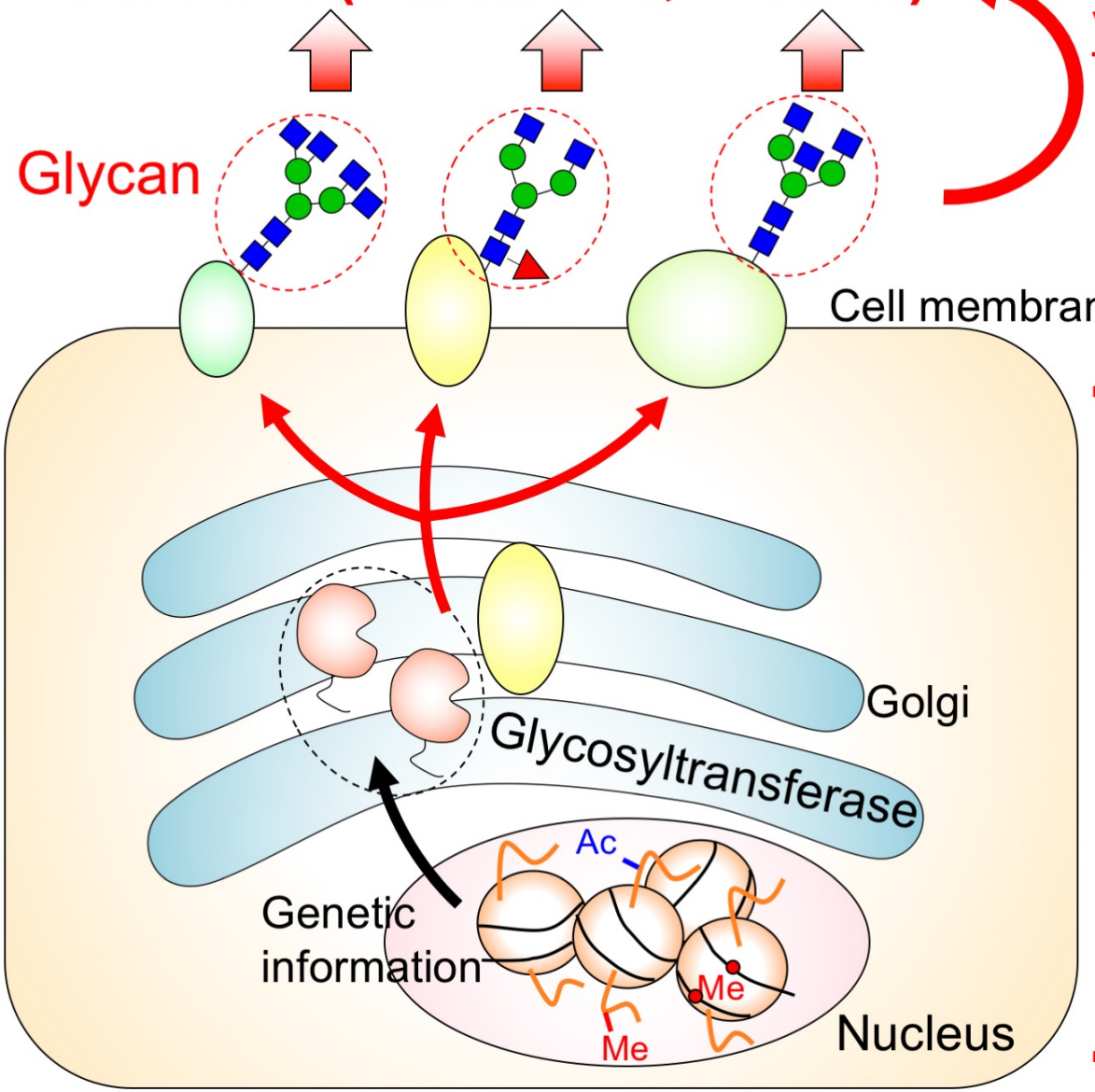




Enzyme reactions are well understood, but
localization mechanisms are poorly understood

Contents

Goal of study

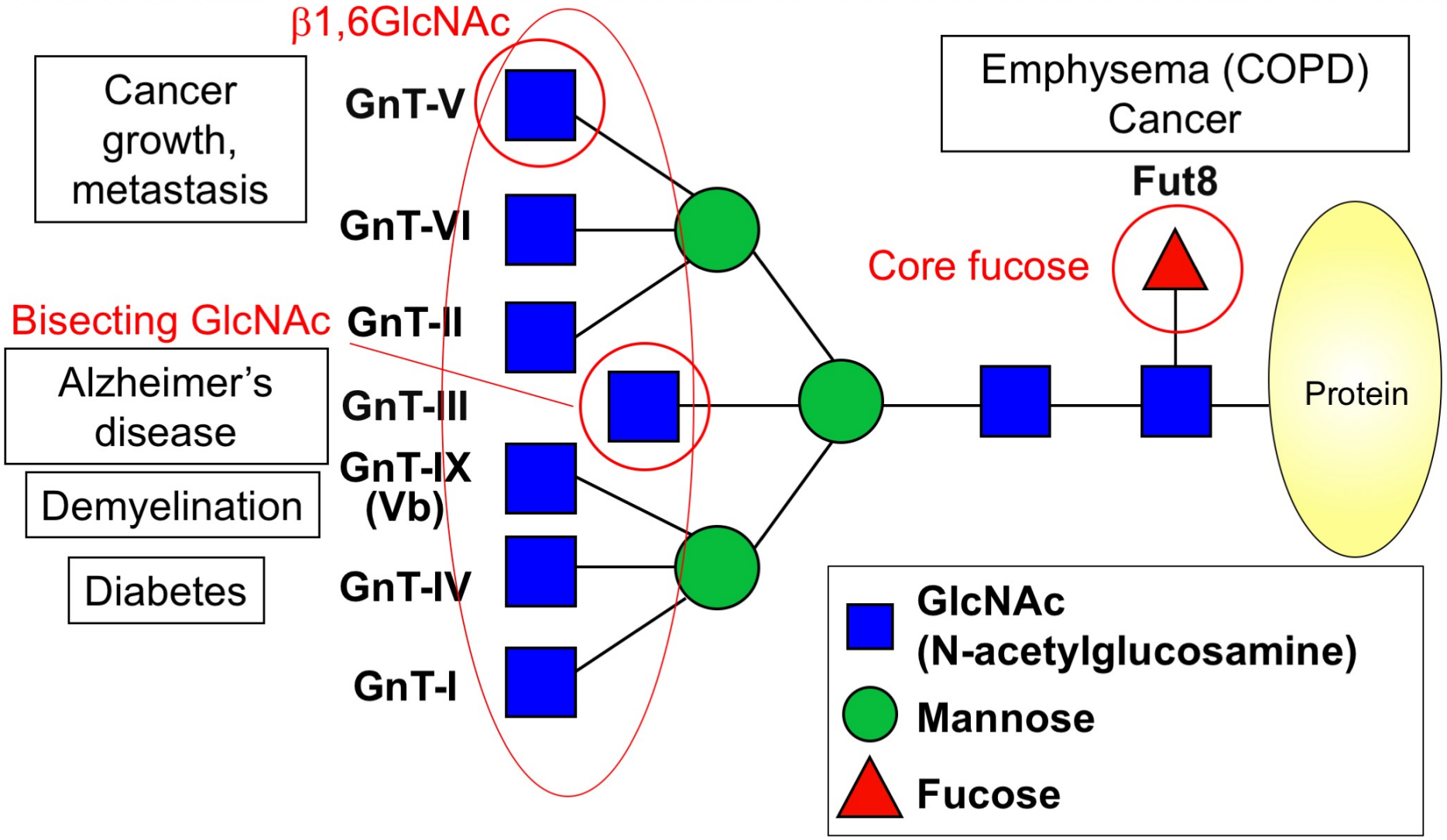


Glycobiology viewpoint

Development of diagnosis and treatment

Basic information of glycans

N-Glycan branching



Each branch has intrinsic functions

Alzheimer's Disease (AD)

Alzheimer's disease (AD)

- Most frequent dementia (60%)
  1 – 2 millions in Japan
- Brain atrophy
  Cognitive and memory impairment
- Almost no effective medicine

Aβ deposition

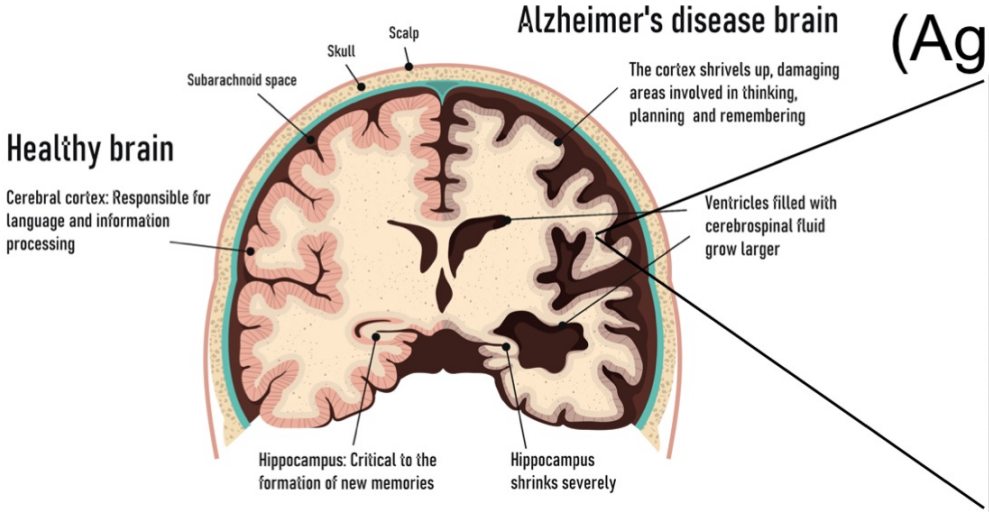
⇩

⇩

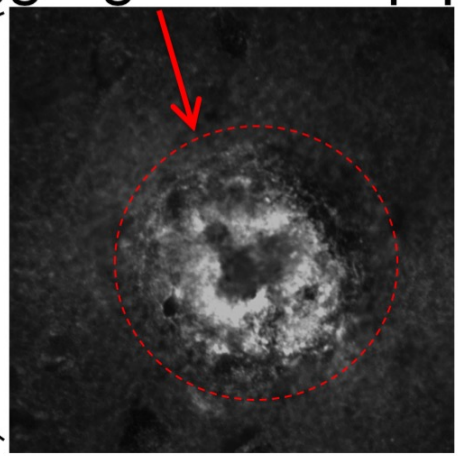
⇩

⇩

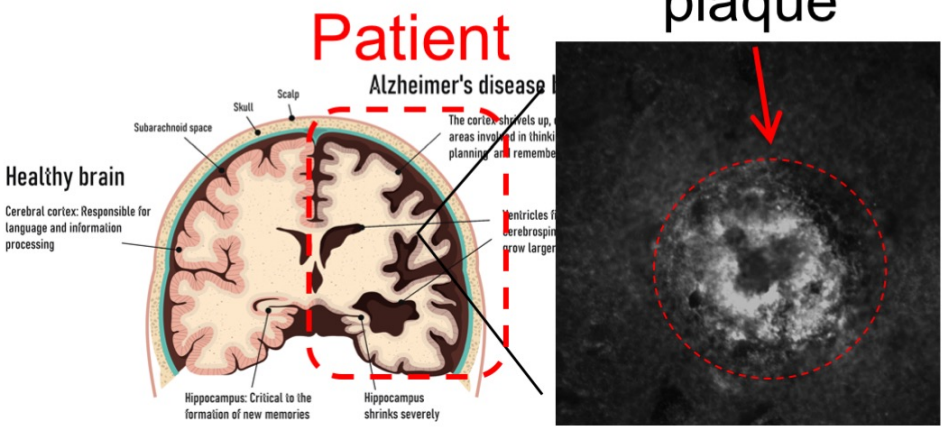
Neuronal death

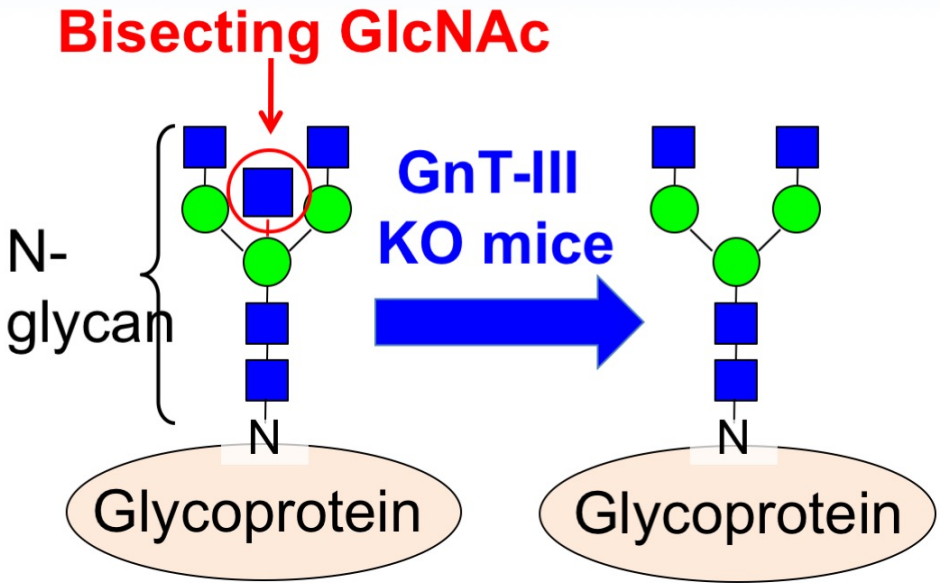


Hallmark: Amyloid (Aβ) plaque
(Aggregates of Aβ peptide)

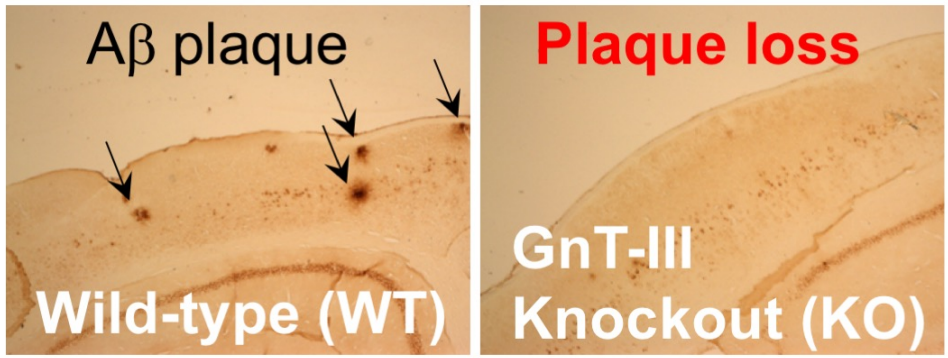


Results summary





Mouse brain

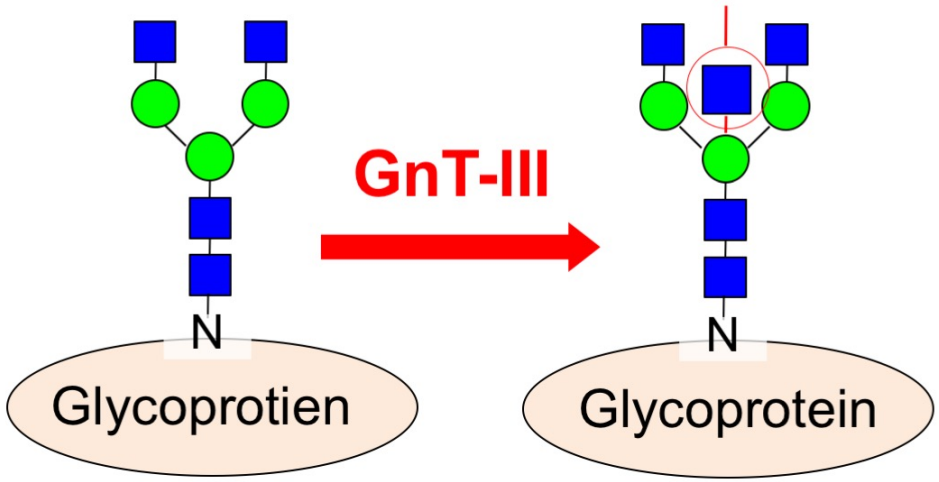


(Kizuka et al., *EMBO Mol. Med.*, 2015, 7, 175)

Short memory

Relationships between GnT-III and AD

Bisecting GlcNAc



(Nishikawa,Taniguchi et al. *J. Biol. Chem.* 1992, 267, 18199)

AD patient brain

GnT-III mRNA is upregulated in AD patient brain

(Akasaka-Manya et al. *Glycobiology* 2010, 20, 99)

Involvement in AD is unknown

Tissue distribution

GnT-III mRNA is most highly expressed in brain

(Miyoshi et al. *Int. J. Cancer* 1997, 72, 1117)

→ Functions in brains are unclear

Purpose

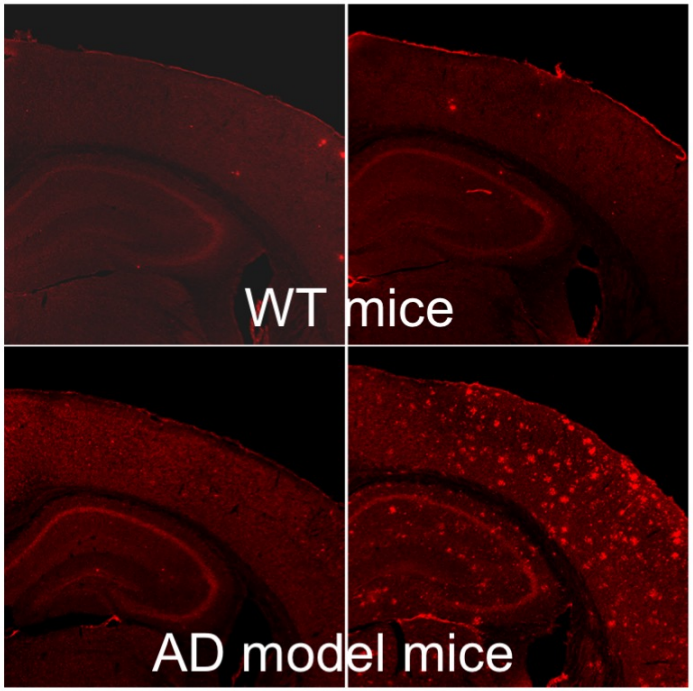
Elucidation of the role of bisecting GlcNAc in AD

→ analysis of GnT-III KO mice

AD model mouse

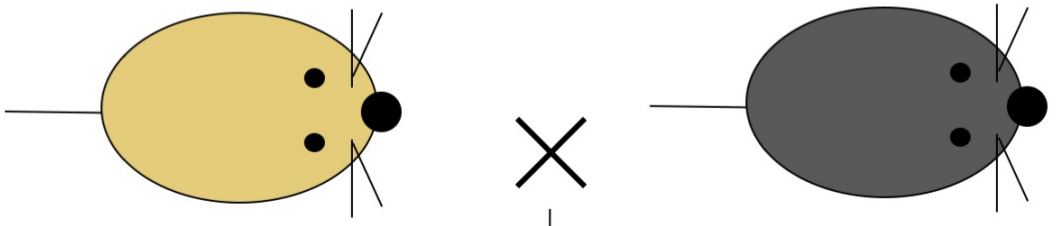
Mice do not develop AD spontaneously due to mutation in Aβ sequence and shorter lifespan than human → AD model mice

Aβ plaque



Aging

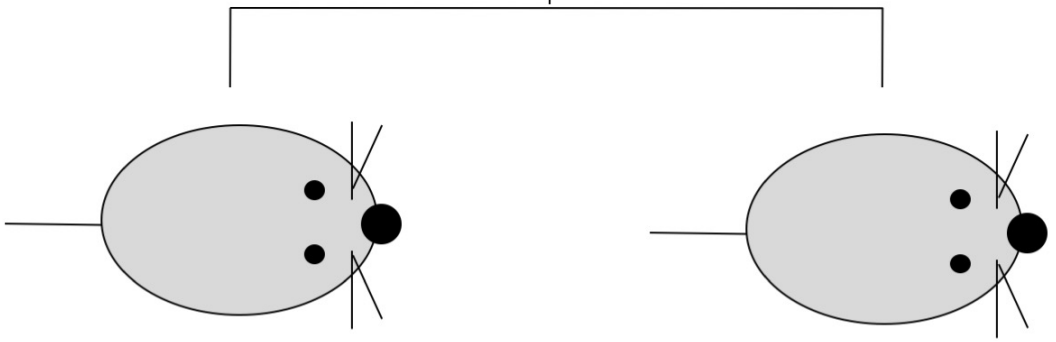
Aβ deposition and memory loss during aging



AD model mice
(human *APP* transgenic)

×

GnT-III KO mice



AD/GnT-III$^{+/+}$
(WT)
Wild Type

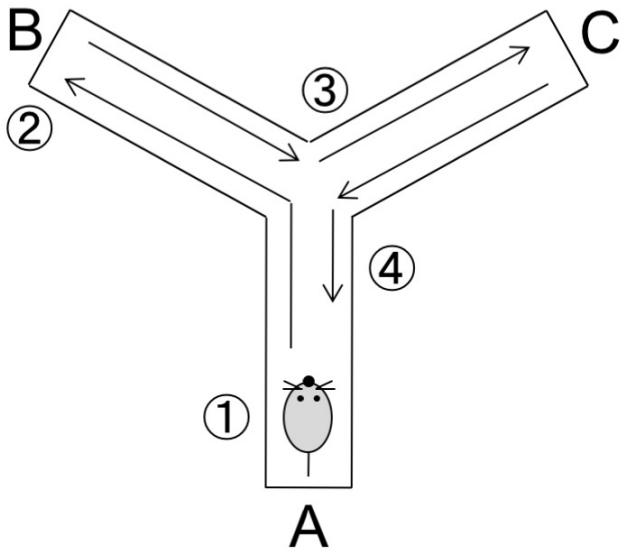
AD/GnT-III$^{-/-}$
(KO)

Aβ is barely deposited in GnT-III KO mice

Mouse brain

(Kizuka et al., *EMBO Mol. Med.*, 2015, 7, 175)

Y-maze task



Remember the latest route

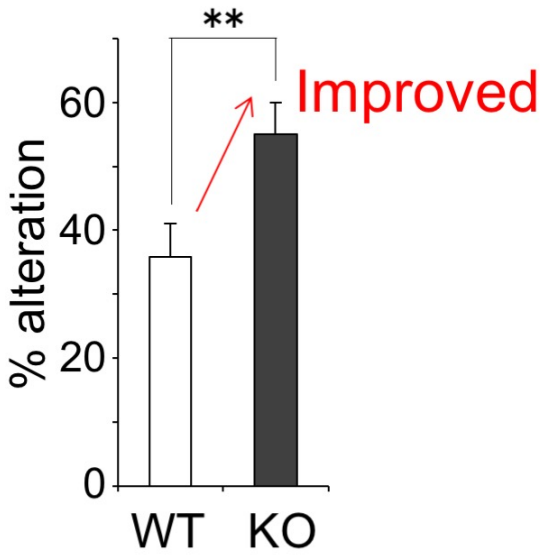
A route of normal mouse

A→B→C→A→B→C

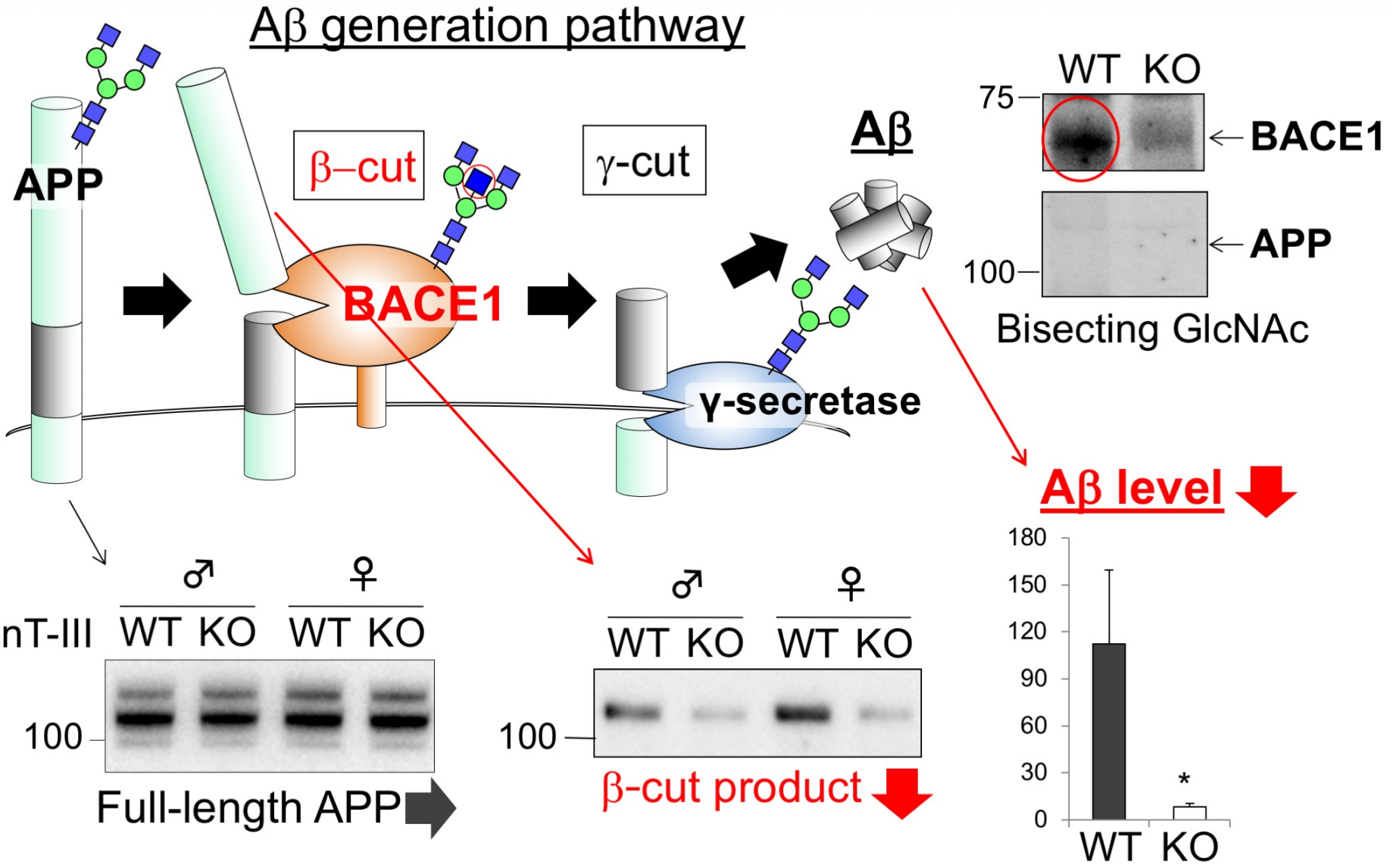
or

A→C→B→A→C→B

Short memory



Why is not Aβ deposited? : BACE1 is a target

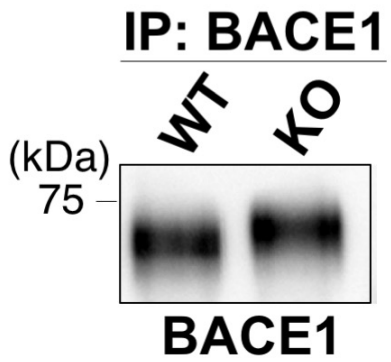


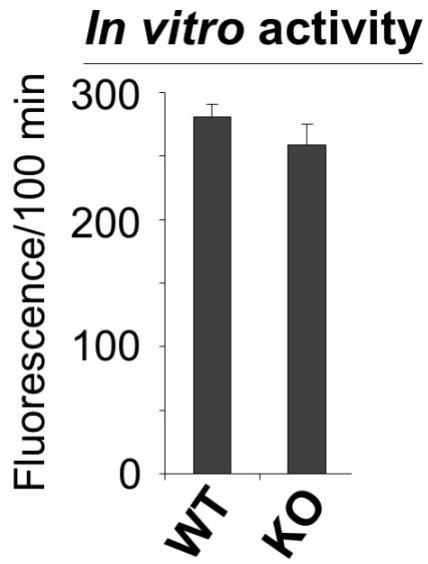
Deletion of bisecting GlcNAc leads to BACE1 dysfunction

(Kizuka et al., *EMBO Mol. Med.*, 2015, 7, 175)

BACE1 distribution is changed

In vitro activity

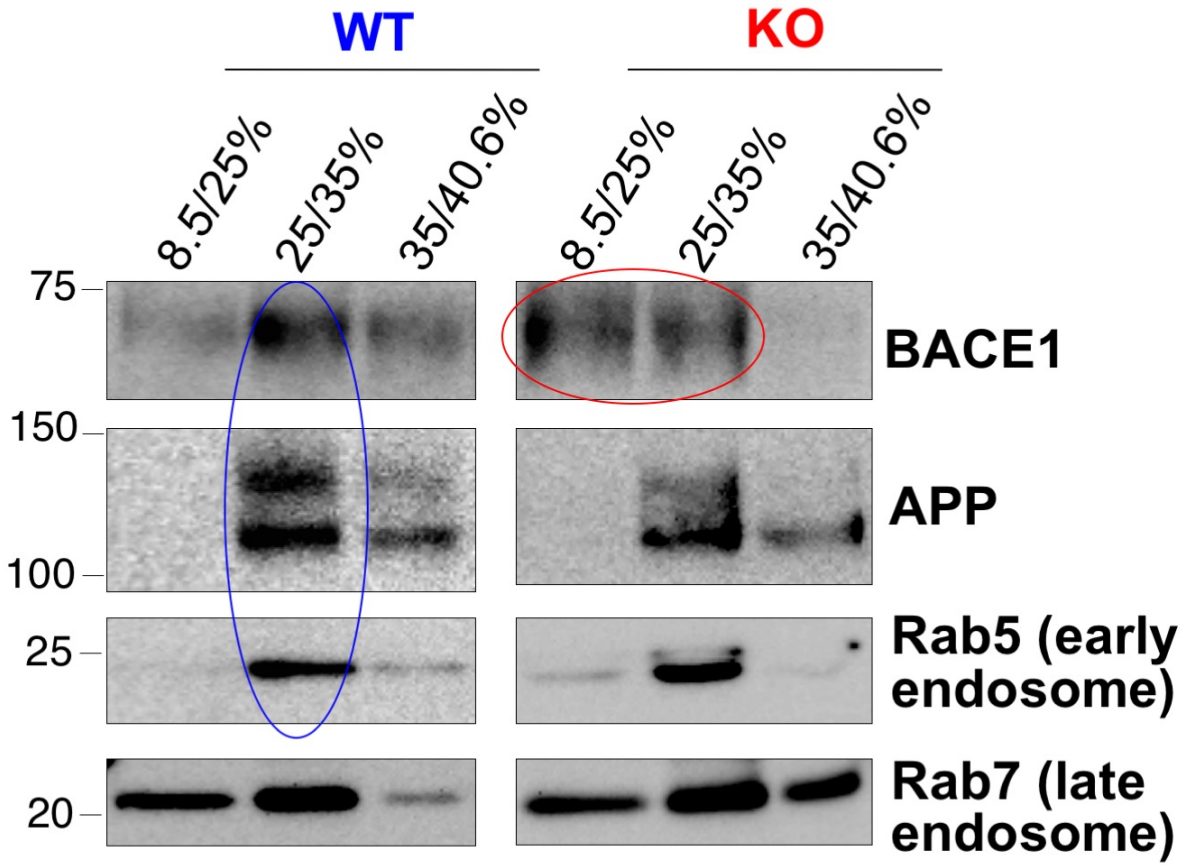




Cleavage activity itself is not changed

Organelle separation

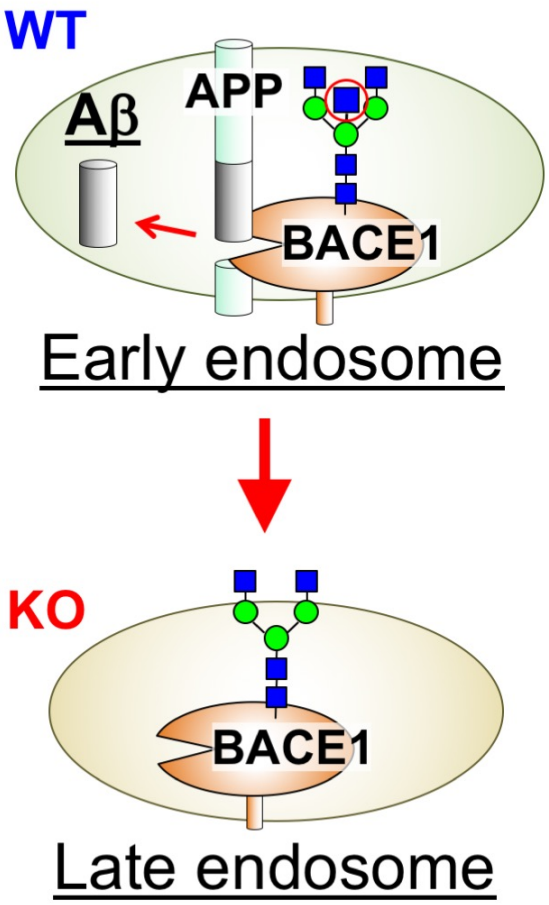
(Sucrose density gradient centrifugation)



BACE1
- WT: early endosome-rich fraction
- KO: early + late endosome

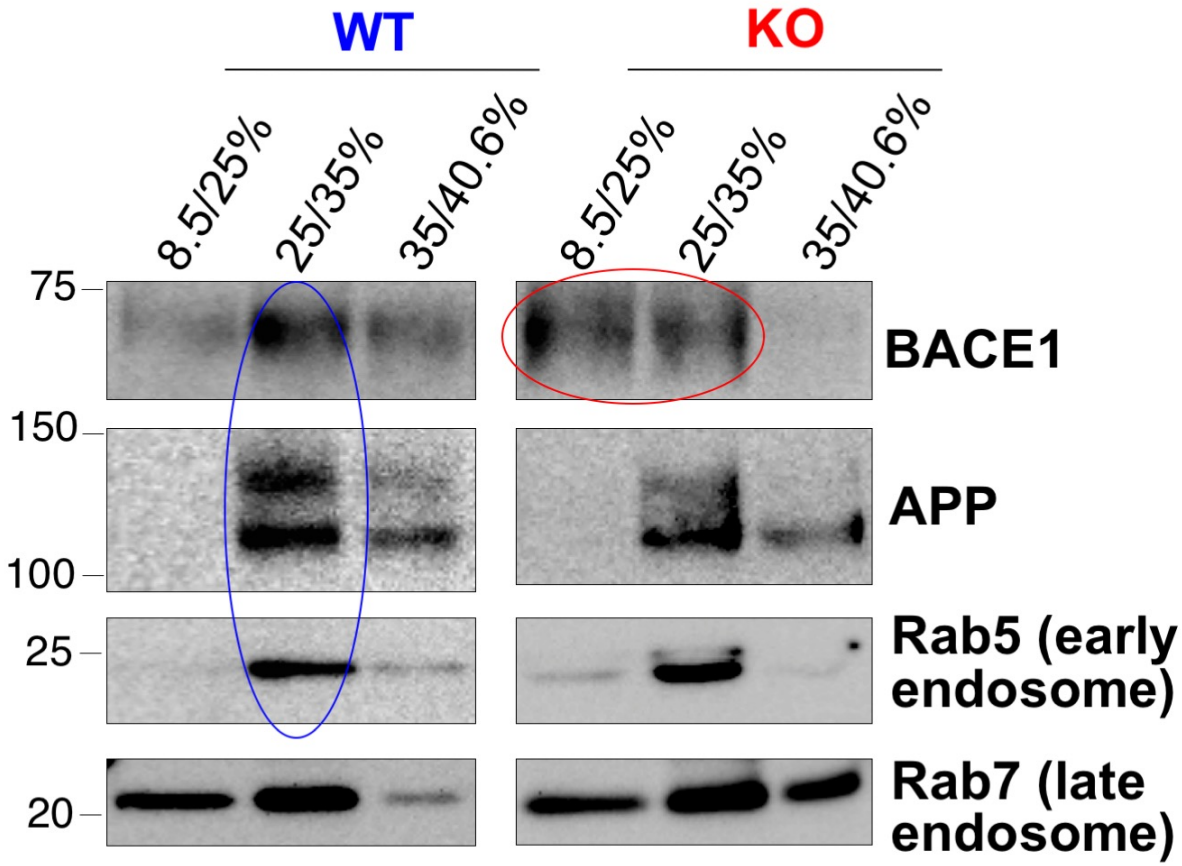
(Kizuka et al., *EMBO Mol. Med.*, 2015, 7, 175)

BACE1 distribution is changed



Organelle separation

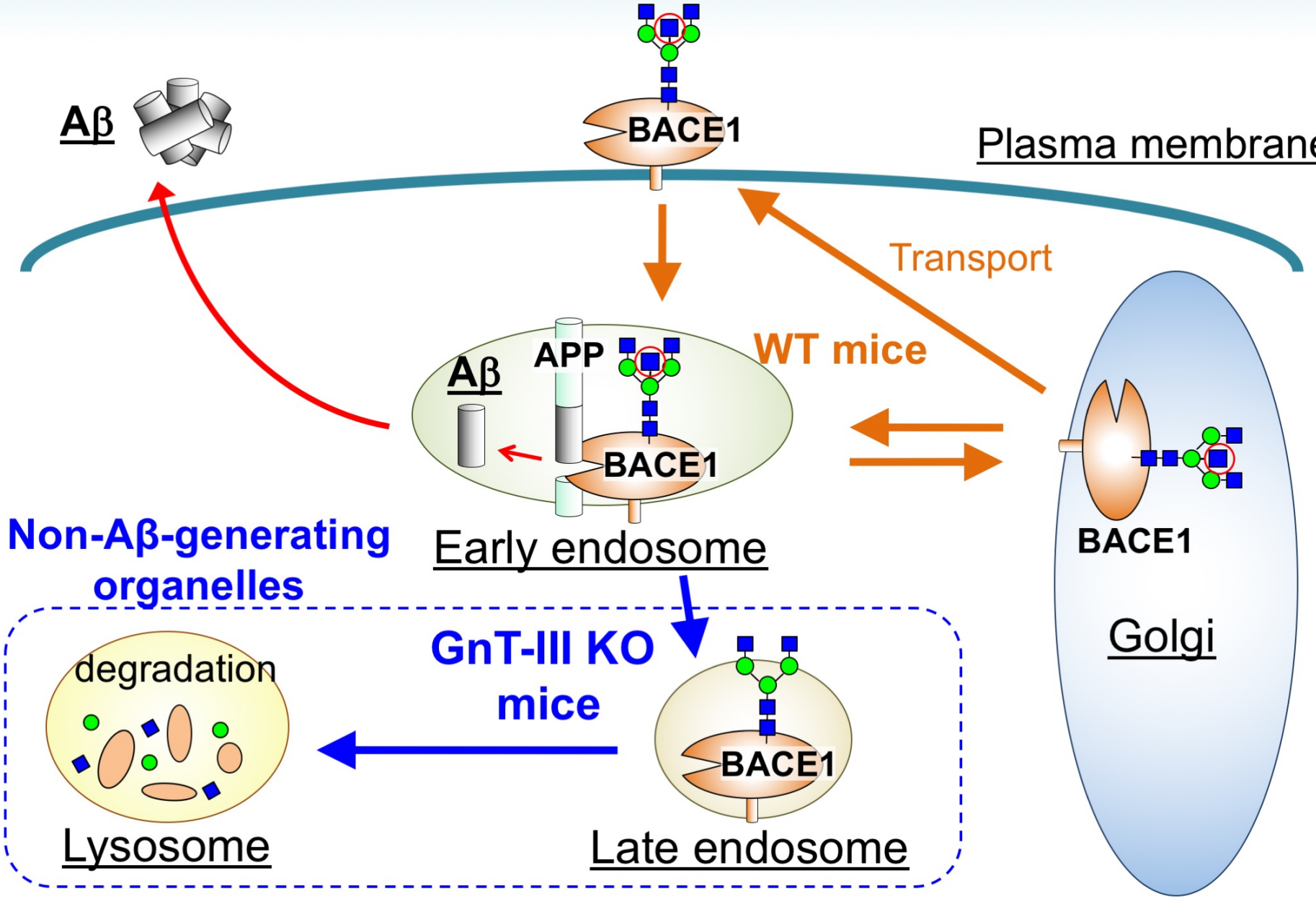
(Sucrose density gradient centrifugation)



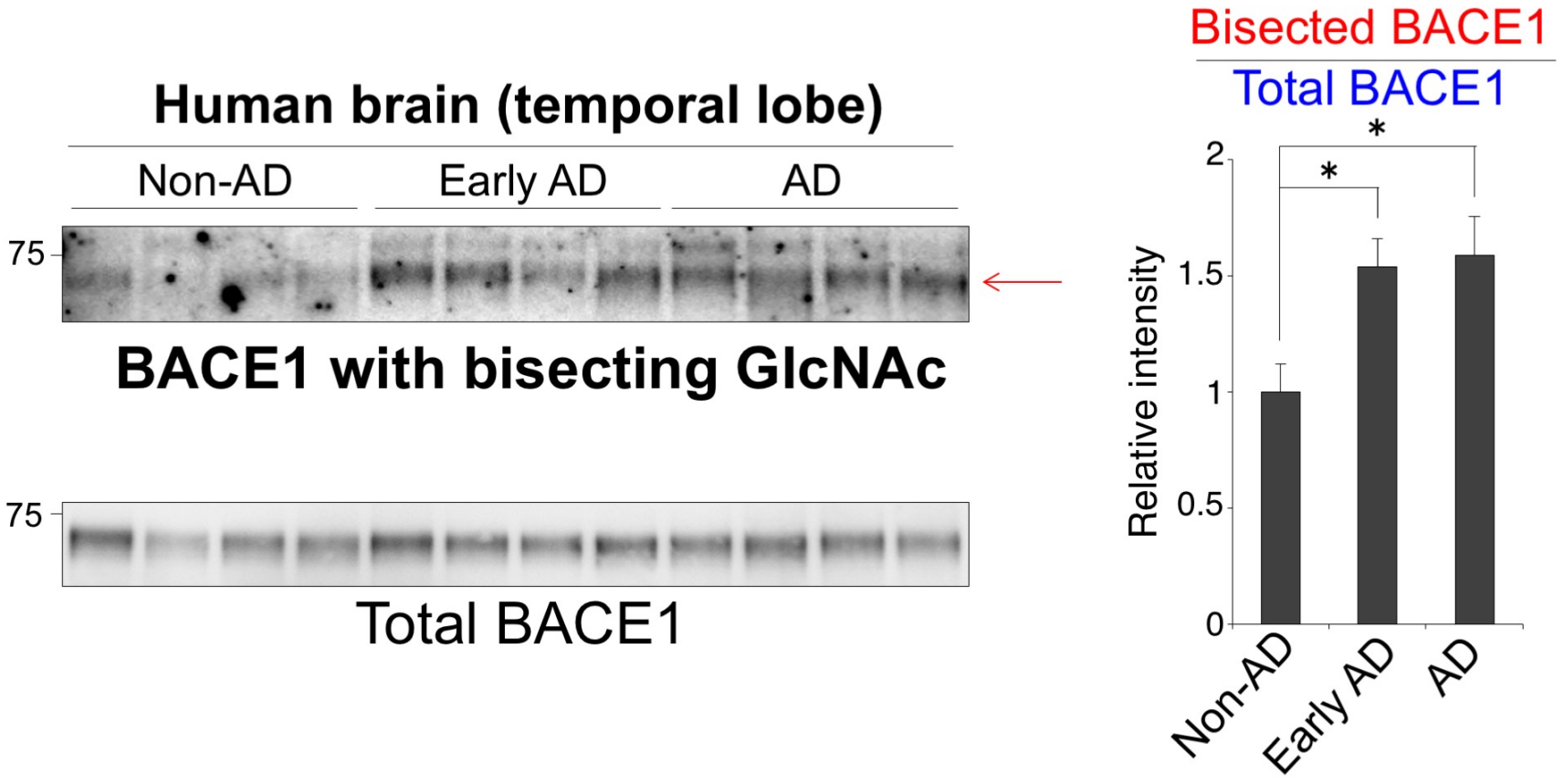
BACE1
- WT: early endosome-rich fraction
- KO: early + late endosome

(Kizuka et al., *EMBO Mol. Med.*, 2015, 7, 175)

Summary of GnT-III KO mice



Also in humans?



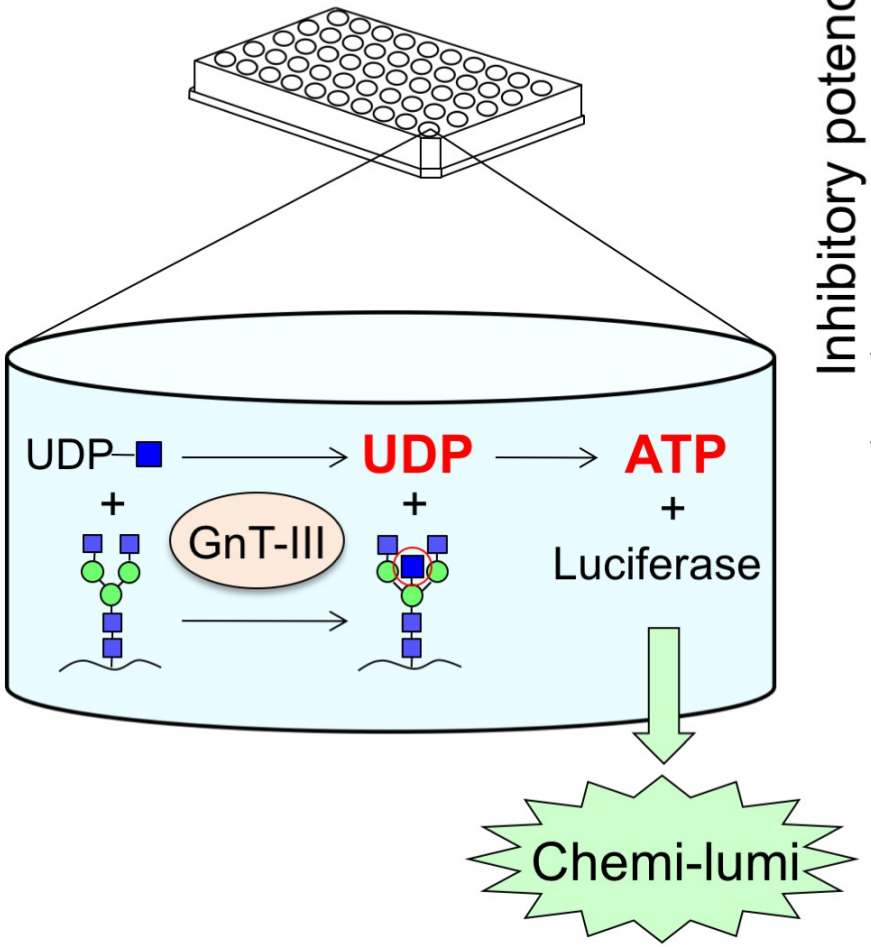
Bisecting GlcNAc on BACE1 is increased in AD brain

→ it suggests that bisecting GlcNAc regulates AD pathogenesis by modulating BACE1 functions

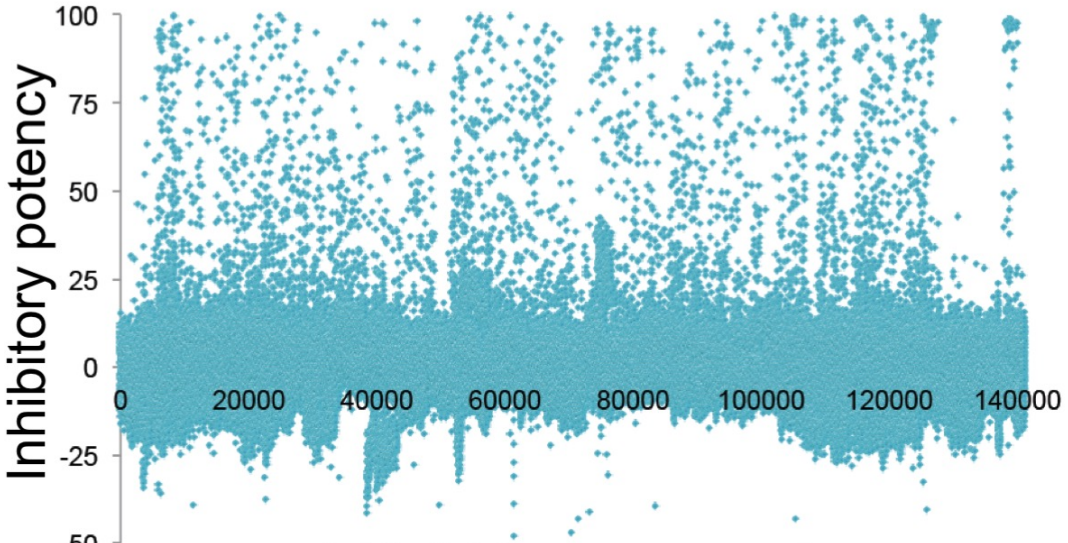
(Kizuka et al., *EMBO Mol. Med.*, 2015, 7, 175)

Search for GnT-III inhibitors

<u>High-throughput assay</u>
(UDP-Glo by Promega)



1st screening



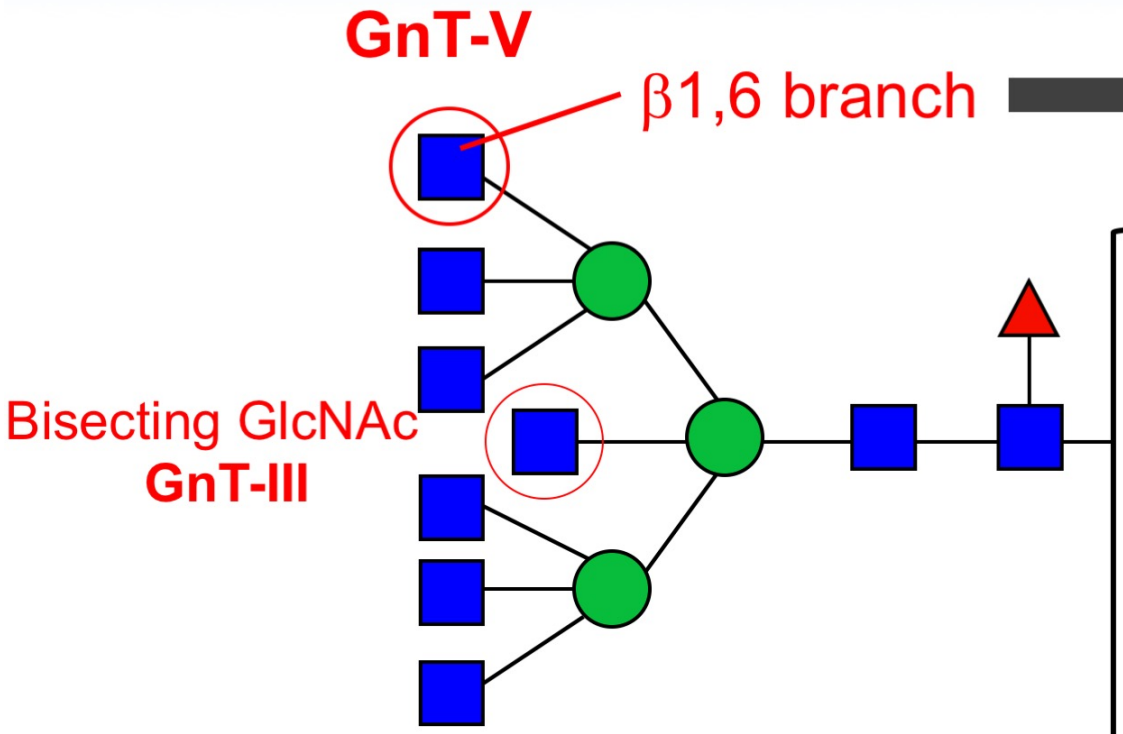
140,000 compounds

2nd screening

Ongoing

Contents

GnT-V and cancer

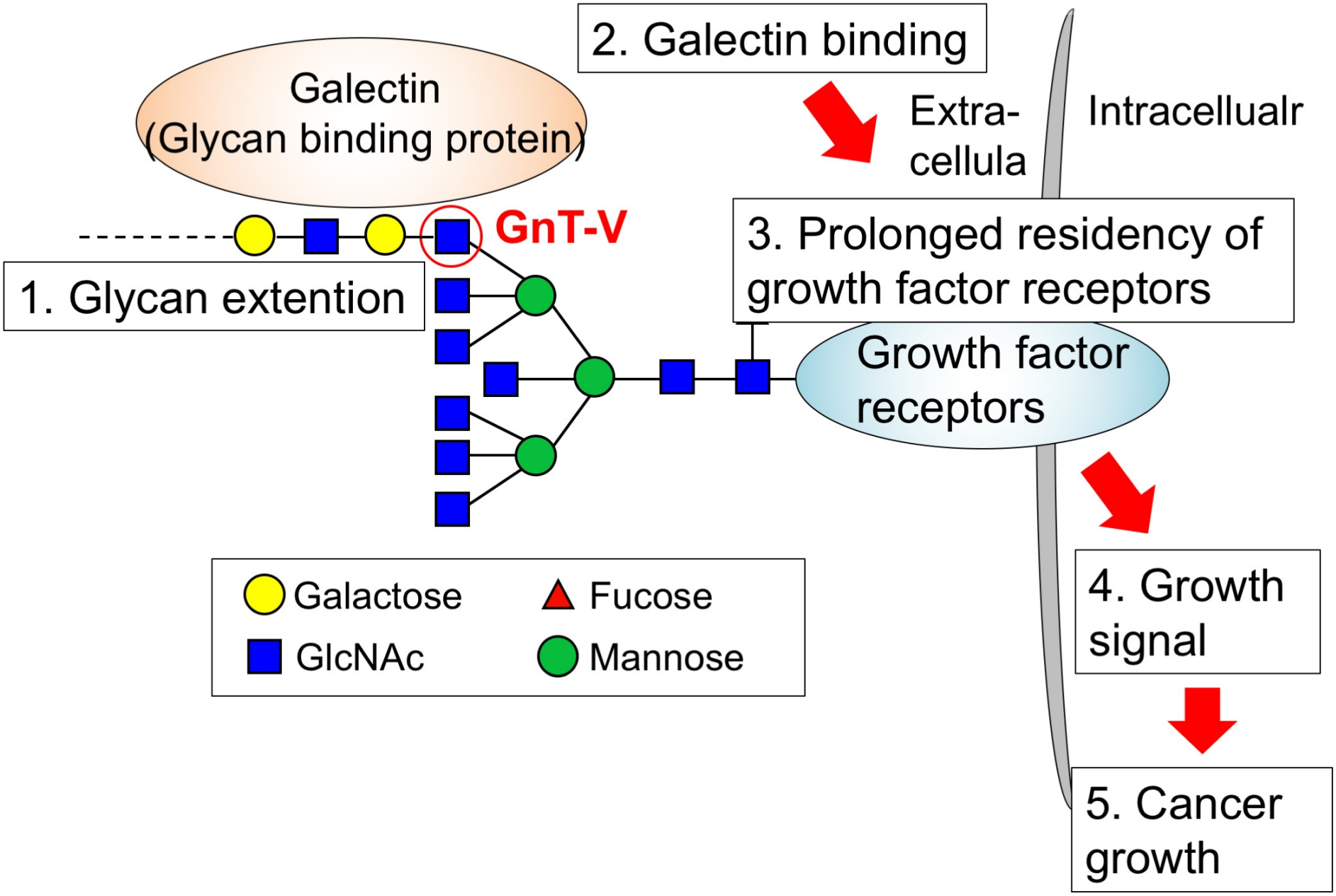


Relationship to cancer

- Upregulated in various cancers
  (Ito et al., *Int. J. Cancer,* 2001, 91, 631)
  (Guo et al., *J. Biol. Chem.,* 2014, 289, 31534)
- Correlation with poor prognosis
  (Murata et al., *Clin. Cancer. Res.,* 2000, 6, 1772)
- GnT-V mRNA is driven by oncogene *ETS1*
  (Buckhaults et al., *J. Biol. Chem.,* 1997, 272, 19575)
  (Chen et al., *Oncogene,* 1998, 17, 2087)
- Suppressed tumor growth and metastasis in GnT-V KO mice
  (Granovsky et al., *Nat. Med.,* 2000, 6, 306)

GnT-V is a drug target in cancer

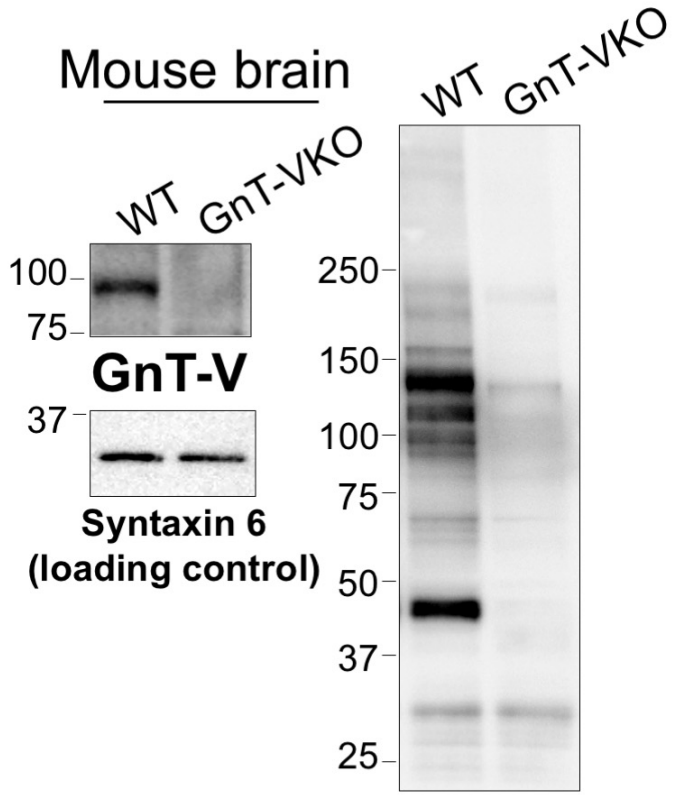
A possible mechanism



Two questions in biosynthesis

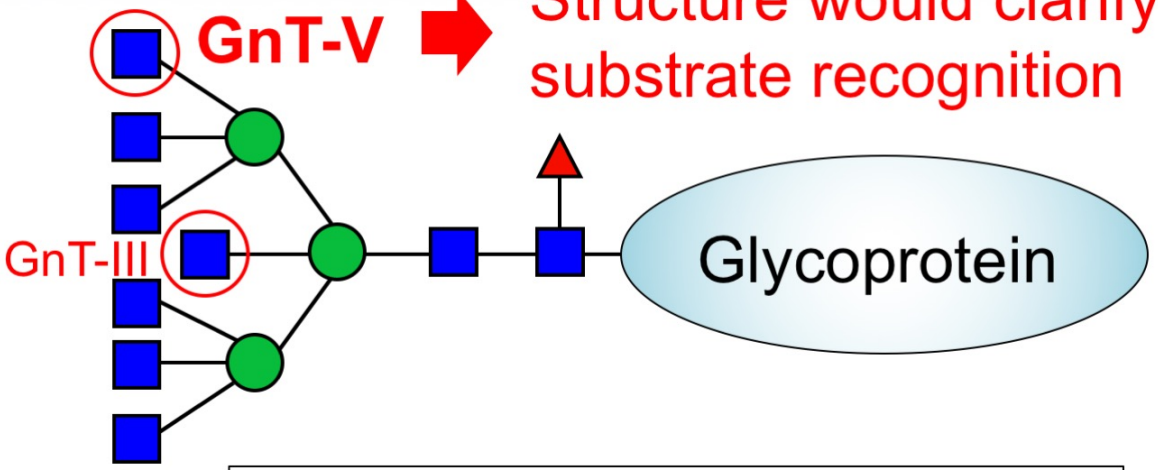
1. Protein selectivity

Mouse brain



β1,6-branch
(L4-PHA lectin)

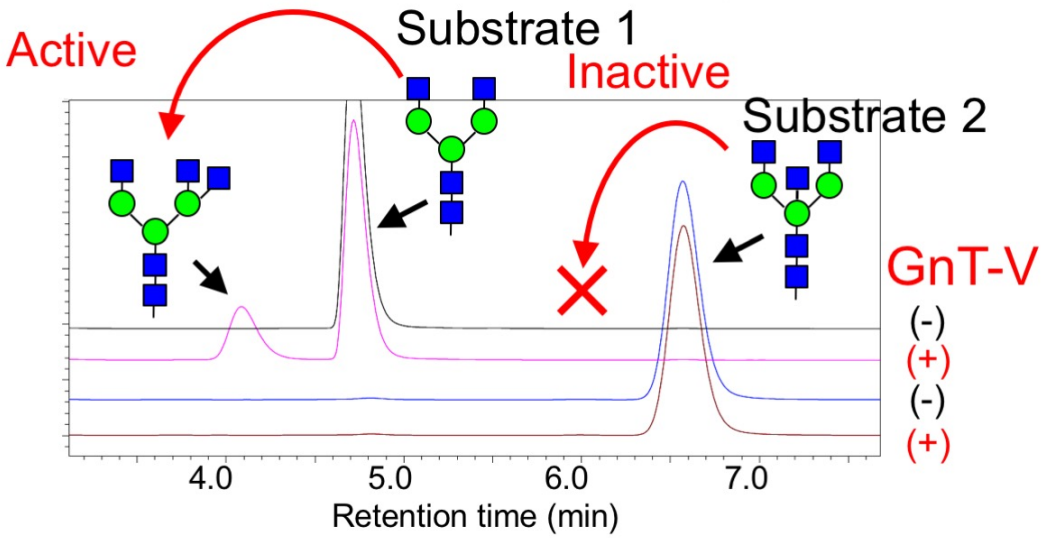
Clearly selects proteins



Structure would clarify substrate recognition

2. Negative regulation by GnT-III

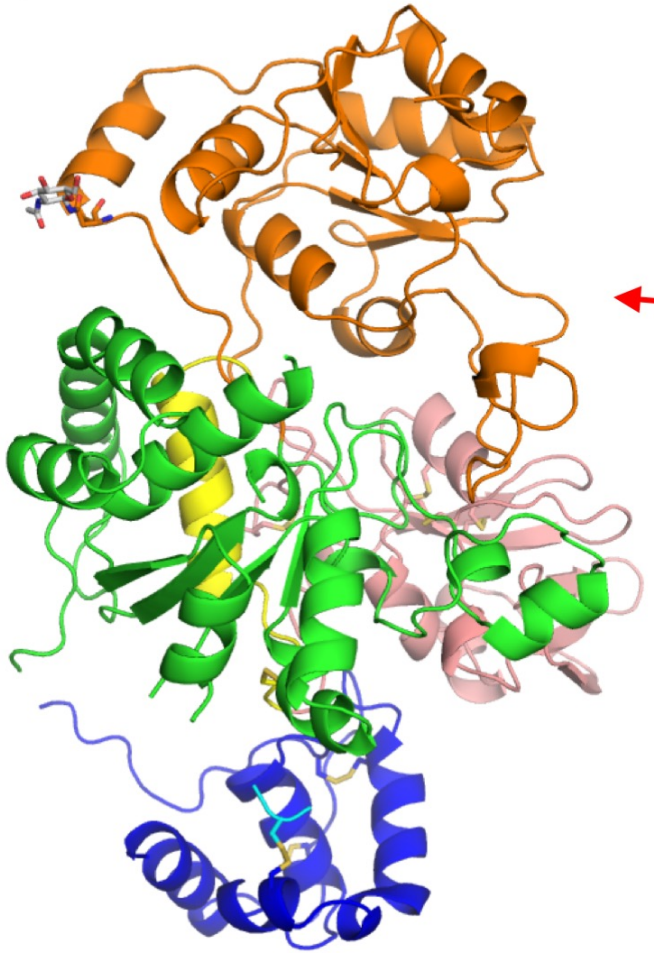
GnT-V activity assay

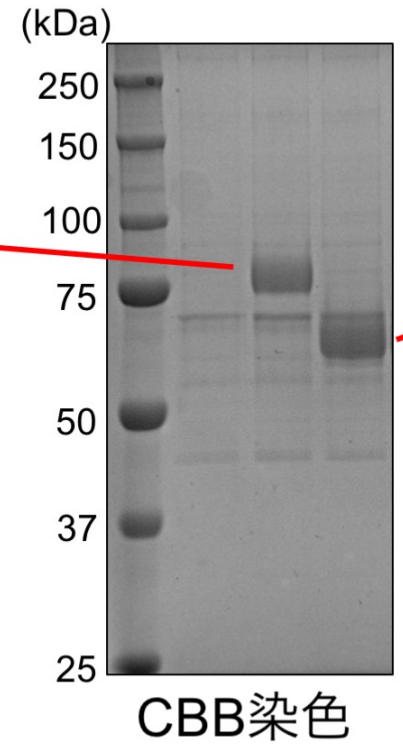


Prior action of GnT-III blocks GnT-V

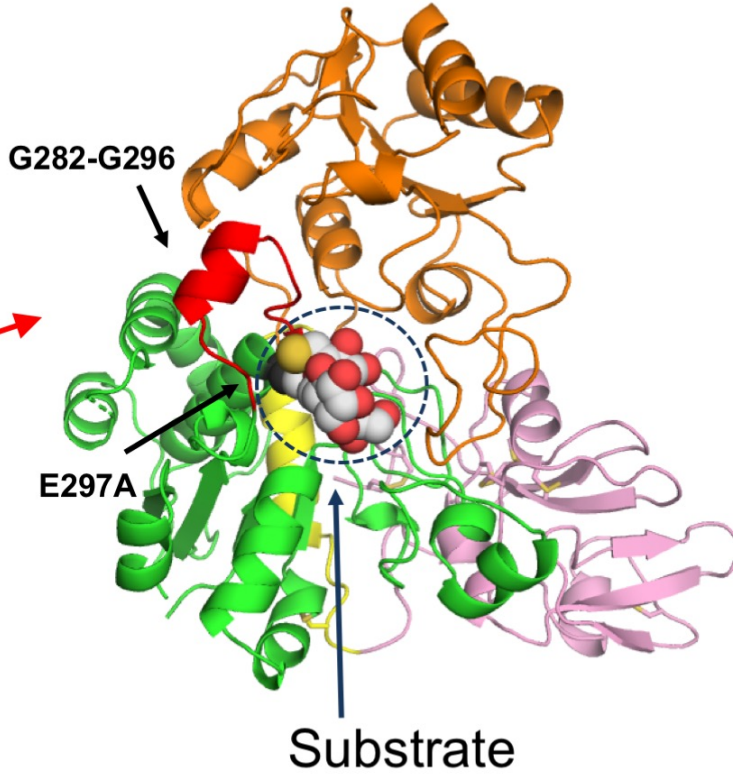
Crystal structure of GnT-V

GnT-V catalytic domain





Modified GnT-V (ver. 4)



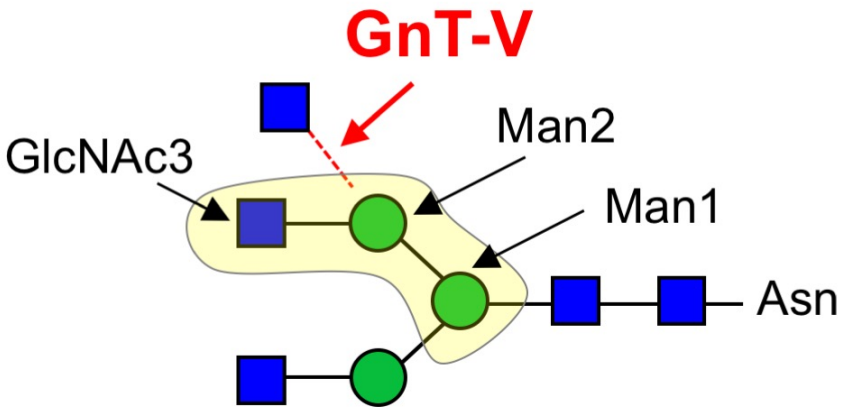
Complex with substrate

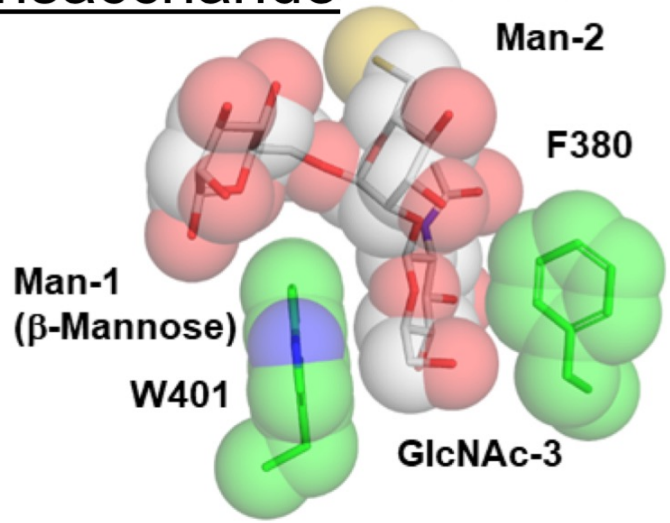
Complex with substrate was not obtained

(Nagae and Kizuka et al., *Nat. Commun.*, 2018, 9, 3380)

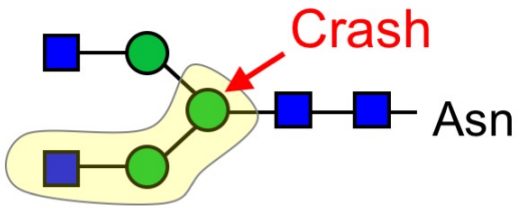
Strict recognition of branch by GnT-V

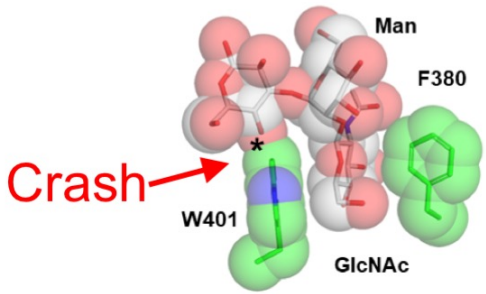
Recognition of trisaccharide



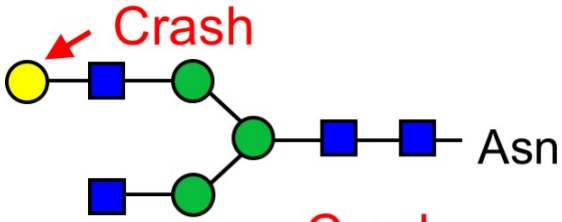


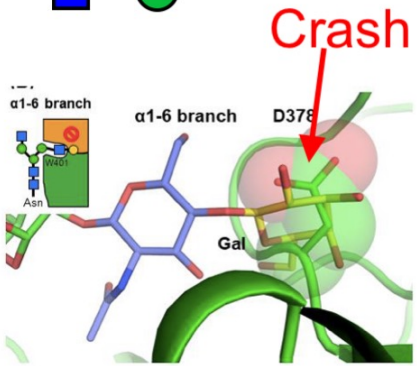
Lower branch



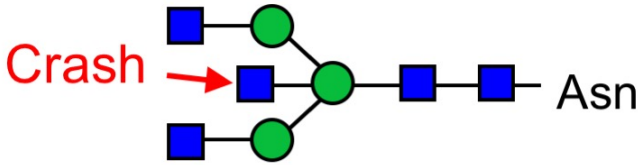


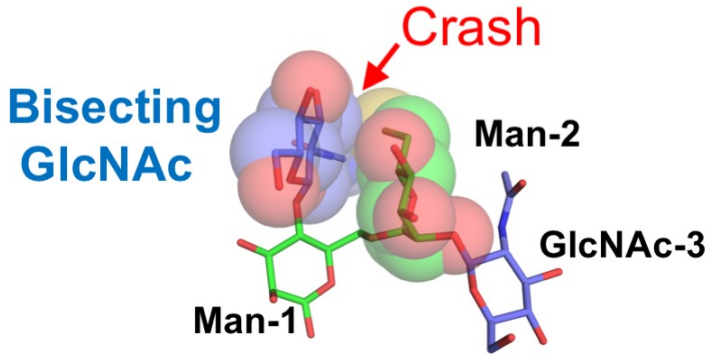
Extention





Bisecting GlcNAc

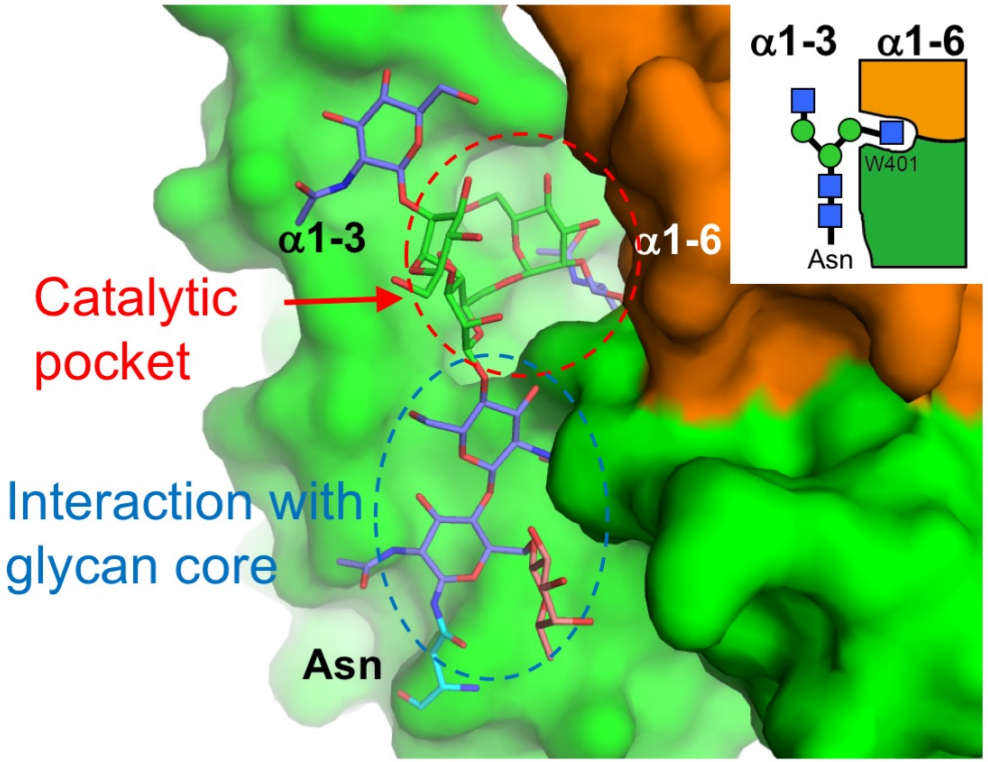




(Nagae and Kizuka et al., *Nat. Commun.*, 2018, 9, 3380)

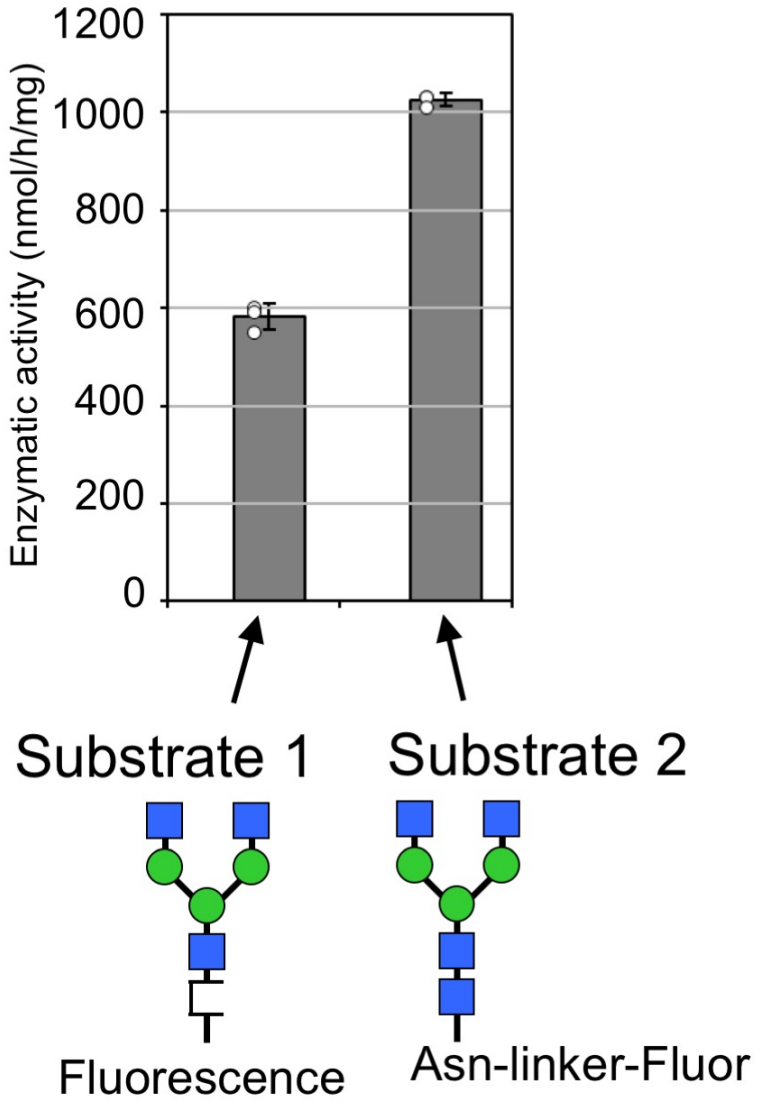
GnT-V looks recognize glycan core and polypeptide

Around catalytic pocket



Activity (substrate recognition) possibly depends on amino acid sequence around Asn

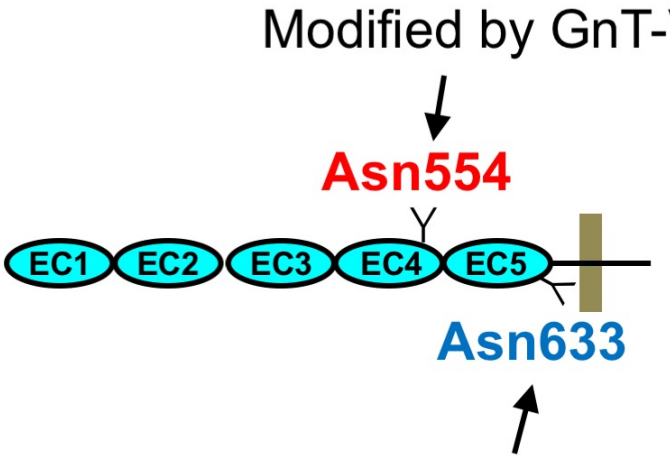
GnT-V activity



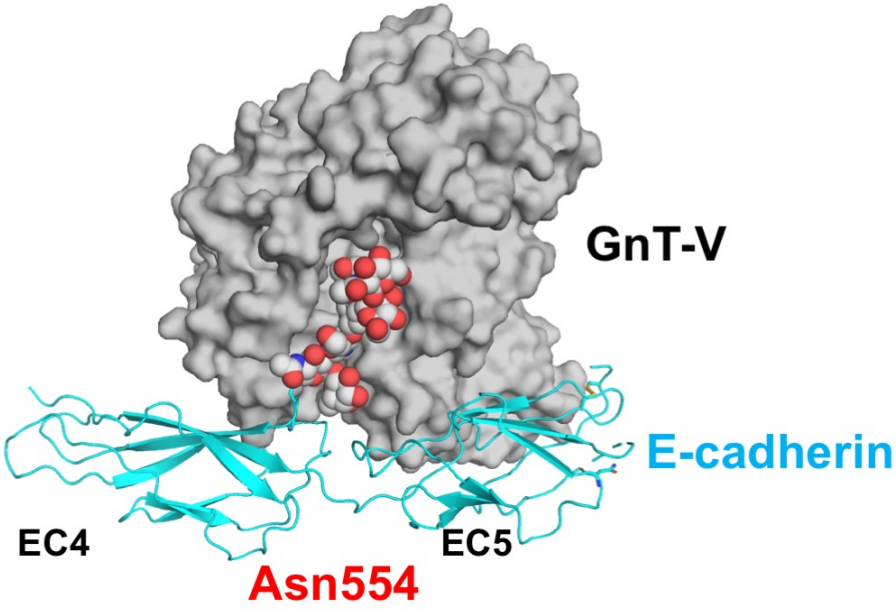
(Nagae and Kizuka et al., *Nat. Commun.*, 2018, 9, 3380)

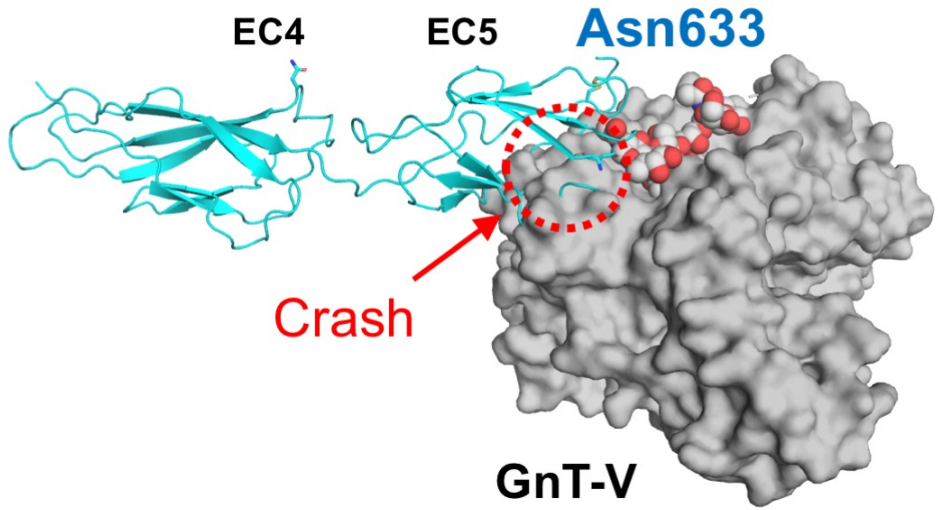
Regulation from a macroscopic viewpoint

E-cadherin



To be modified by GnT-V, GnT-V must avoid protein-protein crash





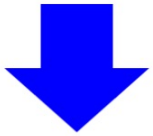
(Nagae and Kizuka et al., *Nat. Commun.*, 2018, 9, 3380)

Short summary

① Structure of cancer-related GnT-V was clarified

② GnT-V strictly recognizes glycan branch and structure

③ Sequence and structure of acceptor proteins are also important factors



Perspective

- Detailed mechanisms of protein selective action
- Development of inhibitors

Summary

① Most glycosyltransferases are Golgi-localized type-II membrane proteins

② Bisecting GlcNAc is a target of Alzheimer's disease

→ Bisecting GlcNAc regulates BACE1 localization and promotes Aβ generation

③ GnT-V selectively acts on its target proteins and synthesizes cancer-related β1,6-branch

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