



Disregulation of Golgi localization of glycosyltransferases alters mucin O-glycosylation and survival or metastatic properties of cancer cells

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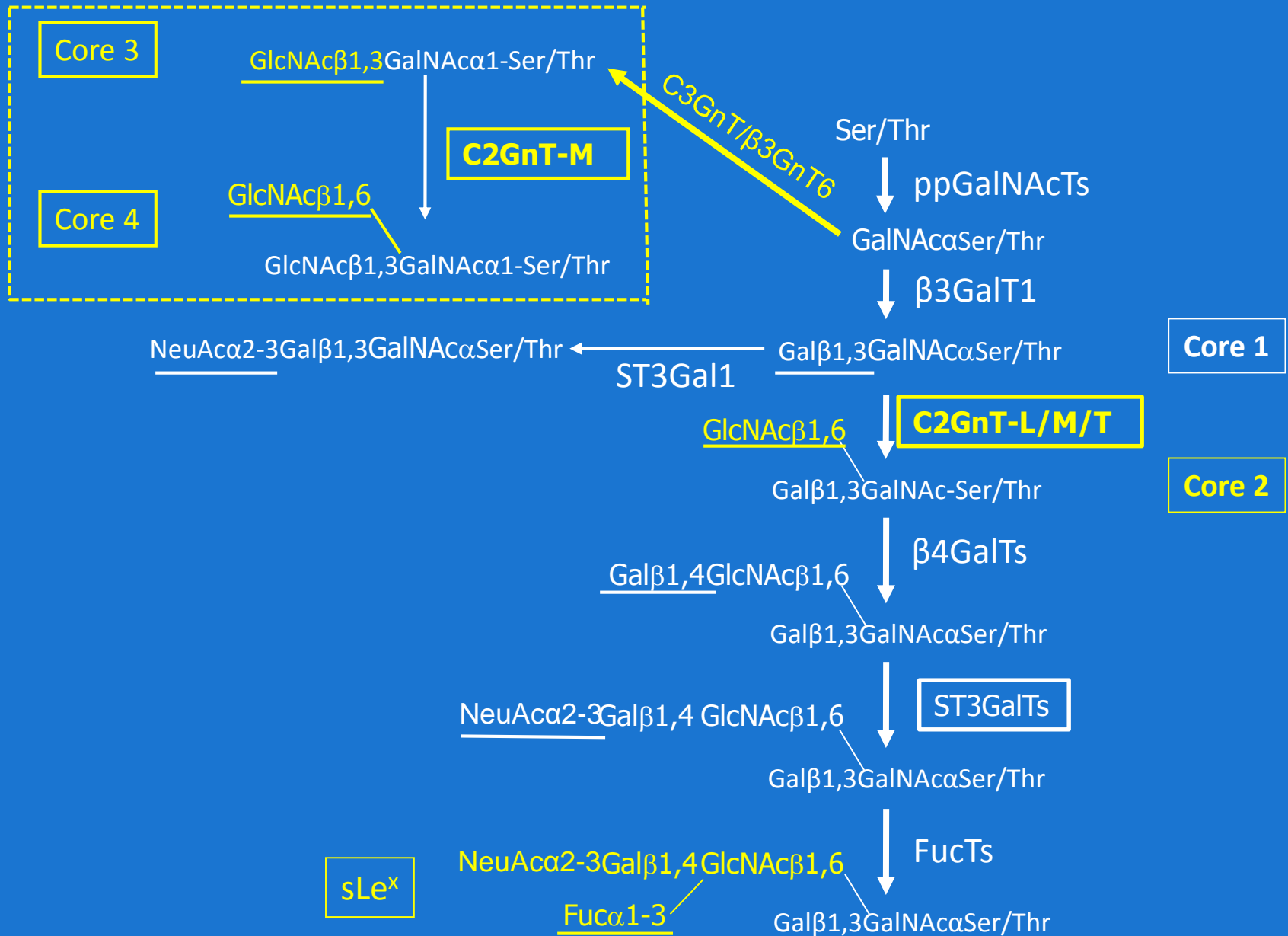
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Outline

- 😊 Structures and functions of mucin O-glycans
- 😊 Factors that regulate glycan biosynthesis
- 😊 Golgi retention of glycosyltransferases
- 😊 Golgi targeting of glycosyltransferases
- 😊 Future study

Major Mucin Core Structures and synthesis of sLe^x on Core 2



Functions of Mucins

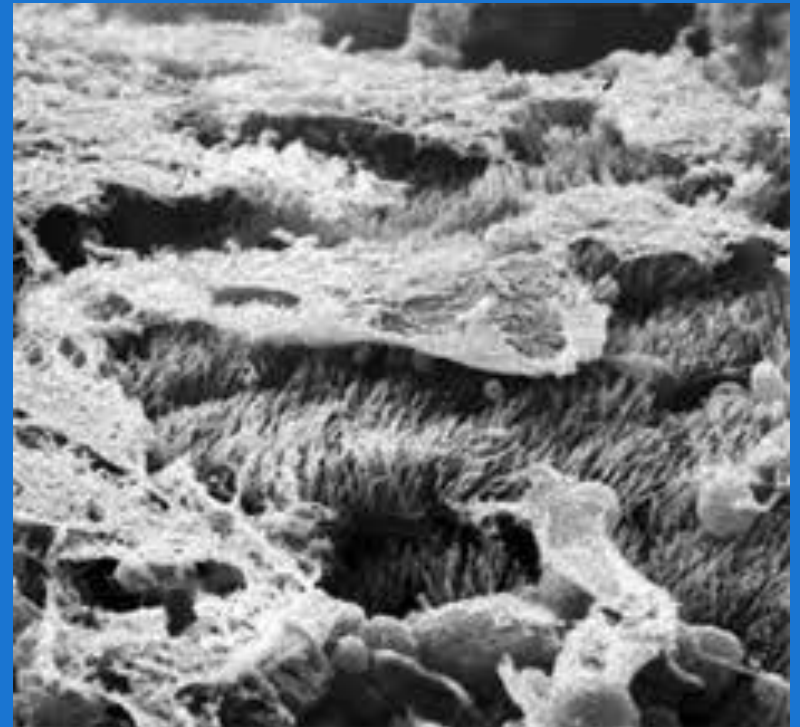
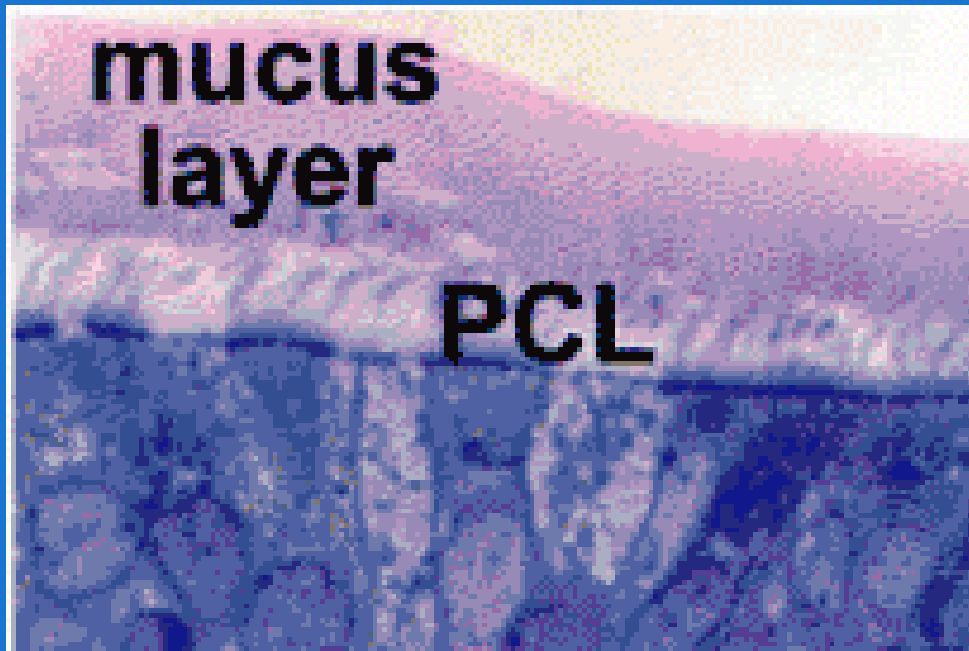
Secreted mucins: Protection of mucus secretory epithelium

1. **Retain water**

High carbohydrate content

2. **Bind and clear inhaled and ingested pathogens**

Heterogeneous carbohydrate structure



Membrane-bound mucins: Signal transduction

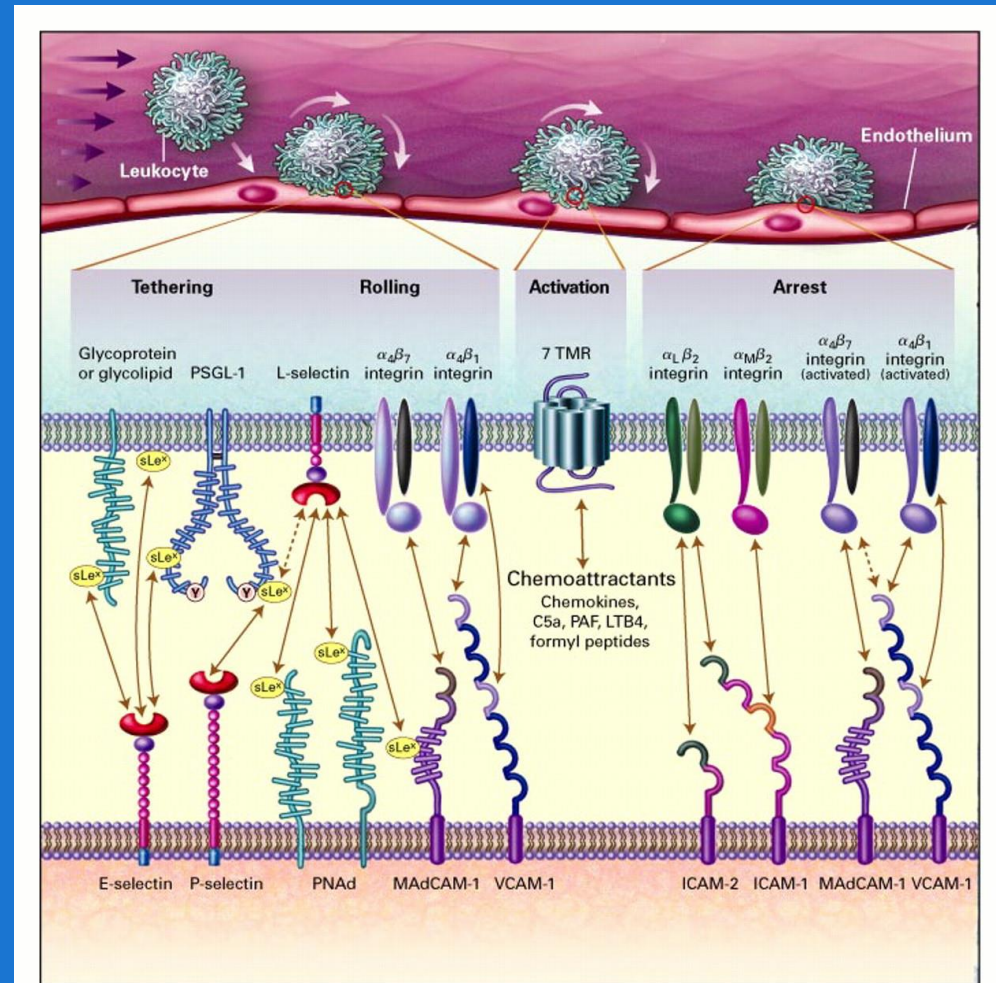
1. **Retain water**: High carbohydrate content
2. **Guide migration of leukocytes/cancer cells**:
sLe^{a/x} and 6-sulfo-sLe^x

Membrane-bound mucins: Signal transduction

1. Retain water: High carbohydrate content
2. Guide migration of leukocytes/cancer cells:
sLe^{a/x} and 6-sulfo-sLe^x

Leukocyte Multistep Adhesion Cascade during Inflammatory Response

(Von Andrian and Mackay, NEJM 343:1020, 2000)



Glycosyltransferase (β 4GalT1)-catalyzed reaction



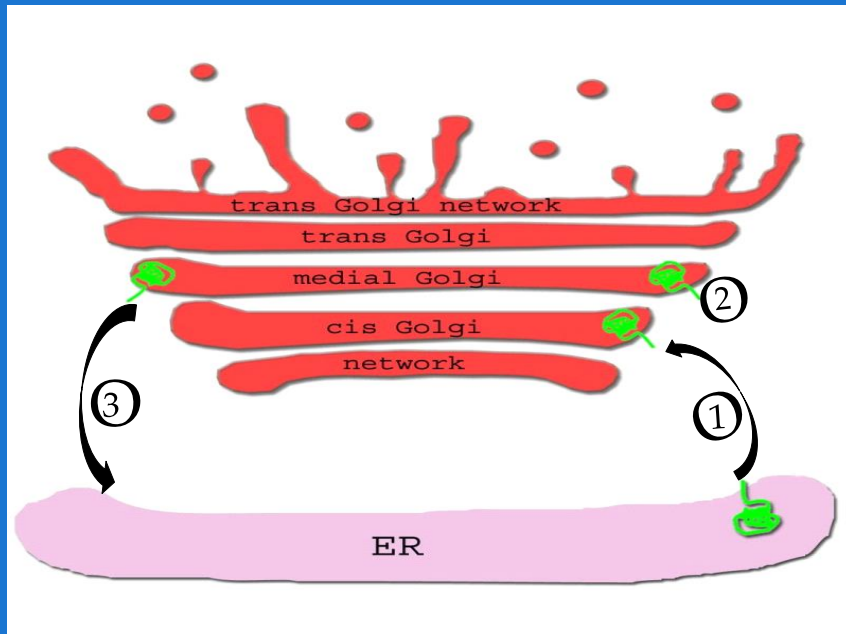
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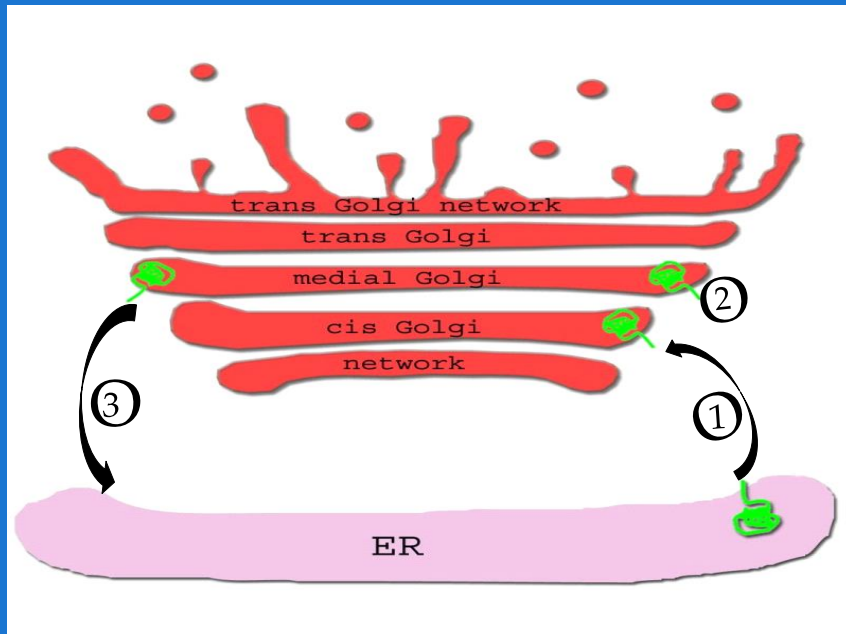
Factors that regulate glycan biosynthesis

1. Substrate (Acceptor) specificity
2. Availability of sugar donor (Nucleotide-sugar)
3. Availability of sugar acceptor
4. Divalent ions and pH
5. Levels of enzymes
6. Golgi localization: Golgi targeting and retention

Golgi Targeting, Retention and Recycling of Glycosyltransferases



Golgi Targeting, Retention and Recycling of Glycosyltransferases



1a. Petrosyan et al: **Glycosyltransferase-specific Golgi targeting mechanisms.** *J Biol Chem* 287:37621-7, 2012.

1b/3d. Petrosyan et al: **Restoration of compact Golgi morphology in advanced prostate cancer enhances susceptibility to galectin-1-induced apoptosis by modifying mucin O-glycan synthesis.** *Mol Cancer Res* 12:1704-16, 2014.

2a. Ali et al: **Golgi phosphoprotein 3 determines cell binding properties under dynamic flow by controlling Golgi localization of C2GnT1.** *J Biol Chem* 287:39564-7, 2012.

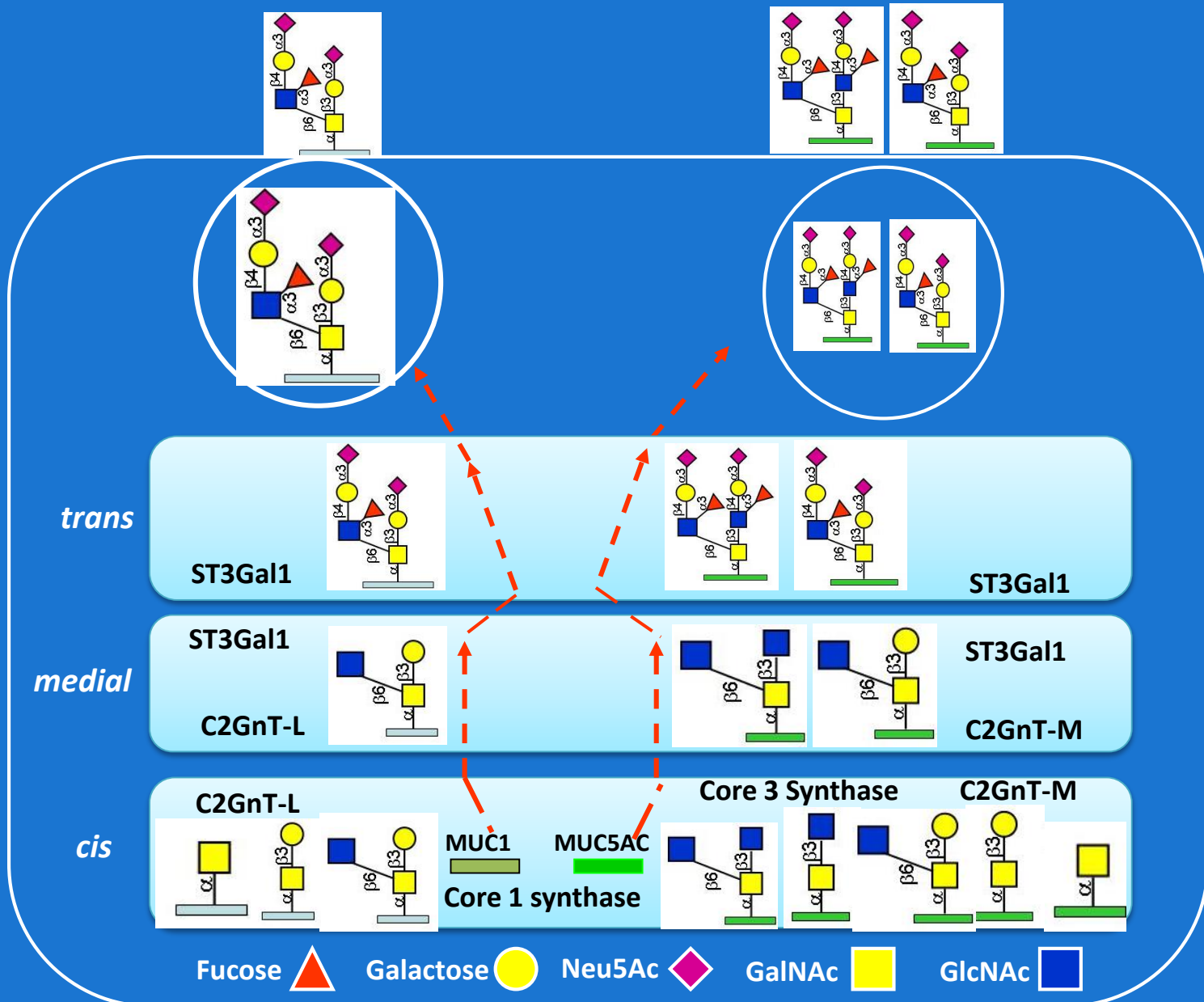
2b. Petrosyan et al: **Keratin 1 plays a key role in Golgi localization of Core 2 N-acetylglucosaminyltransferase M via its cytoplasmic tail.** *J Biol Chem* 290:6256-69, 2015.

3a. Petrosyan et al: **Non-muscle myosin IIA transports a Golgi enzyme to the ER by binding to its cytoplasmic tail.** *Int J Biohem Cell Biol* 44:1153-6, 2012.

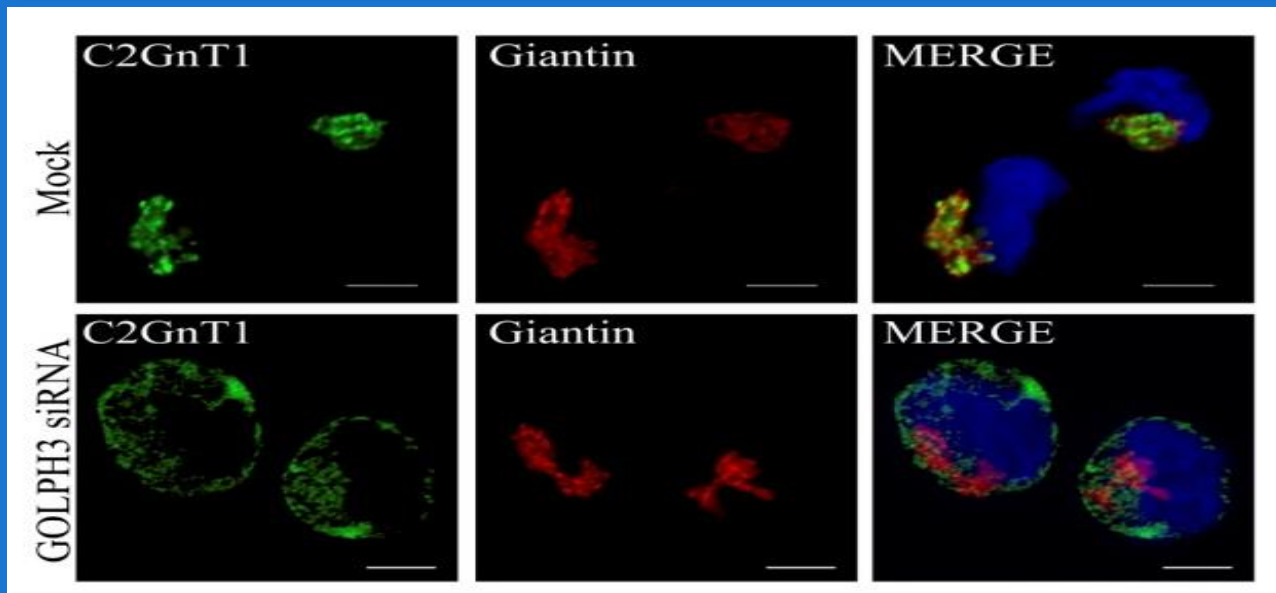
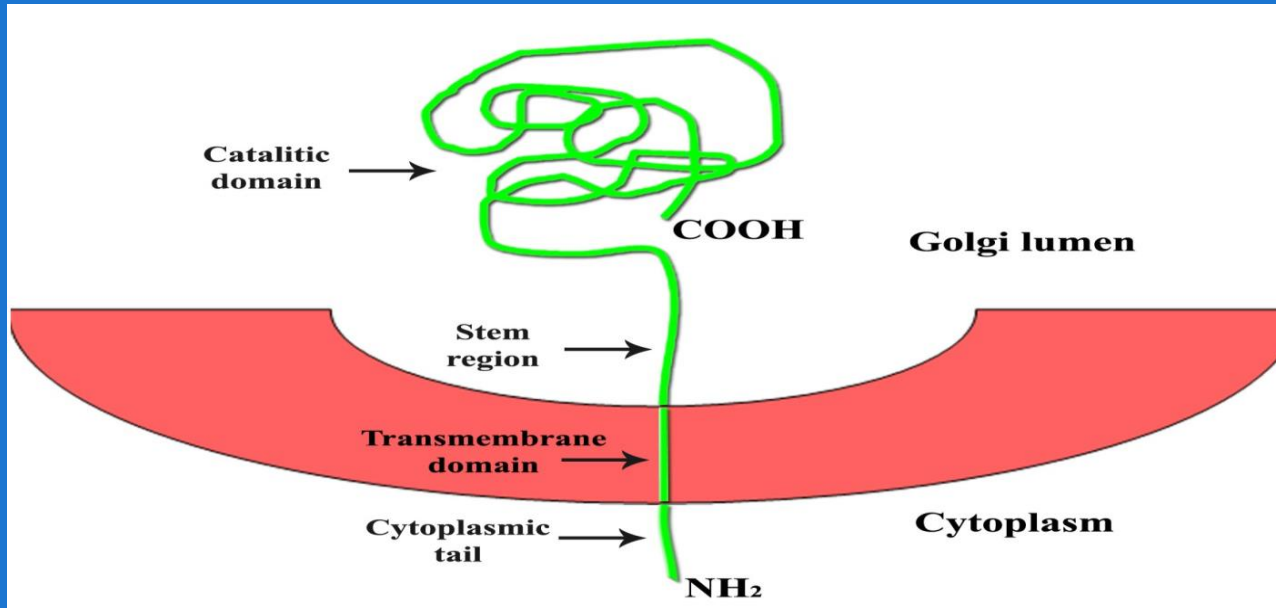
3b. Petrosyan and Cheng: **A non-enzymatic function of Golgi glycosyltransferases: mediation of Golgi fragmentation by interaction with non-muscle myosin IIA.** *Glycobiology* 23:690-708, 2013.

3c. Petrosyan and Cheng: **Golgi fragmentation induced by heat shock or inhibition of heat shock proteins is mediated by non-muscle myosin IIA via its interaction with glycosyl-transferases.** *Cell Stress & Chaperones*. 19:241-54, 2014.

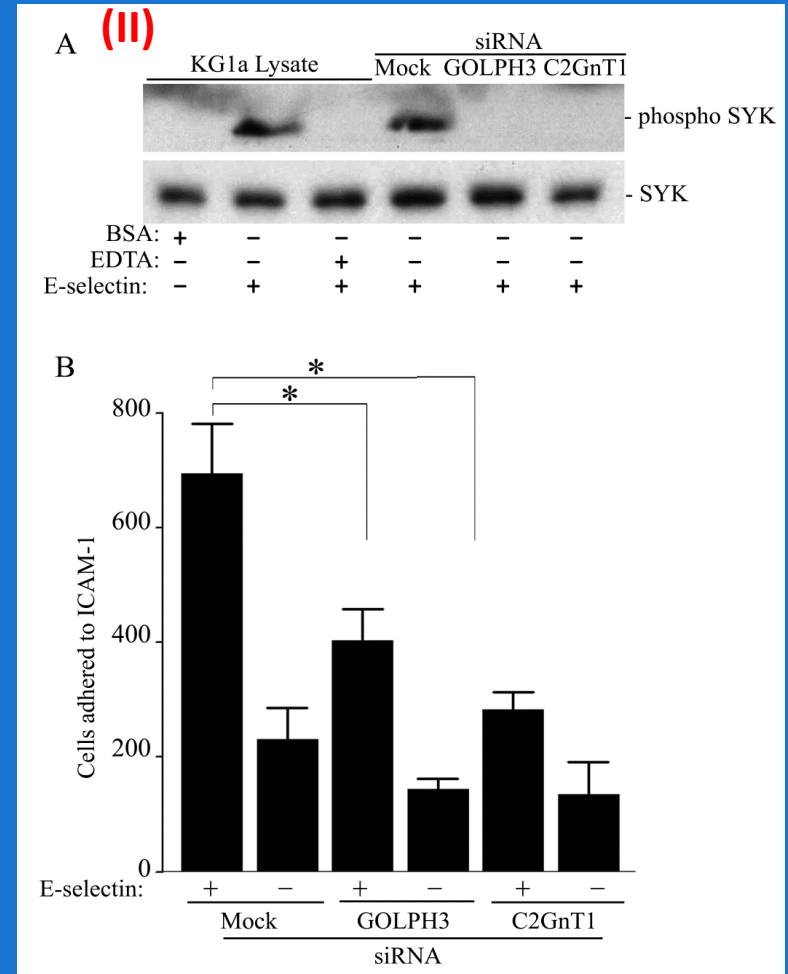
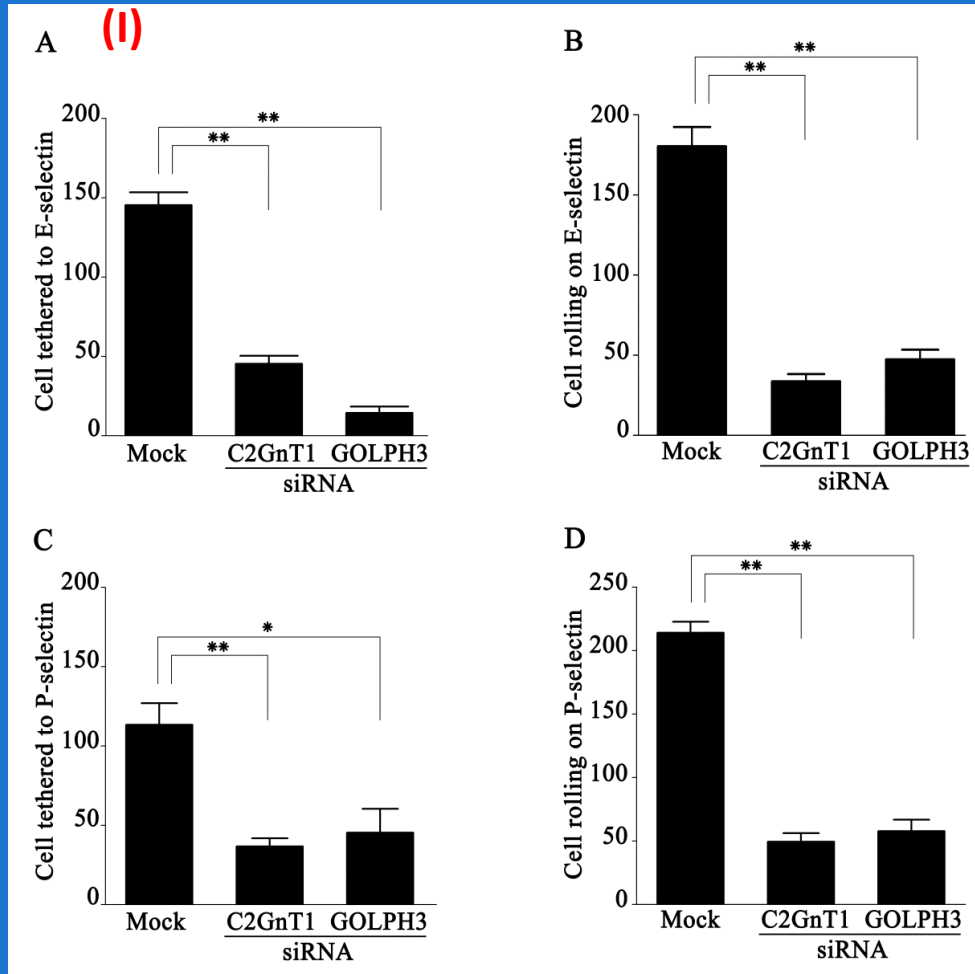
In vivo Glycosylation Scheme



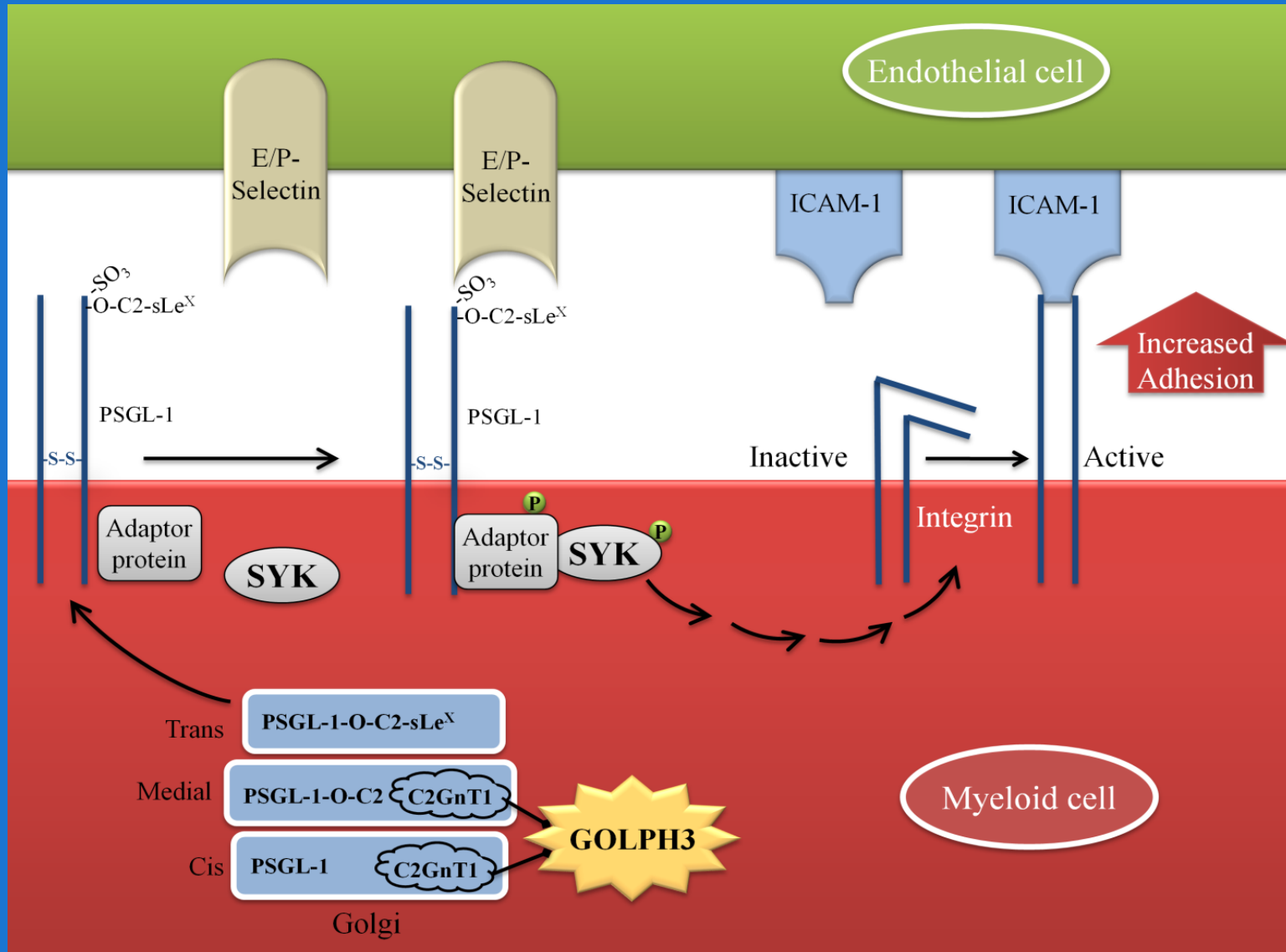
Golgi phosphoprotein 3 (GOLPH 3) is identified as the C2GnT-1/L Golgi-retention protein



Knockdown of C2GnT1 or GOLPH3 in KG1a cells reduces their (I) tethering to and rolling on P or E-selectin and (II) adhesion to ICAM-1 (after activation by E-selectin) under dynamic flow

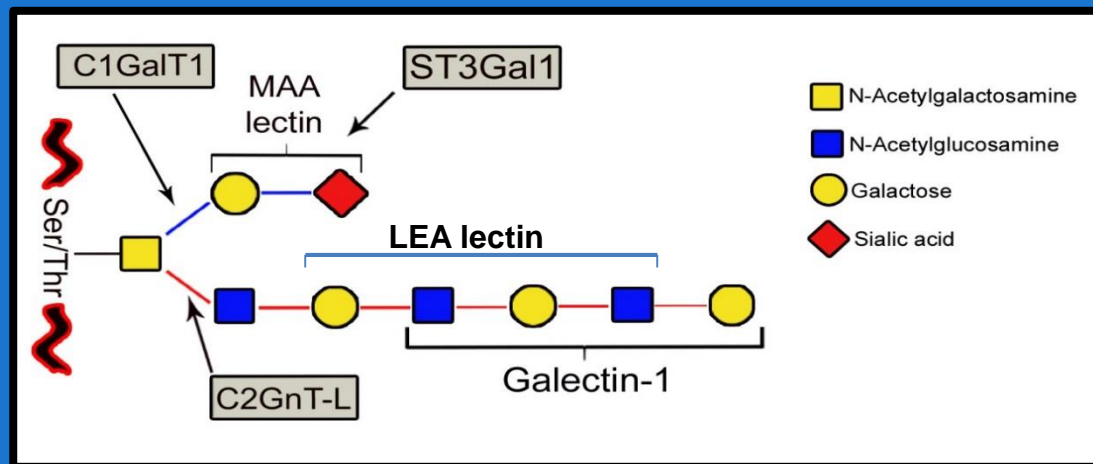


GOLPH 3 regulates the metastatic potential of KG1a cells by controlling Golgi retention of C2GnT1/L

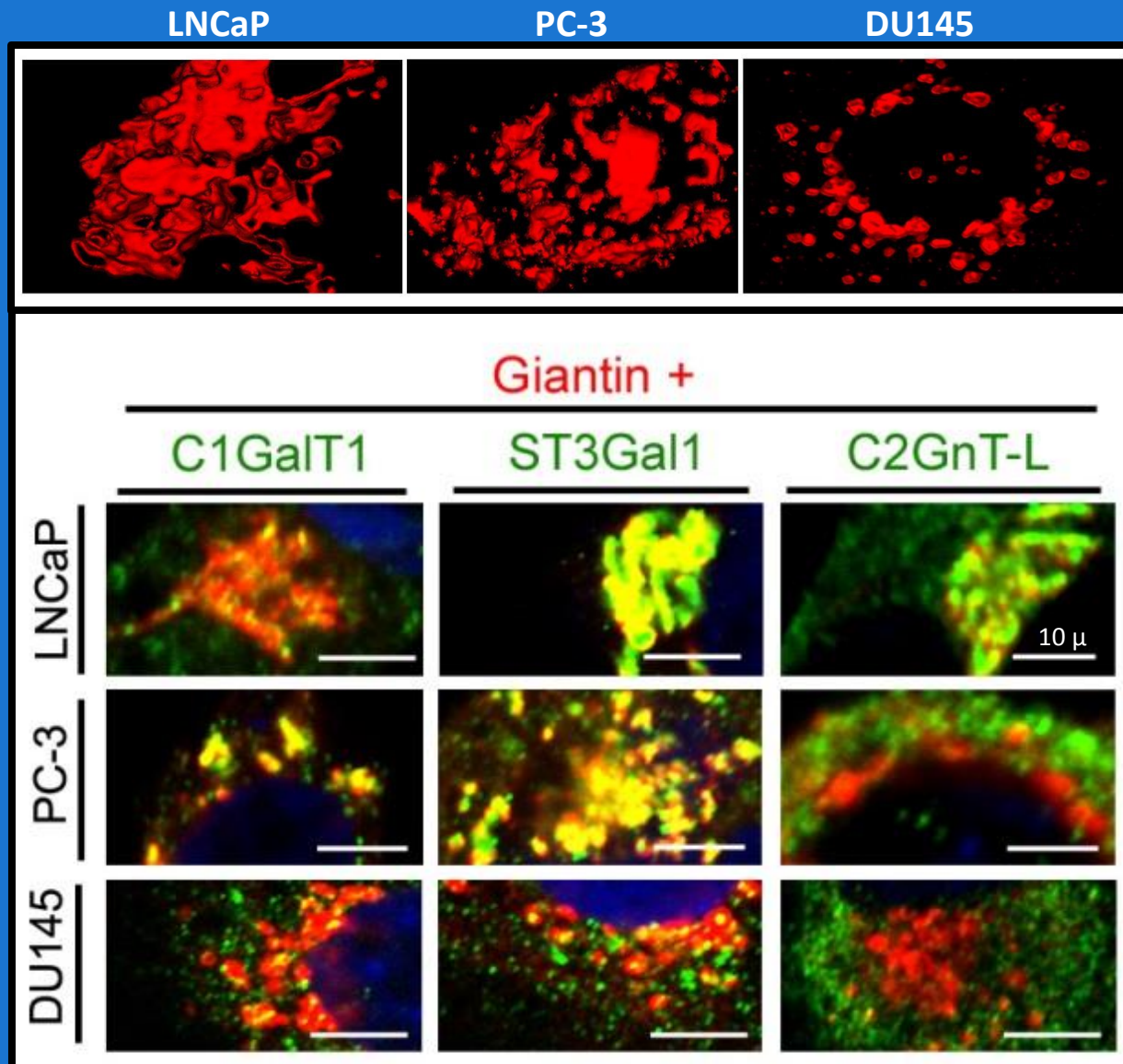


Androgen-sensitive LNCaP cells but not androgen-refractory PC3 and DU145 cells generate Core 2-associated polylactosamine, which renders LNCaP cells susceptible to Galectin 1-induced apoptosis
 (Valenzuela et al. Cancer Res. 67:6155–62, 2007.)

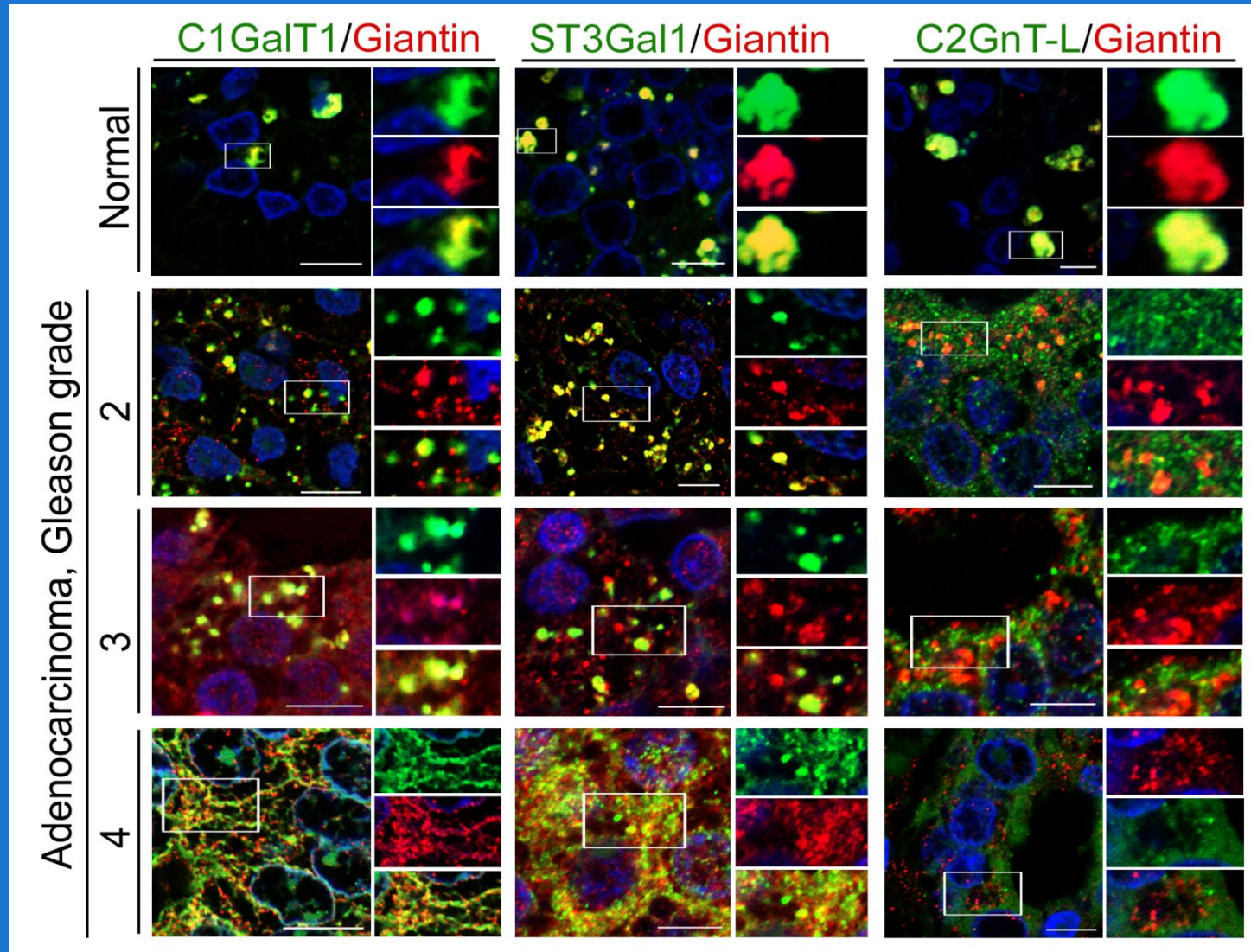
Prostate Cancer Cells	ST3Gal1	C2GnT-1/L	Sialyl-T	Core 2-associated polylactosamine	Galectin 1-induced apoptosis
LNCaP	+	+	-	+	+
PC3/DU145	+	+	+	-	-



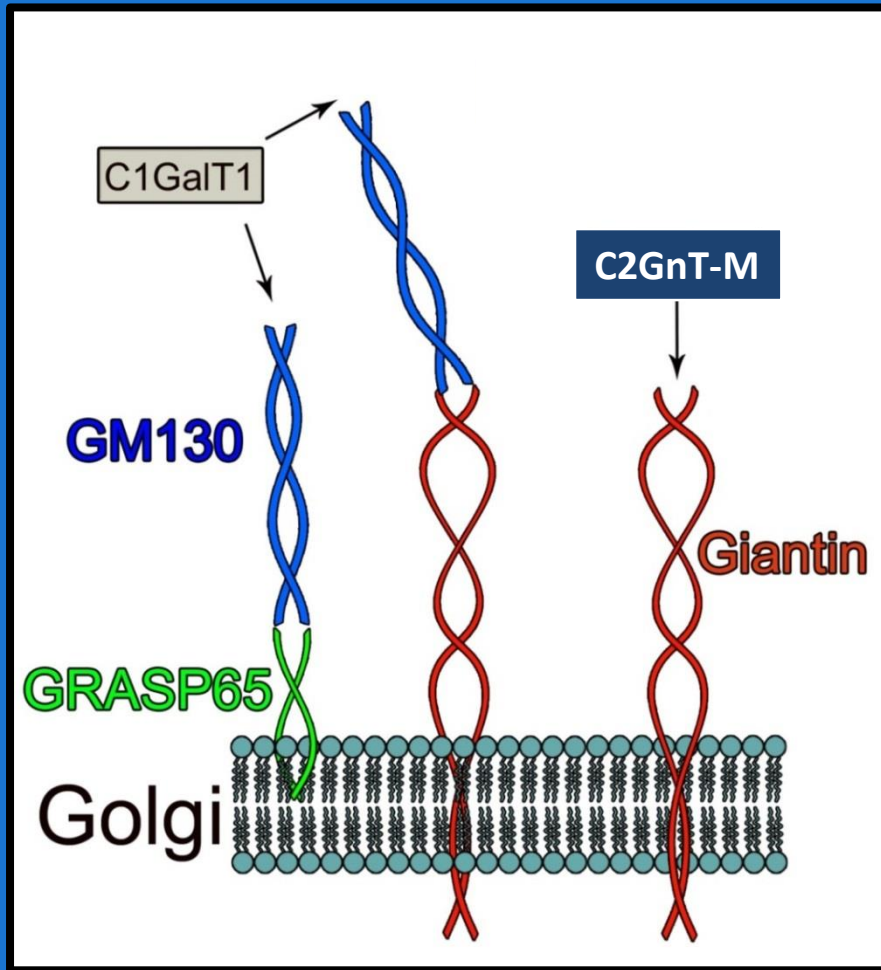
Androgen-sensitive LNCaP prostate cancer cells have compact Golgi, androgen-refractory PC-3 and DU145 cells have fragmented Golgi, and C1GalT1 and ST3GalT1 are in the Golgi of these two cells but C2GnT-L is not



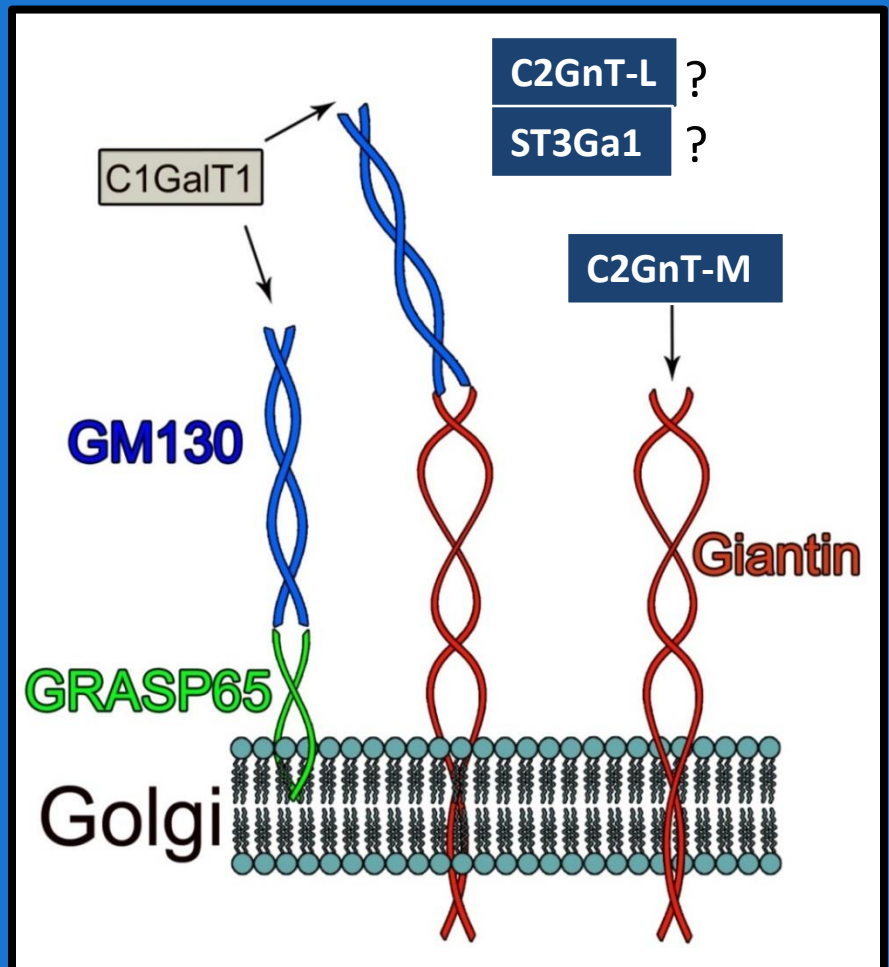
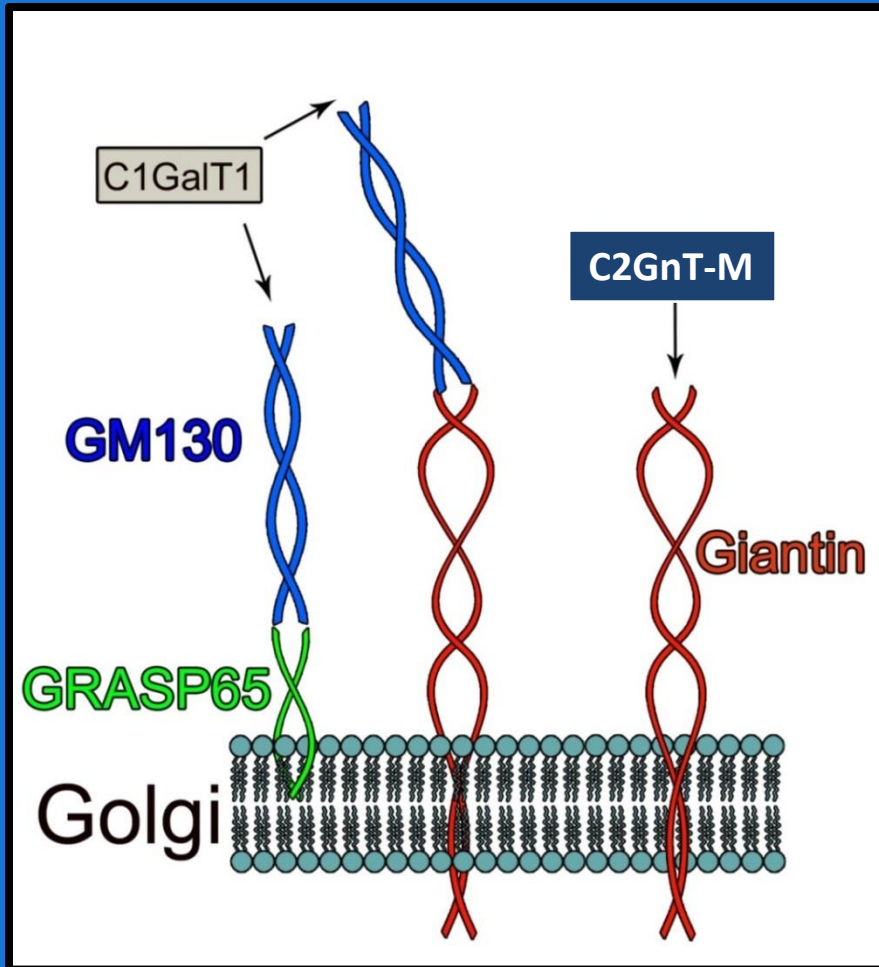
Prostatic tumors exhibit tumor progression-dependent Golgi fragmentation and outside-of-the-Golgi distribution of C2GnT-L



C1GalT1 uses GRASP65-GM130/Giantin for Golgi targeting and C2GnT-M uses Giantin exclusively for Golgi targeting



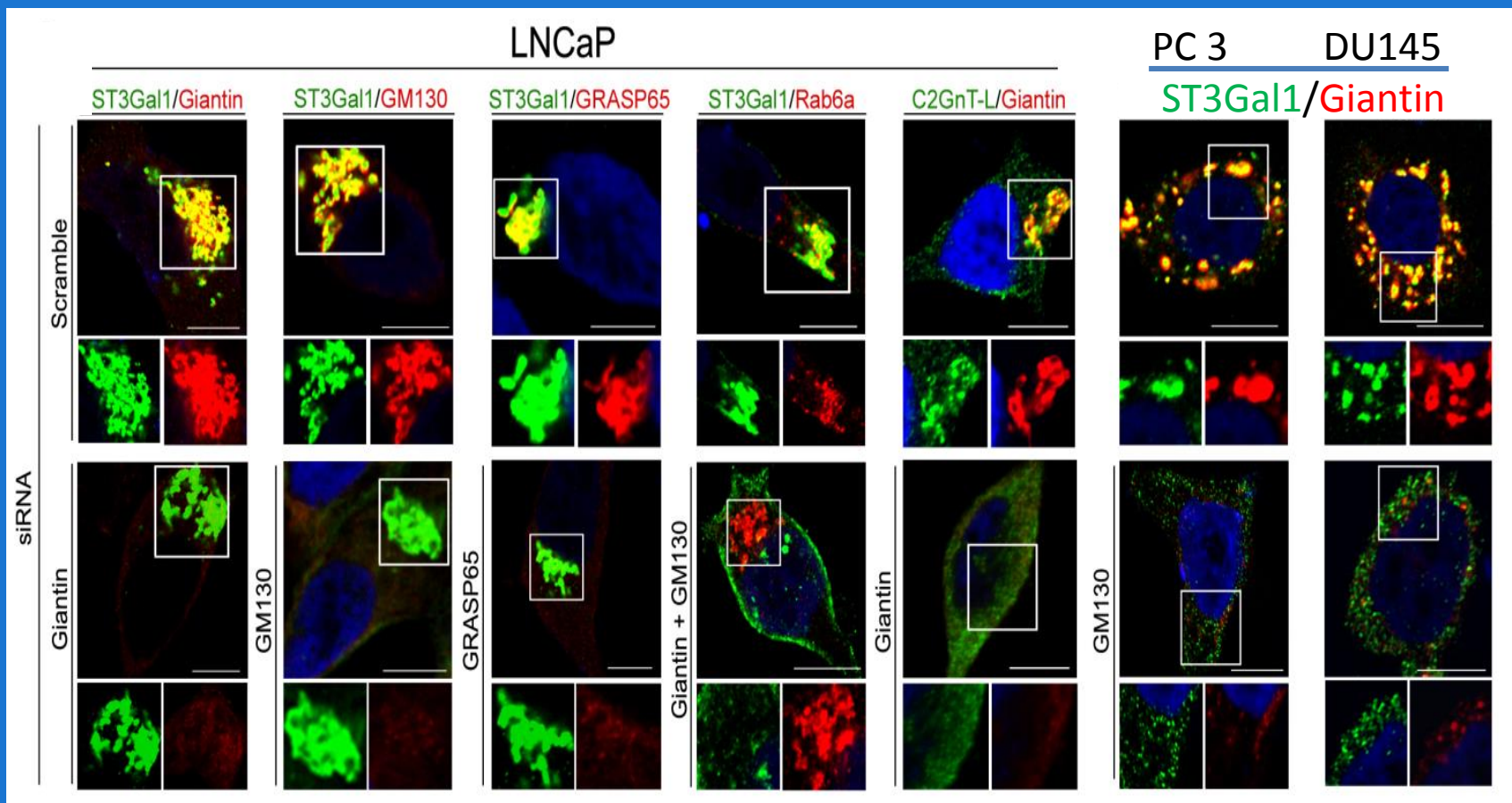
What are the Golgi-targeting sites for ST3Gal 1 and C2GnT-1/L?



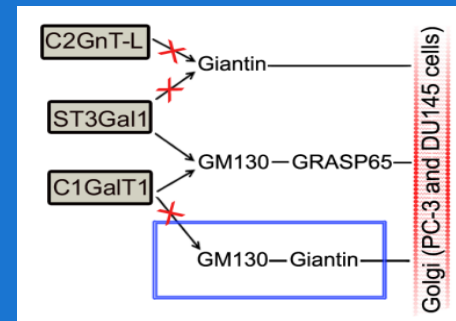
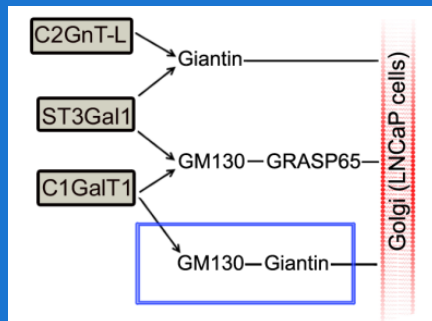
Petrosyan et al: *J. Biol. Chem.* 287:37621-7, 2012.

Petrosyan et al: *Mol. Cancer Res.* 12:1704-16, 2014.

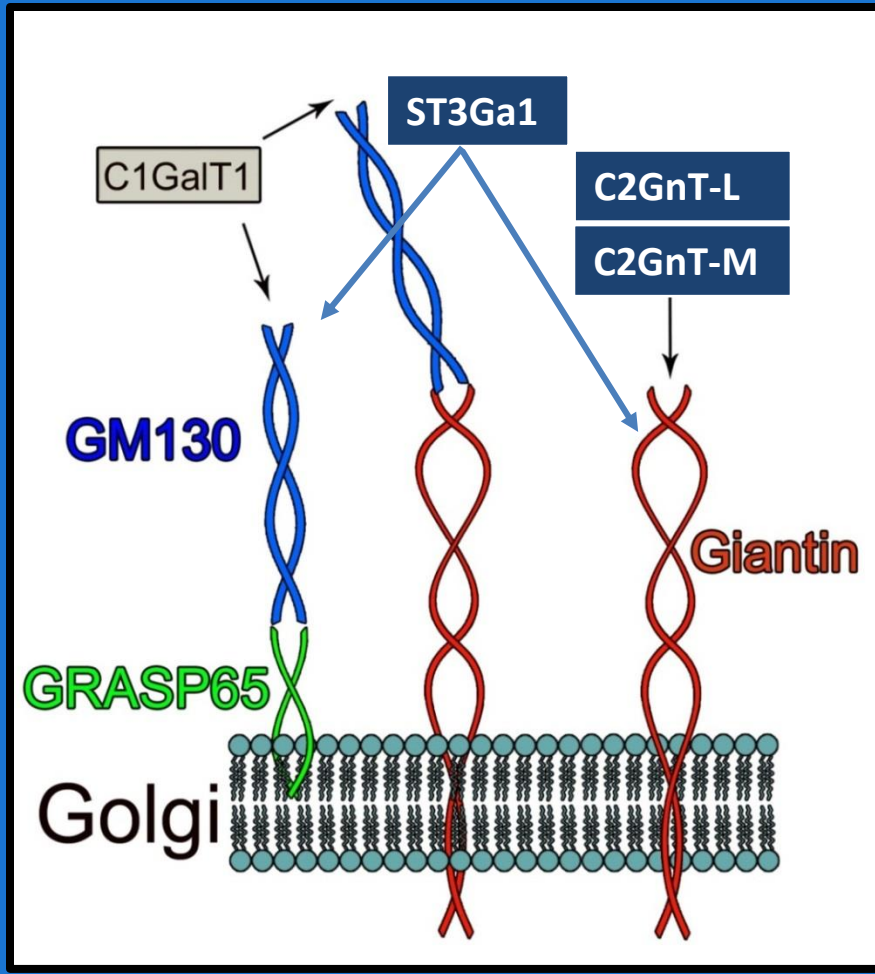
In LNCaP cells, KD of Giantin+C2GnT-L but not Giantin, GM130, or GRASP65 prevents Golgi targeting of ST3Gal1, and KD of Giantin prevents Golgi targeting of C2GnT-L. In PC3 and DU145 cells, KD of GM130 prevents Golgi targeting of ST3Gal1



Petrosyan et al: *Mol. Cancer Res.* 12:1704-16, 2014.



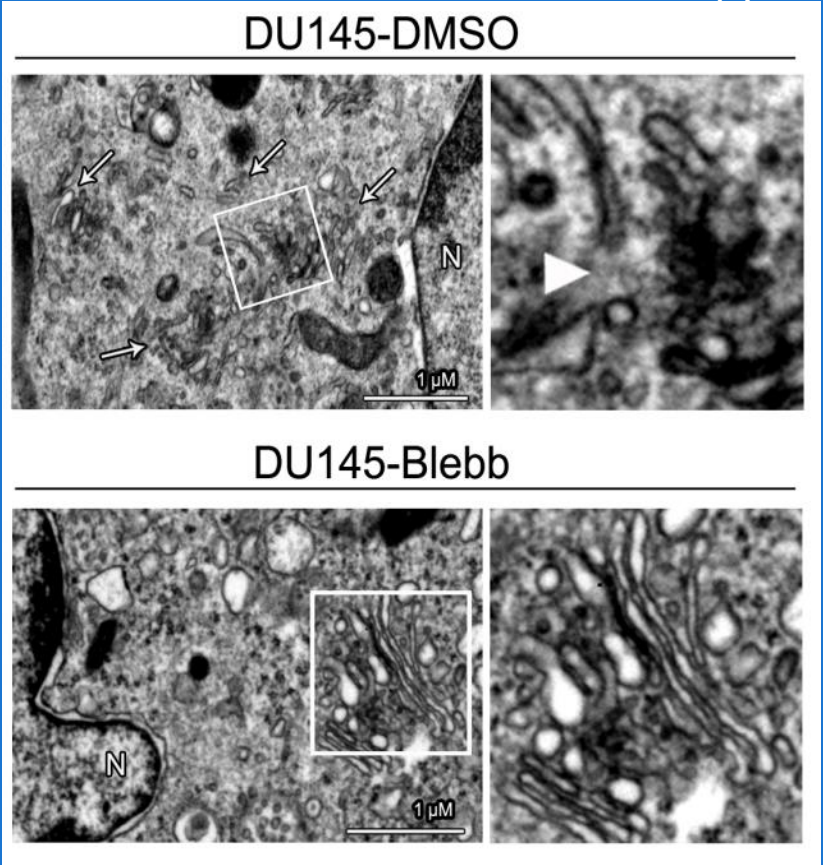
For Golgi targeting, C2GnT-M uses Giantin exclusively, ST3Ga1 uses Giantin and GM130-GRASP65, and C1GaT1 uses GM130-GRASP65



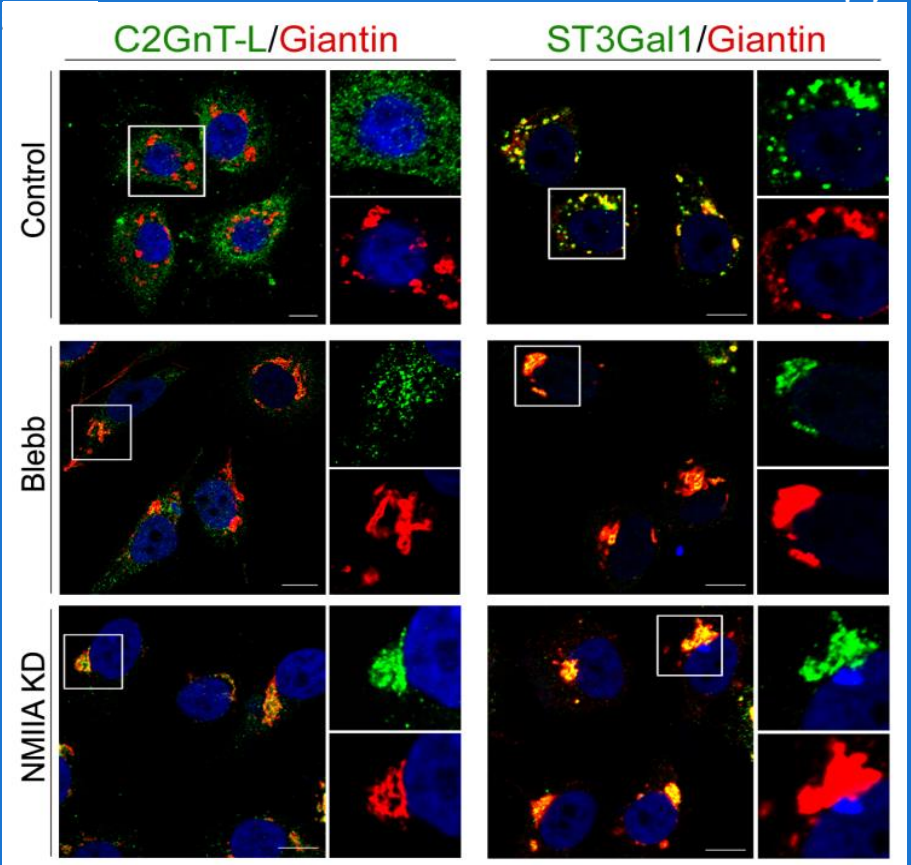
Petrosyan et al: *J. Biol. Chem.* 287:37621-7, 2012.
Petrosyan et al: *Mol. Cancer Res.* 12:1704-16, 2014 .

Inhibition or KD of Non-muscle myosin IIA (NMIIA) in DU145 (also PC3) cells restores compact Golgi morphology (A & B) and Golgi targeting of C2GnT-L (B)

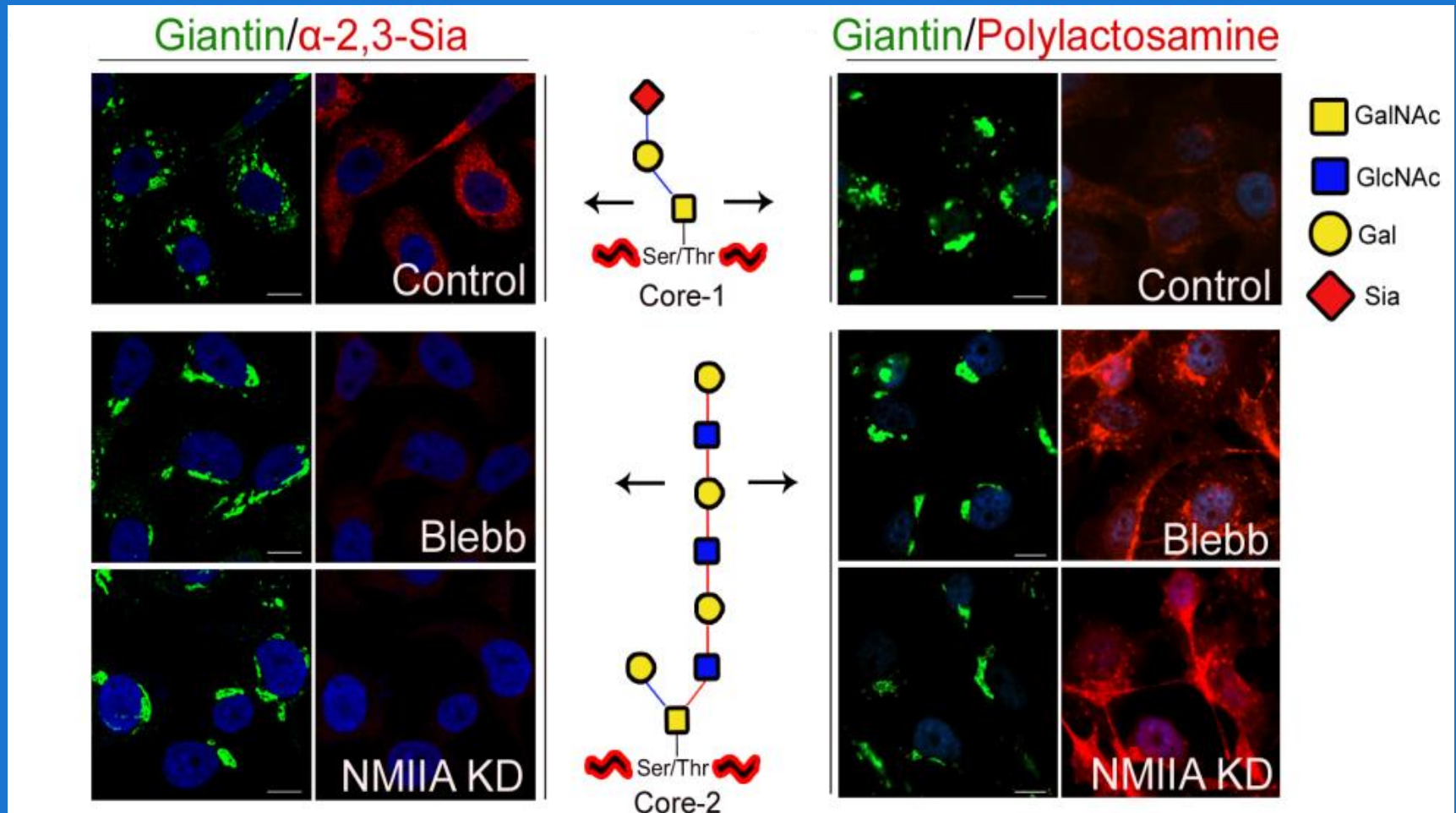
A. Transmission Electron Microscopy



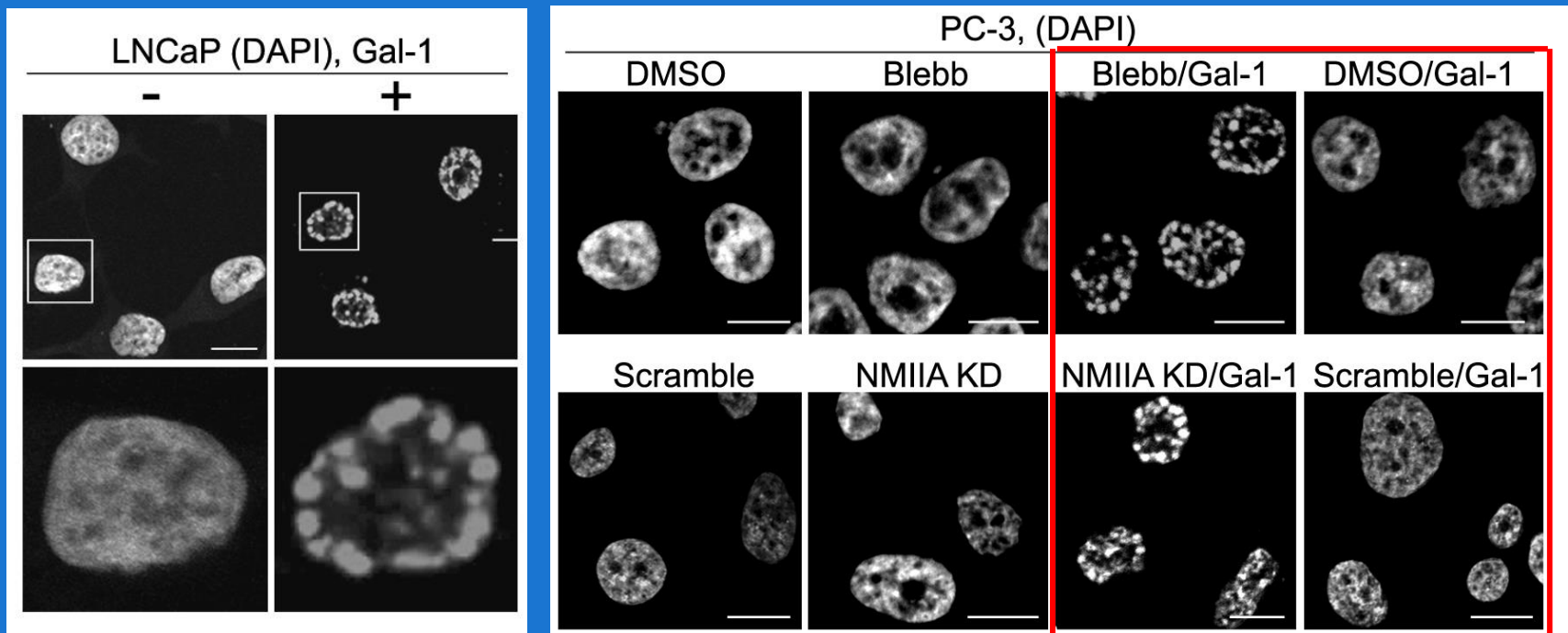
B. Confocal Immunofluorescence Microscopy



Inhibition (Blebb) or KD of NMIIA in DU145 (also PC3) cells converts mucin O-glycan from sialyl-T to Core 2-associated polylactosamine



Galectin-1 induces apoptosis in LNCaP cells but not PC3 (and DU145) cells unless NMIIA is inhibited or knocked down



Petrosyan et al: *Mol. Cancer Res.* 12:1704-16, 2014 .

Conclusions

1. The enzymatic function of a Golgi glycosyltransferase is regulated by its cognate retention protein.
2. The enzymatic functions of C2GnTs are also regulated by giantin, the exclusive Golgi targeting site for these enzymes.

Future study

The effects of altered Golgi targeting of glycosyltransferases induced by stress, such as heat shock, alcohol abuse, cancer malignant transformation etc, on N- and O-glycosylation