Glycoproteins

Foundations in Mammalian Glycobiology

Lecture 2

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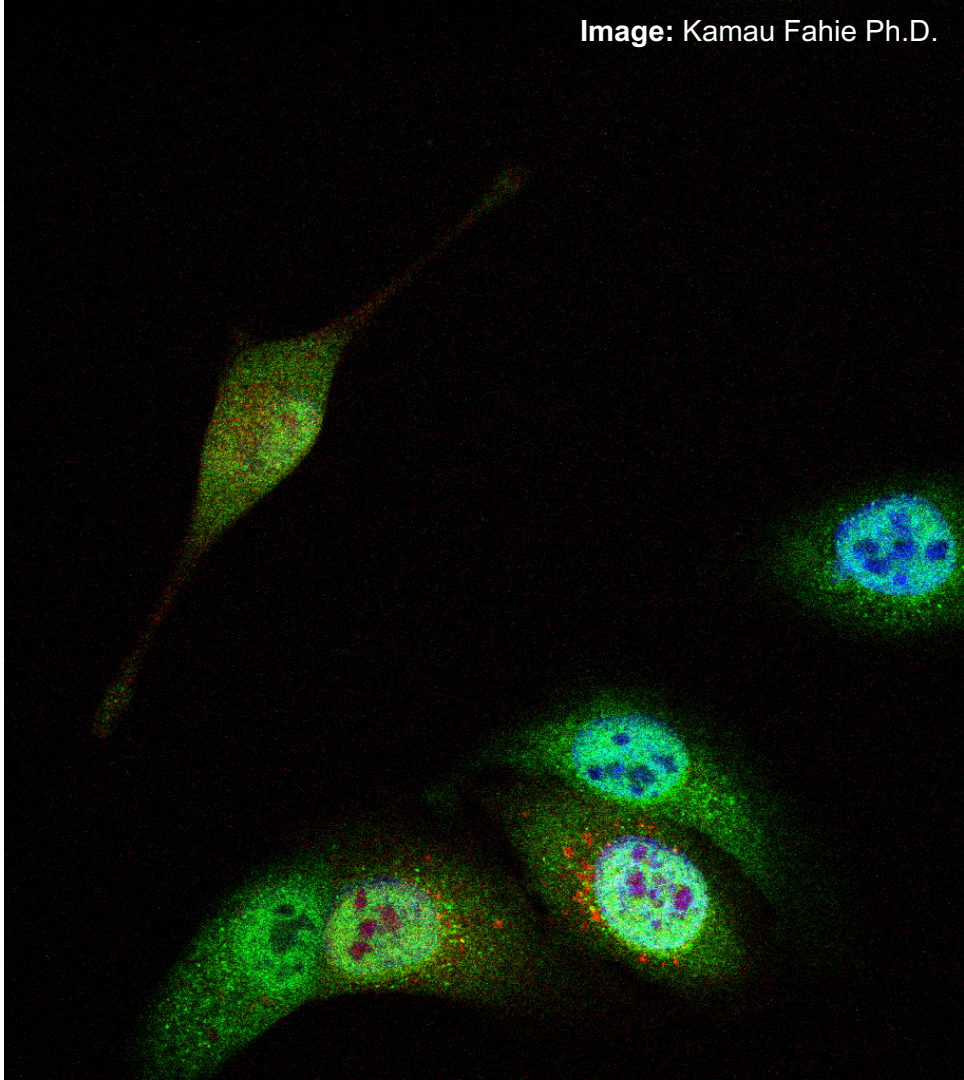
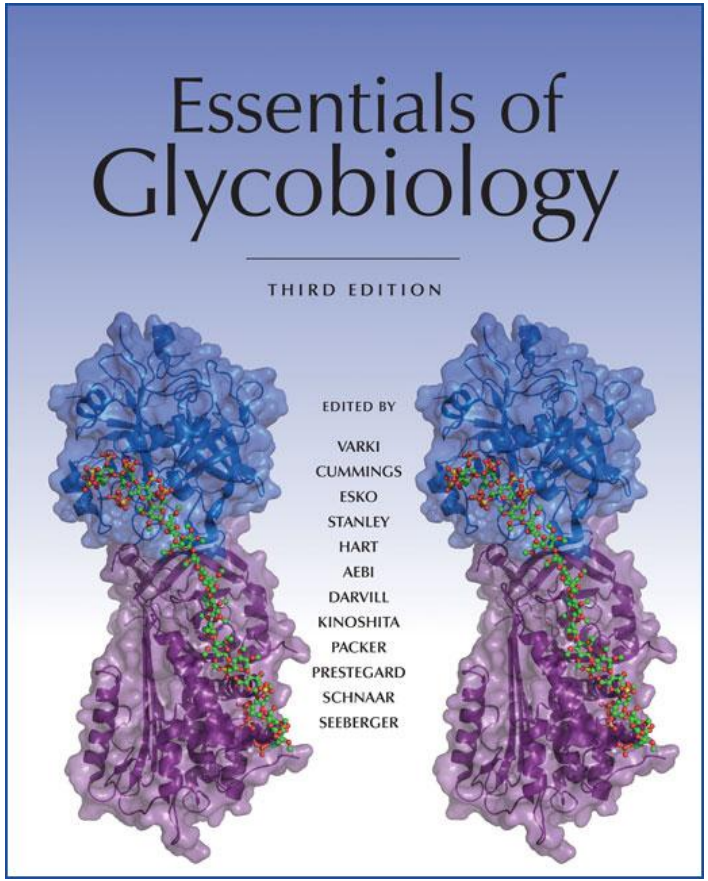


Image: Kamau Fahie Ph.D.

Today

- **Lecture - Glycoproteins:**
  - What is a glycoprotein?
  - What are are the classes of glycoprotein?
  - Examples of glycan function
- **Journal Club:**
  - *Substrate Selectivity of OGT*
- **Practicum:**
  - *Glycosylation of OGT using the Glycoprotein Builder*



Terminology: The Glyco-code

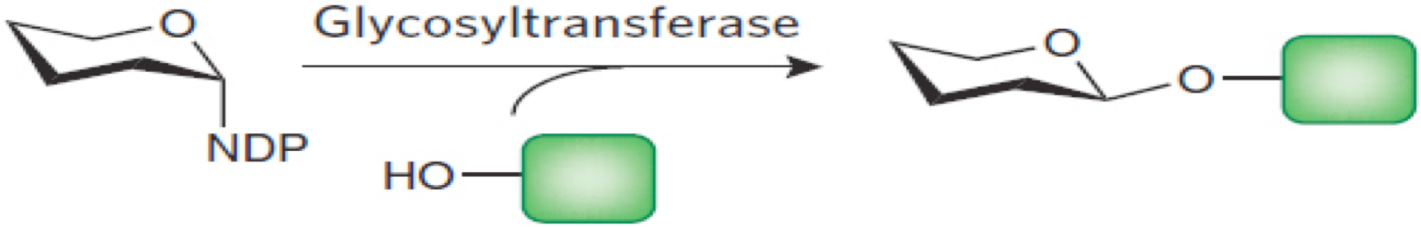
Table 1.

Monosaccharide symbol nomenclature

SHAPE	White (Generic)	Blue	Green	Yellow	Orange	Pink	Purple	Light Blue	Brown	Red
Filled Circle	Hexose	Glc	Man	Gal	Gul	Alt	All	Tal	Ido	
Filled Square	HexNAc	GlcNAc	ManNAc	GalNAc	GulNAc	AltNAc	AllNAc	TalNAc	IdoNAc	
Crossed Square	Hexosamine	GlcN	ManN	GalN	GulN	AltN	AllN	TalN	IdoN	
Divided Diamond	Hexuronate	GlcA	ManA	GalA	GulA	AltA	AllA	TalA	IdoA	
Filled Triangle	Deoxyhexose	Qui	Rha			6dAlt		6dTal		Fuc
Divided Triangle	DeoxyhexNAc	QuiNAc	RhaNAc							FucNAc
Flat Rectangle	Di-deoxyhexose	Oli	Tyv		Abe	Par	Dig	Col		
Filled Star	Pentose		Ara	Lyx	Xyl	Rib				
Filled Diamond	Nonulosonate		Kdn				Neu5Ac	Neu5Gc	Neu	Sia
Flat Hexagon	Unknown	Bac	LDManHep	Kdo	Dha	DDManHep	MurNAc	MurNGc	Mur	
Pentagon	Assigned	Api	Fru	Tag	Sor	Psi				

Glycobiology 25: 1323–1324, 2015. (PMID 26543186).

Glycoprotein(s): Proteins to which one or more carbohydrate has been covalently attached in an enzymatic reaction.



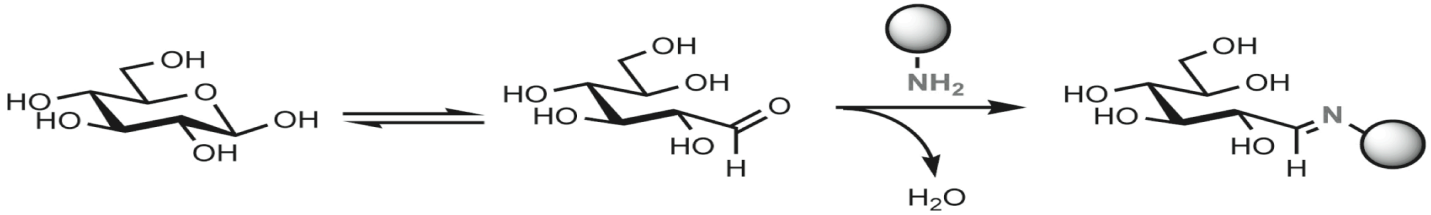
Terminology:

Glycosyltransferase: Enzyme that catalyzes transfer of a sugar from a sugar nucleotide donor to a substrate or aglycone

Protein: aglycone

(**Image: Field RA**. Nat Chem Biol., 2011, PMID: 21931313)

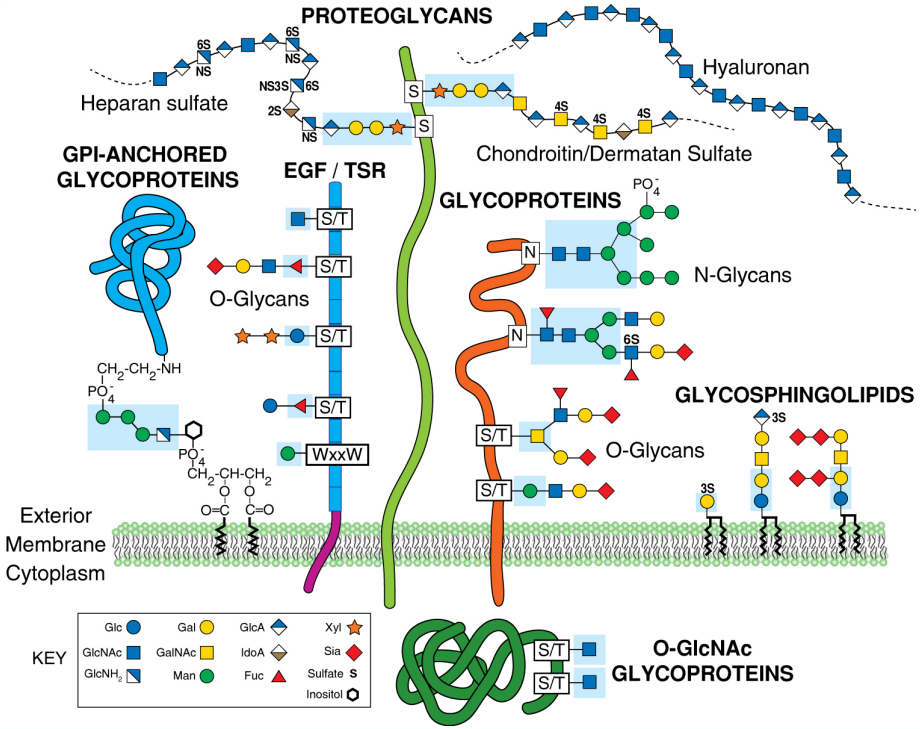
Glycation is NOT Considered a Forms of Glycosylation



Glycation: chemical modification usually through a Schiff-base reaction with the amino group of the side chain of lysine and subsequent Amadori rearrangement to give a stable conjugate.

(**Image:© FASEB Journal**, PMID: 10627289)

Classes of Glycoprotein



Glycoproteins:

N-linked

O-linked

S-linked

C-mannosylation

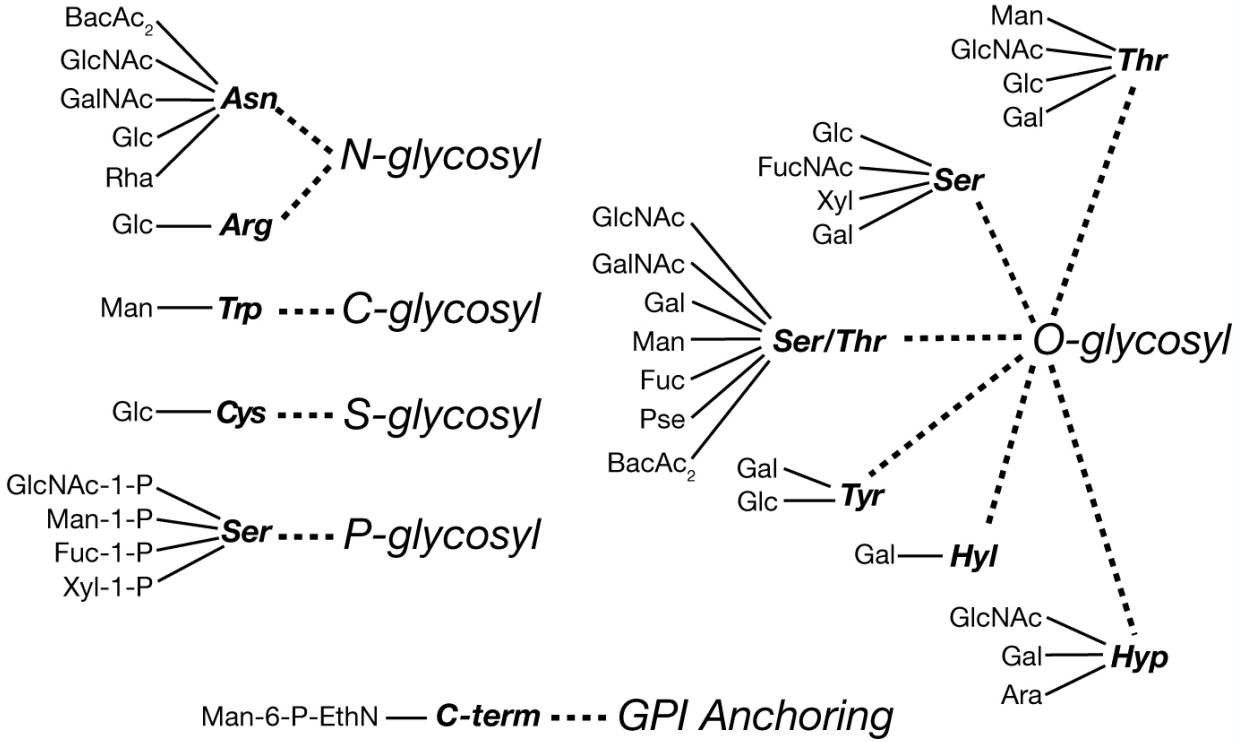
Phospho-linked

GPI anchored

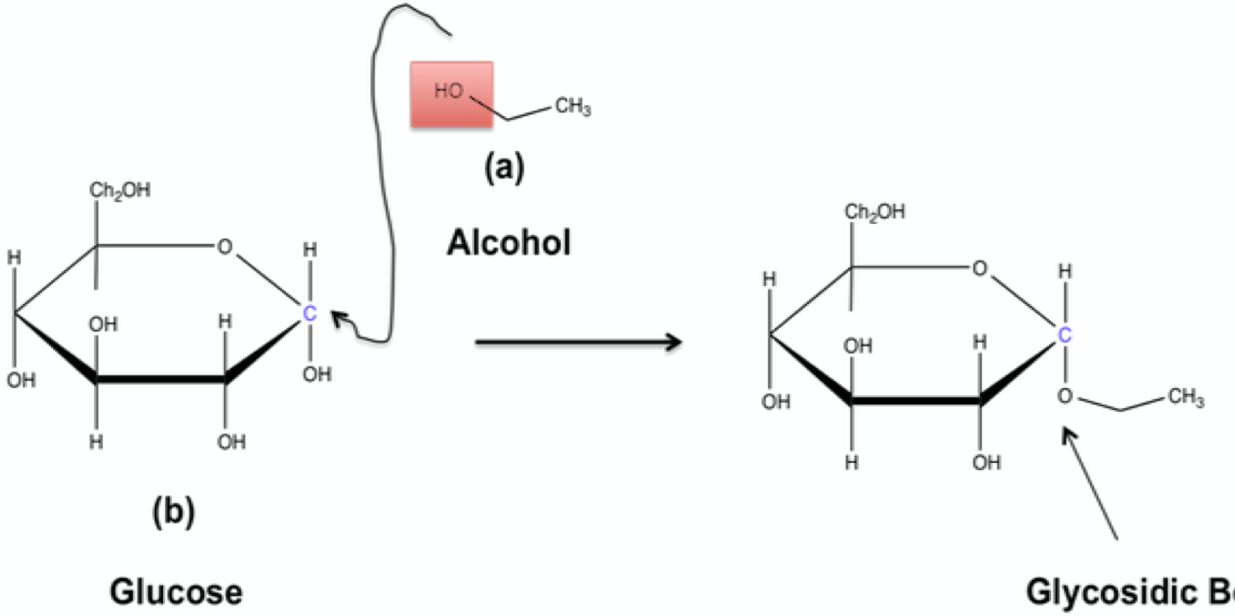
Proteoglycans

Proteins/Glycoproteins modified by one more more glycosaminoglycans

The glycans attached to proteins are characterized by the nature of the linkage between the sugar and the protein, and by the sugar attached



An O-linked glycan is a carbohydrate linked to a through an O-glycosidic bond



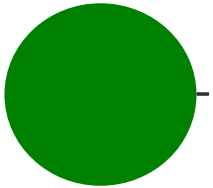
With the exception of O-GlcNAc (O-Mannose in Yeast, O-Fucose in Dictyostelium and some Plants), αGlcNAc-O-HyPro (Dictyostelium) and Glycogen (O-Glucose) –

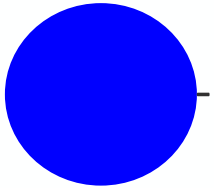
O-linked glycoproteins are secreted, found on the plasma membrane, or in organelles with membranes with the same orientation as the plasma membrane.

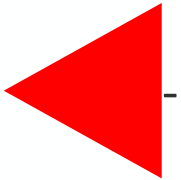
(**Image:** http://study.com/academy/lesson/glycosidic-linkage-definition-lesson-quiz.html)

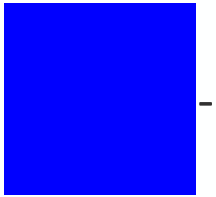
Common forms of Mammalian O-Glycosylation

- O -Ser/Thr


- O -Ser/Thr


- O -Ser

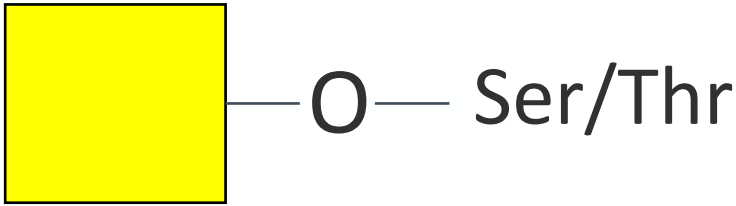

- O -Ser/Thr


- O -Ser/Thr (Cys)


- O -Ser/Thr

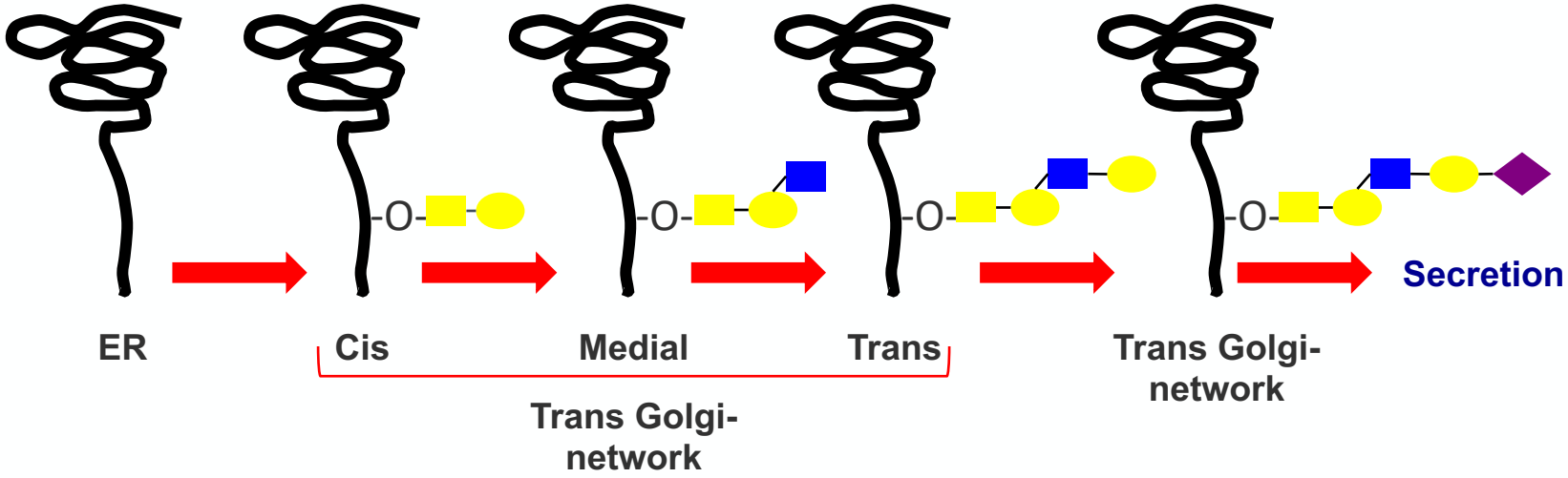
Less common linkages to:
Hydroxyproline
Hydroxylysine
Tyrosine

Mucin-like glycosylation



- The most common form of ER/Golgi-mediated O-glycosylation in mammals
- Mucins – the first known O-linked glycoproteins (1865, E. Eichwald)
- Typically – GalNAc linked to Ser/Thr though an O-glycosidic bond
- Almost always extended into more complex glycans
- Interestingly, in simple eukaryotes such as trypanosomes GlcNAc is used instead of GalNAc; In fungi mannose is often used

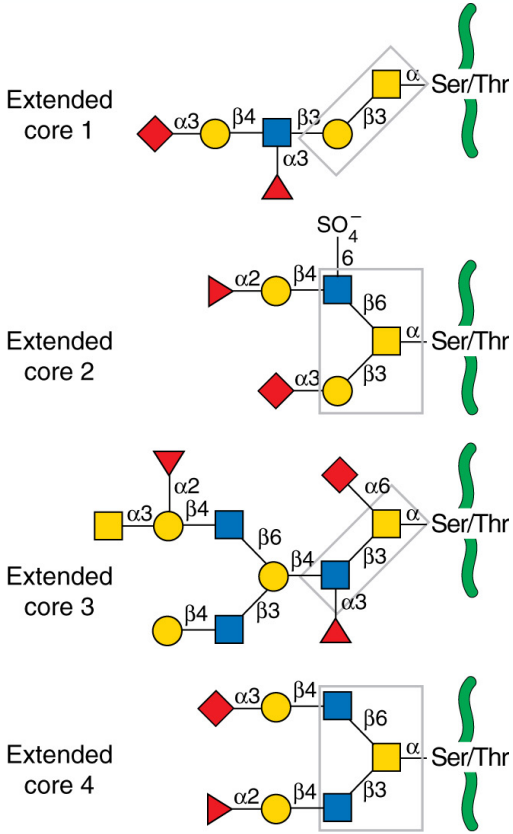
Biosynthesis of O-Glycans in the Golgi



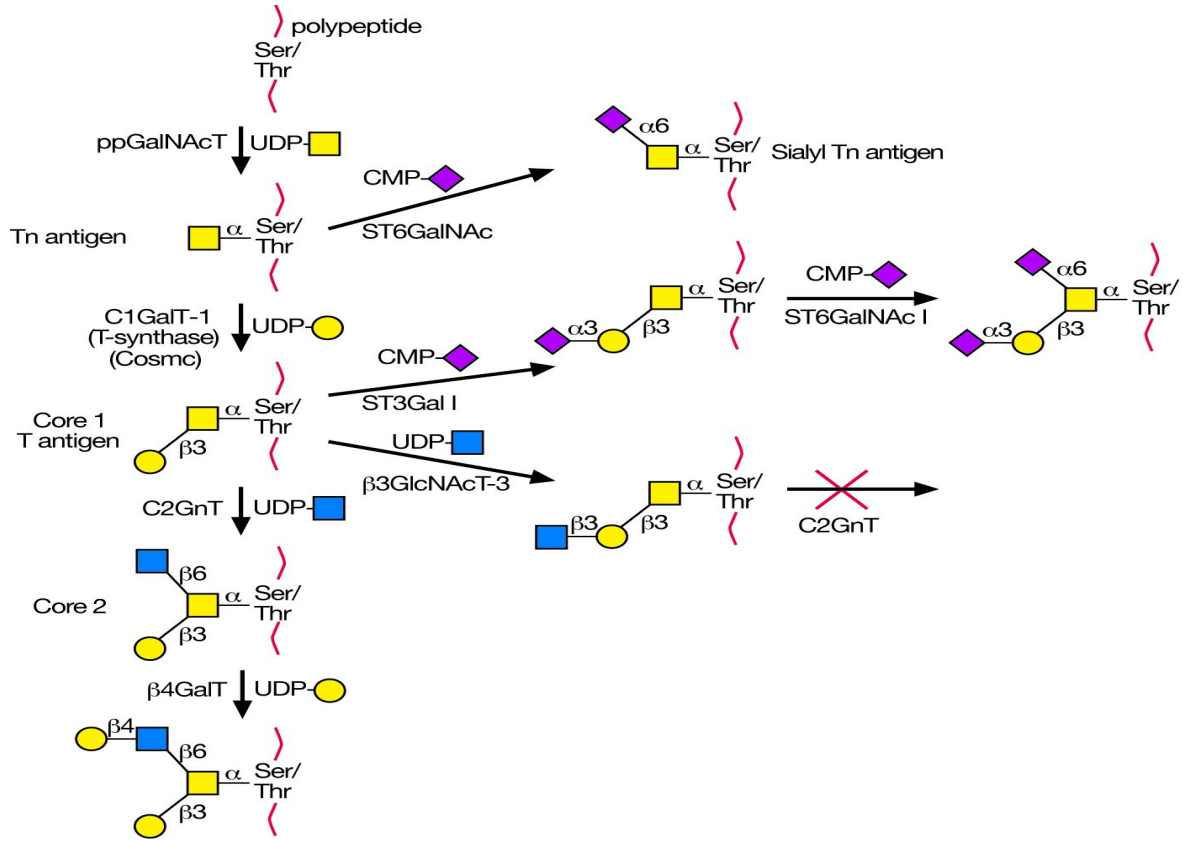
- Mucin-like glycosylation is added in the Golgi apparatus;
- The major sugars found in these O-glycans are GalNAc, GlcNAc, Galactose, Fucose, and Sialic Acid. GalNAc and Galactose can be sulfated
- The structures that are formed are typically determined by the expression, regulation, and localization of glycosyltransferases in the Golgi Apparatus.

There are 8 core structures, although only 1-4 are common

Core	Structure	Antigen
Core 1	Galβ1-3GalNAcα-Ser/Thr	T-Antigen
Core 2	GlcNAcβ1-6(Galb1-3)GalNAcα-Ser/Thr	
Core 3	GlcNAcβ1-3GalNAcα-Ser/Thr	
Core 4	GlcNAcβ1-6(GlcNAcb1-3)GalNAcα-Ser/Thr	
Core 5	GalNAcα1-3GalNAcα-Ser/Thr	
Core 6	GlcNAcβ1-6GalNAcα–Ser/Thr	
Core 7	GalNAcβ16GalNAcα-Ser/Thr	
Core 8	Galα1-3GalNAcα-Ser/Thr	
		Other Antigens
	GalNAcα-Ser/Thr	Tn Antigen
	Sialylα2-6GalNAcα-Ser/Thr	Sialyl-Tn Antigen



Biosynthesis of core 1 and 2 O-GalNAc glycans



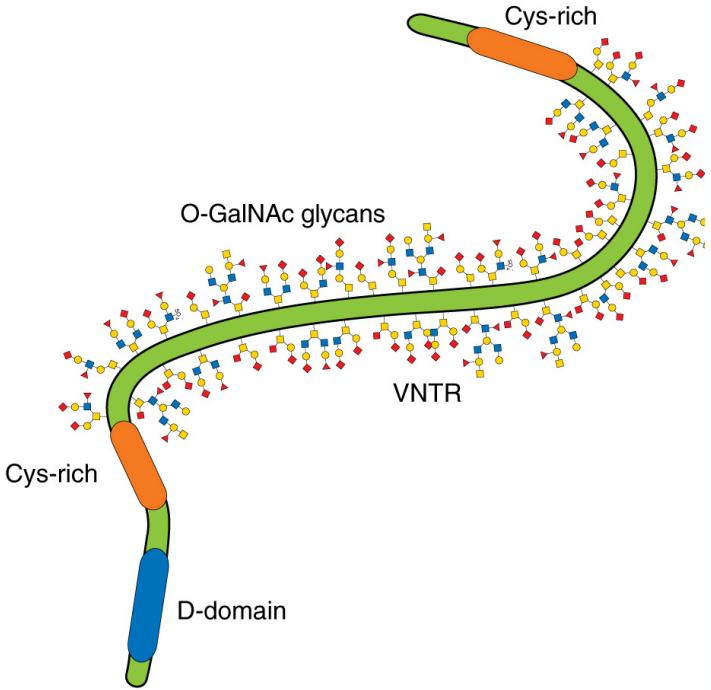
Chapter 9, Figure 3

Essentials of Glycobiology

Second Edition

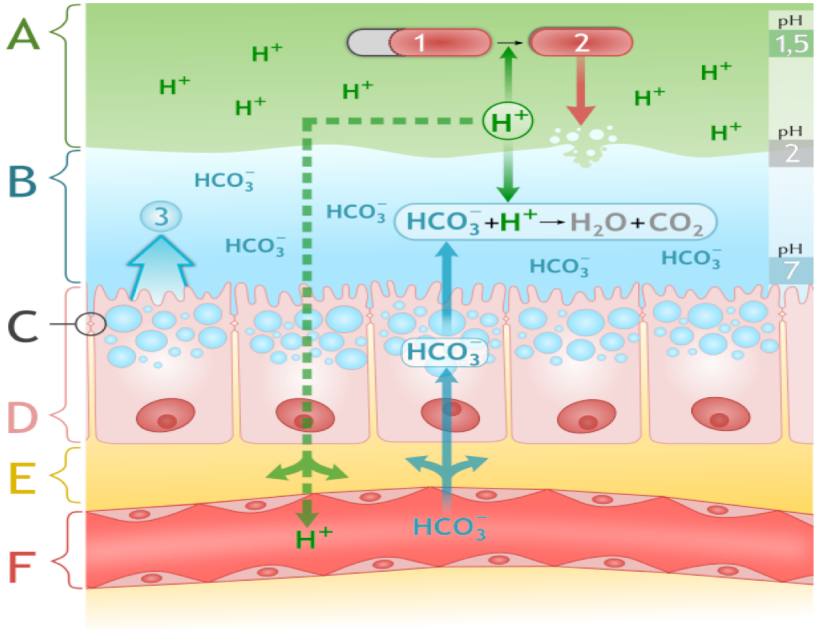
Mucins

- Mucins can be broadly divided into two groups:
  - Polymeric or gel-forming mucins
  - Smaller and monomeric mucins or soluble mucins
- ~20 mucins
- Both classes share characteristic features:
  - Variable number of tandem repeat (VNTR); rich Proline Serine and Threonine
  - Heavily glycosylated
  - Attract water
- Mucins
  - Hydrate and protect the underlying epithelium



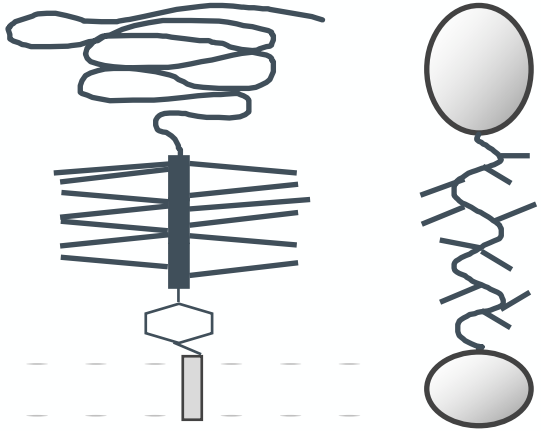
Gel Forming Mucins in the Stomach

- *Mucins (Muc 5AC and Muc 6) are one factor that protects the gastric epithelia from HCl*
- *Mucins, in particular the hydrated gel formed by the glycosylated VNTR, form a diffusion barrier*
- *Bicarbonate ions are held in the mucus layer creating a gradient from pH 2 at the stomach lumen to pH 6–7 at the epithelial-cell surface*



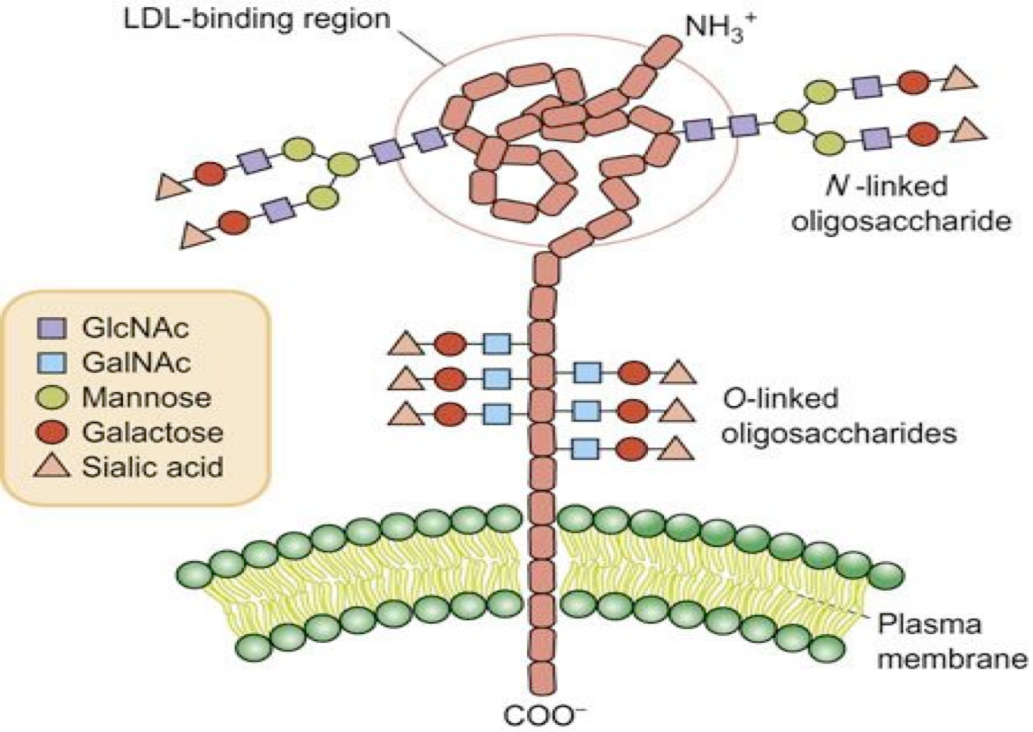
http://en.wikipedia.org/wiki/Foveolar_cell#/media/File:Stomach_mucosal_layer_labeled.svg

Mucin-like domains



- Often contain variable numbers of tandem repeats;
- Sugars project domains from each other or away from the plasma membrane;
- Protect the extended protein sequence from proteases;
- Sugars can play additional functional roles.

How does GalNAc/Gal regulate the expression of the LDL receptor



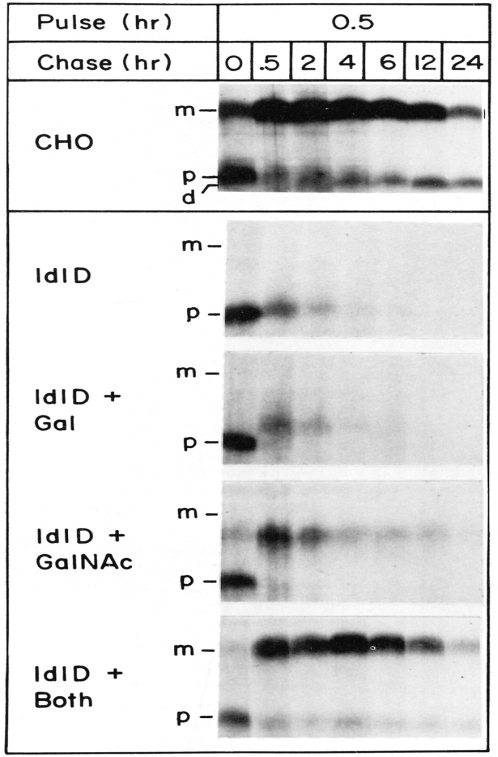
LDLR, which are present on the plasma membrane of most cells, bind particles that contain APOB or APOE proteins, such as chylomicron remnants, VLDL and LDL.

An alternative to de novo synthesis for cellular cholesterol acquisition is uptake from lipoproteins from the extracellular milieu through receptor-mediated mechanisms.

Glycosylation regulates the stability of the LDL-Receptor

- *LDL receptor is synthesized as a 125,000 dalton precursor (p) that was rapidly converted to a 155,000 dalton mature form (m)by extensive processing of N- and O-linked carbohydrate chains.*

- *The mature form of the receptor was relatively stable and could still be detected after chase times as long as 24 hr*



Kozarsky K, Kingsley D, Krieger M.. Proc Natl Acad Sci U S A. 1988 Jun;85(12):4335-9. PMID:3380796

Glycosylation regulates the stability of the LDL-Receptor

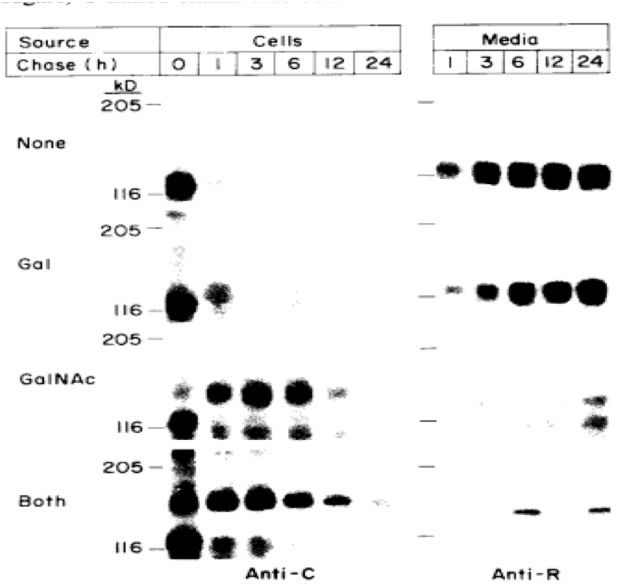
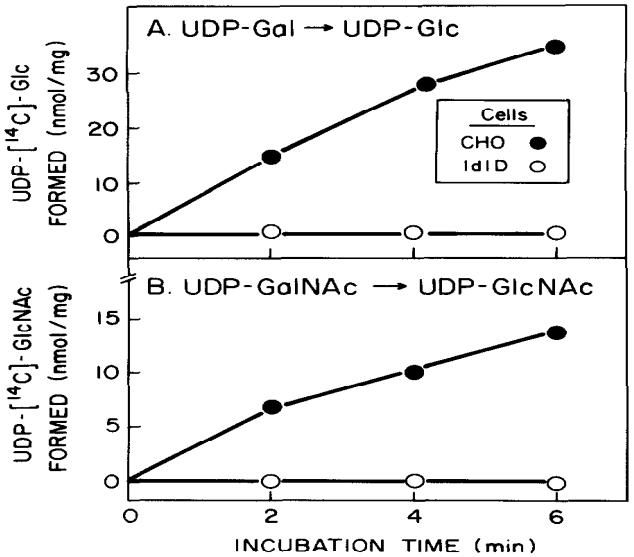
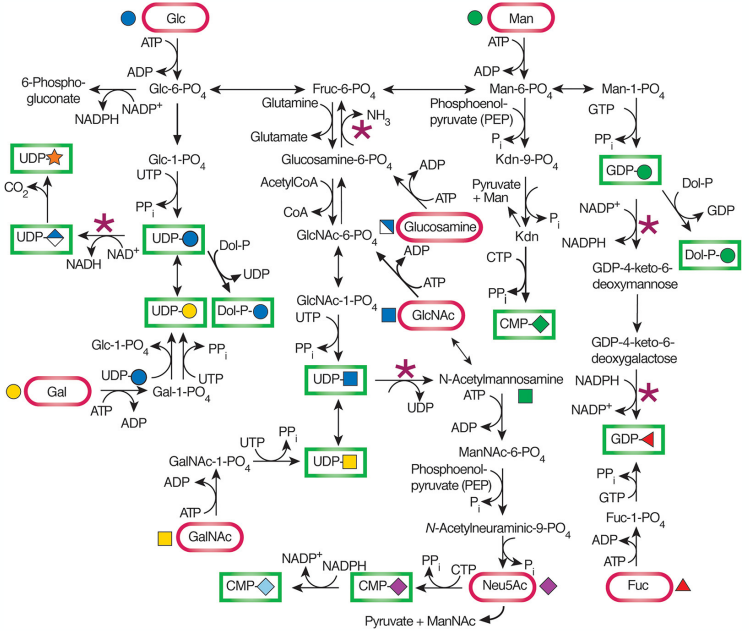


FIG. 5. Effects of sugar additions on the release of LDL receptors into the culture media. *LDLD* cells were plated, grown, pulse-labeled for 30 min with 450 μCi of [^{35}S]methionine per ml, and chased in unlabeled medium for the indicated times, all in media containing the indicated additions of galactose (20 μM) and/or GalNAc (200 μM) as described. Comparable amounts of cells and media were collected and subjected to immunoprecipitation procedures, electrophoresis, and autoradiography as described.

Kozarsky K, Kingsley D, Krieger M.. Proc Natl Acad Sci U S A. 1988 Jun;85(12):4335-9. PMID:3380796

Interestingly, somewhere in evolution we went from having two epimerases to a bifunctional epimerase





They demonstrated that IdID cells are deficient in the enzyme UDP-galactose and UDP-N-acetylgalactosamine (GalNAc) 4-epimerase.

(Kingsley DM, Cell. 1986 Mar 14;44(5):749-59. PMID: 3948246; Ikonen Nature Reviews Molecular Cell Biology 2008; 9, 125-138.)

O-Mannosylation

-O-Ser/Thr

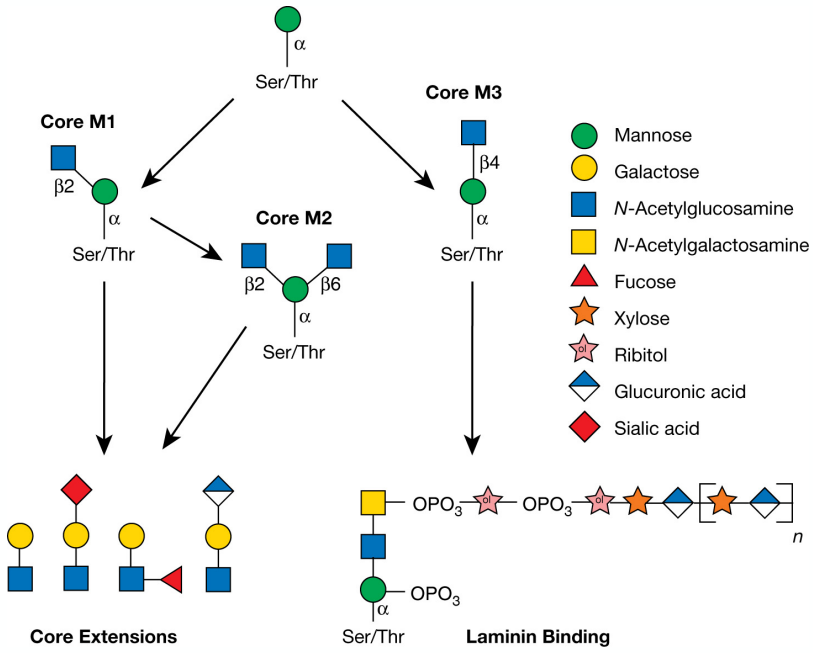
O-Mannose-linked glycans are complex

O-Mannose glycans account for up to one-third of all O-glycans in some mammalian tissue

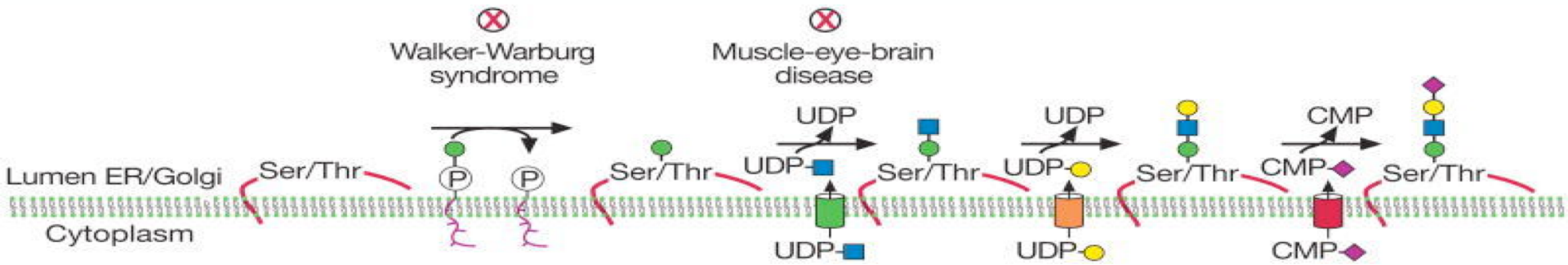
Structures are predominantly based on a common core of Siaα3Galβ4GlcNAcβ2Manα-Ser/Thr;

Can be fucosylated and sialylated;

When modified by the 3-O-sulfated glucuronic acid, the structure formed is the HNK1 epitope. This epitope is implicated in neuronal cell adhesion.



O-Mannosylation



5 forms of congenital Muscular Dystrophy result from mutations which alter the O-Mannosylation biosynthetic pathway.

The first mannose residue is added in the ER, and the glycan is extended in the Golgi

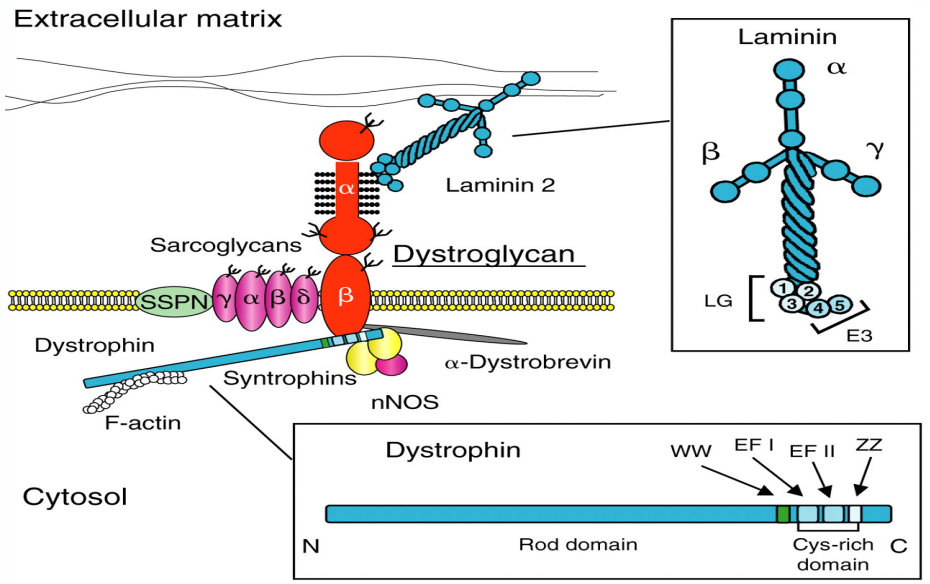
Glycosylation is thought to improve protein solubility in the ER

Muscular Dystrophy

A heterogeneous group of inherited disorders associated with muscle weakness and wasting

Many mutations effect Dystrophin, whose interactions with the extracellular matrix and the actin cytoskeleton stabilize the sarcolemma during muscle contraction

Laminin-2 binds glycans on αDystroglycan. Disrupting glycosylation of αDystroglycan disrupts the formation of the dystrophin-glycoprotein complex, and thus compromises the integrity of sarcolemma

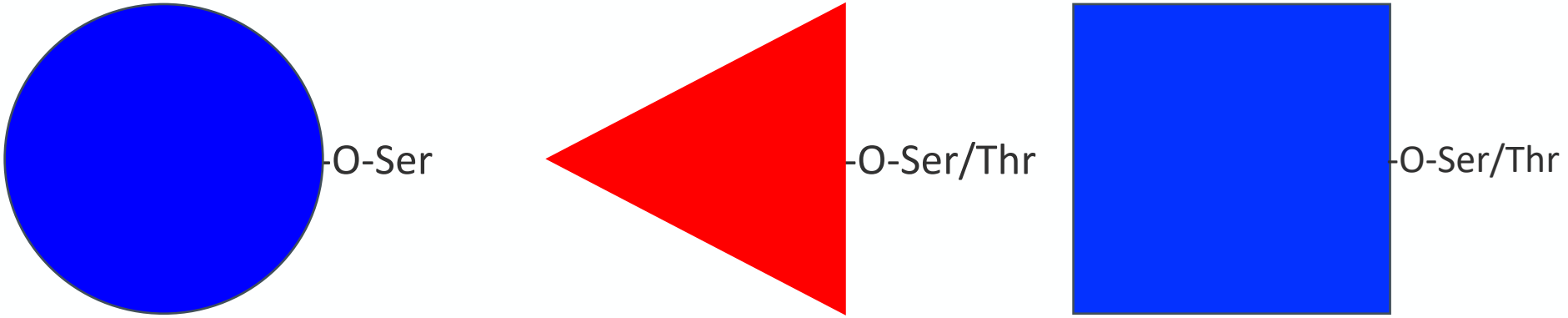


Sarcolemma: Plasma membrane of a striated muscle fibre

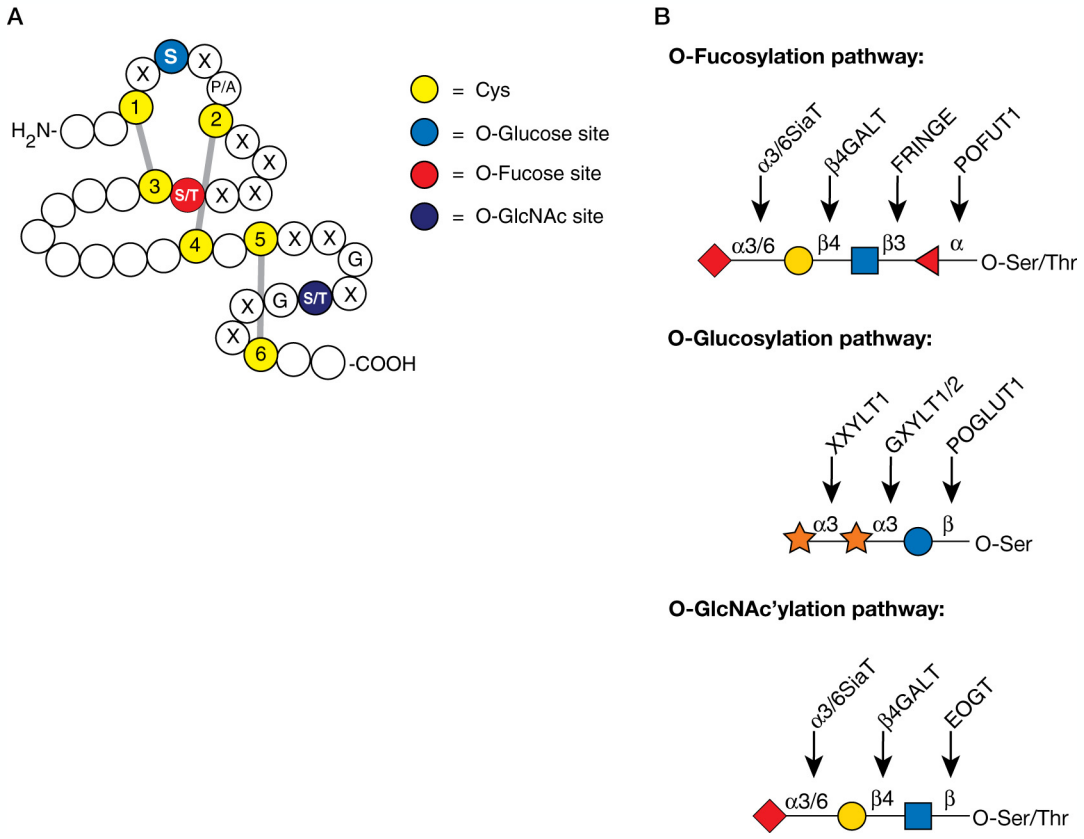
Barresi R , and Campbell K P J Cell Sci 2006;119:199-207

Journal of Cell Science
jcs.biologists.org

O-Glucose, O-Fucose, eO-GlcNAc

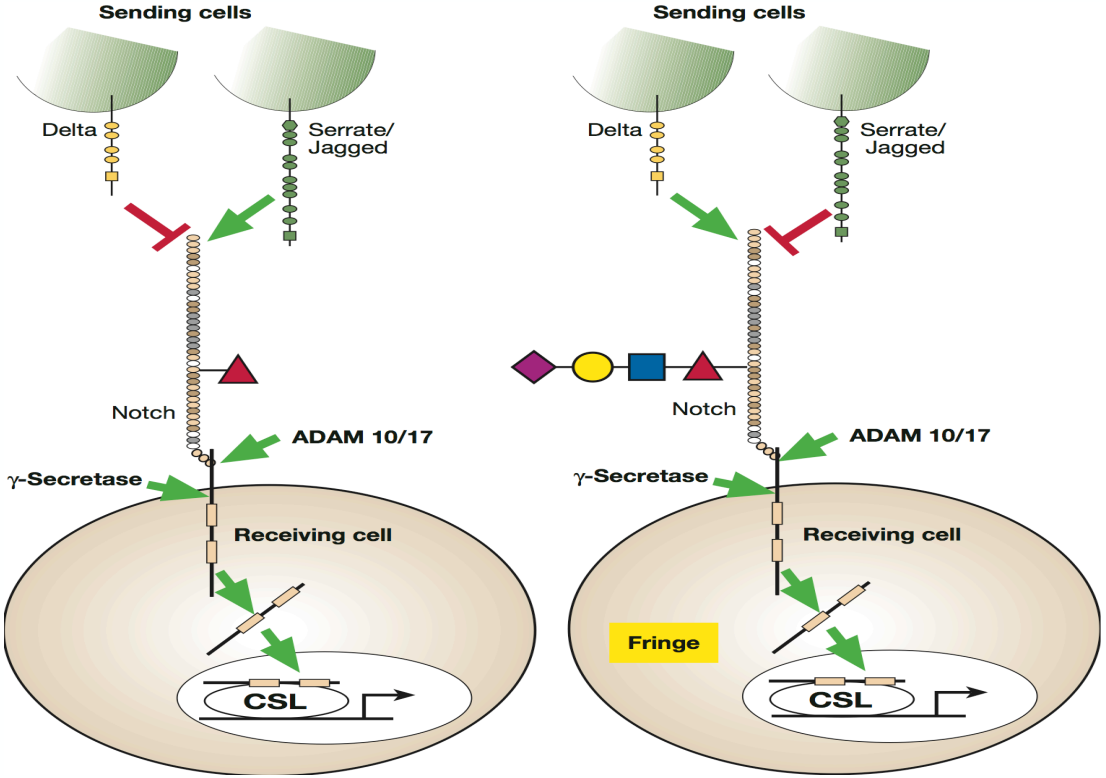


Epidermal growth factor (EGF)-like repeats can be modified by either O-fucose or O-glucose

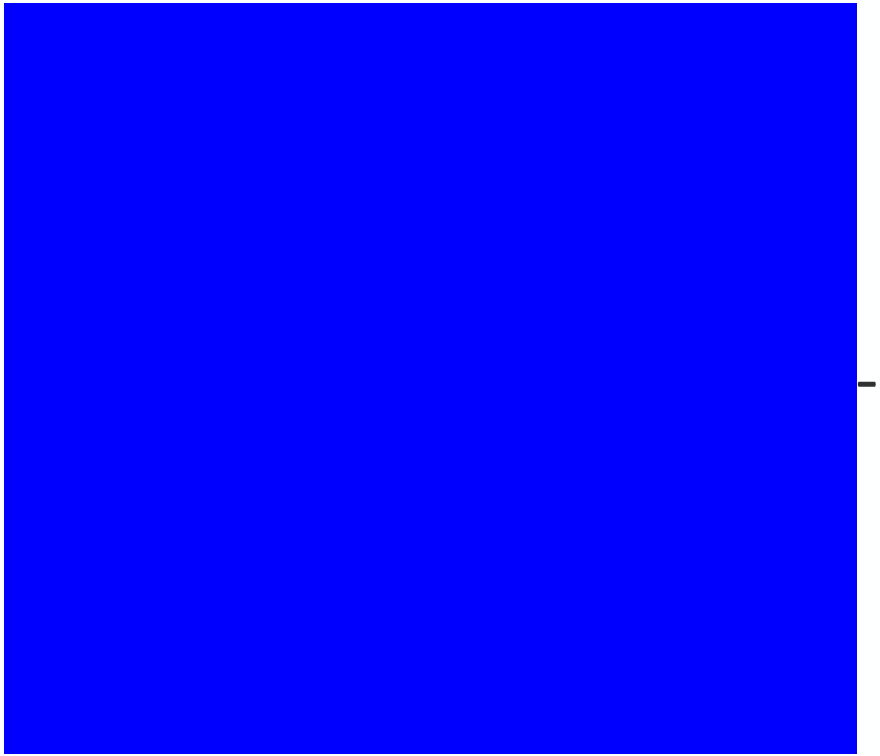


Also found on Thrombospondin like (TSR) repeats

Notch signaling pathway



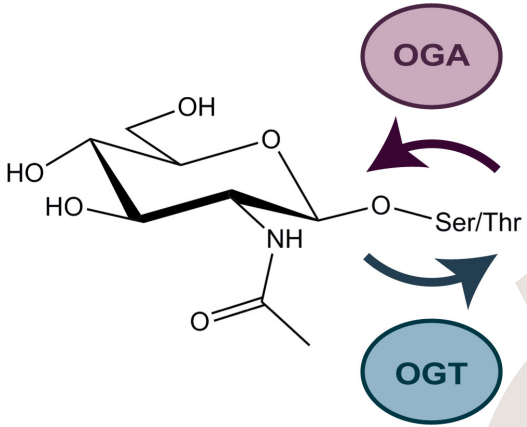
O-GlcNAc



-O-Ser/Thr (Cys)

O-GlcNAc: O-linked β-N-acetylglucosamine

a dynamic, post-translational, modification of intracellular proteins



Histones
Chromatin modifiers
RNA Polymerase II
mRNA processing proteins
Transcription factors
Ribosomal proteins
Proteosomal proteins
Vesicle transport proteins
Metabolic enzymes
Cytoskeletal proteins
Mitochondrial proteins
Kinases, HDACs, PRMTs
Chaperones

Activity
Stability
Structure
Localization
PTMs

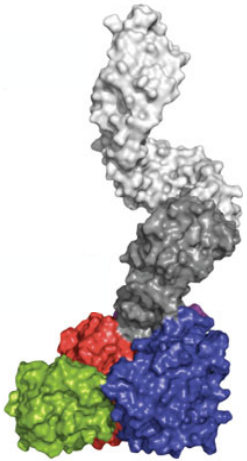
Epigenetics
Transcription
mRNA Processing
Translation
Proteostasis
Protein degradation
Signaling
Metabolism
Architecture
Cell cycle
Stress

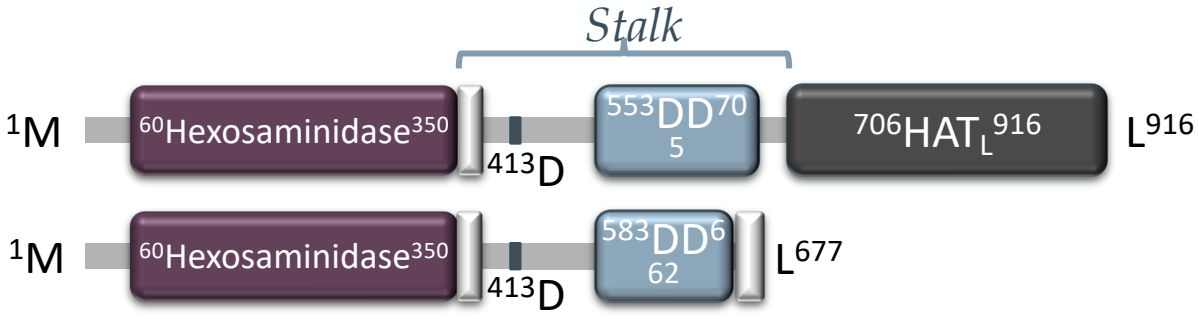
Cancer
Heart failure
Neurodegeneration
Metabolic syndrome
X-linked disability
Hypertension
Aging

Reviewed in **Groves**, **Lee**..Zachara (2013) *Cell Stress and Chaperones,* 18:535-58;
Martinez..Zachara (2017) *Biochemical Society Transactions,* 45:237-249.

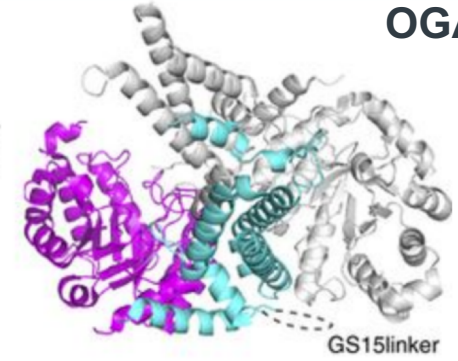
OGT and OGA

OGT



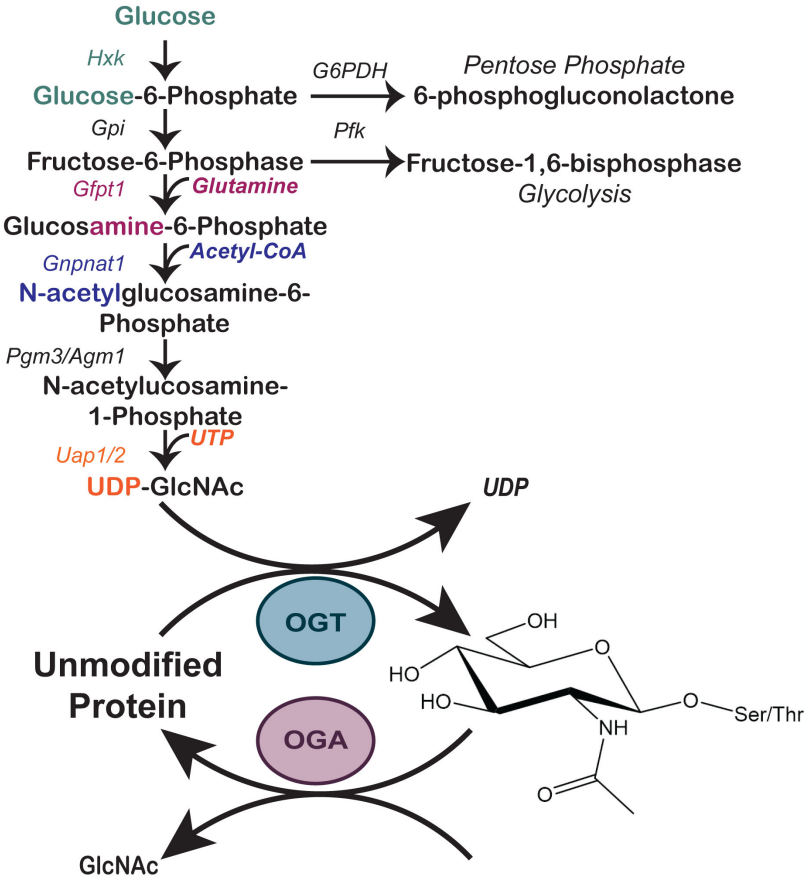


OGA

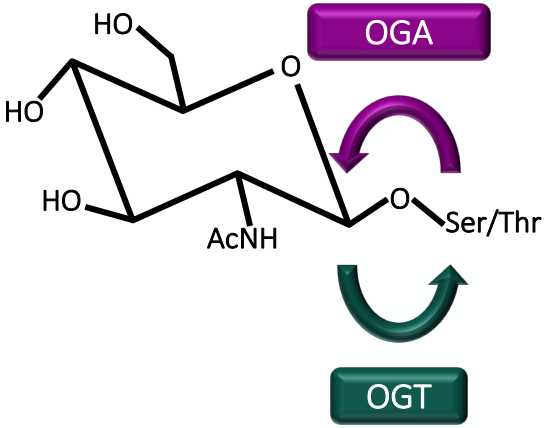


OGT cloning: **Kreppel** *et al.* (1997, PMID:9083067); **Lubas** *et al.* (1997, PMID: 9083068); **Hanover** *et al.* (2003, PMID: 12504895) *OGA cloning:* **Gao** *et al.* (2001, PMID: 11148210)
OGT structure: **Lazarus** *et al.* (2010, PMID:21240259) *OGA structure:* **Li** *et al.* (2017; PMID: 28319083), **Roth** *et al.* (2017; PMID: 28346405); **Elsen** *et al.* (2017, PMID:28346407)

O-GlcNAc


Glucose
Hxk
Glucose-6-Phosphate
G6PDH
Pentose Phosphate
6-phosphogluconolactone
Gpi
Fructose-6-Phosphase
Pfk
Fructose-1,6-bisphosphase
Glycolysis
Gfpt1
Glutamine
Glucosamine-6-Phosphate
Gnpnat1
Acetyl-CoA
N-acetylglucosamine-6-Phosphate
Pgm3/Agm1
N-acetylucosamine-1-Phosphate
Uap1/2
UTP
UDP-GlcNAc
UDP
OGT
Unmodified Protein
OGA
GlcNAc
OH
O
HO
HO
NH
O
O
Ser/Thr

O-GlcNAc as a regulator of protein function



Regulating protein half-life

Han and Kudlow, 1997, PMID: 9111324; Jinag et al., 1997, PMID: 8999954
Sümegi et al., 2003, PMID: 14652013; Zhang et al., 2003, 14675536

Regulating protein complex formation

Lercher et al., 2015, PMID: 26305776

Toleman et al., 2018, PMID: 29784830

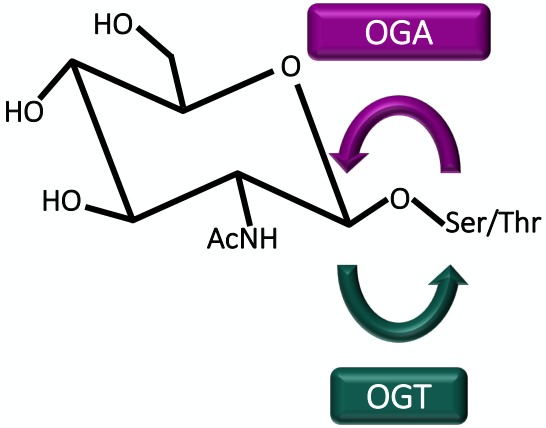
Preventing protein aggregation

Lim et al., 2006, PMID: 16879824; Yuzwa et al., 2010, PMID: 22366723

Regulating protein half-life

Han and Kudlow, 1997, PMID: 9111324; Jinag et al., 1997, PMID: 8999954
Sümegi et al., 2003, PMID: 14652013; Zhang et al., 2003, 14675536

O-GlcNAc as a regulator of protein function



O-GlcNAc cycles rapidly & in response to signals

Roquemore et al., 1996, PMID: 8639509
Kearse and Hart, 1991, PMID: 2000378;

Sites-modified are similar to those utilized by Proline-directed map kinases

Hart et al., 1995, PMID: 859

Competing with phosphorylation

Kelly et al., 1993, PMID: 848669; Comer and Hart, 2001, PMID: 11425311
Wang et al., 2010, PMID: 20068230; Zhong et al., 2015, PMID: 25263469

Regulation of protein Kinases *Dias et al., 2009, PMID: 19506079*

Blocking ATP binding *Dias et al., 2012, PMID: 22564745*

Kinase complex formation *Ku et al., 2010, PMID: 20729838*

Altering Allosteric regulation *Yi et al., 2012 PMID22923583*

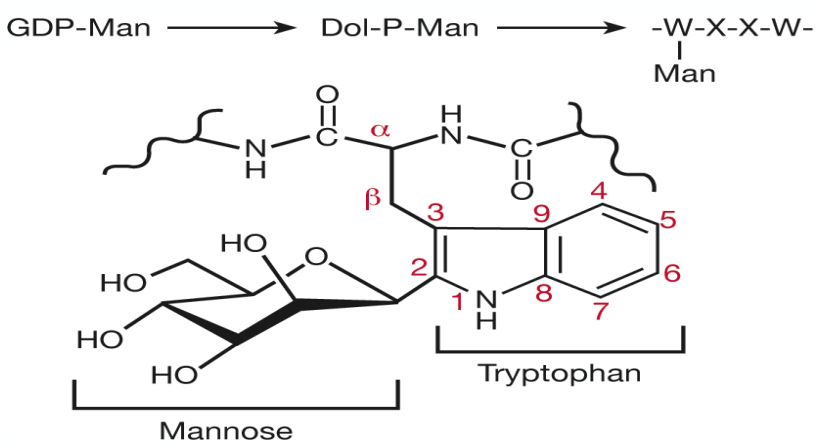
Substrate Specificity *Tarrant et al., 2012, PMID: 22267120*

C-Mannosylation

-C-Tryptophan

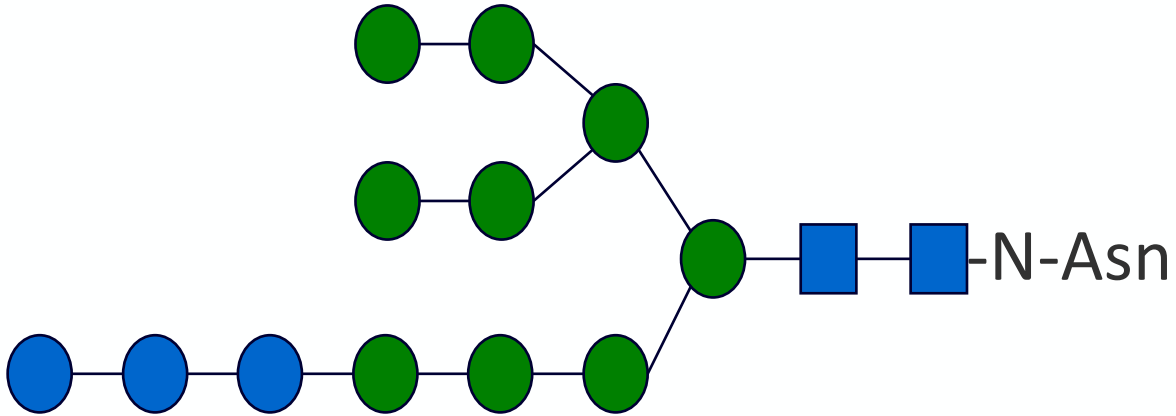
C-mannosylation

- *The C-1 atom of a single mannose residue is added in an a-linkage to the C-2 atom of the indole moiety of tryptophan 7 in RNase 2;*
- *Absent in yeast, arthropods and bacteria;*
- *>300 mammalian proteins contain the consensus motif ($_m$W-X-X-W)for C-mannosylation, may be more commonly modified in proteins which contain TSR and EGF repeats;*
- *Of these predicted sites, at least 49 are known to be modified;*
- *Initiated in the ER, and may aid in protein folding.*



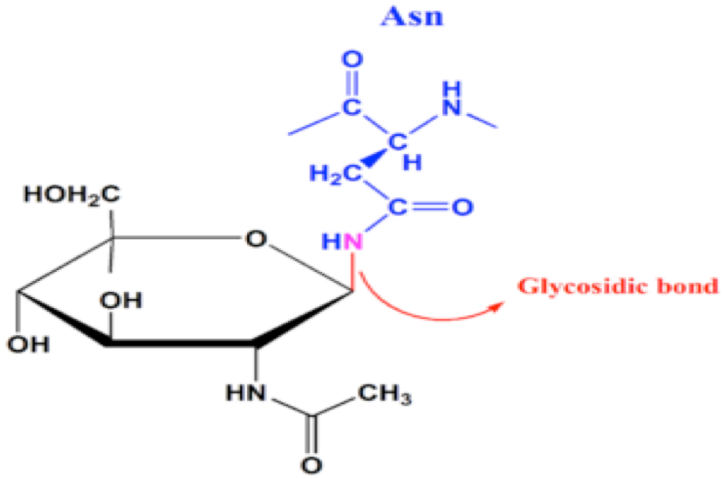
Acta Biochim Pol. 2000;47(3):781-9. PMID: 11310977

N-Linked Glycosylation

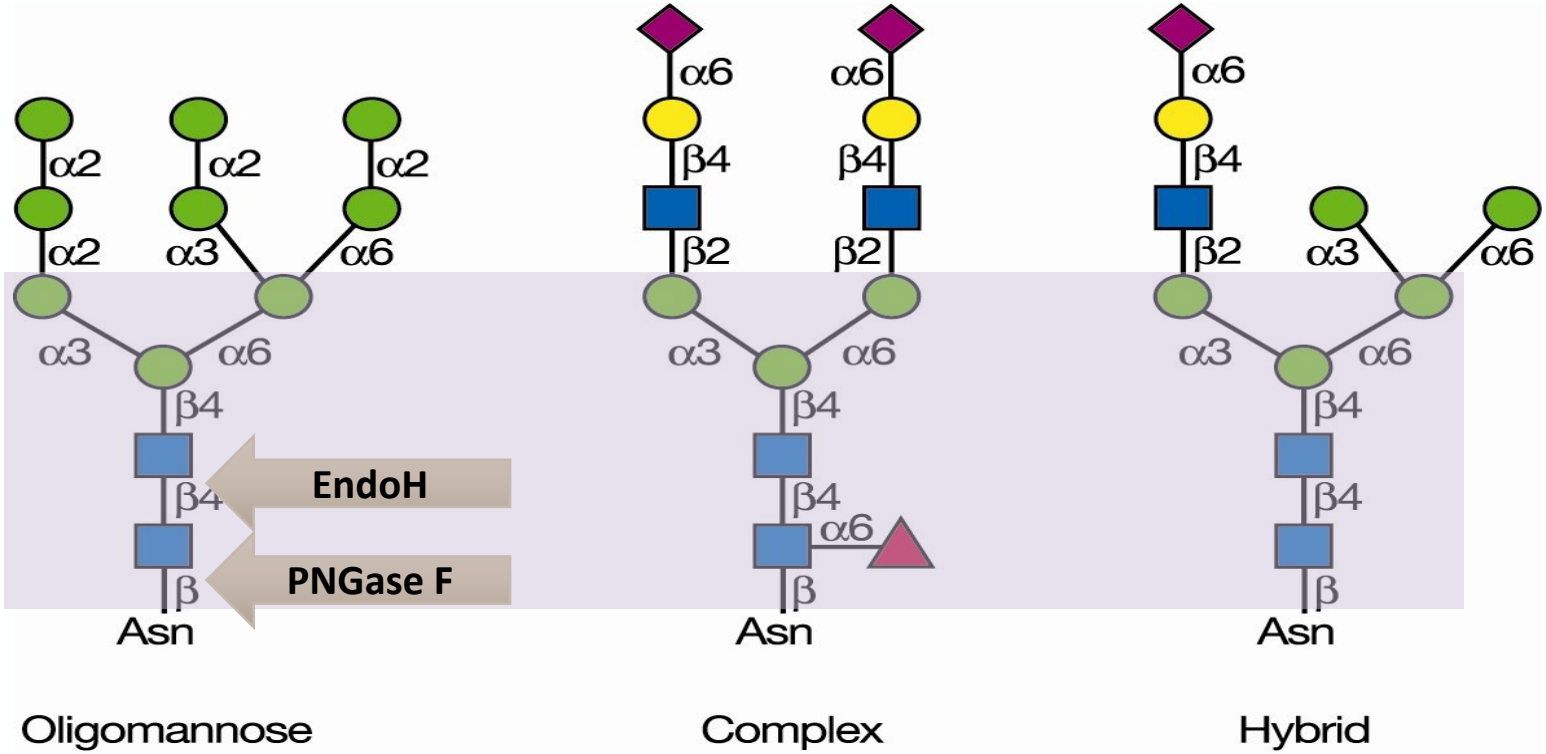


N-linked Glycosylation

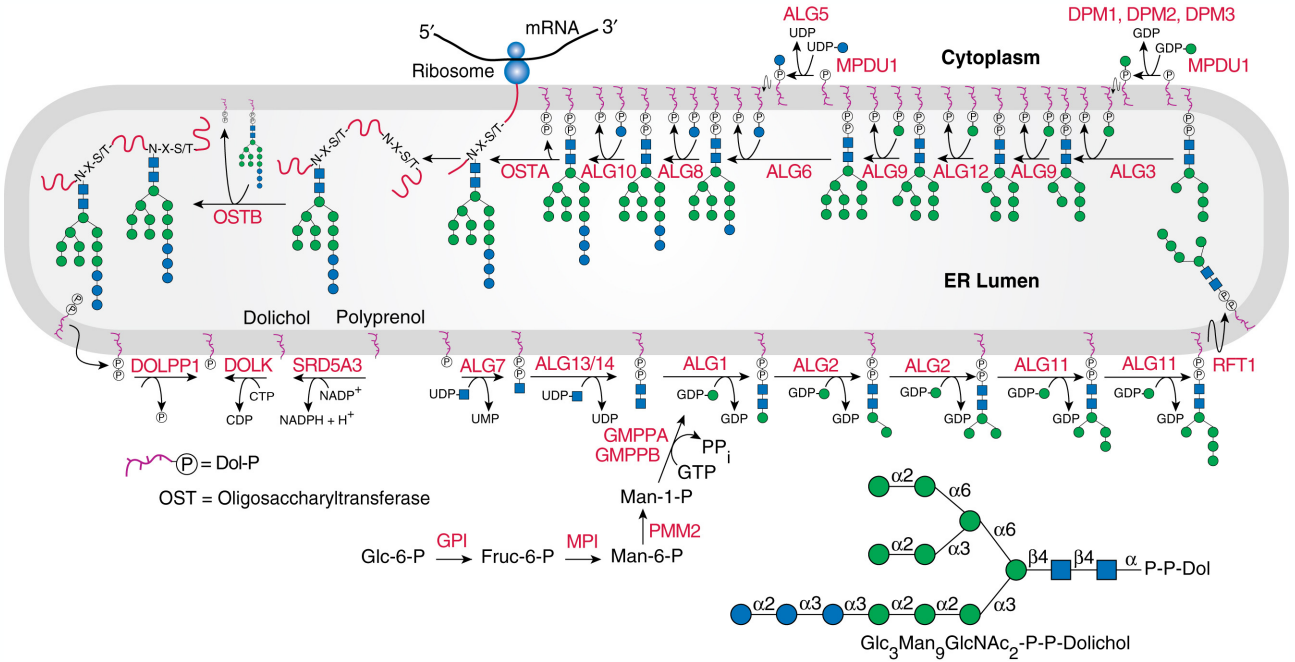
- *N-linked glycans are attached to the amide nitrogen of asparagine;*
- *Typically to asparagine's in the motif Asn-Xaa-Ser/Thr (Xaa ≠ Pro);*
- *With few exceptions the first residue is a residue of GlcNAc in the β-configuration;*
- *Added co-translationally in the ER;*
- *Only found in the nucleus and cytoplasm in certain situations:*
  - *Export from the ER via the ERAD pathway;*
- *N-linked glycosylation affects conformation, solubility, signaling, antigenicity and protein-protein interactions.*



N-Linked Glycans Contain a Similar Core Structure

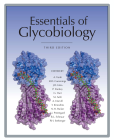


Synthesis of Dolichol-P-P-GlcNAc$_2$Man$_9$Glc$_3$

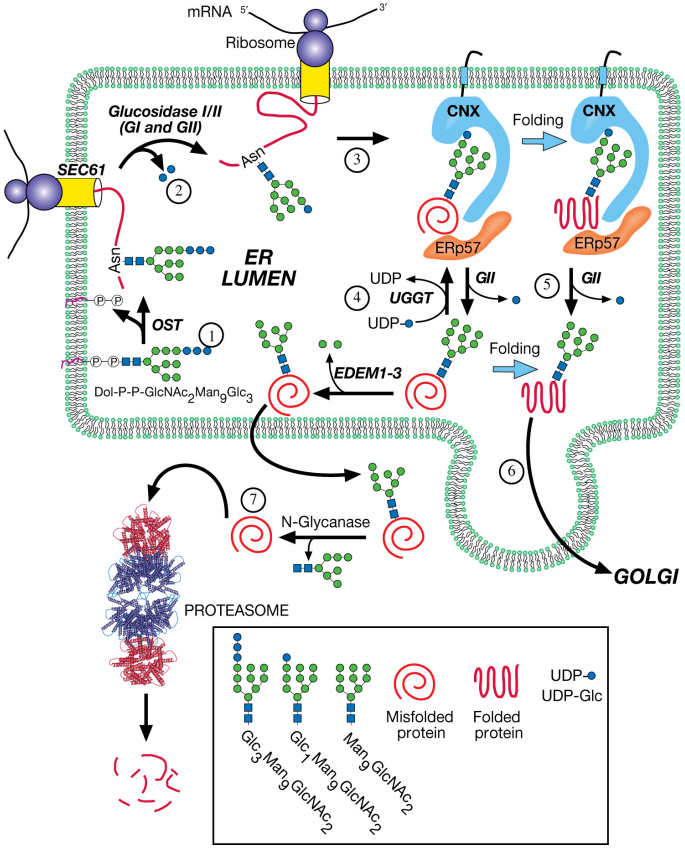


Chapter 9, Figure 3. Essentials of Glycobiology, **Third Edition**

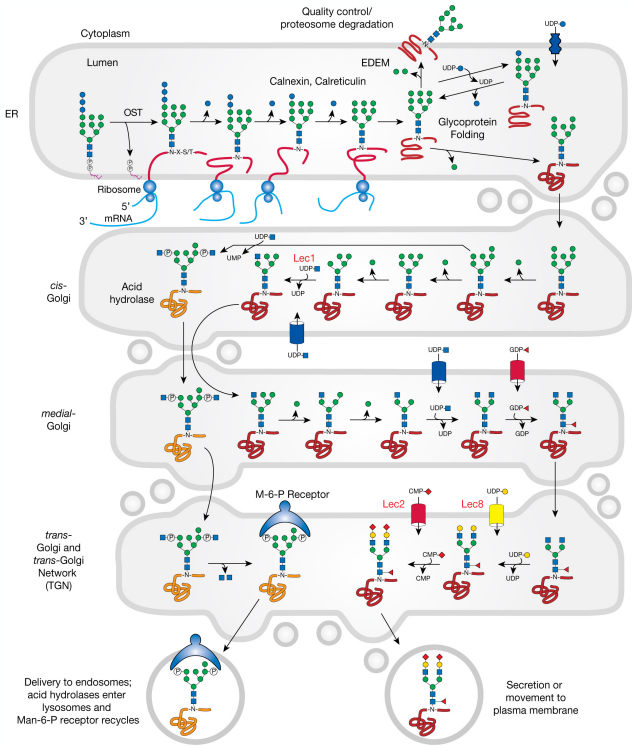
Symbol Nomenclature for Glycans (SNFG)



ER Associated Degradation

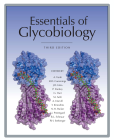


Processing and Maturation of an N-Glycan

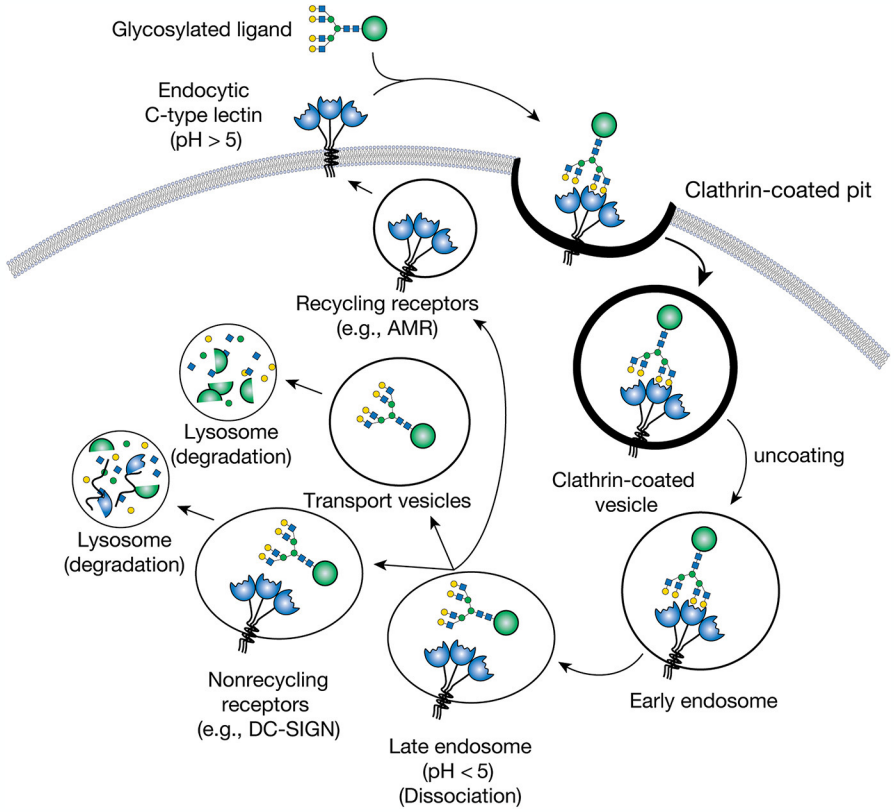


Chapter 9, Figure 4. Essentials of Glycobiology, **Third Edition**

Symbol Nomenclature for Glycans (SNFG)

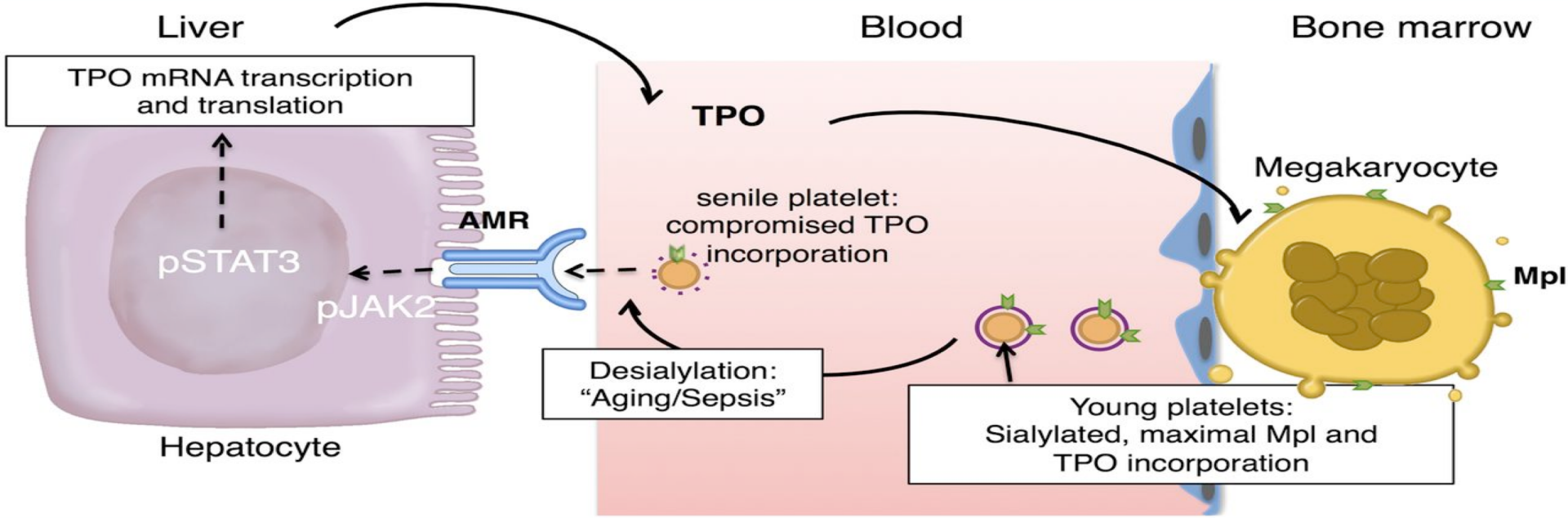


THE ASHWELL–MORELL RECEPTOR or the Asialoglycoprotein Receptor



Chapter 34, Figure 4. Essentials of Glycobiology, Third Edition

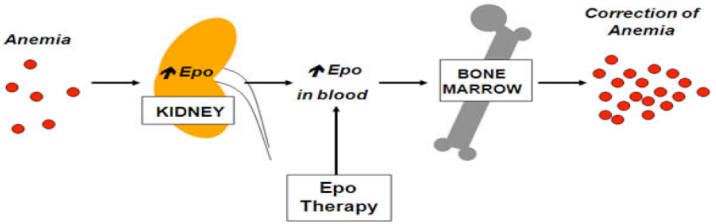
Glycans can also regulate the half-life of cells in serum

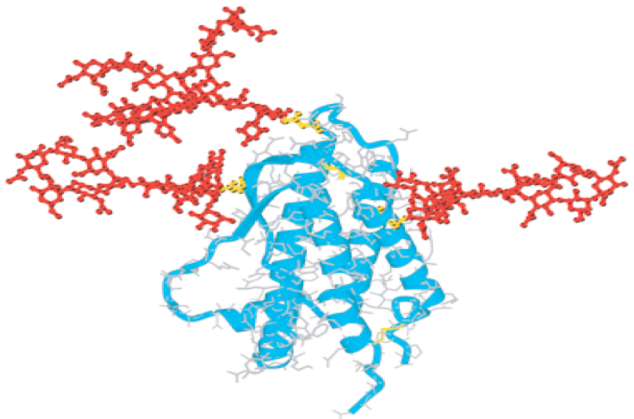


Grozovsky R, Giannini S, Falet H, Hoffmeister KM. Regulating billions of blood platelets: glycans and beyond. Blood. 2015 Oct 15;126(16):1877-84. PMID: 26330242

One function of N-linked glycosylation can prolong the half-life of a protein in serum

- *Erythropoietin a circulating cytokine that binds to the erythropoietin receptor, inducing proliferation and differentiation of erythroid progenitors in the bone marrow;*
- *Natural and recombinant forms of erythropoietin carry three sialylated complex N-glycans and one sialylated O-glycan;*
- *In vitro the activity of deglycosylated erythropoietin is comparable to that of the fully glycosylated molecule;*
- *Activity in vivo is reduced by about 90%, because poorly sialylated erythropoietin is rapidly cleared by filtration in the kidney;*





http://www.hematology.org/Publications/50-Years-in-Hematology/4726.aspx; Nature Biotechnology 25, 145 - 146 (2007)
Published online: 1 February 2007 | doi:10.1038/nbt0207-145

Shared Glycans

Repeating Units

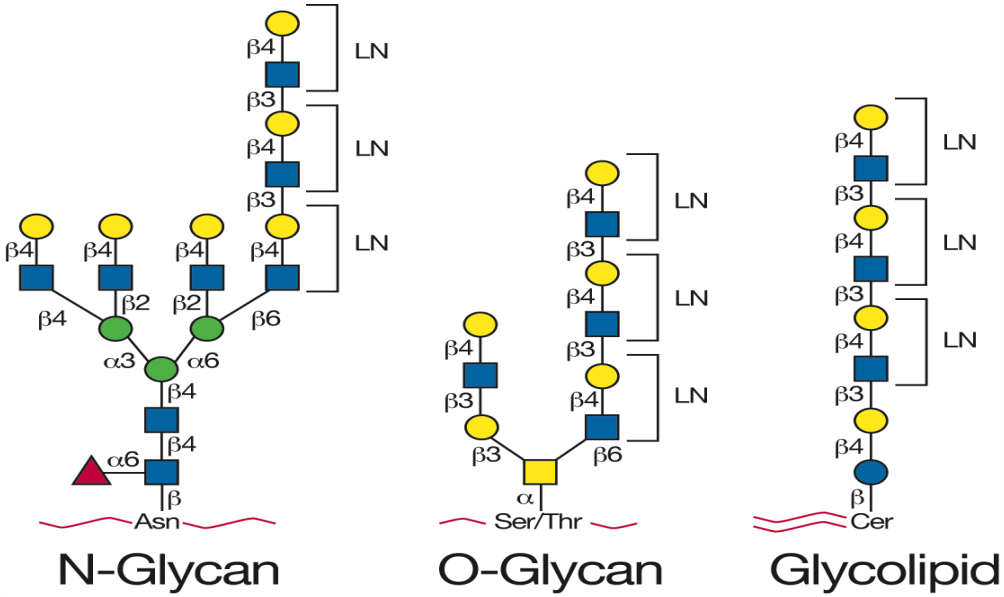
Type-2 (β1-4) Glycan units

Gal**β1-4**GlcNAcβ1-3 (LacNAc)

GlcNAc**β1-4**GlcNAcβ1-3 (LacdiNAc)

Type-1 (β1-3) Glycan Units

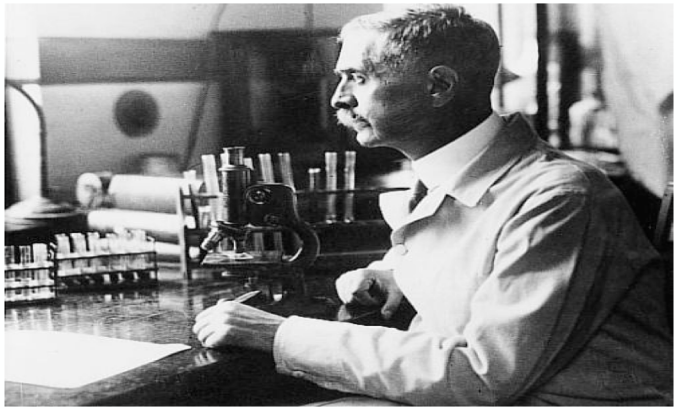
Gal**β1-3**GlcNAcβ1-3 (LacNAc)



Note, the enzymes used to extend O-Glycans are also used to extend N-linked glycans and glycolipids

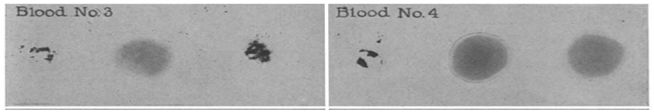
Many antigens that define self, such as the ABO blood group antigens, depend on carbohydrate

- *The ABO system was discovered in the twentieth century by Karl Landsteiner and colleagues*
- *They took sera from different patients and looked at the ability of sera to agglutinate blood of another patient*
- *"Curiously enough the reactions with normal human isoagglutinins do not occur in a, so to speak, haphazard manner but they separate the human bloods into four sharply defined groups designated as O, A, B, and AB." Landsteiner and Levine, 1928*



Karl Landsteiner

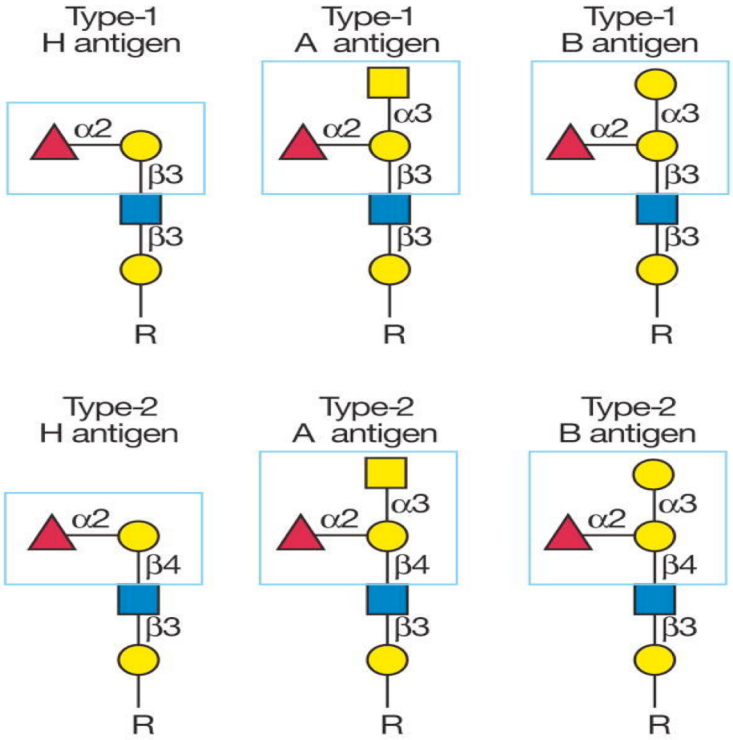
Nobel Prize in Physiology or Medicine in 1930.



Landsteiner K, Levine P. J Exp Med. 1928 Apr 30;47(5):757-75. PMID: 19869442
http://americangothgirl.tumblr.com/post/24128164591/todays-badass-jew-3-is-karl-landsteiner-who

LacNAc chains are derivatized to form the ABO blood group antigens

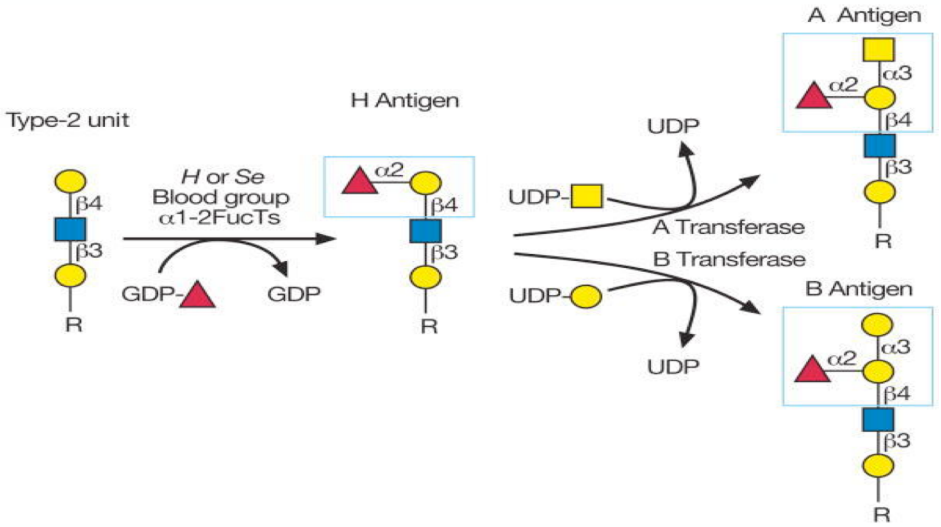
- *ABO antigens found on type 1 and type 2 LacNAc Chains;*
- *On each red blood cell approximately 1–2 million ABH determinants (~80% of the total) are attached to the anion transport protein;*
- *These are not only found on the surface of red cells, but in other tissues including the vascular endothelium and a variety of epithelia.*



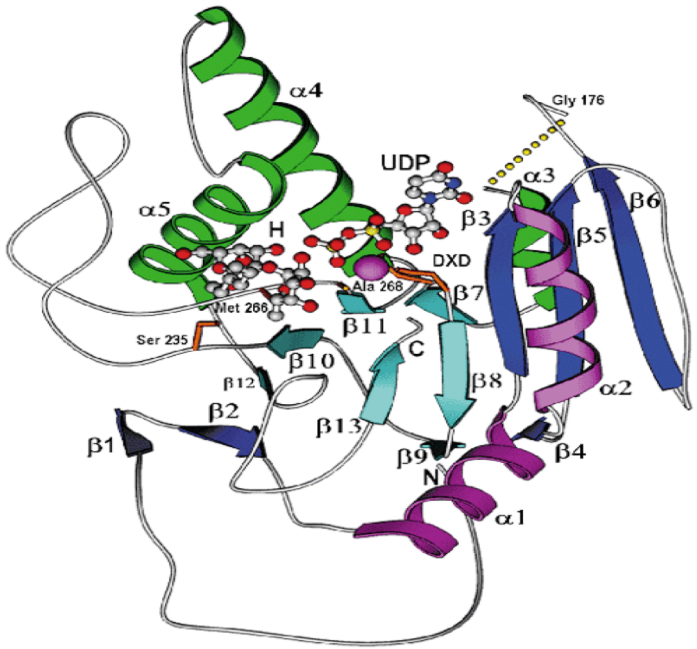
The ABO Blood groups are Defined by Carbohydrates

Structures are determined by the inheritance of genes that encode glycosyltransferases with different activities;

The A, B, and H antigens are formed by the sequential action of glycosyltransferases encoded by three genetic loci: the ABO, H, and Secretor loci;



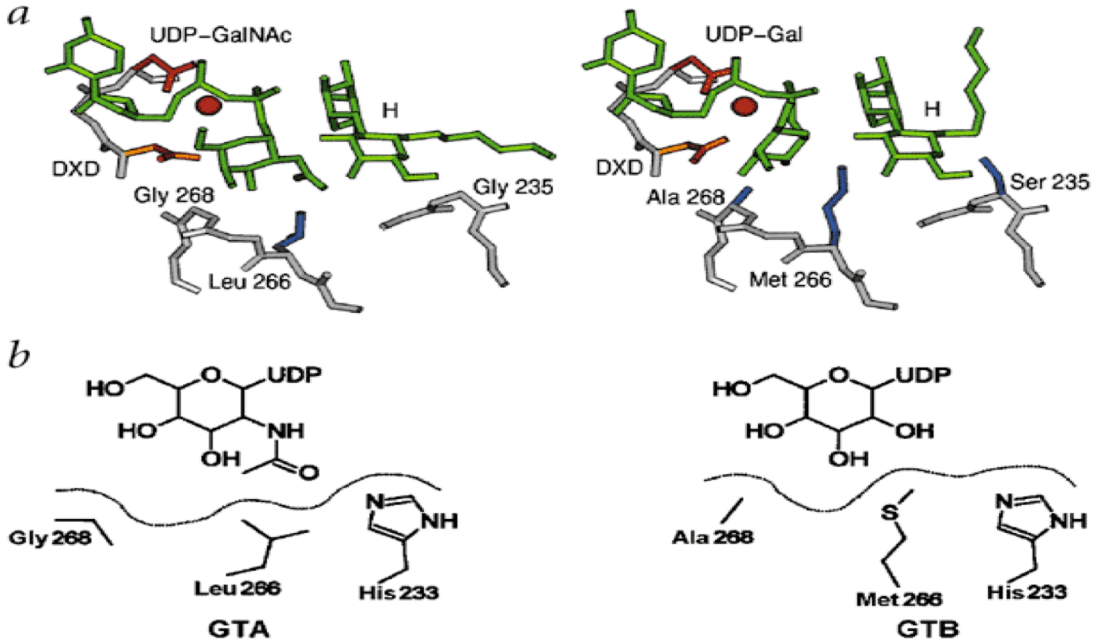
GTA and GTB, which are 354 amino acids in length, differ only in 4 amino acids



GTA/GTB: Arg/Gly^{176}, Gly/Ser^{235}, Leu/Met^{266}, and Gly/Ala^{268} differ between GTA and GTB

Yamamoto et al., Nature. 1990 May 17;345(6272):229-33., PMID: 2333095; Patenaude et al., Nat Struct Biol. 2002 Sep;9(9):685-90. PMID: 12198488 **Bay et al.,** Glycoconj J. 2014 Oct;31(6-7):469-73. PMID: 25117515; **Patnaik et al.**, Nucleic Acids Res. 2012 Jan;40(Database issue):D1023-9. PMID: 22084196

GTA and GTB, which are 354 amino acids in length, differ only in 4 amino acids

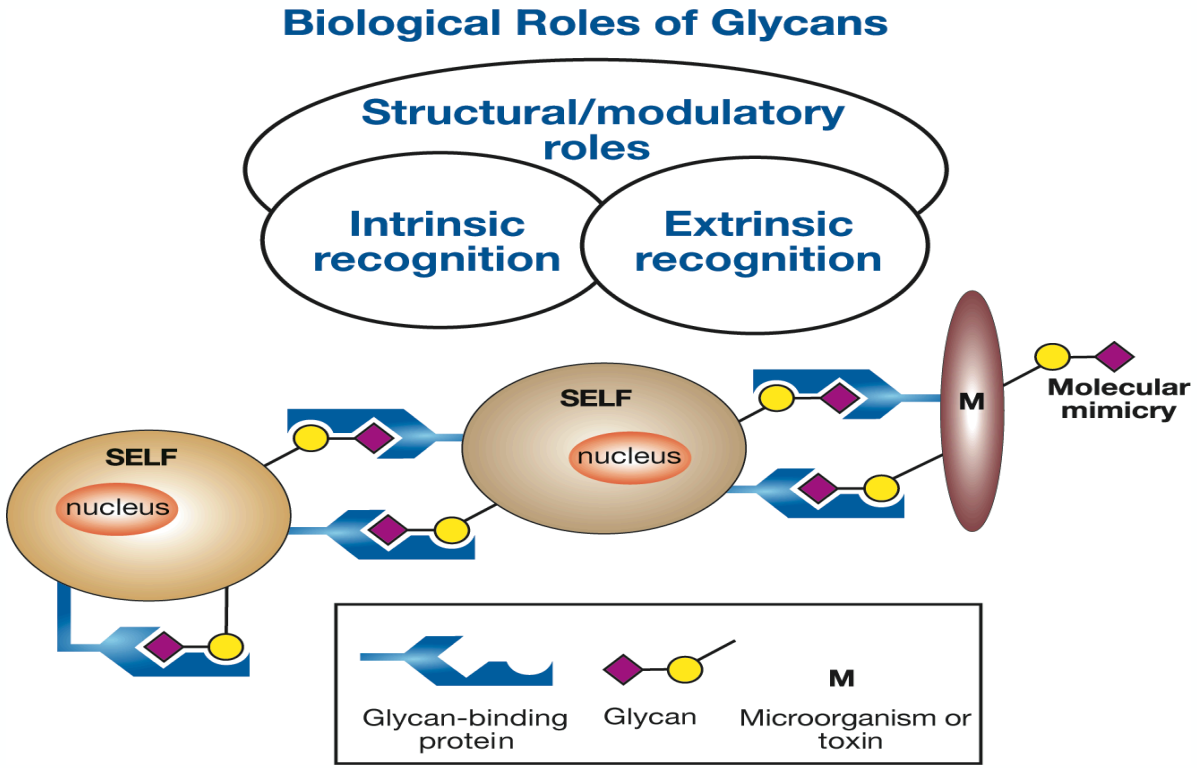


Leu/Met^{266} and Gly/Ala^{268} have been shown to be responsible for discrimination between the two donor molecules.

Gly/Ser^{235} and Leu/Met^{266} impact acceptor recognition .

Yamamoto et al., Nature. 1990 May 17;345(6272):229-33., PMID: 2333095; Patenaude et al., Nat Struct Biol. 2002 Sep;9(9):685-90. PMID: 12198488 **Bay et al.,** Glycoconj J. 2014 Oct;31(6-7):469-73. PMID: 25117515; **Patnaik et al.,** Nucleic Acids Res. 2012 Jan;40(Database issue):D1023-9. PMID: 22084196

Blood Groups may have evolved to evade pathogens

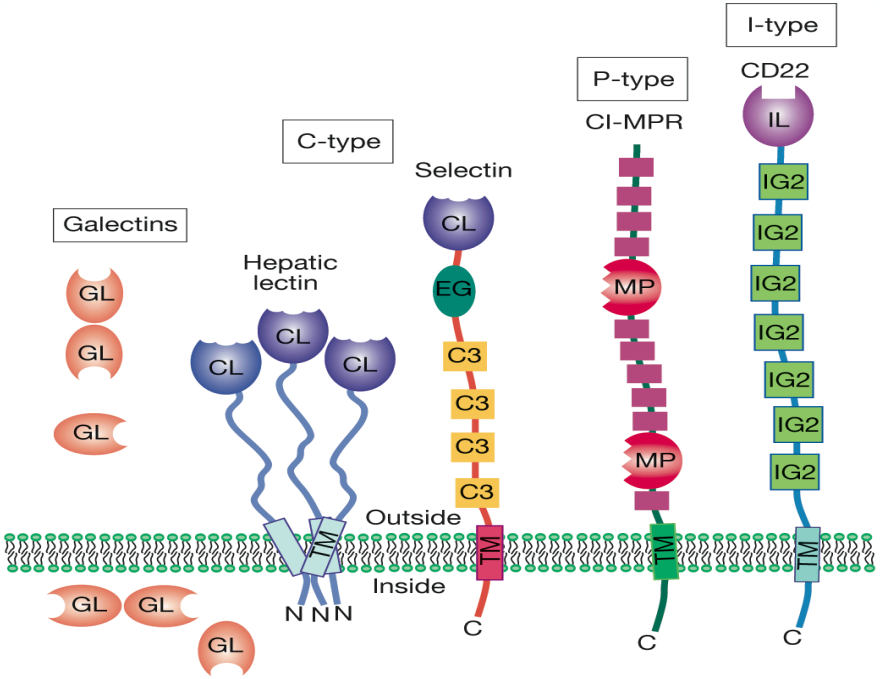


One role of sugars is to act as ligands for glycan binding proteins

Mammalian glycan binding proteins are grouped into:

Glycosaminoglycan binding proteins

Lectins (pictured right) – which contain conserved carbohydrate binding domains



Essentials of Glycobiology
Second Edition

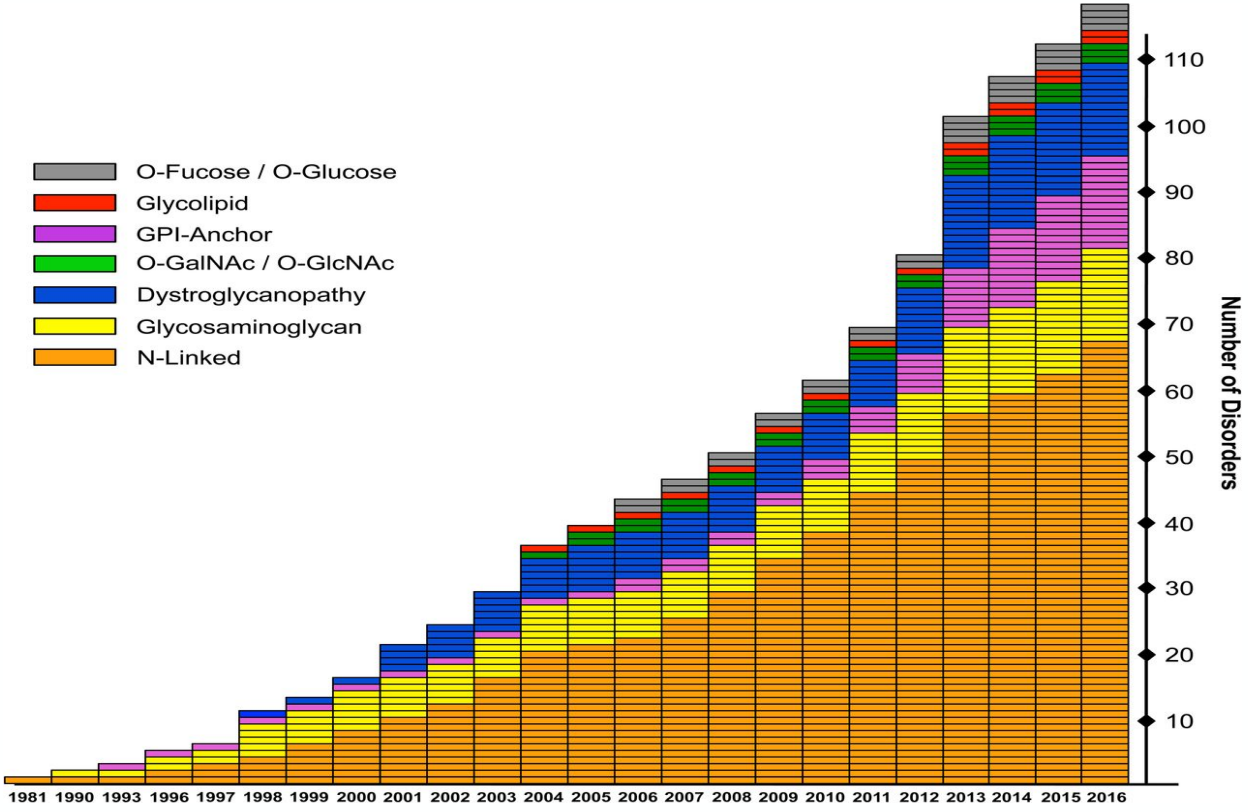
Chapter 26, Figure 1

Roles of Glycans on Proteins

- Protein Folding and Stability
- Glycans can alter protein structure
- Protein and Cell Localization
- Glycosylation can regulate the half-life of glycoproteins and cells
- Identification of Self
- Regulation of signal transduction
- Glycans can mediate interactions between proteins, as well as between cells and the ECM (For example, Dystroglycan)
- Glycans can act as ligands
- Regulation of protein function

The Essential Roles of Glycan is underpinned by the number and severity of congenital disorders of glycosylation

GLYCOBIOLOGY



Varki A, Glycobiology 2017; PMID: 27558841

Tools and Databases

- Glycans @ the NCBI:
  - https://www.ncbi.nlm.nih.gov/glycans/

- Lots of tools & Resources
  - https://commonfund.nih.gov/glycoscience
  - http://www.functionalglycomics.org/
  - http://glycam.org/
  - https://www.glygen.org/resources.html (list of “glycol resources” at glygen)
  - https://glygen.ccrc.uga.edu/glygen/

GlyGen Webinar

A Computational and Informatics Resources for Carbohydrate and Glycoconjugate Related Data

May 5, 2020

11 a.m.

To register email: jeetvora@gwmail.gwu.edu

- Have you ever wondered whether there was a resource you could access to get an answer to a question like “What kind of glycans have been reported to be linked to specific glycosylation sites on my glycoprotein of interest?” Or, “What glycoproteins have been shown to carry a particular glycan structure that I am really interested in?” GlyGen can help you with these sorts of questions (https://www.glygen.org).

- GlyGen is a data integration and dissemination resource funded by the NIH Common Fund Tools for Glycoscience initiative. While so many of us are being kept away from our laboratories, GlyGen developers and investigators thought it would be a good time to offer a webinar to introduce the capabilities and features of this resource.
- We will offer this free webinar on Tuesday, May 5 at 11:00 AM for 1 hour and we most cordially invite you to attend. In order to gauge interest and plan appropriately, please drop an e-mail to the following address if you are interested: jeetvora@gwmail.gwu.edu

- We will respond to your e-mail by sending a link on May 4, the day before the webinar. Please forward this announcement to colleagues that you think might also be interested.

Sequence Motifs or Sequons for the Addition of O-Glycans

Glycopeptide bond	Consensus sequence or peptide domain
GalNAc-α-Ser/Thr	Repeat domains rich in Ser, Thr, Pro, Gly, Ala (Golgi)
Man-α-Ser/Thr	Ser/Thr rich domains (ER)
Fuc-α-Ser/Thr	EGF modules (Cys-X-X-Gly-Gly-Thr/Ser-Cys)
Glc-β-Ser	EGF modules (Cys-X-Ser-X-Pro-Cys)
GlcNAc-β-Ser/Thr	EGF modules
Gal-β-Hyl	Collagen repeats (X-Hyl-Gly)
GlcNAc-β-Ser/Thr	Ser/Thr/Pro rich domains (Cytosol, Nucleus, Mitochondria)