HARVARD MEDICAL SCHOOL
Beth Israel Deaconess Medical Center

National Center for Functional Glycomics

Background Information on Glycoconjugates

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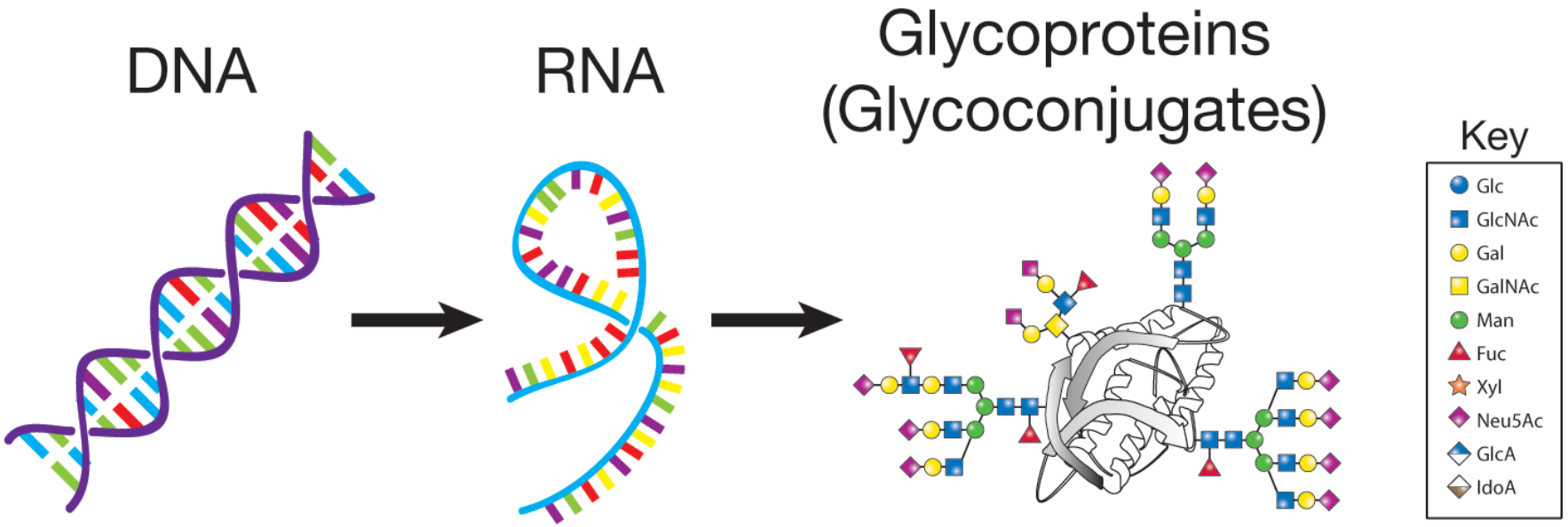
For General Reference On-Line See: *Essentials of Glycobiology* (2nd Edition) Varki, Cummings, Esko, Freeze, Stanley, Bertozzi, Hart and Etzler) http://www.ncbi.nlm.nih.gov/books/NBK1908/

From Small Sugars Come Big Things



Glycoconjugates are Essential to Normal Health and Development

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From Small Sugars Come Big Things

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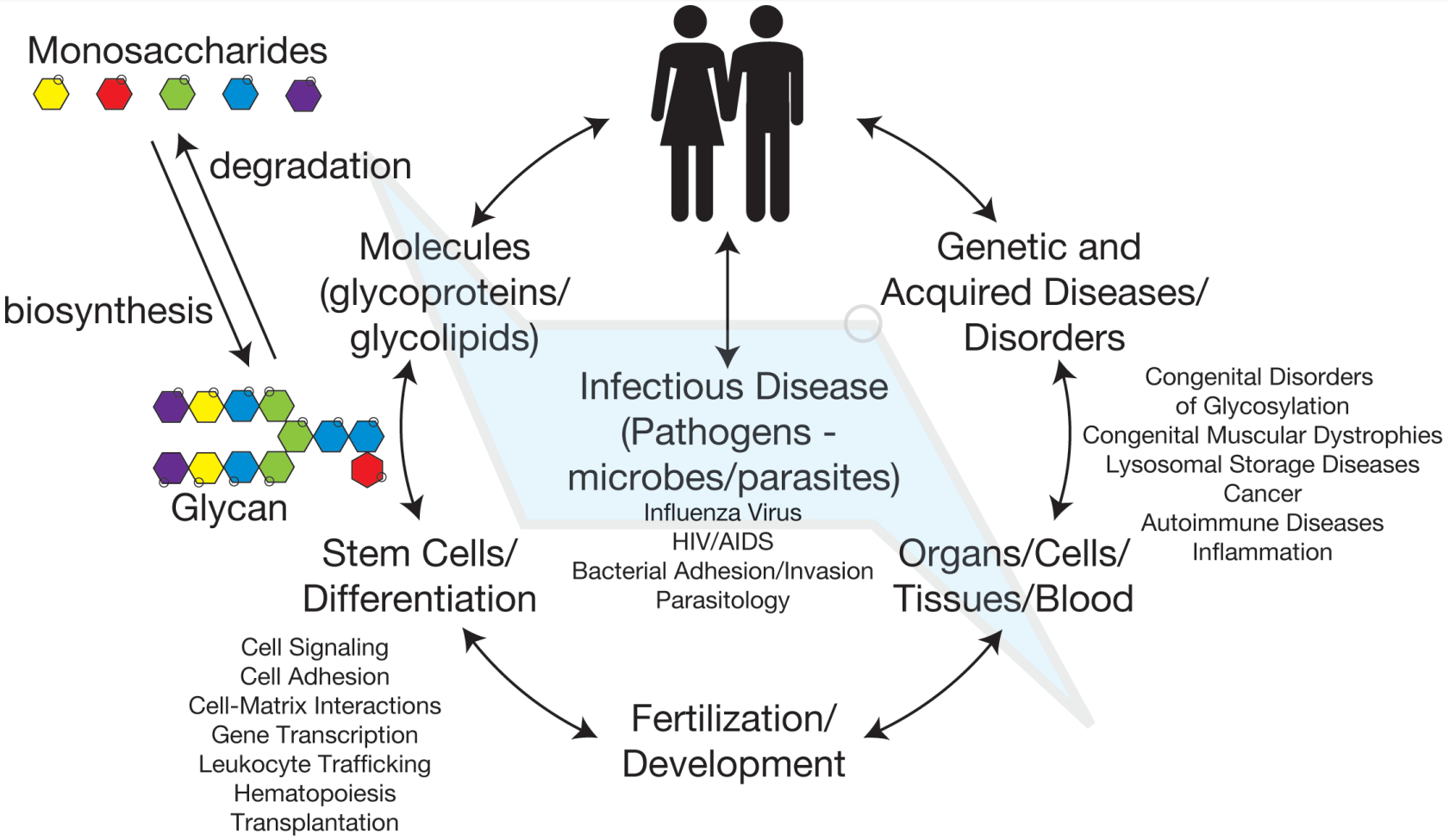
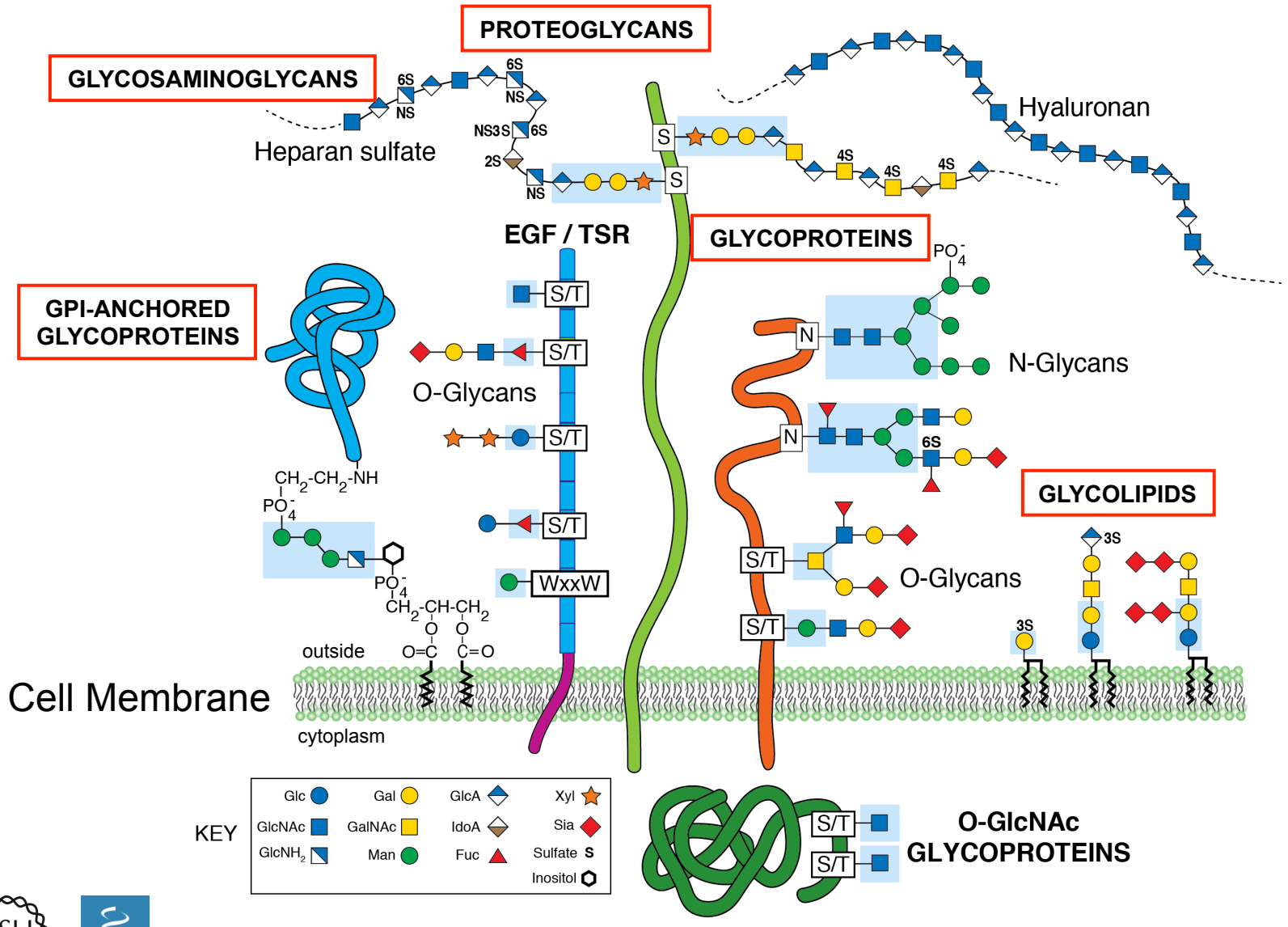


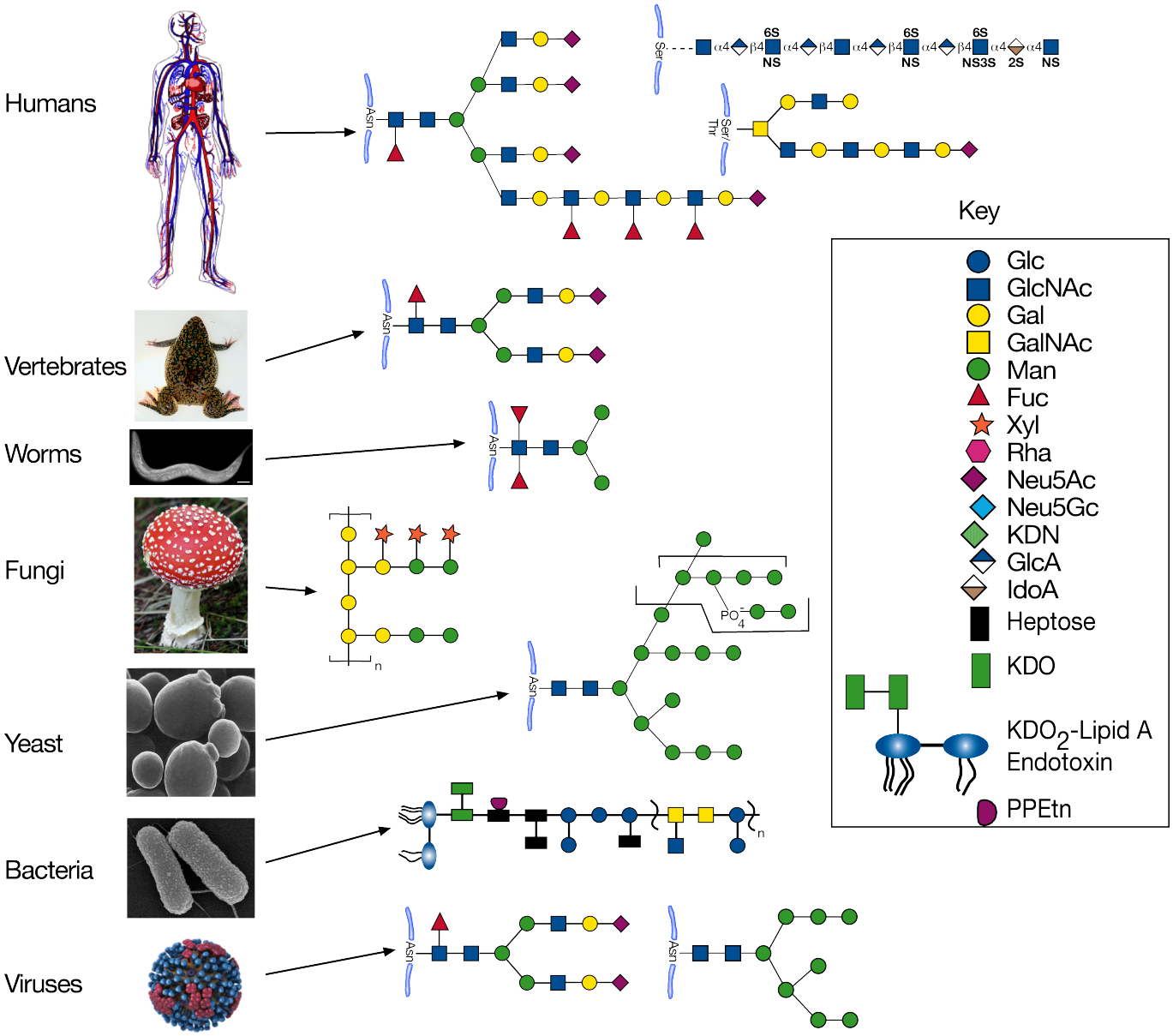
Glycobiology/Glycoscience are areas of research that explore the structures, functions, and biosynthetic regulation of glycoconjugates.

Glycoconjugates, Which are Molecules Containing Sugars (Monosaccharides) Linked Within Them, are the Major Constituents of Animal Cell Membranes (*Glycocalyx*) and Secreted Material: See Different Classes of Glycoconjugates Below in Red Boxes

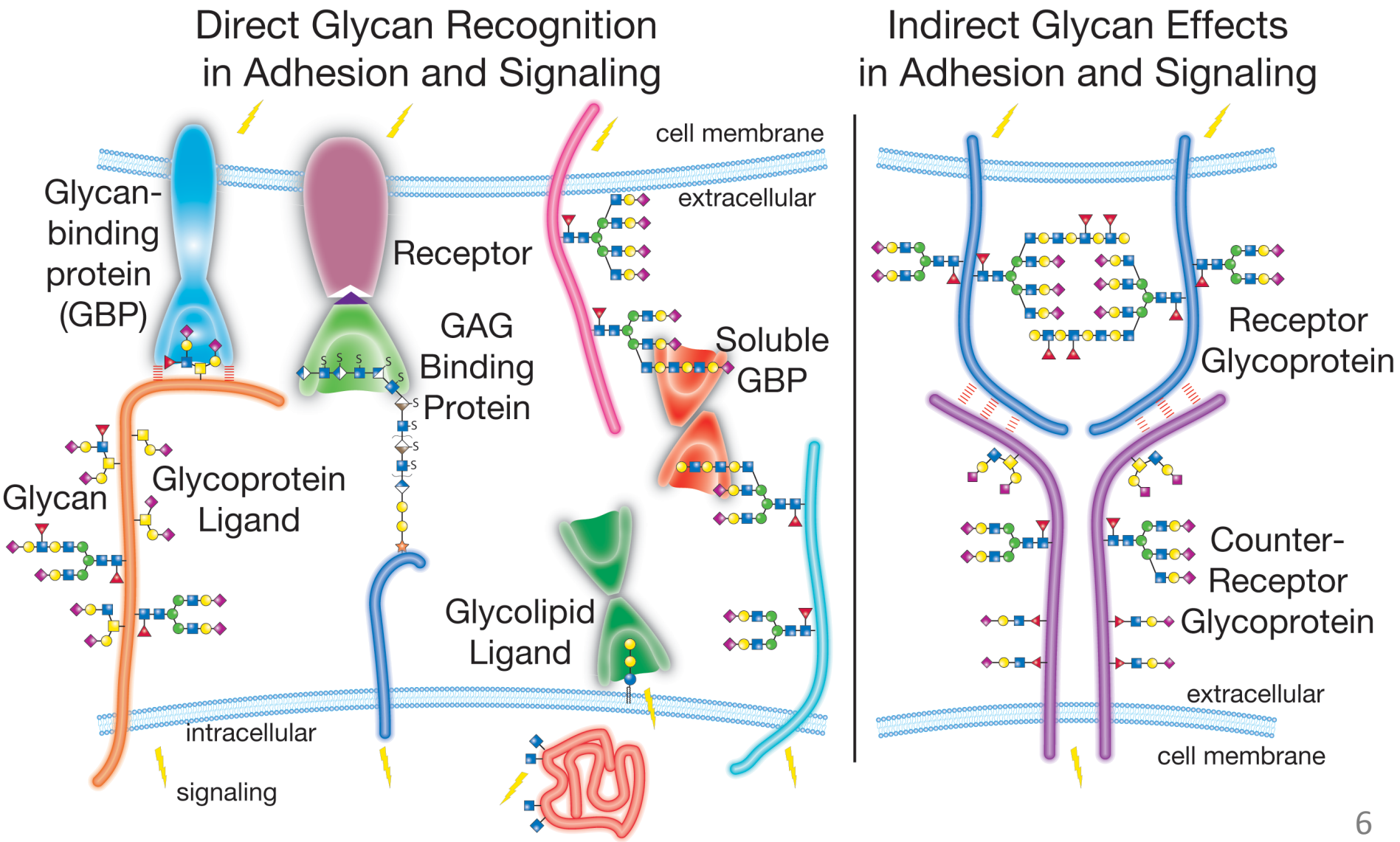


Essentials of Glycobiology, 3rd Edition
Chapter 1, Figure 6

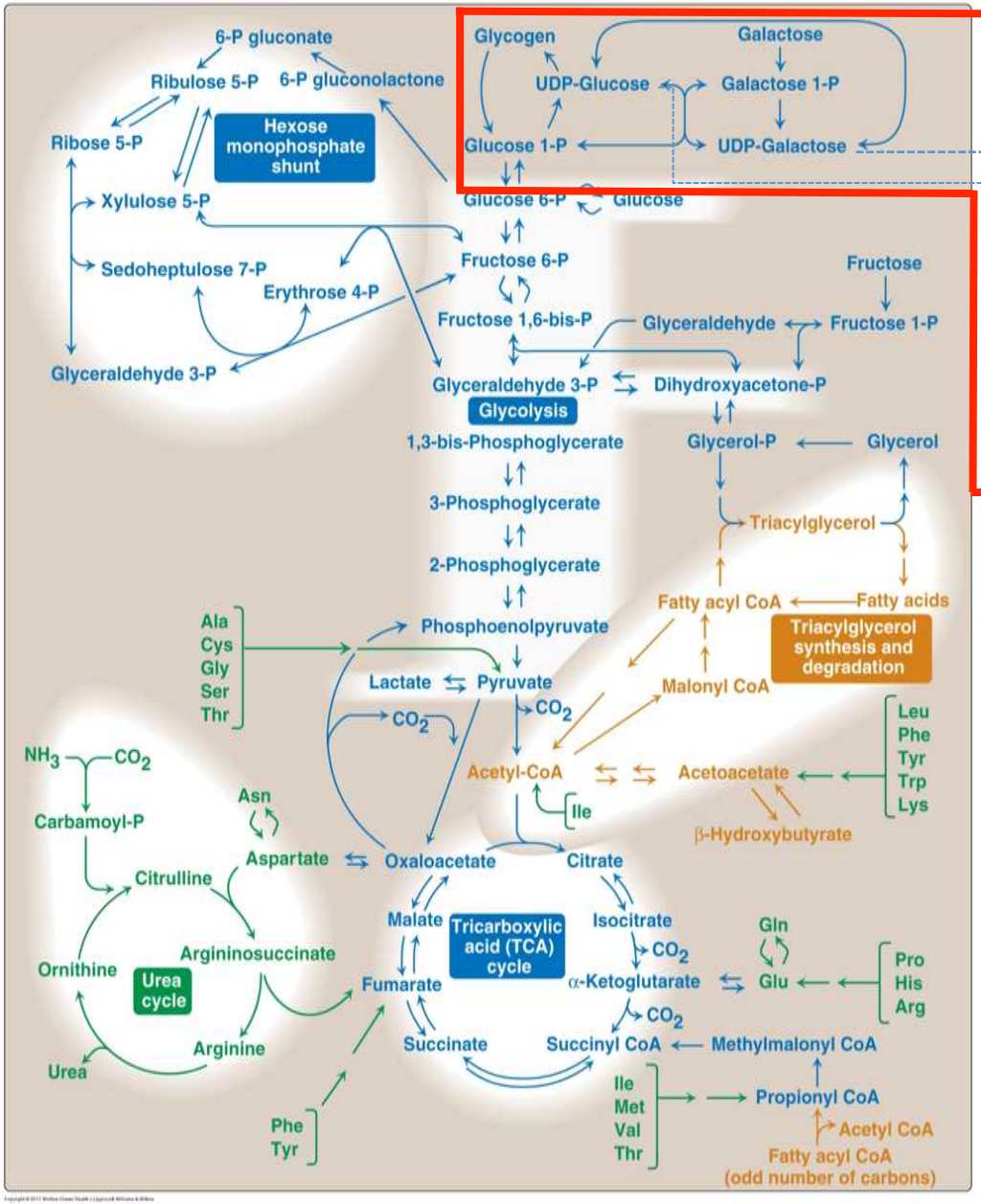
Glycans are as Ubiquitous as DNA/RNA and Appear to Represent Greater Molecular Diversity



General Roles of Glycans in Glycoprotein Functions



Big Picture: Connection of Glycoconjugate Biosynthesis to Intermediary Metabolism



Nucleotide Sugars

- UDP-Glc,
- UDP-Gal,
- UDP-GlcNAc,
- UDPGalNAc,
- UDP-GlcA,
- UDP-Xyl,
- GDP-Man,
- GDP-Fuc,
- CMP-Neu5Ac

used for synthesizing glycoconjugates, e.g, glycoproteins & glycolipids

7

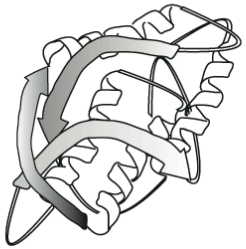
Important Topics to Consider

1. The different types of monosaccharides found in animal cell glycoconjugates
2. The different types of glycoconjugates and their differences, e.g. glycoproteins, glycolipids
3. The nucleotide sugars, glycosyltransferases, glycosidases, transporters, endoplasmic reticulum, and Golgi in terms of their roles in glycoconjugate biosynthesis and turnover
4. The general steps in biosynthesis of glycoprotein N-glycans and O-glycans
5. The general steps in biosynthesis of glycosaminoglycans and glycolipids
6. The human blood group antigens and the basis for acceptable or unacceptable donors of blood and plasma
7. The Congenital Disorders of Glycosylation (CDGs)
8. I-cell disease and the consequences on lysosomal hydrolase targeting to lysosomes.
9. The bases of Lysosomal Storage Disorders (LSDs) and Mucopolysaccharidoses

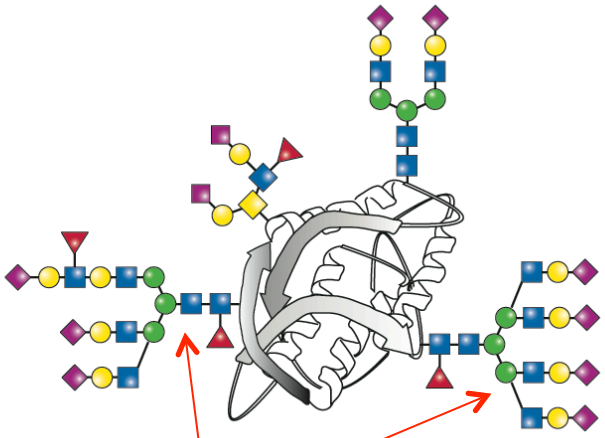
- **Glycoconjugates** and their **carbohydrate** residues represent one of the 4 classes of macromolecules in organisms.
- Recently glycoconjugates have become structurally defined and biosynthetically understood, especially in terms of human diseases, and are accessible to new drug, diagnostic, and therapeutic developments.
- **Glycobiology** is the study of the biological functions, synthesis, and structures of glycoconjugates.
- **Glycomics** is the study of the repertoire of **glycans** found in cell-derived **glycoproteins** and **glycolipids** and in free fluids, e.g. milk, urine, etc.

DNA/RNA

Protein



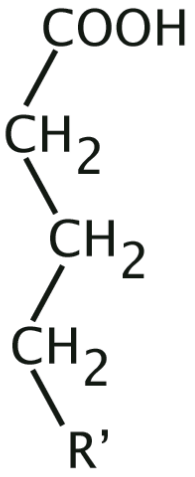
Polysaccharides/ Glycoconjugates



Carbohydrates attached to a protein

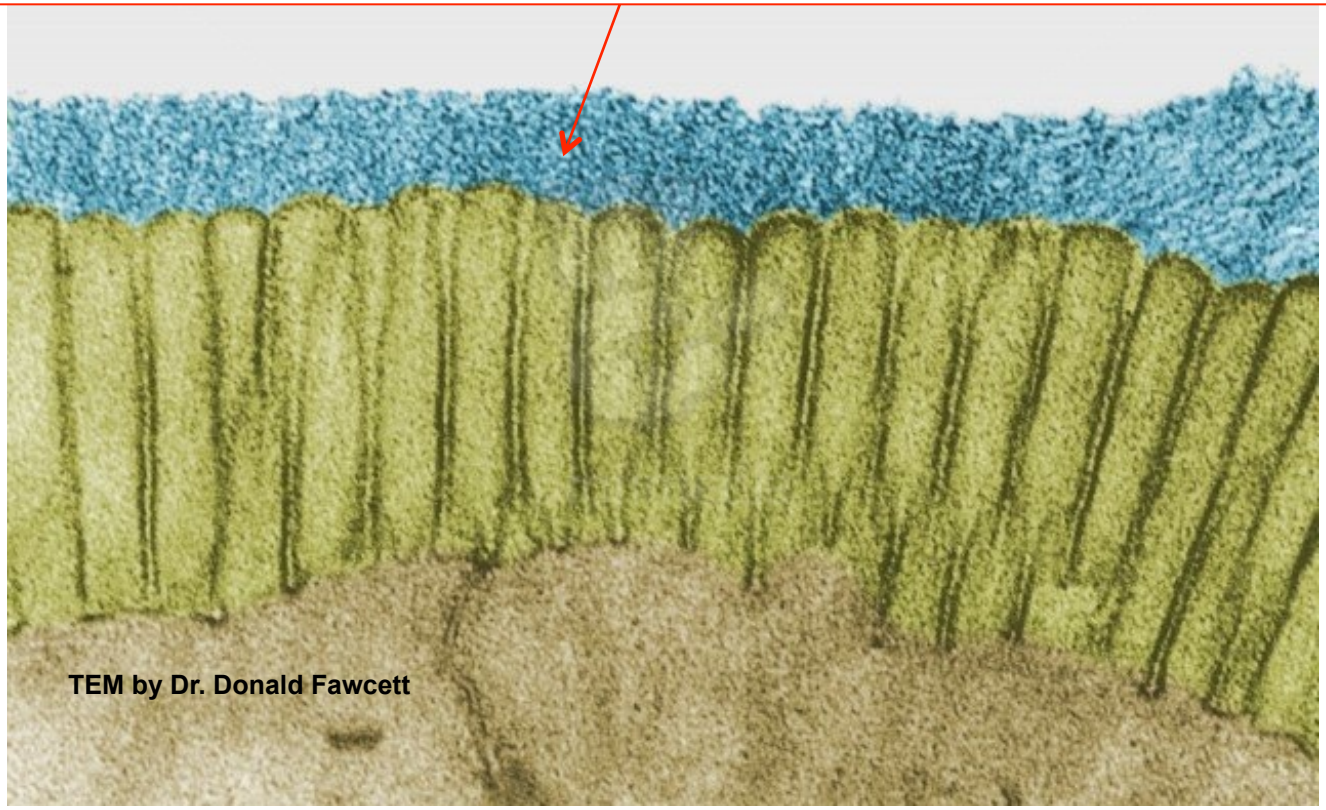
Example of a *Glycoprotein*

Lipids

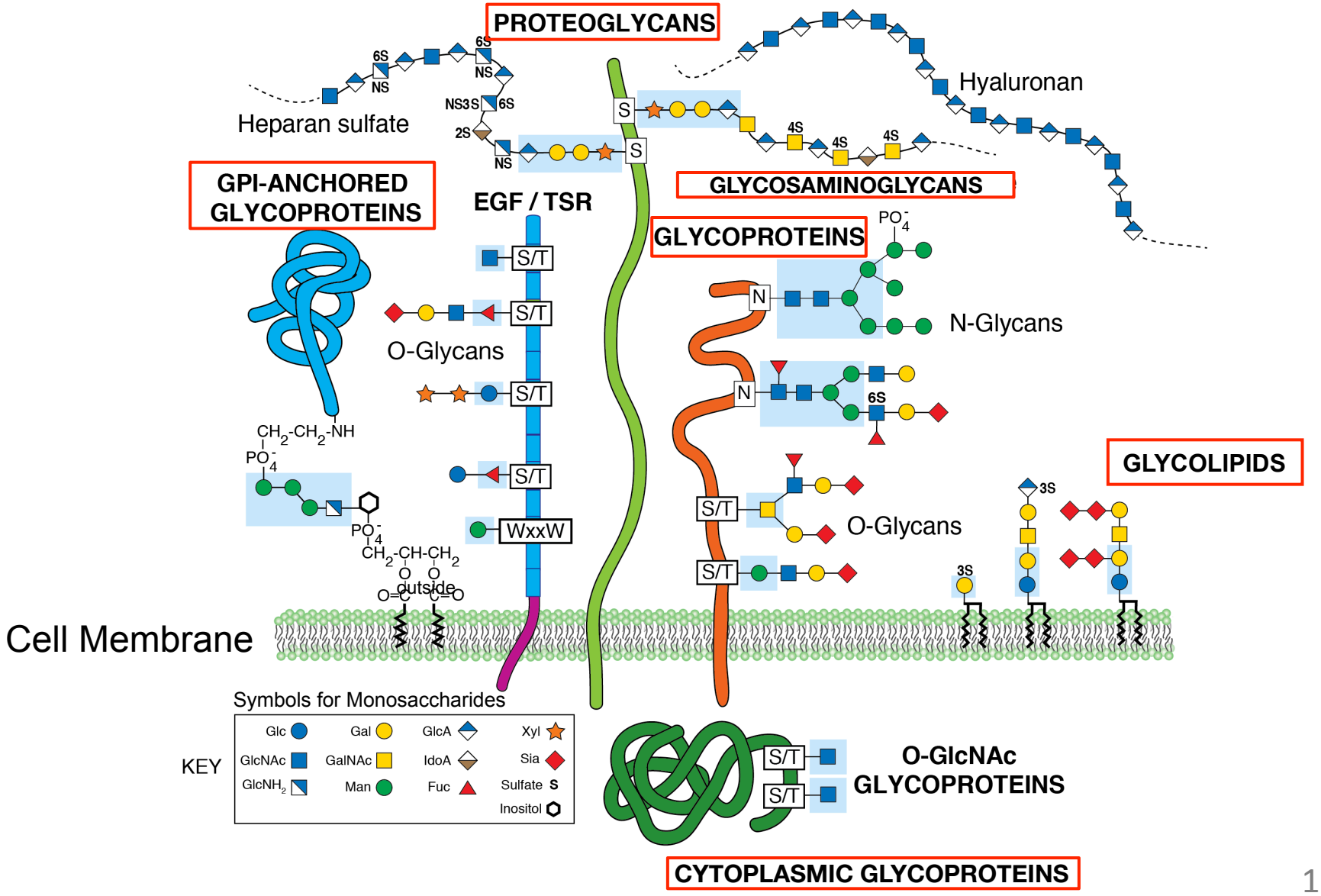


Plasma membranes of all animal cells contain a very high density of glycoconjugates, often termed the **glycocalyx**, which include all types of glycoproteins, that occur as receptors, transporters, adhesion molecules, and mucins

Brush border of the intestinal epithelium showing numerous microvilli and a prominent glycocalyx stained blue

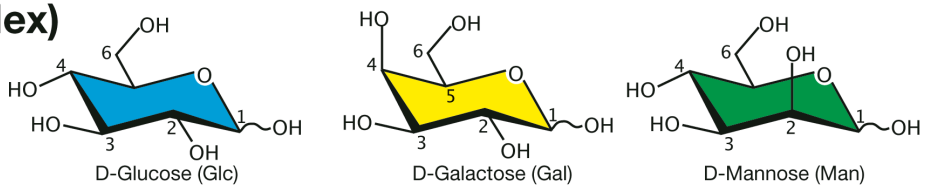


Glycoconjugates, Which are Molecules Containing Sugars (Monosaccharides) Linked Within Them, are the Major Constituents of Animal Cell Membranes (*Glycocalyx*) and Secreted Material: See Different Classes of Glycoconjugates Below in Red Boxes

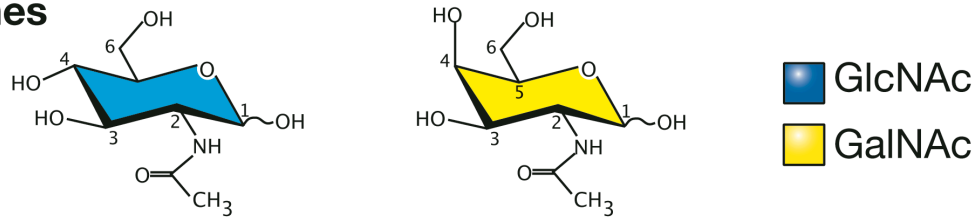


The Major 10 Monosaccharides Found in Human Glycans: Comprised of Hexoses, Hexosamines, Pentose, Uronic Acids, Deoxyhexoses, and Sialic Acids

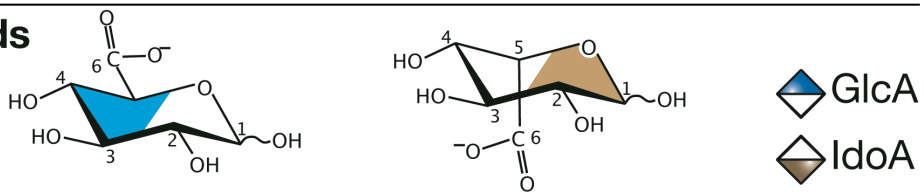
Hexoses (Hex)



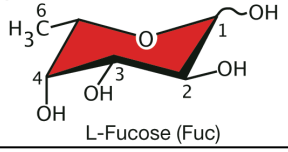
Hexosamines (HexNAc)



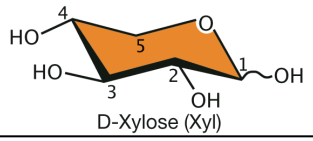
Uronic Acids



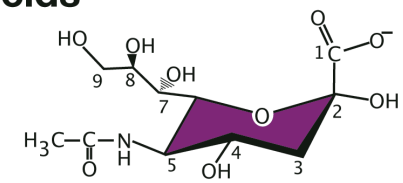
Deoxyhexose (dHex)

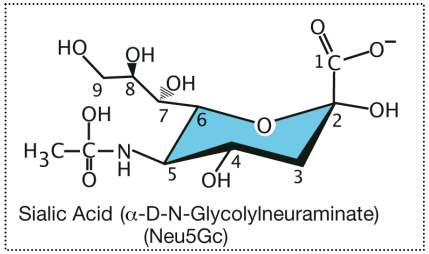


Pentose (Pent)



Sialic acids (Sia)





Triose – 3 carbons
Tetrose – 4 carbons
Pentose – 5 carbons
Hexose – 6 carbons
Heptose – 7 carbons
Octose – 8 carbons
Nonose – 9 carbons
Decose – 10 carbons

Note the **epimeric** relationships among the hexoses, carbon numbering, different properties of each class of monosaccharide

Epimers are molecules that differ only in the spatial arrangement around a single carbon atom

Note: **Neu5Gc** is not synthesized by humans and most birds, but is made by most other mammals and is found in cows, pigs, and sheep. Human consumption of glycoconjugates containing **Neu5Gc** can lead to its incorporation into human glycoconjugates.

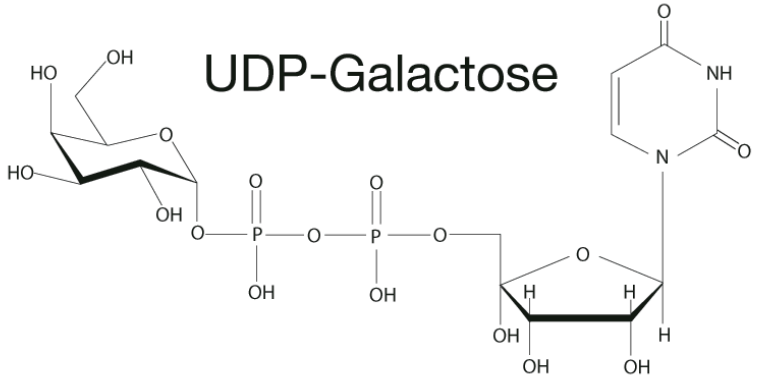
Sugar Phosphates, e.g. Glc-1-P, and Gal-1-P, and Nucleotides, e.g. UTP, and GTP, are Precursors for Nucleotide Sugars, which are Required for Glycoconjugate Biosynthesis

The Different Nucleotide Sugars in Human Based on Uridine Diphosphate, Guanine Diphosphate, and Cytosine Monophosphate

UDP- UDP- GDP- CMP-

UDP- UDP- GDP-

UDP- UDP-



Key Point! *

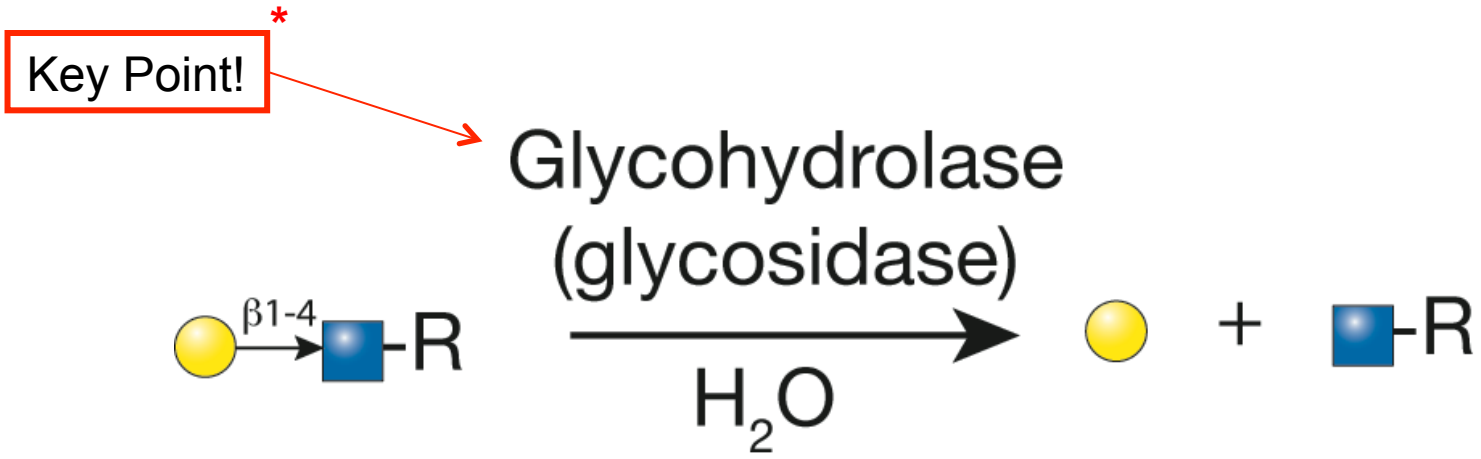
Glycosyltransferase

Nucleotide Sugar + Acceptor ⟶ Nucleotide diphosphate + Sugar-Acceptor

UDP-▪-R ⟶ UDP ● β1-4 ▪-R

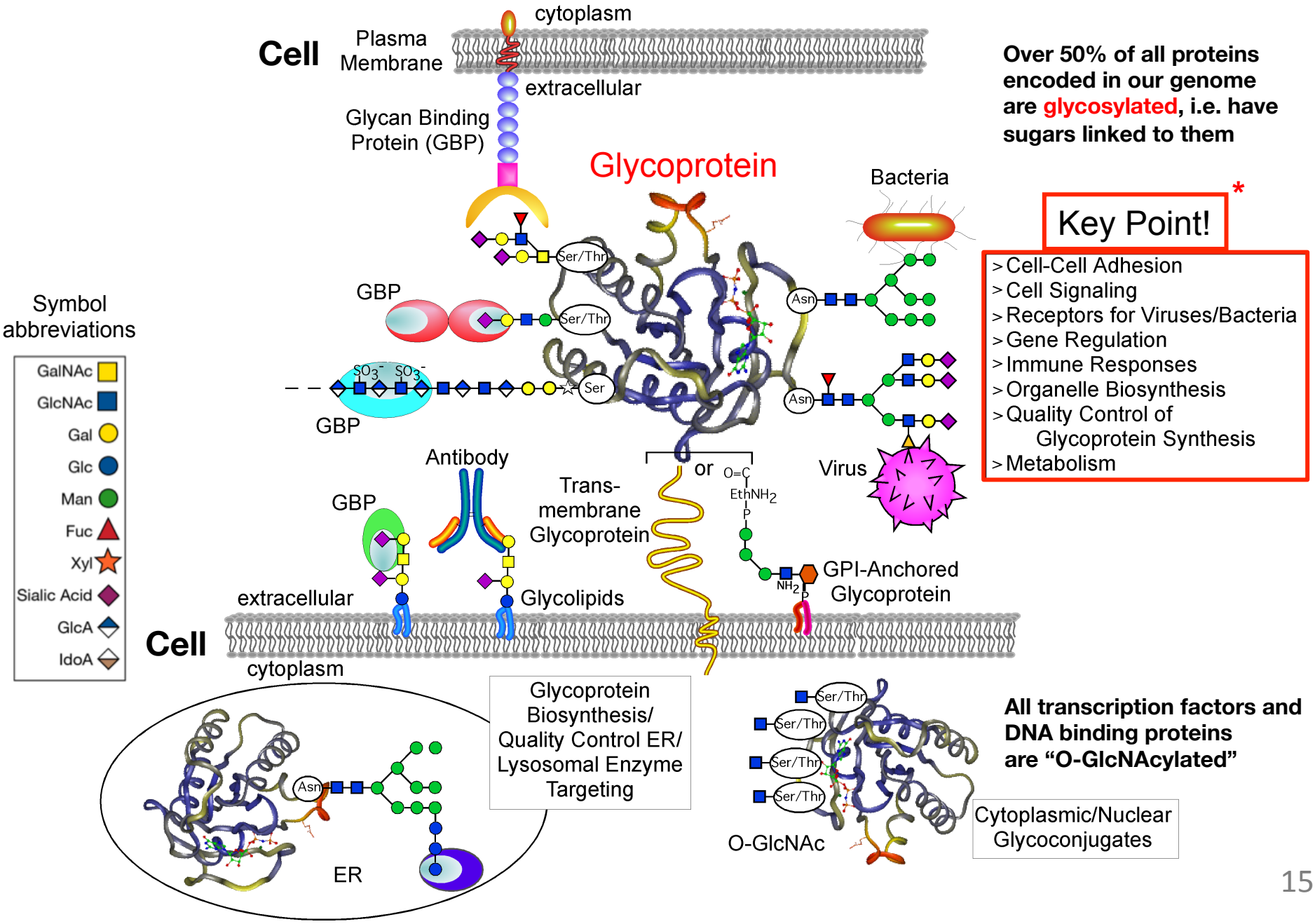
- **Each reaction occurs in order one sugar at a time.**
- **The donor sugar must be in a activated, i.e. pyrophosphoryl, form, as a nucleotide sugar.**
- **Each unique glycan linkage formed uses a different enzyme.**

Exo-Glycosidase Reaction



- **Each reaction occurs in order, with one sugar at a time being released from the non-reducing end of a glycoconjugate.**
- **Degradation of a heterogenous polysaccharide with multiple monosaccharides and linkages may require multiple enzymes and multiple reactions until it reaches the reducing end.**
- **The reaction requires water and is typically reversible.**
- **Each glycan linkage to be cleaved uses a different enzyme.**

Some Biological Functions of Glycoconjugates



Over 50% of all proteins encoded in our genome are glycosylated, i.e. have sugars linked to them

Key Point! *

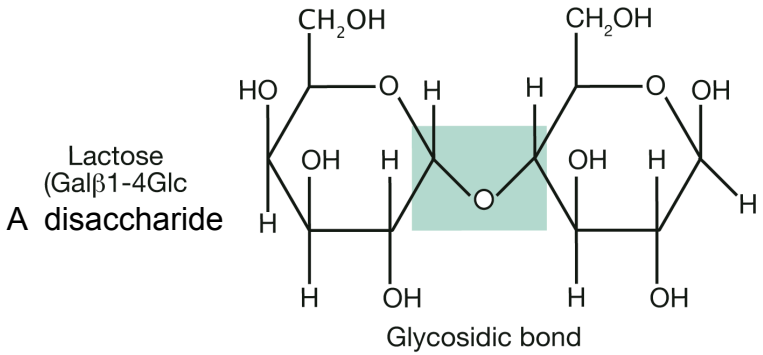
- Cell-Cell Adhesion
- Cell Signaling
- Receptors for Viruses/Bacteria
- Gene Regulation
- Immune Responses
- Organelle Biosynthesis
- Quality Control of Glycoprotein Synthesis
- Metabolism

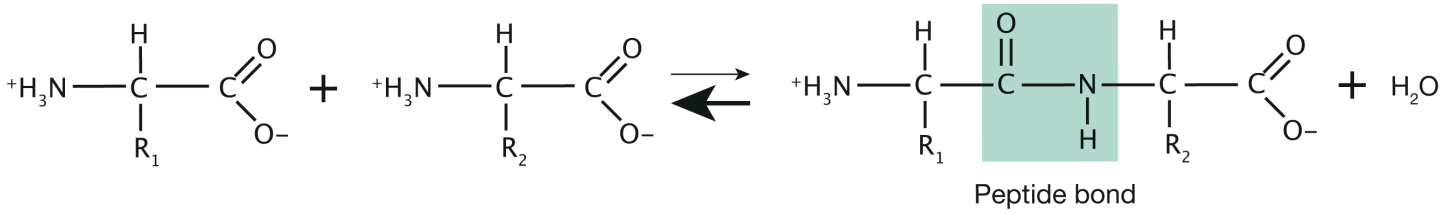
All transcription factors and DNA binding proteins are "O-GlcNAcylated"

Sugars in Glycans are Linked to Each Other by a Glycosidic Bond: Differences between a Glycosidic Bond and a Peptide Bond

Key Point! *

The glycosidic bond is the bond formed between simple sugars, which is very different from a peptide bond that is the bond formed between amino acids.



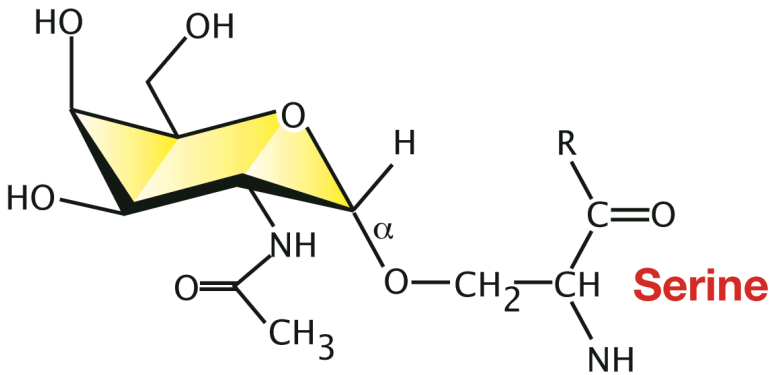


Oligosaccharide is a glycan containing more than 1 monosaccharide with a defined length, typically up to 30-40 residues;
Polysaccharide is usually reserved for glycans containing ≥ 30 monosaccharides lacking defined length and having a repeating structure;
Glycan is a general term denoting all kinds of saccharides linked to each other or to an aglycone (non-carbohydrate)

Glycoproteins Typically Have Two Major Linkage Types of Sugars to Amino Acids

O-Glycan (to Serine and Threonine)

Typical O-glycan Linkage
GalNAcα1-Ser/Thr

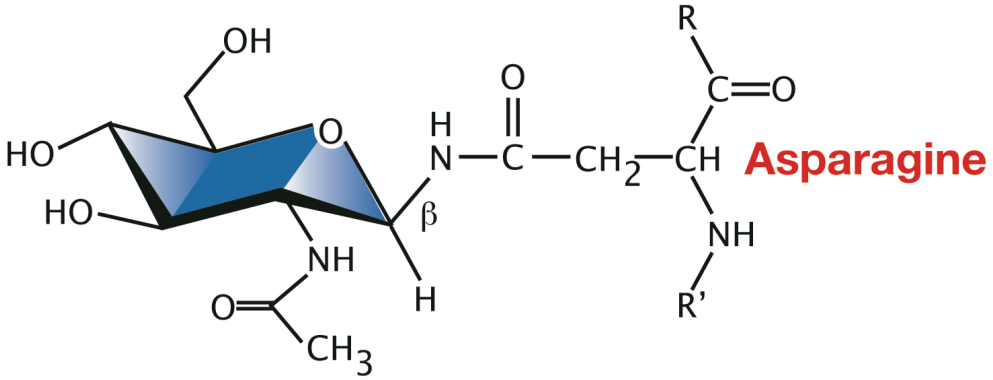


N-acetylgalactosamine
(GalNAc)

No consensus sequence, but modified Serine or Threonine (Ser/Thr) residues are often adjacent or near Pro residues

N-Glycan (to Asparagine)

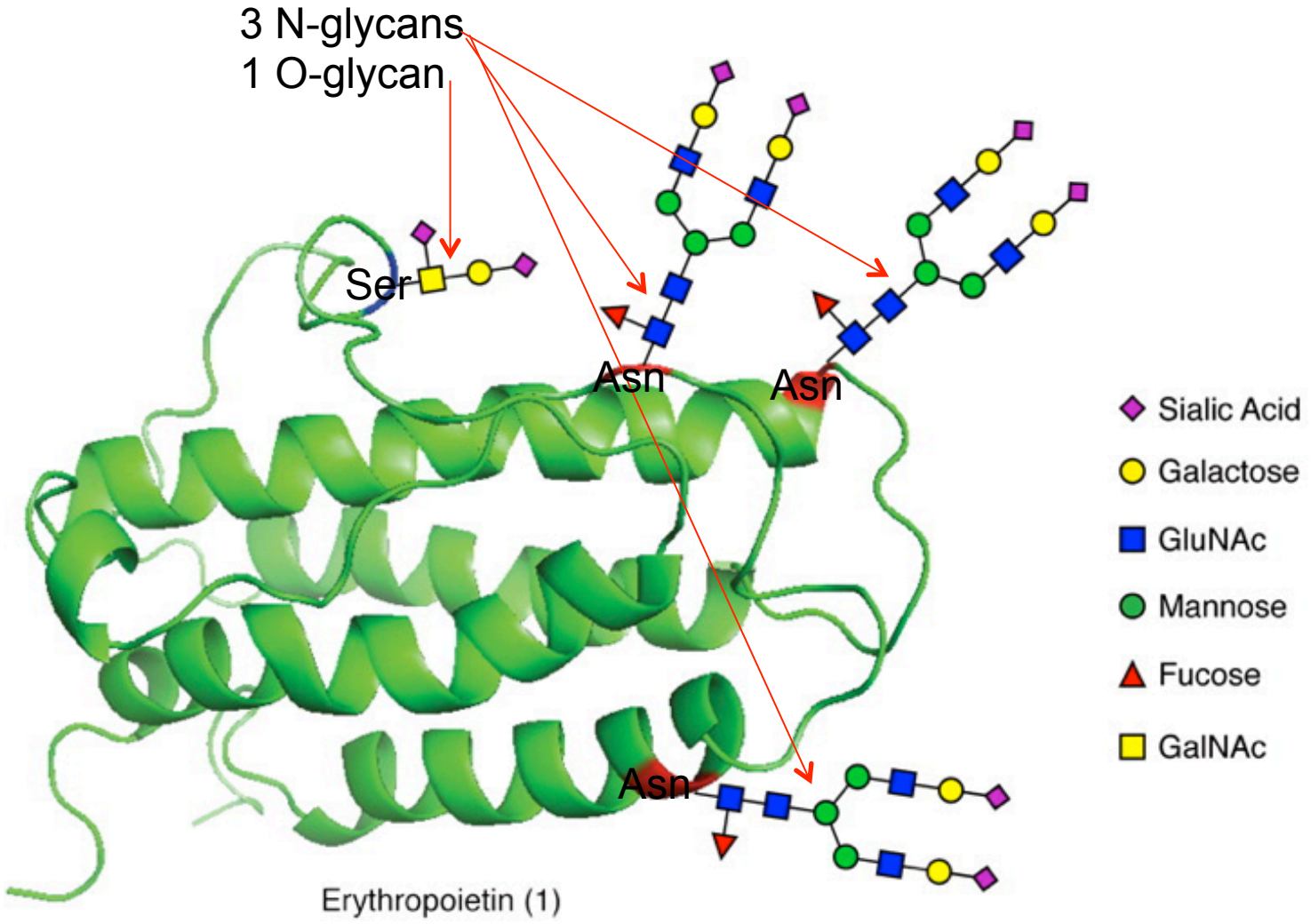
Typical N-glycan Linkage
GlcNAcβ1-Asn



N-acetylglucosamine
(GlcNAc)

Always in consensus sequence
---Asn-X-Ser/Thr---
Where X = any amino acid except Pro

Example of a Glycoprotein: Erythropoietin is a Glycoprotein Hormone That Controls Erythropoiesis



O- versus N-glycosylation

- Some glycoproteins have a single O-glycan, whereas others, e.g. mucins, may have hundreds to thousands of O-glycans

- Some glycoproteins have 1 or more N-glycans and lack O-glycans, and vice versa; some glycoproteins have numerous N- *and* O-glycans

- The N-glycans are added co-translationally to proteins in the endoplasmic reticulum (ER) of cells using pre-assembled lipid-linked oligosaccharide donors [*see upcoming discussion*]

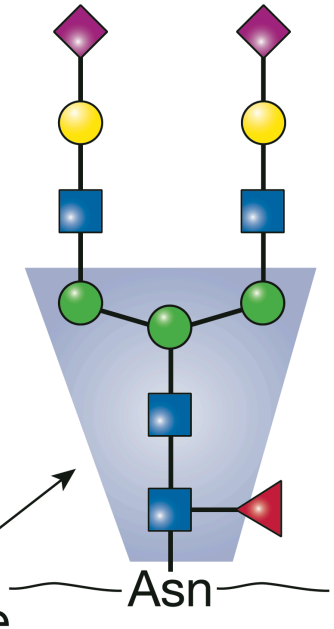
- The O-glycans in general are added post-translationally to proteins in the Golgi apparatus by single step additions of sugar from nucleotide sugar donors; no other precursors are involved

- O-glycans can be on adjacent Ser/Thr residues, whereas it is not possible for adjacent Asn residues to be N-glycosylated, although they can be near each other in sequence (the consensus sequence for N-glycosylation is –Asn-X-Ser/Thr-).

NOTE: In a non-enzymatic process free sugars, typically serum glucose, which may be elevated in disease conditions, can covalently modify lysine residues in a process termed "**glycation**" (which is a very different term from glycosylation) as seen in HbA_{1c} in diabetes.

Glc + Lysine-Protein ⟶ Glc-Lys-Protein (glycated)

Two Common Types of N-glycans in Glycoproteins



1. Complex-type N-glycan

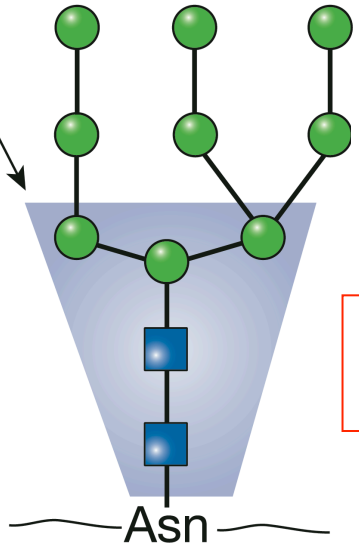
Has no terminal Mannose and has additional sugars beside Mannose and GlcNAc

Key

- GlcNAc
- Gal
- Man
- Neu5Ac (sialic acid)
- Fucose

Key Point!

Common pentasaccharide core



2. High Mannose-type N-glycan

Mainly Mannose and GlcNAc

Biosynthesis of Glycoproteins – in both the ER and Golgi:
The 5 Major Steps in N-Glycosylation of Proteins in Animal Cells

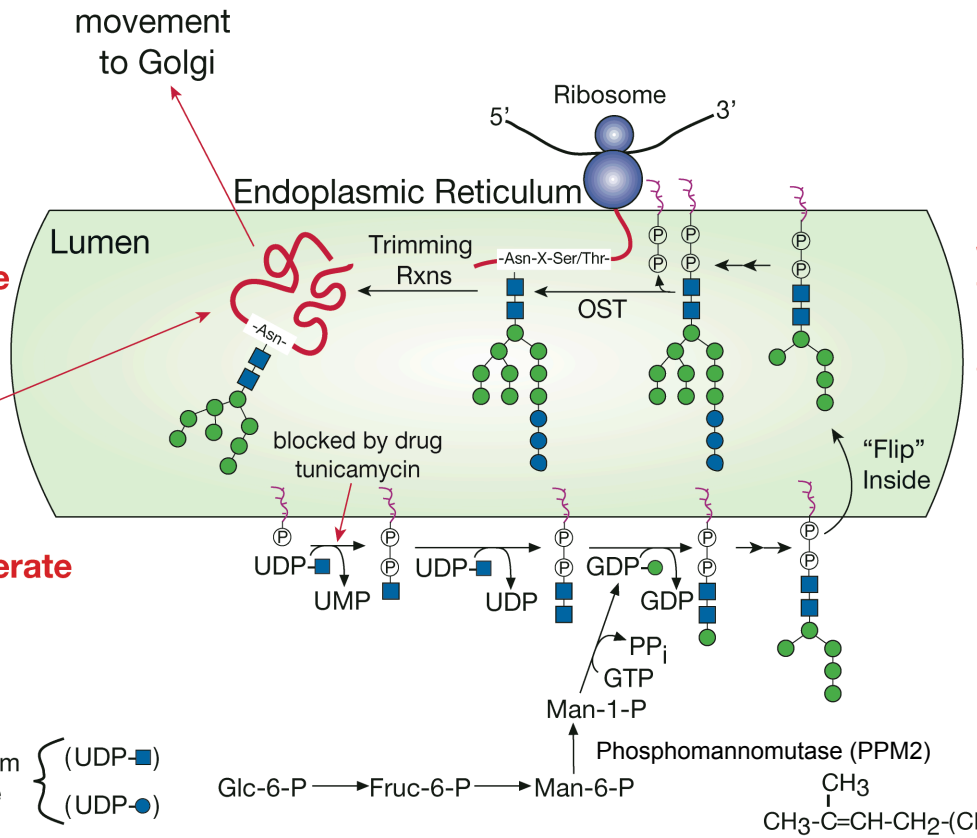
Steps 1-4 in the Endoplasmic Reticulum

Key Point! *

Step 3: Transfer of $Glc_3Man_9GlcNAc_2$ by the Oligosaccharyltransferase (OST) to Nascent Polypeptide in the ER

Newly Synthesized Glycoprotein in ER with an N-glycan

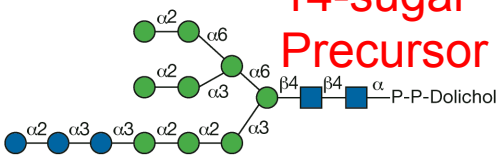
Step 4: Trimming reactions in ER to generate $Man_8GlcNAc_2$ on properly folded glycoprotein



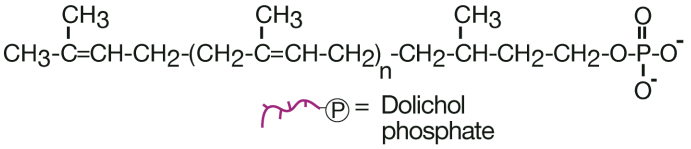
Step 1: Build Precursors in Cytoplasm

Step 2: Build Lipid-linked Oligosaccharide ($Glc_3Man_9GlcNAc_2$-P-P-Dolichol) on and in the ER membrane (Unique 14-sugar precursor to N-glycosylation)

14-sugar Precursor



$Glc_3Man_9GlcNAc_2$-P-P-Dolichol



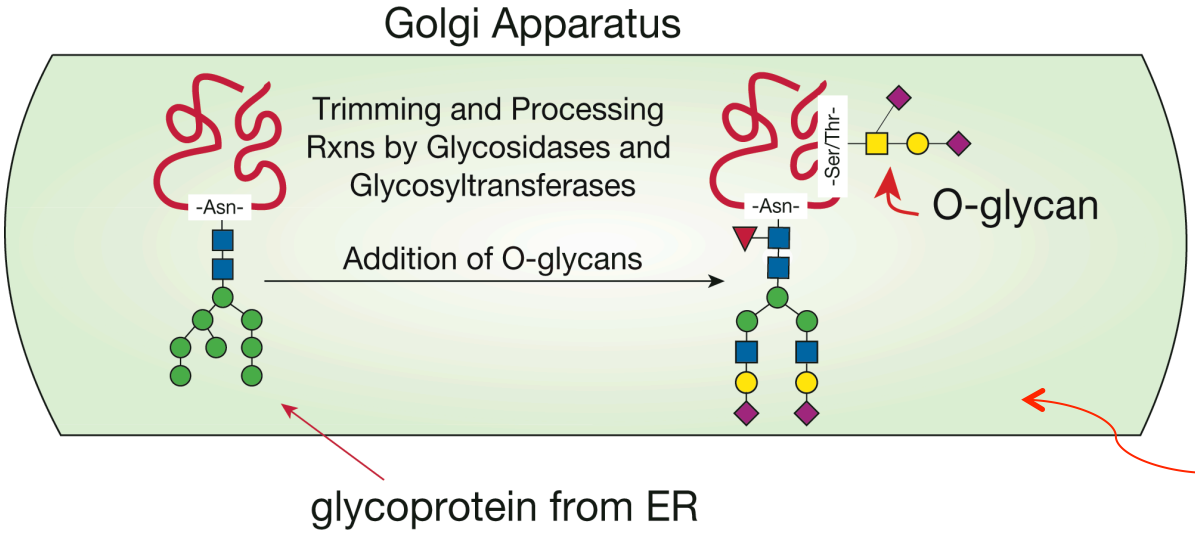
In the dolichol cycle, Dol-P-P generated after protein glycosylation is converted back to Dol-P

Biosynthesis of Glycoproteins – in both the ER and Golgi:

The 5 Major Steps in N-Glycosylation of Proteins in Animal Cells

Step 5 in the Golgi Apparatus

Key Point! *



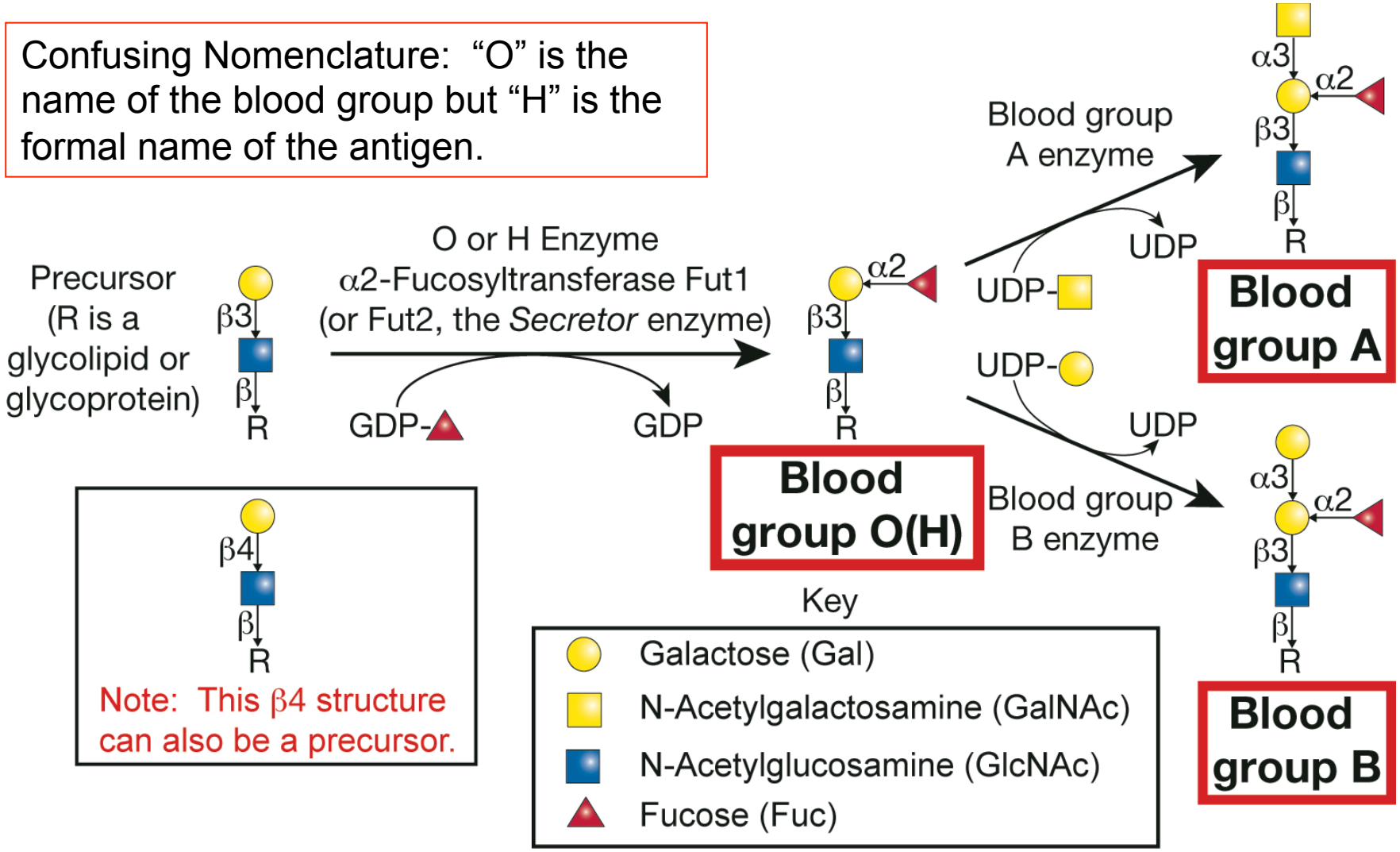
Step 5: Further trimming and processing (extension) in Golgi to generate complex-type N-glycan on mature glycoprotein

Specific transporters in Golgi membrane import nucleotide sugars into Golgi; there are specific transporters for UDP-Gal, UDP-Glc, GDP-Fuc, etc.

Note: Addition of O-glycans to glycoproteins occurs in Golgi by single step sugar additions from nucleotide sugars and no preformed intermediates

The Extended Termini of Some Glycans in Glycoproteins and Glycolipids Mainly in Red Blood Cells and Epithelial Cells, Contain the ABO(H) Blood Group Antigens. Each Person may also Generate Antibodies to these Blood Group Antigens

Confusing Nomenclature: "O" is the name of the blood group but "H" is the formal name of the antigen.



Key Point! – ABO are different carbohydrate structures on red blood cells *

The ABO(H) Blood Group Antigens

- The ABO(H) blood group antigens are carbohydrate structures synthesized on glycoproteins and glycolipids in the Golgi apparatus of red blood cell precursors, megakaryocytes, and many types of epithelial cells, and occur on cell surfaces and in secretions.

- Biosynthesis occurs through a series of enzymatic reactions that add a single sugar from a nucleotide sugar donor to an acceptor as shown.

- The nucleotide sugars used are GDP-Fucose, UDP-Galactose, and UDP-N-acetylgalactosamine.

- The products of the reactions of Fut1 (H-enzyme) or Fut2 (secretor enzyme) become the acceptors for the Blood group A or B enzymes, to create the human A or B antigens, respectively.

- People with blood group A, have both the A enzyme and the Fut1 enzyme, whereas people with blood group B, have the B enzyme and the Fut1 enzyme.

- People with blood group O(H) lack the A and B enzyme and have Fut1 enzyme.

- The secretor H structure is inherited independently (Fut2) and some people are non-secretors (meaning no blood antigens are in saliva, etc., and some are secretors, where they can make blood group antigens in secretions.

Antigens on Erythrocytes and Serum Antibodies to Blood Groups

Blood Type	Erythrocyte Antigens	Serum Antibodies that can Agglutinate other Erythrocytes
A	A	Anti-B
B	B	Anti-A
A/B	A & B	none
O	H	Anti-A & Anti-B

Key Point! *

- People of Type O are Universal red cell donors, since individuals of Type A, Type B, and Type AB lack antibodies to Type O and thus are able to receive transfusions of Type O blood.

- By contrast, Type AB individuals are Universal plasma donor, since their plasma lacks antibodies to the ABO(H) antigens.

- Type AB individuals are also Universal recipients, since they lack antibodies to ABO(H) and they can receive blood cells from any donor.

Inheritance of ABO

Parent Alleles	A	B	O
A	AA (A)	AB (AB)	AO (A)
B	AB (AB)	BB (B)	BO (B)
O	AO (A)	BO (B)	OO (O)

Key Point! *

Note: Parentheses denotes the phenotypes of offspring.

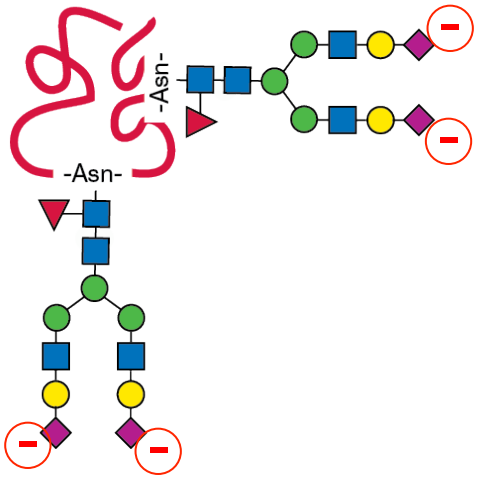
Alterations in Biosynthesis of Glycans Occurs in **Congenital Disorders of Glycosylation (CDGs)**

- The Congenital Disorders of Glycosylation (CDG) is a group of autosomal recessive diseases that affect the synthesis of glycoproteins, typically affecting N-glycosylation – 36 known genes so far (specific steps in Slides 16 and 17). Key Point! *
- These disorders (frequency estimated to be 1/20,000) are characterized by neurological involvement that can be associated with multivisceral involvement.
- Symptoms range from severe developmental delay and hypotonia with multiple organ system involvement to hypoglycemia and protein-losing enteropathy with normal development, and thus is often un- or mis-diagnosed
- The biological diagnosis is commonly based on the demonstration of abnormal glycosylation of serum glycoproteins, such as serum transferrin, based on isoelectric focusing, the measurement of leukocyte enzyme activities responsible, and the search for mutations in the corresponding genes.
- CDGs are associated with different enzymatic deficits of which the most common is a phosphomannomutase (PMM2) deficit (corresponding to CDG Ia and representing 70% of the CDG syndromes) (specific steps in Slides 16 and 17). Key Point! *

Congenital Disorders of Glycosylation (CDGs)

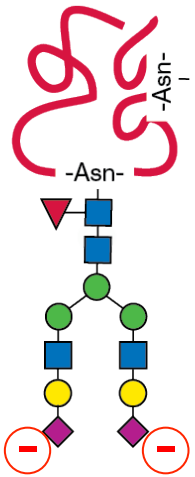
- **Type I CDG** have defects in assembly of $Glc_3Man_9GlcNAc_2$-P-P-Dolichol or defects in efficiency of transfer of the oligosaccharide to protein, thus synthesizing glycoproteins *deficient in numbers of glycans*. Key Point! *
- **Type II CDG** exhibit defects in trimming or processing of $Man_8GlcNAc_2$ after transfer to Protein and thus have altered N-glycan structures (loss of sialic acid, galactose, etc), but the *number of N-glycans on a protein are normal*

Normal glycoprotein



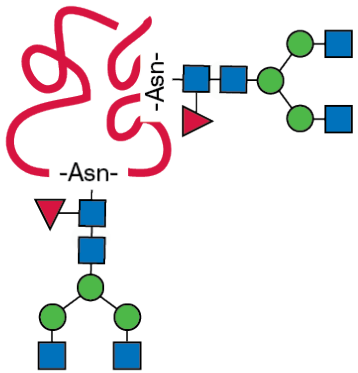
4 net negative charges due to Sialic acid

Type I CDG



2 net negative charges due to Sialic acid

Type II CDG



0 net negative charges due to Sialic acid

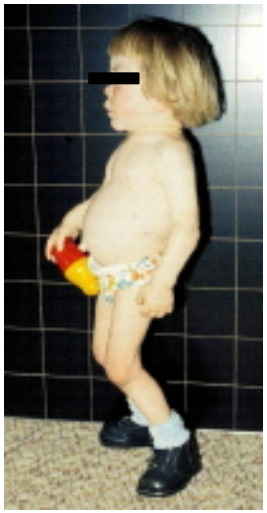
Other Disorders in Glycoprotein Biosynthesis - **I-Cell Disease (Mucolipidosis Type II):**

Patients with **I-Cell Disease** are characterized by deficiency in dozens of different lysosomal hydrolases in the lysosomes, and instead elevations of them in their serum.

The patients are characterized by skeletal abnormalities, restricted joint movement, coarse facial features, and severe psychomotor impairment; death usually occurs by age 8.

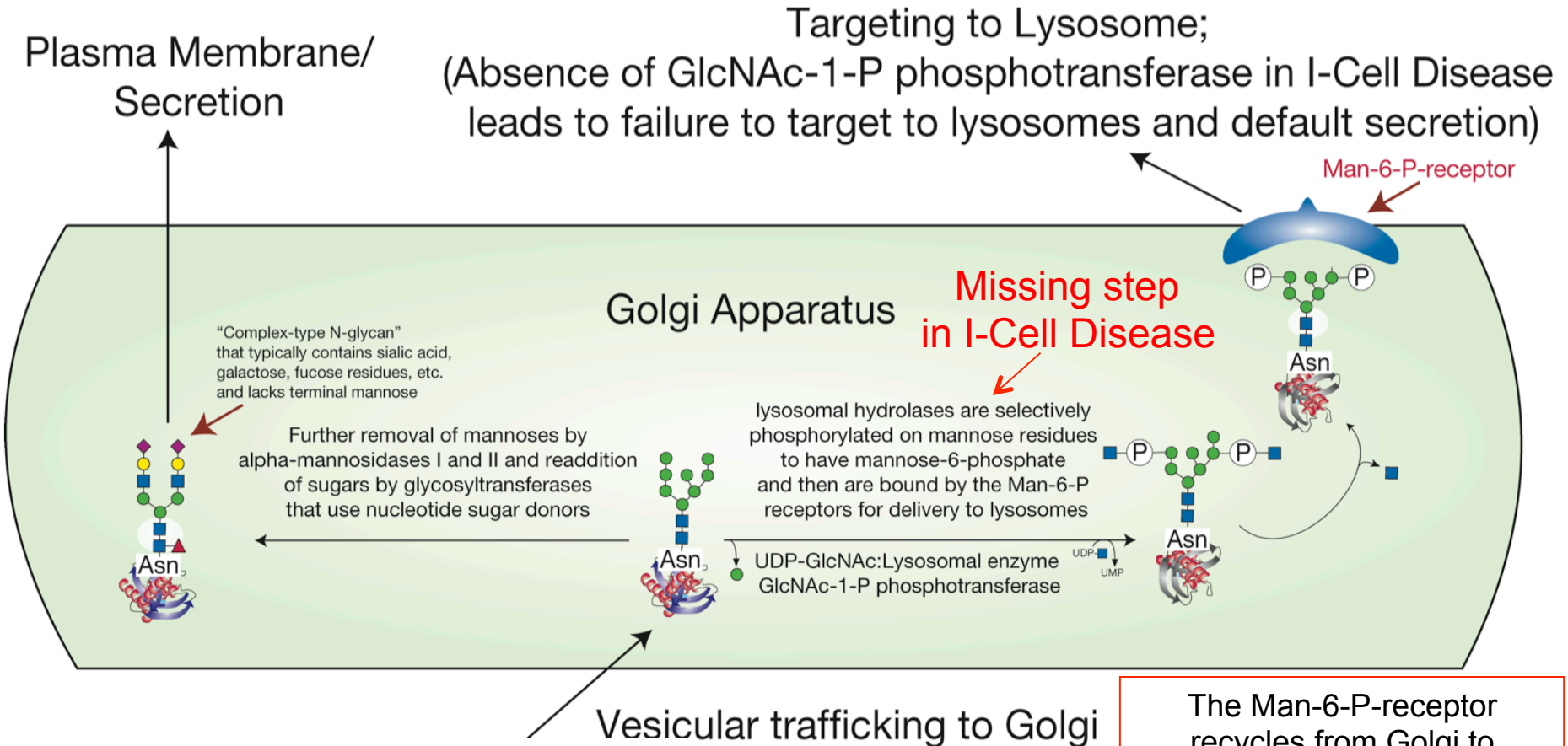
The loss of lysosomal enzyme is due to deficiency of a Golgi enzyme (the GlcNAc-1-phosphate Phosphotransferase) to generate mannose-6-phosphate on the N-glycans of lysosomal hydrolases. Key Point! *

Lack of their Man-6-P moiety leads to inability to bind the Mannose-6-phosphate Receptor, which is responsible for removing hydrolases from the secretory pathway and directing them to endosomes and subsequently lysosomes.

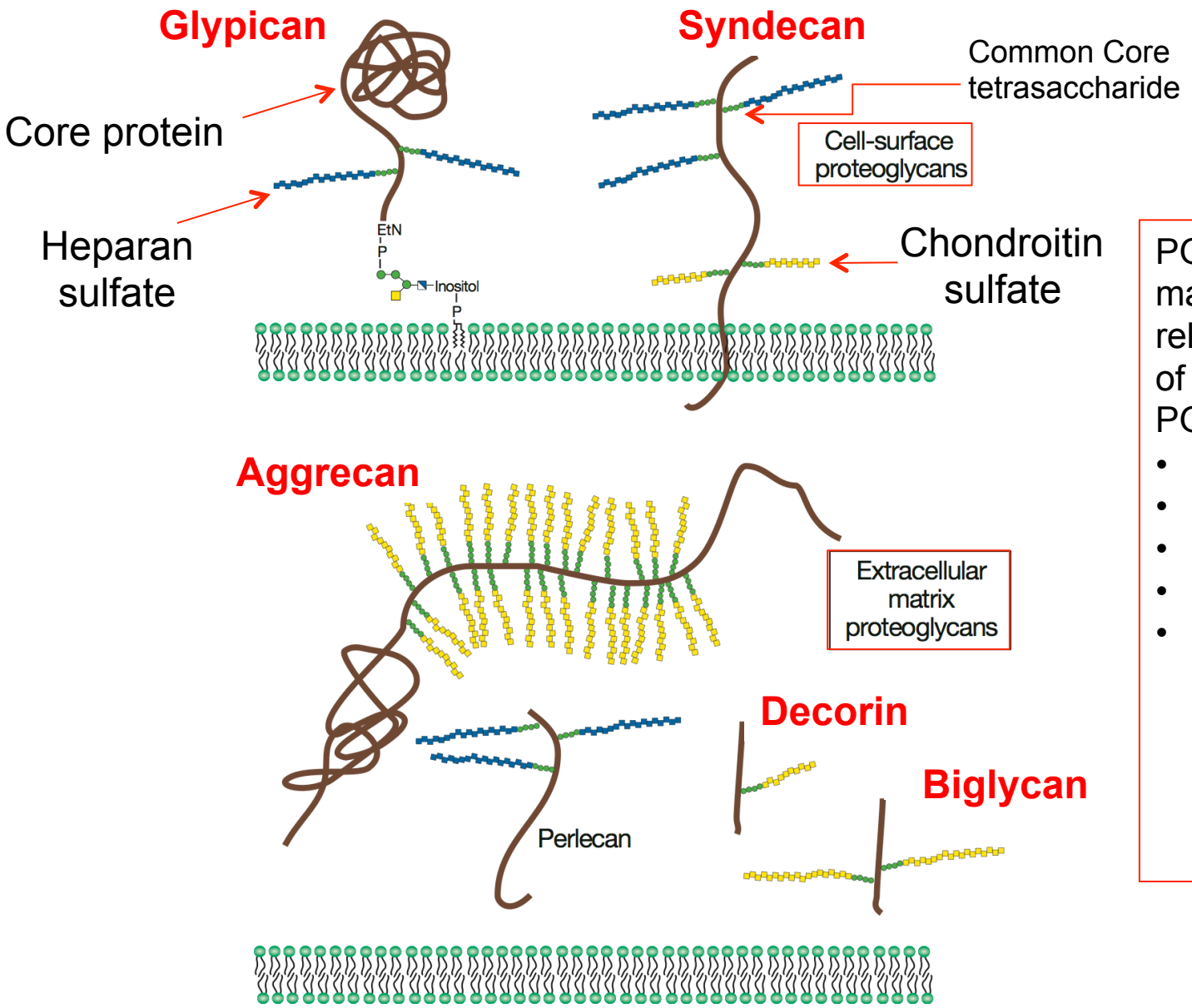


Profile view of 3-year-old with I-cell disease. Growth ceased more than one year earlier. Note small orbits, proptotic eyes, full and prominent mouth caused by gingival hypertrophy, short and broad hands, stiffening of small hand joints, prominent abdomen with umbilical hernia, and limited extension of the hips and knees.

Lysosomal Hydrolases are Glycoproteins that Are Processed Differently from Non-Lysosomal Hydrolases: I-Cell Disease Caused by Absence of Mannose-6-Phosphate



Proteoglycans (PGs) (old term is Mucoproteins) Consist of a Protein Core and One or More Covalently Attached Glycosaminoglycans: PGs Make up All Connective Tissue



Key Point! *

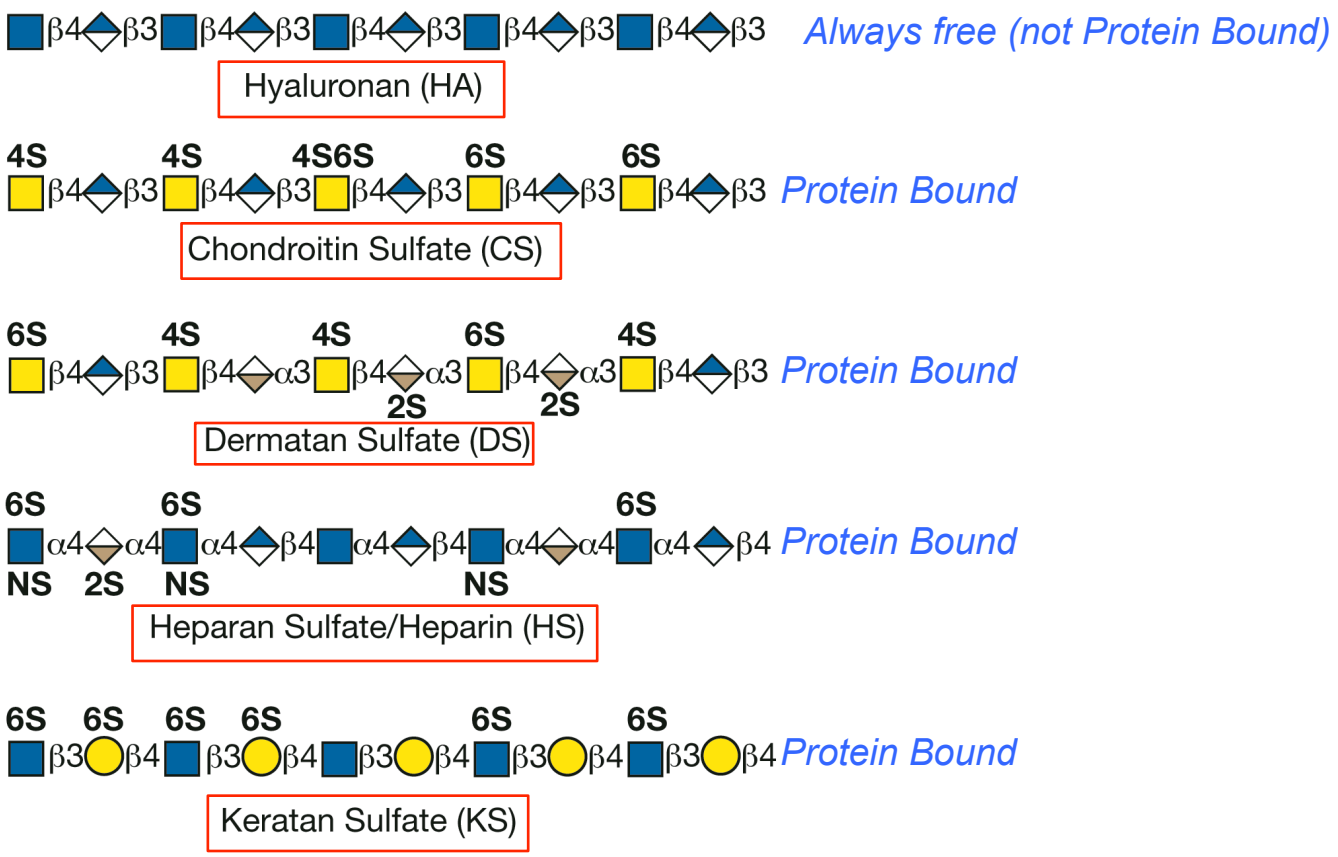
PGs are composed mainly of sugars and relatively small amount of protein;
PGs function to provide:

- tensile strength,
- rigidity
- resilience
- elasticity
- and function as signaling receptors and "docking sites" for growth factors, cytokines, etc.

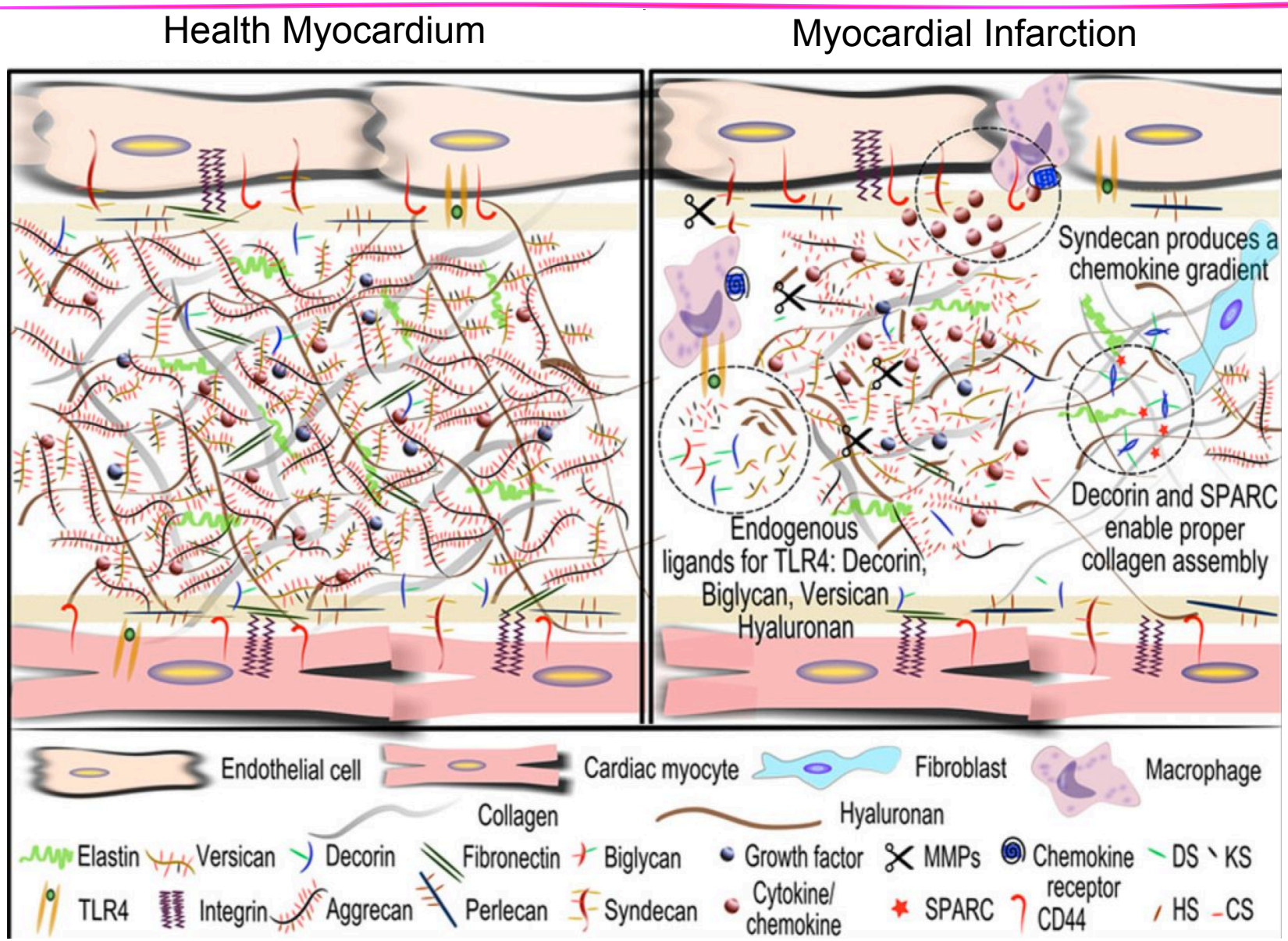
Proteoglycans (PGs) are Proteins with attached Glycosaminoglycans (GAGs)

GAGs are linear polymers of disaccharide units of one acidic and one amino sugar

5 types of Glycosaminoglycans



Proteoglycans (PGs) (old term is Mucoproteins) are Major Components of Connective Tissue Throughout the Body: In Disease or Damage Alterations in Proteoglycans and Extracellular Matrices Occur - Example shown is Myocardium



Rienks et al (2014) *Circulation Research* **114**:872

Mucopolysaccharidoses (Lysosomal Storage Diseases – LSDs)

- The mucopolysaccharidoses (lysosomal storage diseases or LSDs) are a group of *inherited metabolic diseases in which a defective or missing glycohydrolase enzyme causes accumulation of complex sugars* (GAGs in many cases) to accumulate in harmful amounts in the body's cells and tissues.

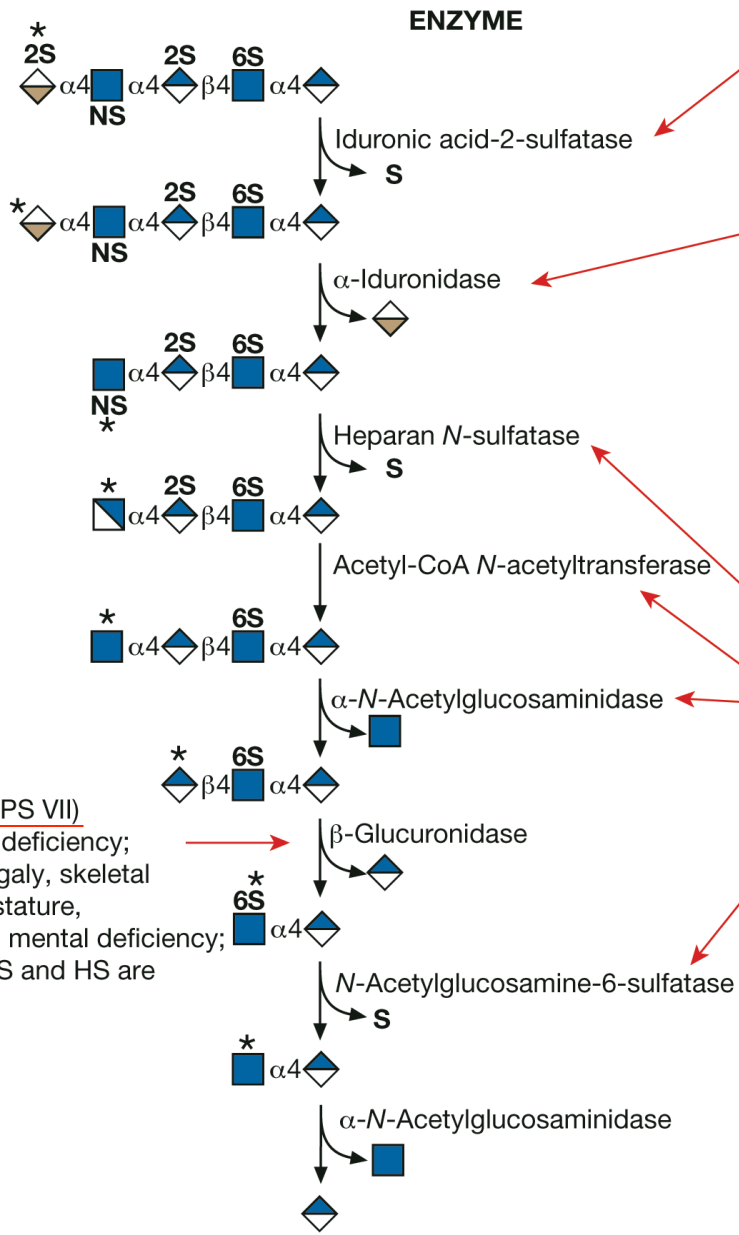
- This accumulation causes permanent, progressive cellular damage that affects appearance, physical abilities, organ and system functioning, and, in most cases, mental development.

- Depending on the type of mucopolysaccharidosis (MPS (x)), affected individuals may have normal intellect or may be profoundly retarded, may experience developmental delay, or have severe behavioral problems.

- Physical symptoms generally include coarse or rough facial features, thick lips, an enlarged mouth and tongue, short stature with a disproportionately short trunk (dwarfism), abnormal bone size or shape (and other skeletal irregularities), thickened skin, enlarged organs such as the liver or spleen, hernias, and excessive body hair growth.

Mucopolysaccharidoses Result from Defects in Specific Glycohydrolases (Glycosidases) Affecting the Stepwise Degradation of Glycosaminoglycans

Key Point! *



Hunter Syndrome (MPS II)
Iduronate-2-sulfatase deficiency - wide range of severity - no corneal clouding, but physical deformity and mental retardation is mild to severe

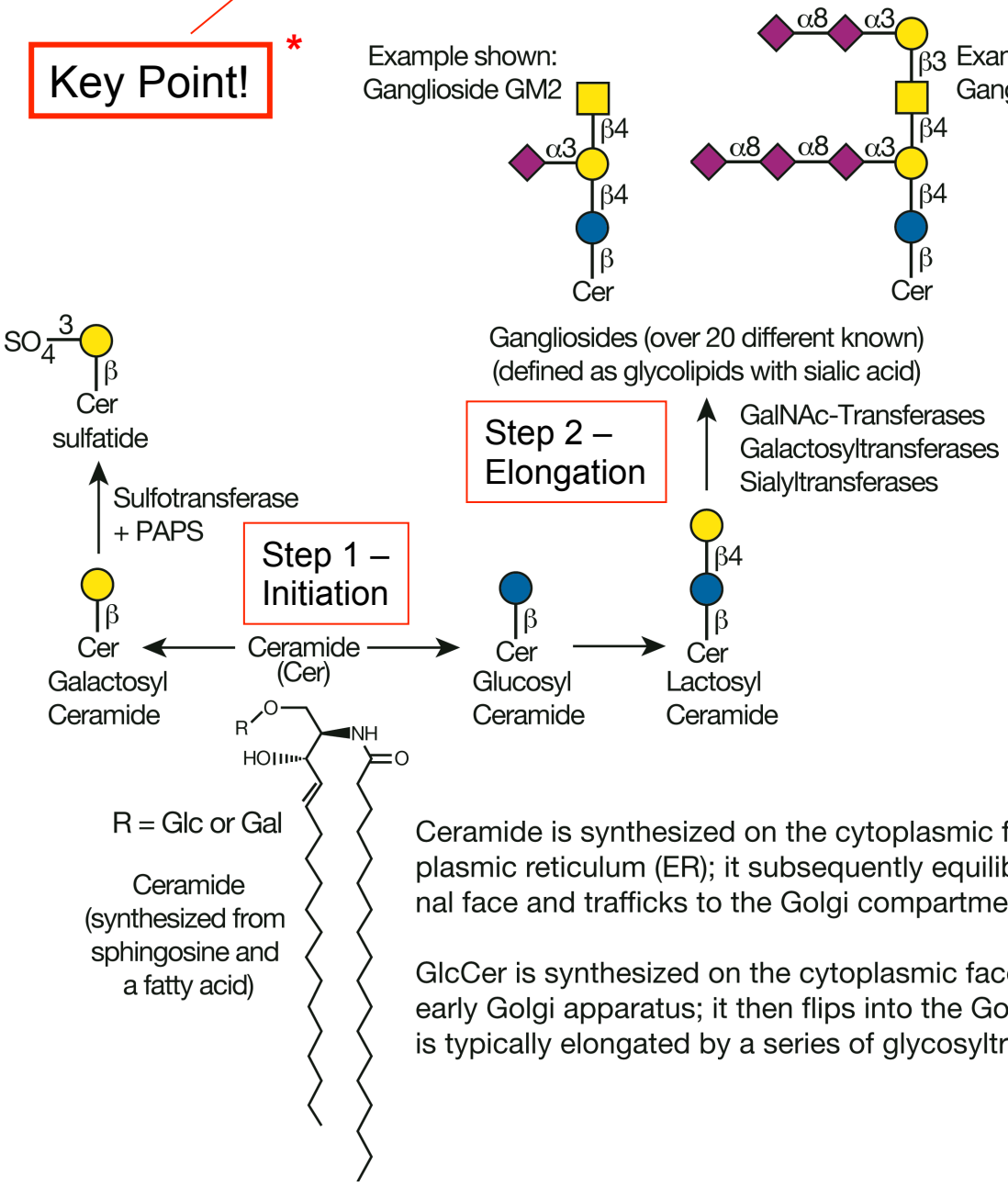
Hurler Syndrome (MPS I H)
α-Iduronidase deficiency - corneal clouding, mental retardation, dwarfing, coarse facial features, upper airway obstruction; degradation of DS and HS are affected; Deposition in coronary artery leads to ischemia and early death; disease can be trated by bone marrow transplant before age 18 months

Sanfilippo Syndrome types A-D (MPS III)
four enzymatic steps are necessary for removal of N-sulfated or N-acetylated-glucosamine residues from HS;
Type A: Heparan *N*-sulfatase
Type B: α-*N*-Acetylglucosaminidase
Type C: Acetyl-CoA *N*-acetyltransferase
Type D: *N*-Acetylglucosamine-6-sulfatase
Severe nervous system disorders and mental retardation

Sly Syndrome (MPS VII)
β-Glucuronidase deficiency; hepatosplenomegaly, skeletal deformity, short stature, corneal clouding, mental deficiency; degradation of DS and HS are affected

2 Major Steps in Biosynthesis of Glycosphingolipids (Glycolipids)

Key Point! *



Defects in degradation of glycolipids are also associated with LSDs; example is Tay-Sachs Disease, which is caused by mutations in a hexos-aminidase and results in the accumulation of the ganglioside GM2 in cellular lysosomes and inclusion bodies, culminating in irreversible, fatal deterioration of brain function

Ceramide is synthesized on the cytoplasmic face of the endo-plasmic reticulum (ER); it subsequently equilibrates to the lume-nal face and trafficks to the Golgi compartment.

GlcCer is synthesized on the cytoplasmic face of the ER and early Golgi apparatus; it then flips into the Golgi lumen, where it is typically elongated by a series of glycosyltransferases.

Summary

- *Glycoproteins* contain *N-glycans*, *O-glycans*, or both *(and although not discussed (see slide 5) some also are GPI-anchored glycoproteins)*;
- N-glycosylation of proteins occurs in the Endoplasmic Reticulum (ER) by addition of a glycan containing 14 sugars that is added *co-translationally* to proteins using a *dolichol-linked glycan precursor*;
- *O-glycosylation* occurs in the *Golgi* by the stepwise addition of sugars to Ser/Thr residues without a preformed precursor;
- *Blood group antigens* are antigenic carbohydrate on glycoproteins and glycolipids found mainly in erythrocytes, epithelial cells, and epithelial cell secretions;
- *Glycosaminoglycans* are long, linear polymers of repeating disaccharide units (typically containing uronic acid sugars) linked to protein (*proteoglycans)*;
- *Glycolipid* biosynthesis occurs in the ER/Golgi using ceramide as the lipid moiety
- Defects in biosynthesis of glycoproteins causes *Congenital Disorders of Glycosylation*;
- Defect in synthesis of Mannose-6-phosphate in lysosomal hydrolases is the cause of *I-Cell Disease*;
- Defects in the degradation of GAGs or glycolipids are causes of *lysosomal storage diseases*.