

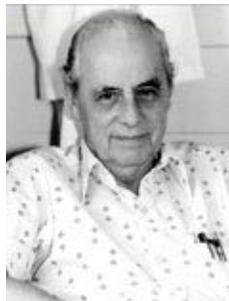
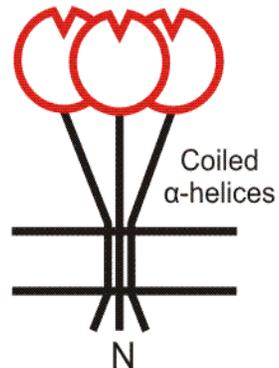
NIH Glycosciences: A rich and lasting heritage



Claude Hudson
The founder of basic carbohydrate research at the NIH (Chief-1952)



Hewitt G. Fletcher
Chief, 1951-1973



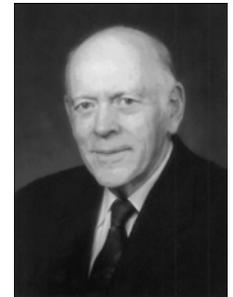
G. Gilbert Ashwell
Discovery of Mammalian Lectins
Chief, LBM, 1978-1983



Elizabeth Neufeld
Chief, GBB 1979-1983



Victor Ginsburg
Chief, Lab Structural Biology
1986-1991



Roscoe Brady
NINDS
1972 to 2006

Glycoscience Interest Group
Undiagnosed Disease Program

Rare Diseases Provide Rare Insights

“People with rare genetic diseases give humanity so much, scientifically and spiritually, that we owe them a huge debt of gratitude. In fact, they make us more human”

Dr. William Gahl





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Kids who don't cry: New genetic disorder discovered

By **Jacque Wilson, CNN**

🕒 Updated 2:53 PM ET, Thu March 20, 2014



Grace Wilsey was born with NGLY1 deficiency, which is caused by two mutations in the NGLY1 gene.

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MEDICAL DISPATCH | JULY 21, 2014 ISSUE

ONE OF A KIND

What do you do if your child has a condition that is new to science?

BY SETH MNOOKIN



MEDICAL DISPATCH ONE OF A KIND

What do you do if your child has a condition that is new to science?

BY SETH MNOOKIN

Met Myles and Cristina Cristina was the only female geneticist in the country. That afternoon, when Myles was able to check his phone, he saw that Cristina had left several messages. To kick him up, he told her he was on his way. "I didn't know he'd be here," she said. "I didn't know he'd be here."



Unusually, Myles and Myles was the only female geneticist in the country. That afternoon, when Myles was able to check his phone, he saw that Cristina had left several messages. To kick him up, he told her he was on his way. "I didn't know he'd be here," she said. "I didn't know he'd be here."

They began dating. Within a year, they were married. The couple had their first child, Myles, on December 9, 2007, not long after Myles started his Ph.D. in computer science at the University of California, San Diego. They named him Myles because of the British children's author and mathematician Myles Borel. After a few hours of work, the new parents began to

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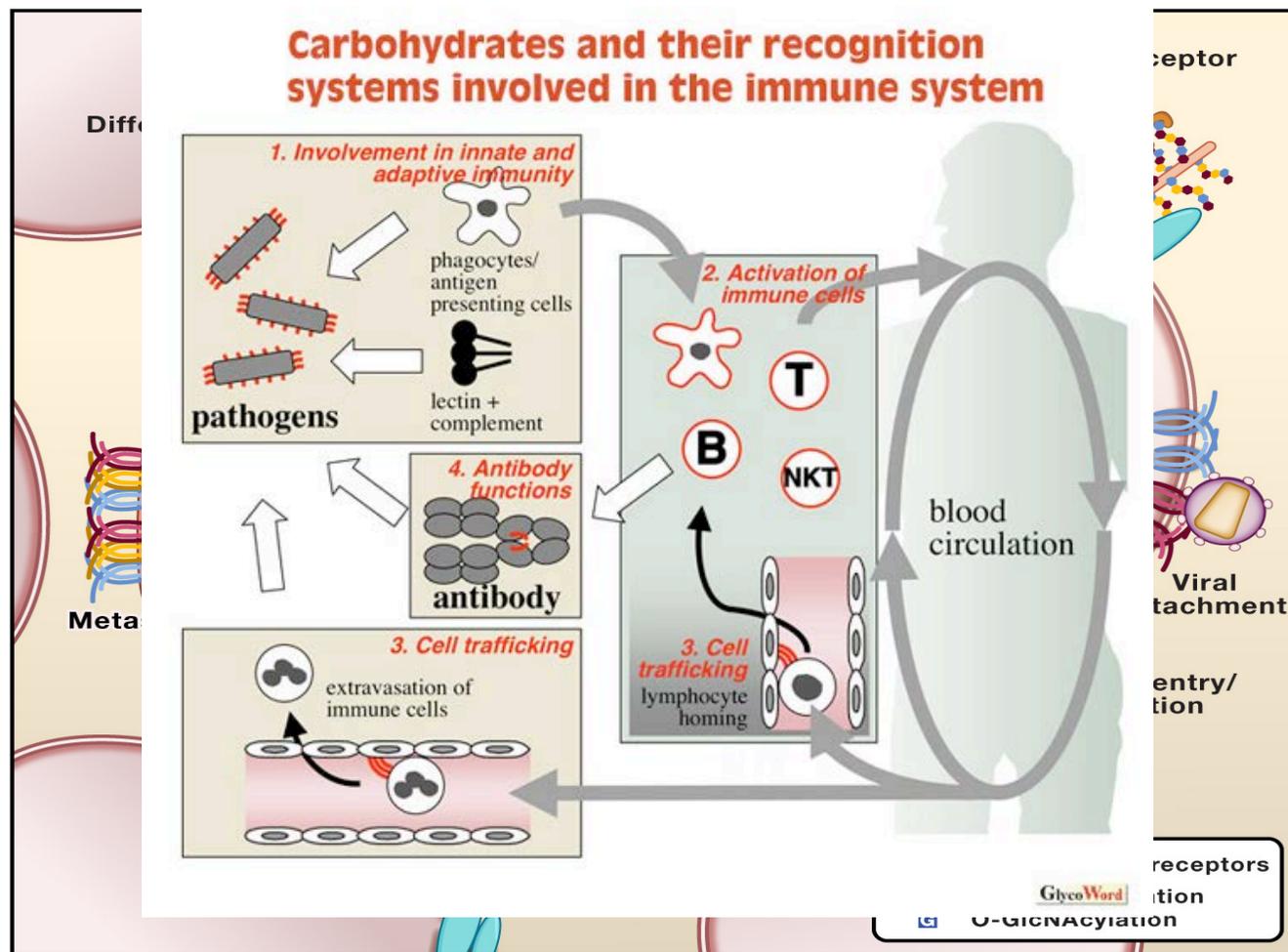


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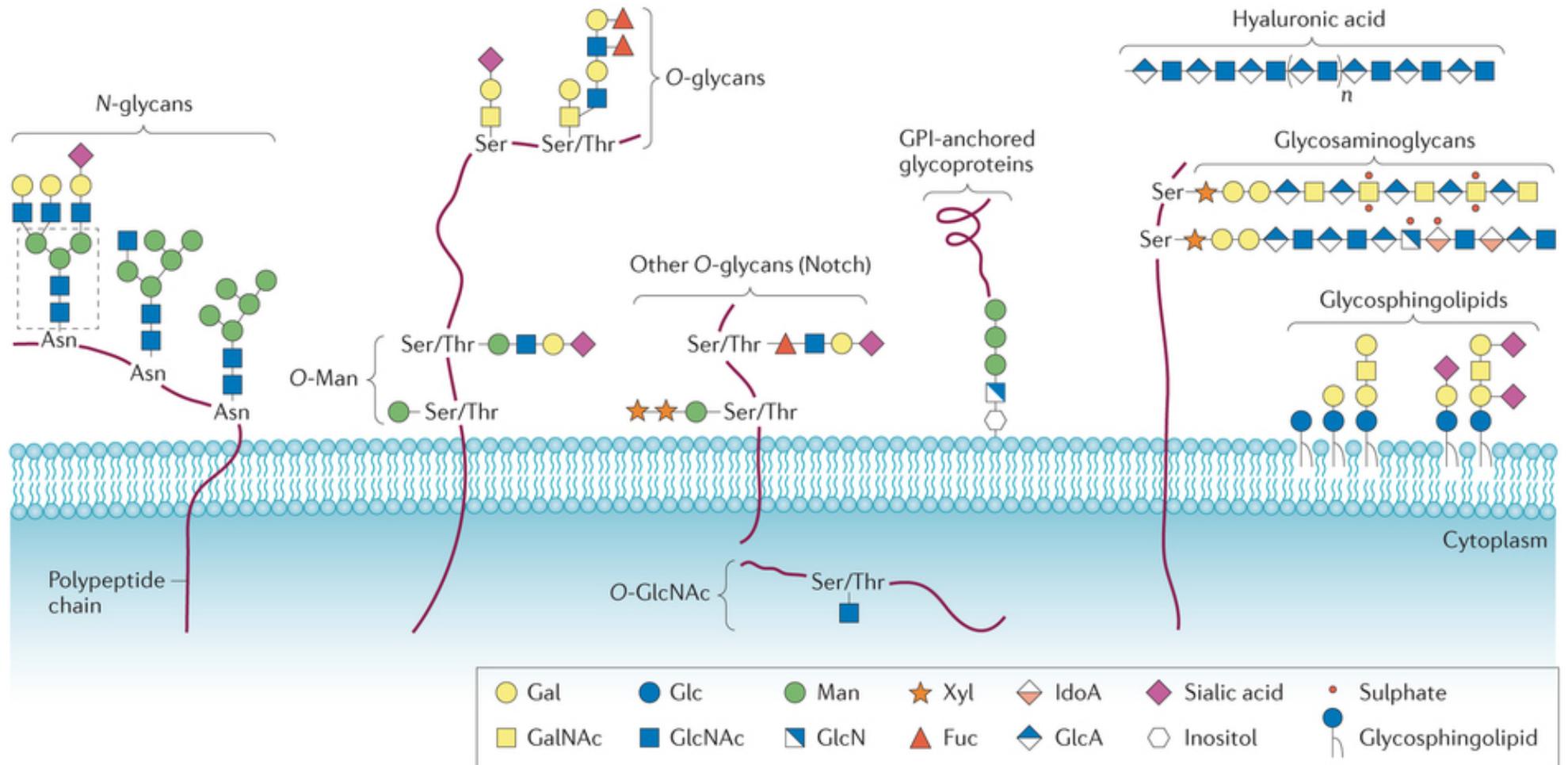


Glycoprotein Diseases: Glycoproteins, Allergy, and Other Diseases

- Glycoproteins in Physiology and Disease
- Glycoprotein Biogenesis and CDGs
- Nucleotide Sugars
- PGM3 Deficiency

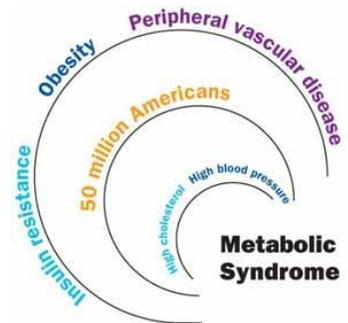
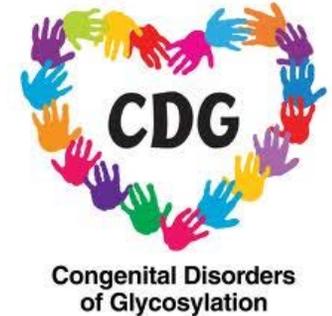


Mammalian Glycoconjugates



Glycans play a major role in human disease:

- Rarity/Severity (~1/20,000) of genetic diseases highlight importance of glycans
- Some Examples of Glycans and Disease:
 - Defective O-glycosylation in Muscular Dystrophy
 - O-GlcNAcylation: Diabetes, Alzheimer's, Cancer, Heart Disease.
 - Notch Signaling by Glycans
 - Selectins and Inflammation
 - Siglecs and Regulation of Immunity
 - Galectins role in immunity
 - Proteoglycans: growth factors, microbe binding, morphogenesis
 - Microbes and Viruses: Glycans role in entry and defense
 - Heparin – this 'drug' is a GAG.
 - Monoclonal Therapeutics – Glycoforms
 - Cell Surface Glycans in Tumor Metastasis – Cancer Biomarkers.
 - Vaccines to Infectious Organisms – Many (Most) are glycans.

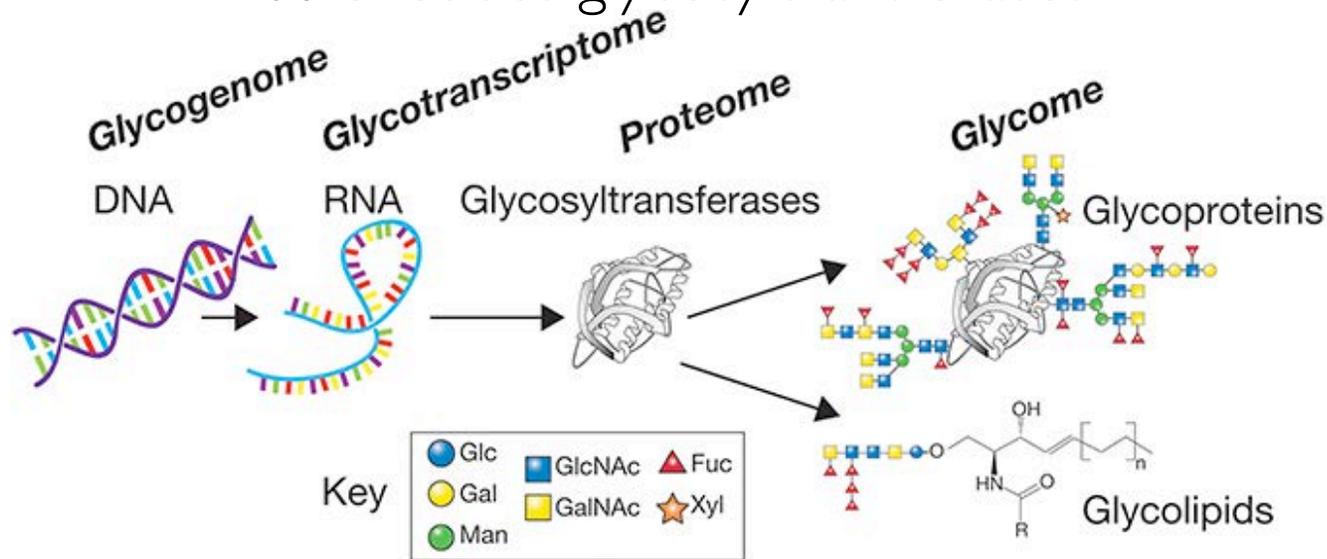


The Glycogenome represents a substantial target

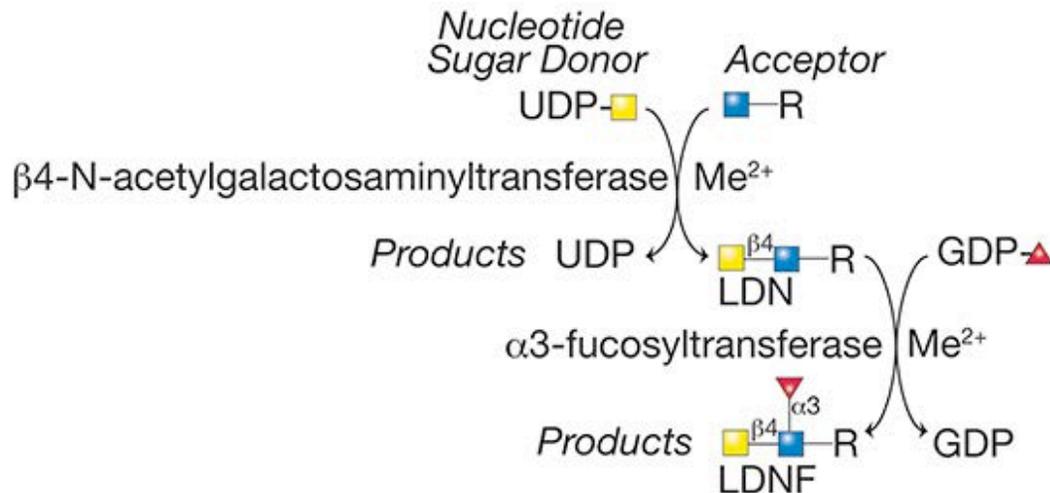
Human Genome:

~5 % encodes carbohydrate active enzymes

~2 % encodes glycosyltransferases

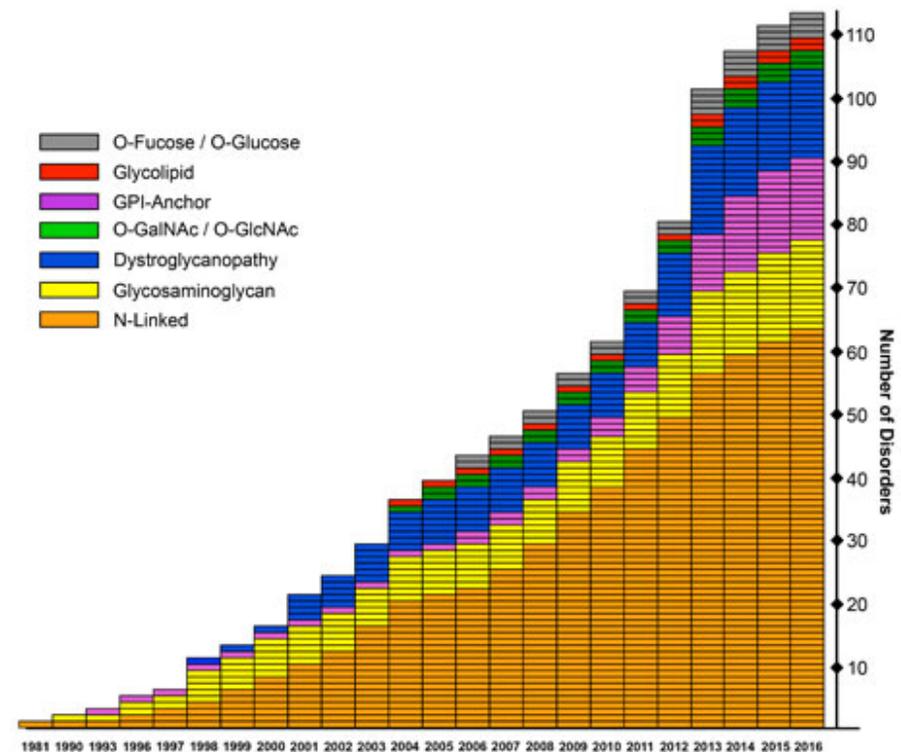
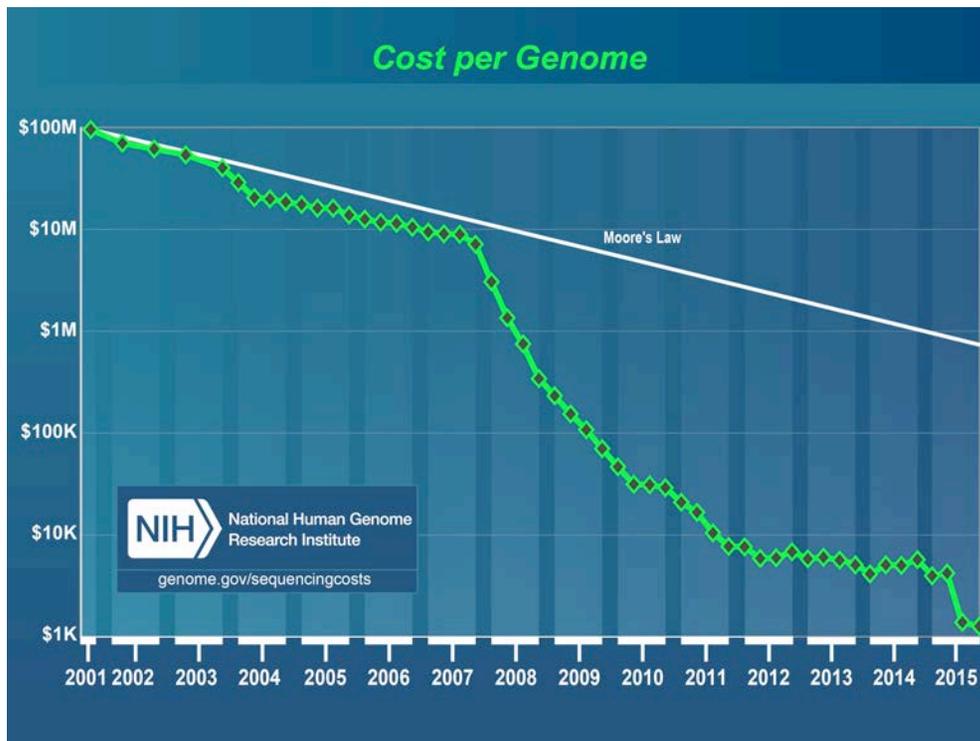


Examples of Glycosyltransferase Reactions



Exome and Genome Sequencing has accelerated CDG identification

- Genome Project fueled growth in CAZy database (Microbiome)
- Exome sequencing costs have plummeted
- CDGs are rare (~1/20,000)
- Estimates suggests that ~20 % of the population have a CDG allele



Clinical Features of CDG

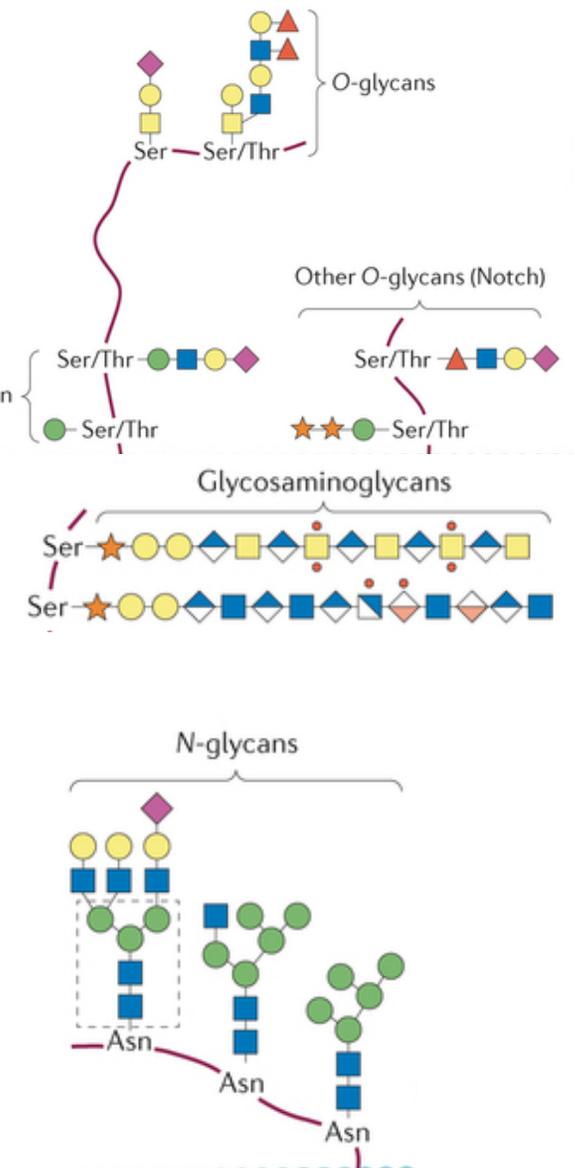
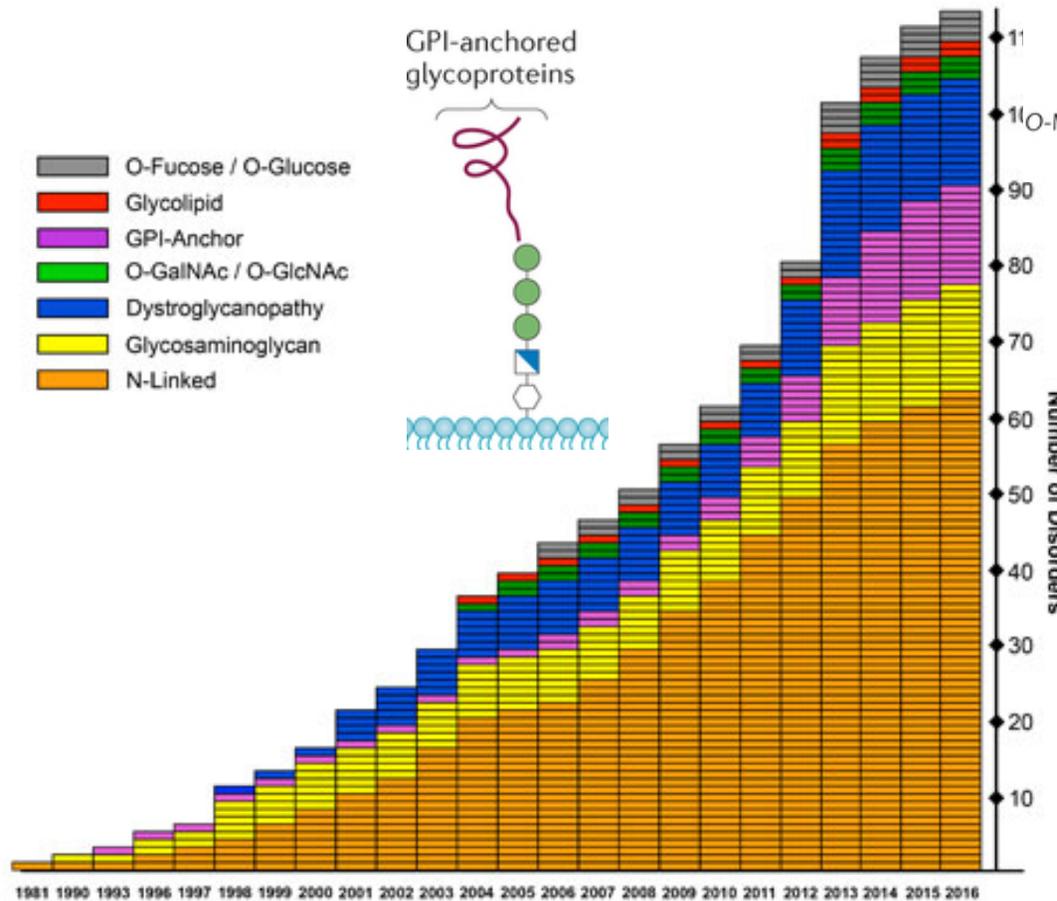
Table 2 Clinical features seen in different types of CDG

Type	Features
Ia	Hypotonia, variable psychomotor retardation, seizures, peripheral neuropathy, stroke-like episodes, strabismus, cardiomyopathy
Ib	Normal development, hypoglycemia, coagulopathy, protein-losing enteropathy, hepatic fibrosis, cyclic vomiting
Ic	Hypotonia, psychomotor retardation, seizures, strabismus, feeding problem, coagulopathy
Id	Hypotonia, severe psychomotor retardation, seizures, microcephaly, optic atrophy
Ie	Hypotonia, severe psychomotor retardation, seizures, delayed myelination, Blindness
If	Hypotonia, severe psychomotor retardation, seizures, blindness, dry skin, low food intake, vomiting
Ig	Hypotonia, severe psychomotor retardation, seizures, feeding difficulties, facial dysmorphism, coagulopathy
Ila	Hypotonia, severe psychomotor retardation, frequent infections, widely spaced nipples
Ilb	Hypotonia, generalized edema, hypoventilation, apnea, hepatomegaly, demyelinating polyneuropathy
Ilc	Hypotonia, psychomotor retardation, elevated peripheral leukocytes, failure to thrive, short arms and legs
Ild	Hypotonia, hydrocephalus, myopathy, coagulation abnormalities

Discovery of Congenital Disorders of Glycosylation

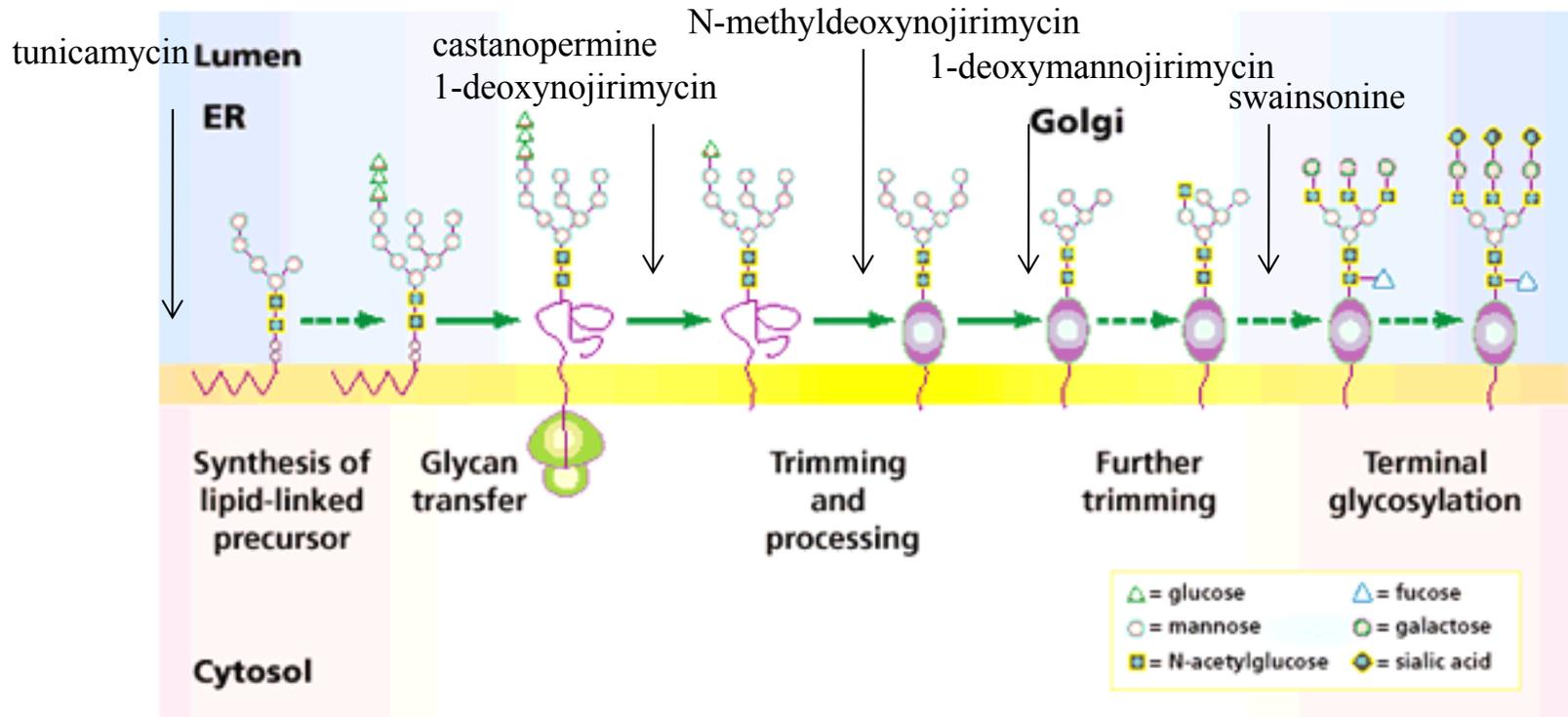
Table 1. Biochemical Markers for Various Glycosylation Pathways

Disorder Type	Biomarker(s)	Sample Type
N-glycan	transferrin ^{39,40}	serum, plasma
GPI anchor	CD59, CD55, CD16b, ALP, and a GPI-binding toxin, aerolysin (FLAER) ^{41,42}	granulocytes, platelets, fibroblasts
α -dystroglycanopathies	α -DG antibody (IIH6) ⁴³	muscle biopsy, fibroblasts



N-Glycan Biosynthetic Pathway: A System to Generate Diversity.

What do we know?

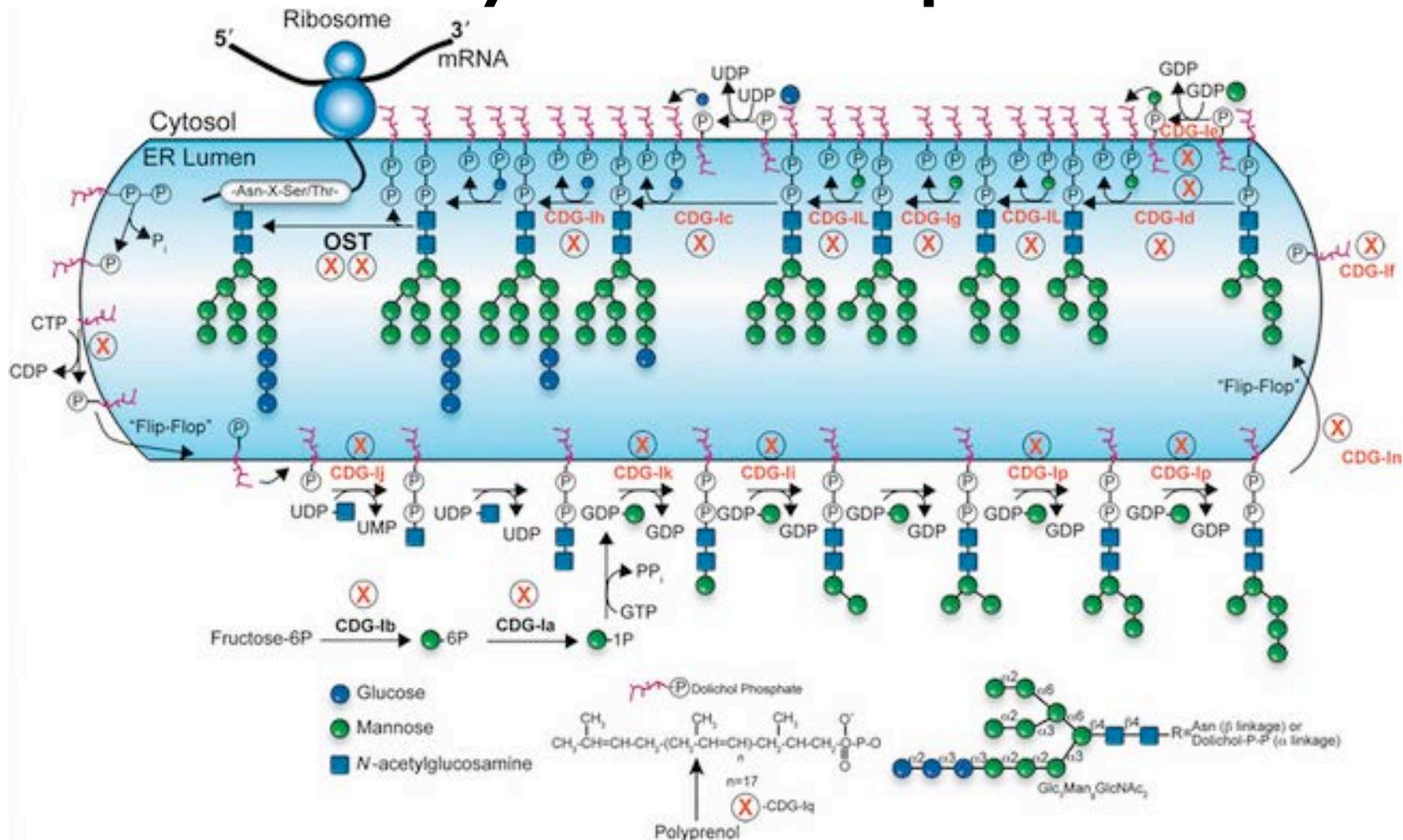


How did we learn it?

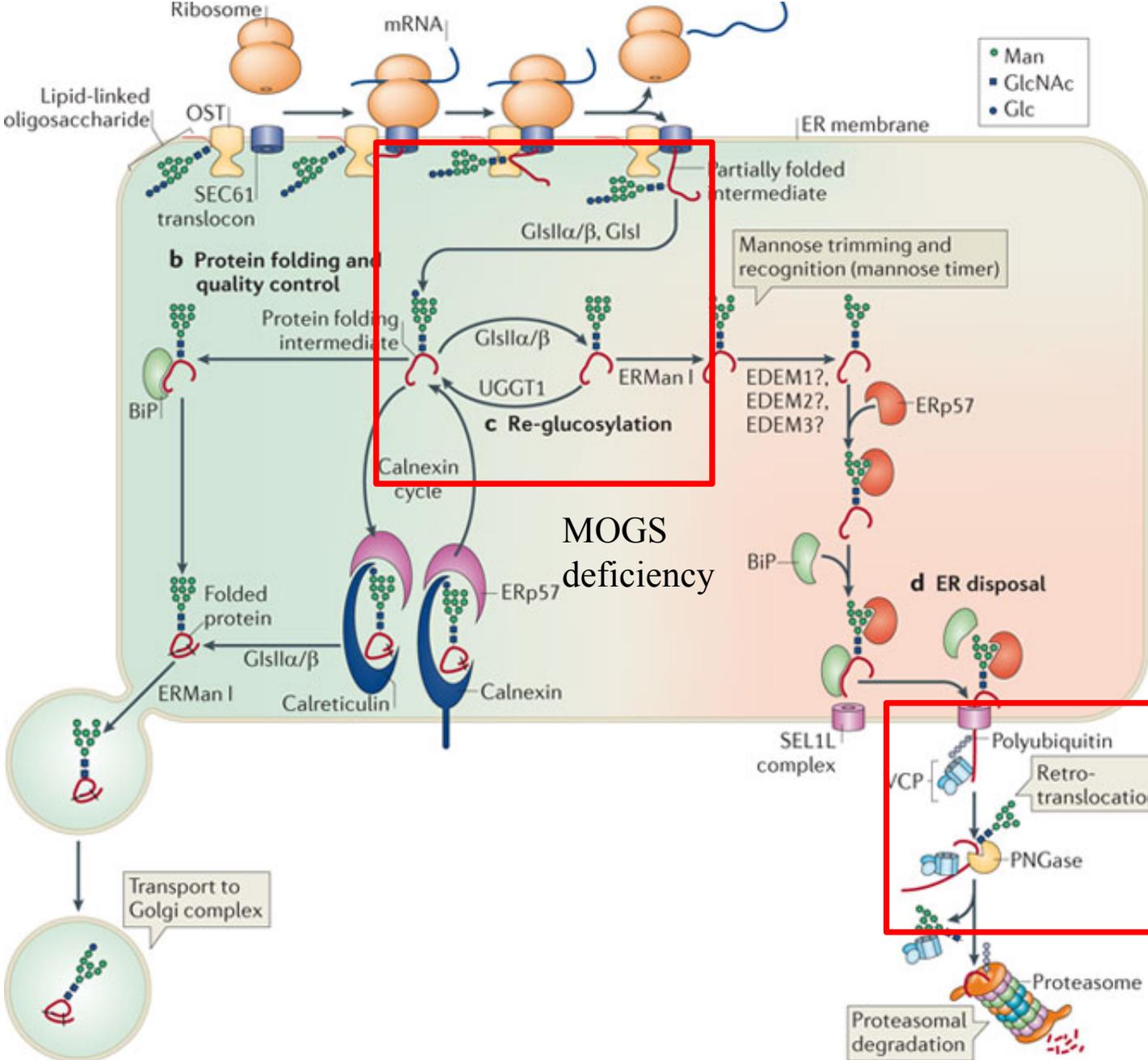
1. Biochemistry
2. Inhibitors
3. Yeast and Somatic Cell genetics
4. Congenital Disorders of Glycosylation

Congenital Disorders of Glycosylation: N-linked

- **Most common CDG**
- **Multiple steps**
- **Assembly of a common precursor**



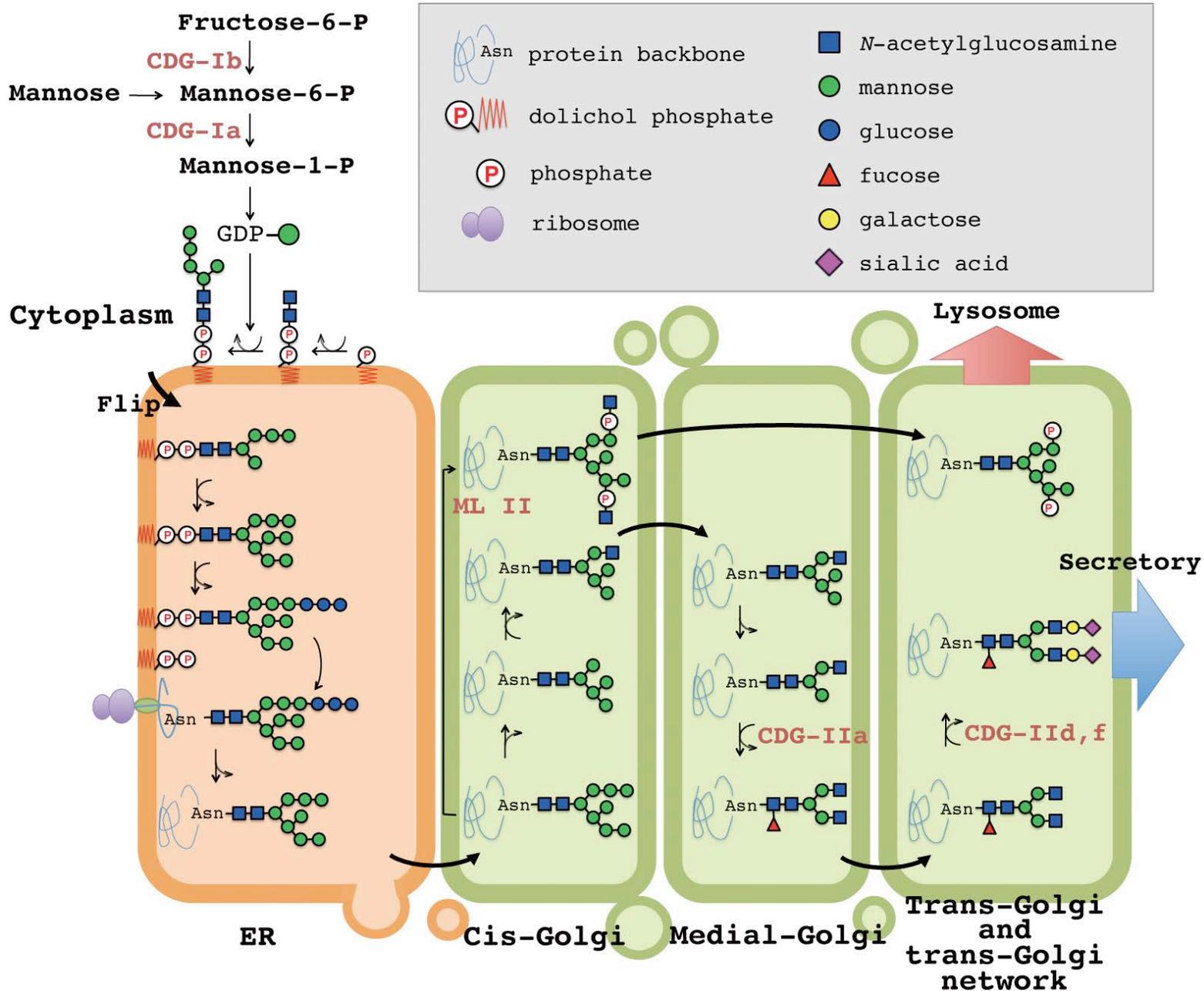
N-Glycan Biosynthetic Pathway and ER Quality Control



MOGS deficiency

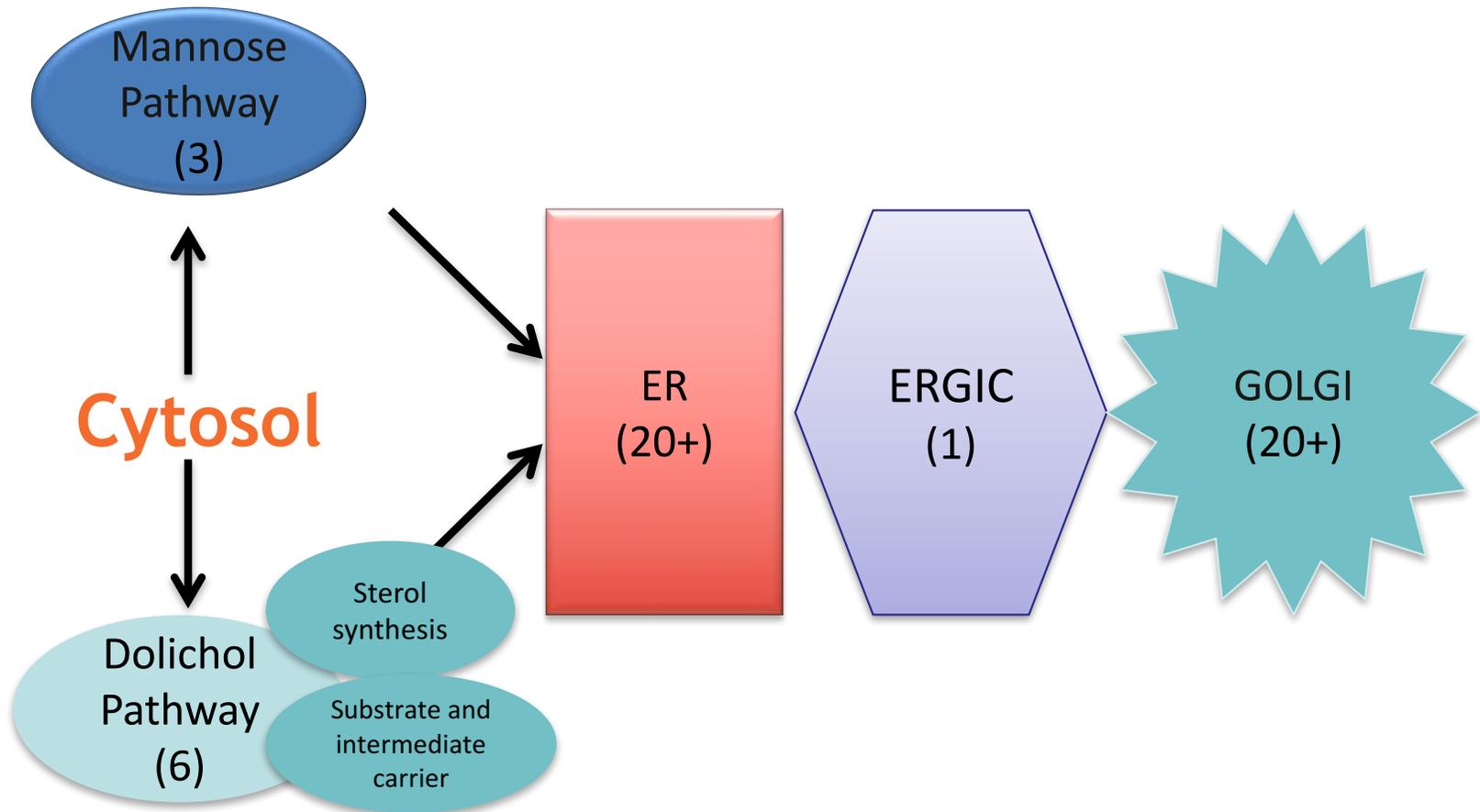
NGLY1 deficiency

Glycoprotein Biogenesis and the Congenital Disorders of Glycosylation



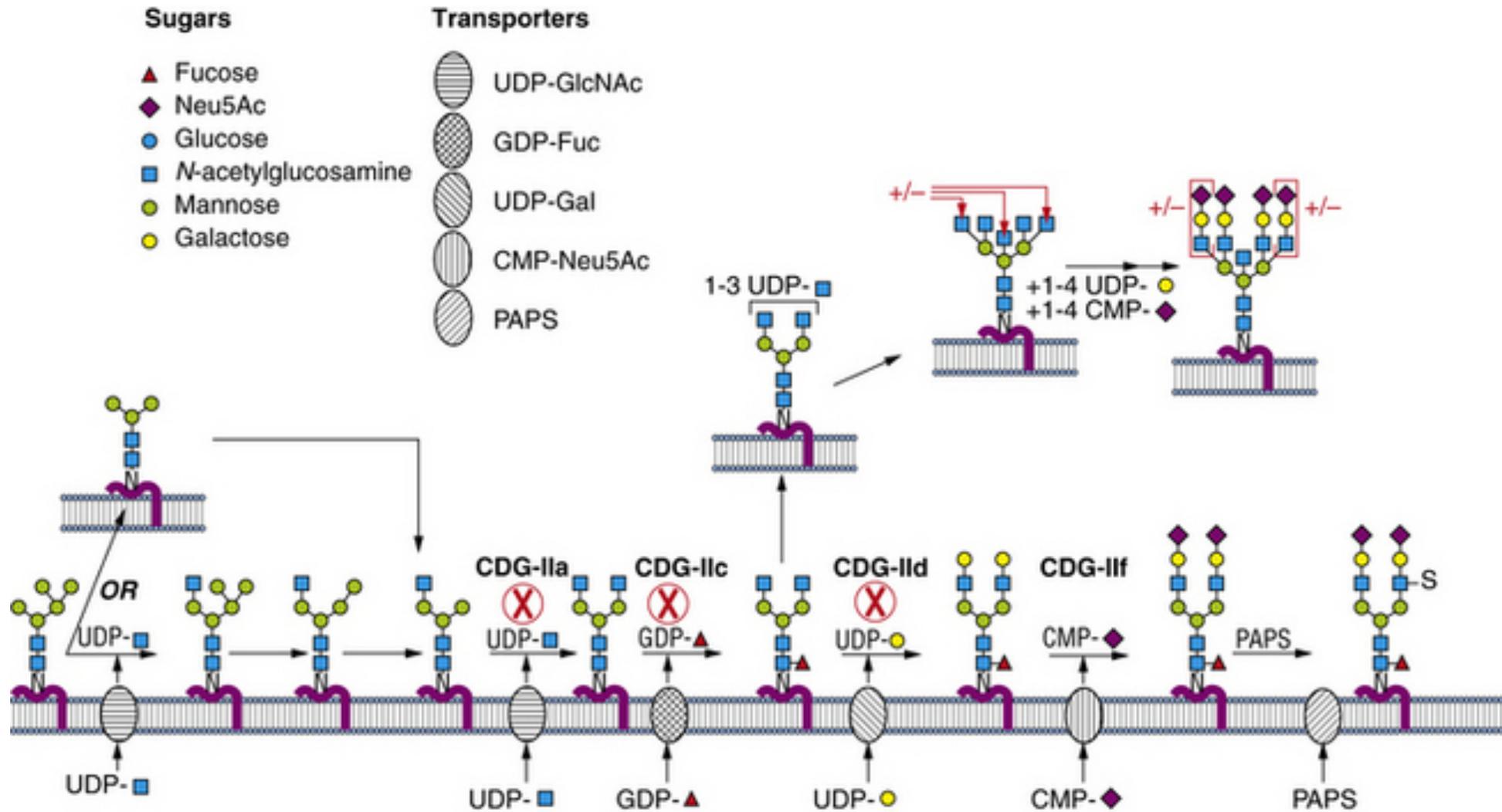
CDG Disorders by Compartment

- Roughly corresponds to CDG allele frequency
- Probably a gross underestimate of disease burden



Adapted from J Inherit Metab Dis (2011) 34:853-858
Thanks to Lynne Wolfe NP and Donna Krasnewich, MD, NGMS

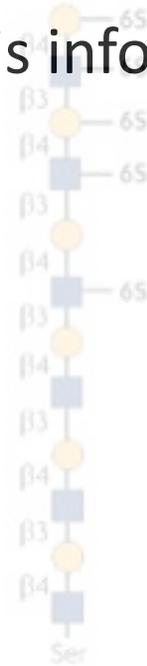
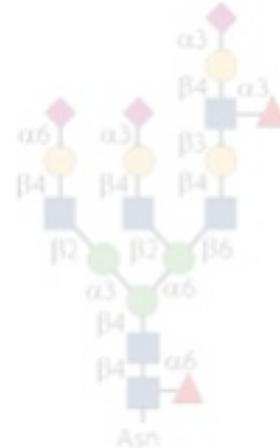
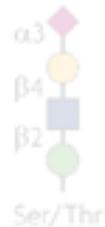
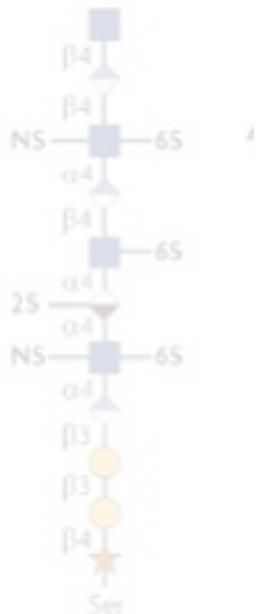
The Nucleotide sugar precursors



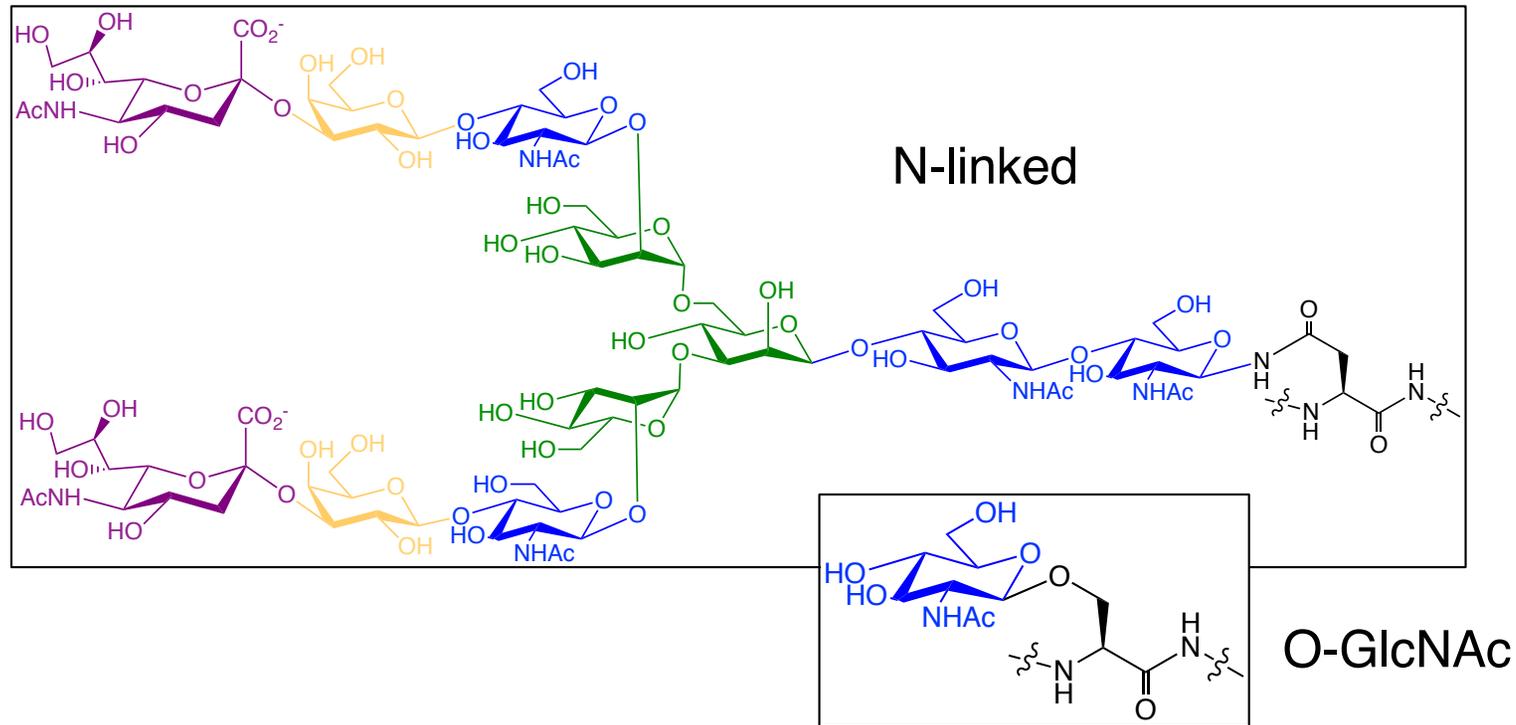
Glycan diversity requires multiple sugar donors and enzymes



- Glycans can be complex structures and dynamically altered
- Focusing on chemical details of biological sugars is informative for defining details of disease

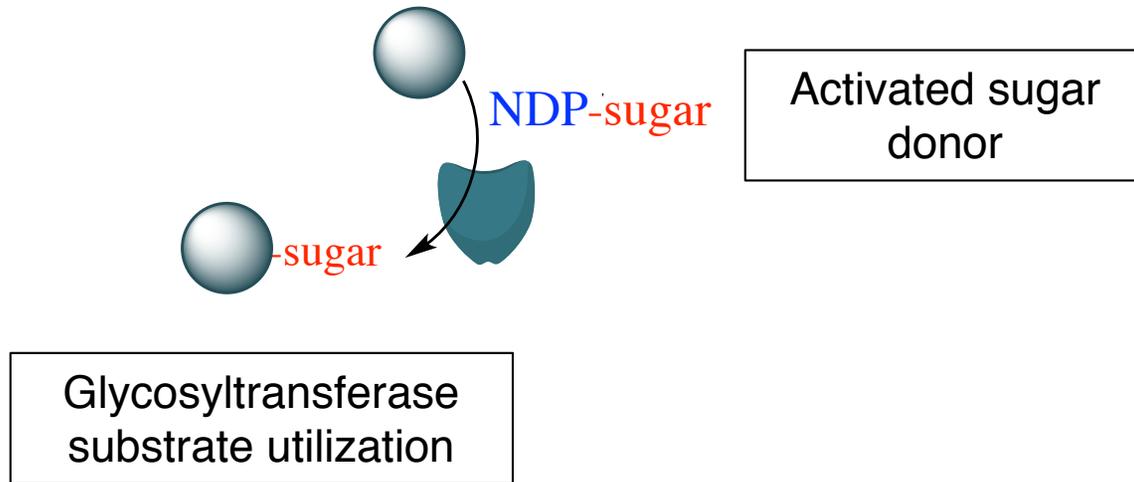


Multiple components are required for glycan synthesis

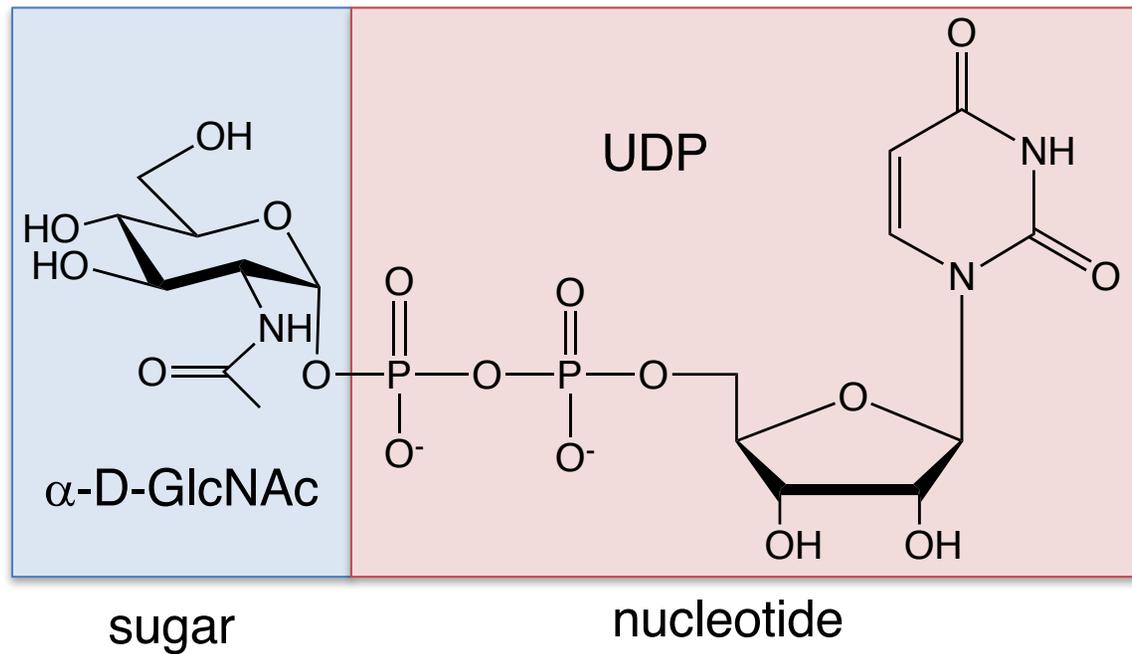


- ☑ Glycosyltransferases
- ☑ Glycoconjugate acceptors
- ☑ Activated sugar donors
- ☑ Nucleotide sugar transporters
- ☑ Glycan remodeling enzymes (e.g., glycosidases)

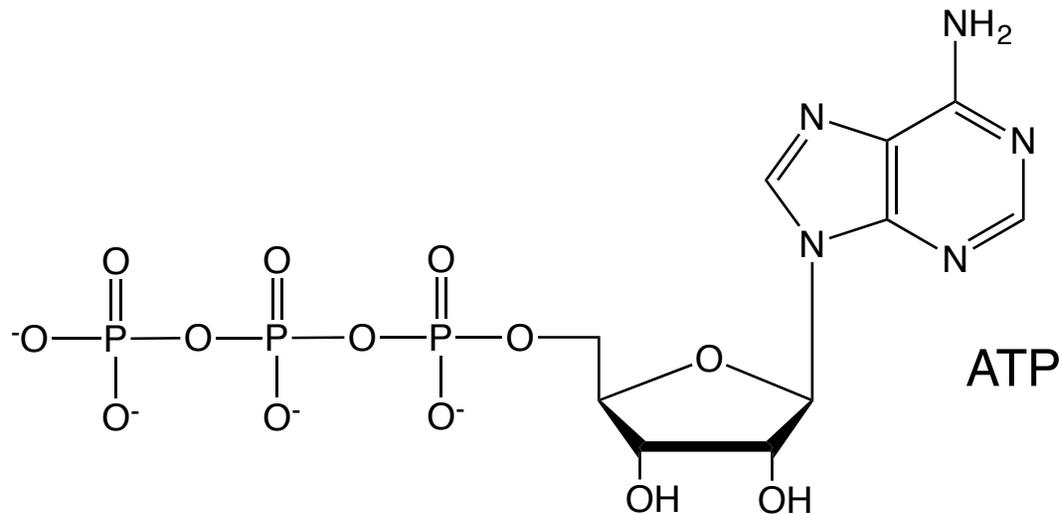
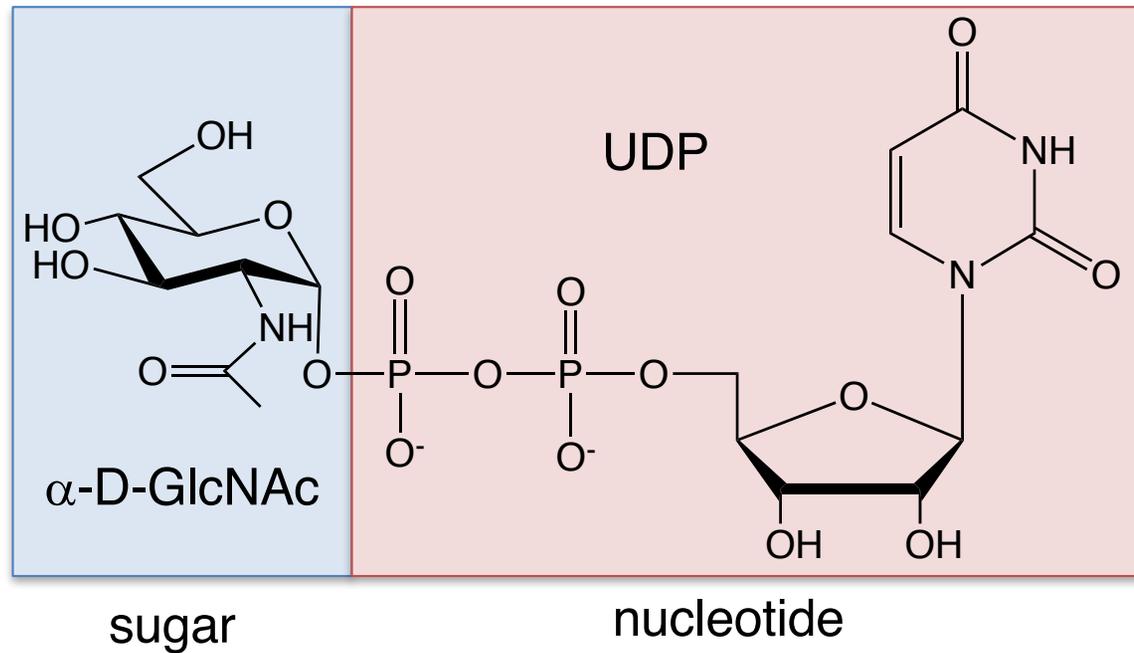
NDP-sugars are utilized in multiple cellular locations



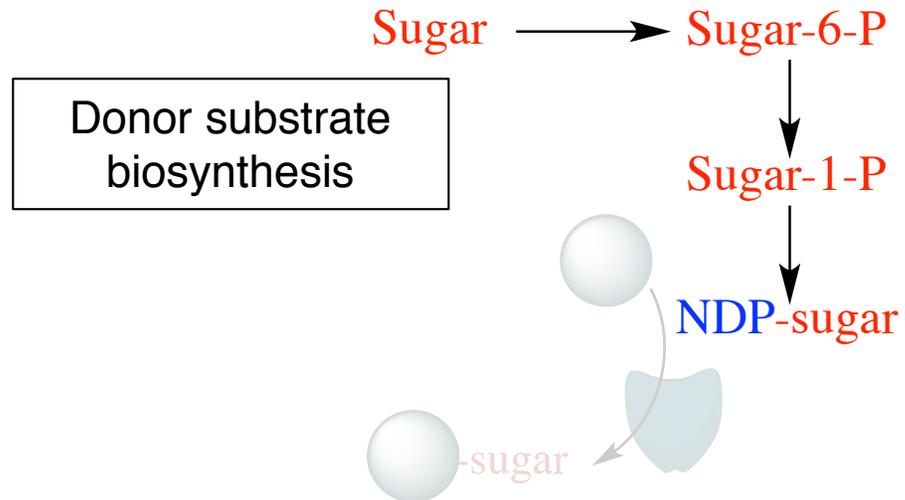
Glycan synthesis requires high-energy, activated donors



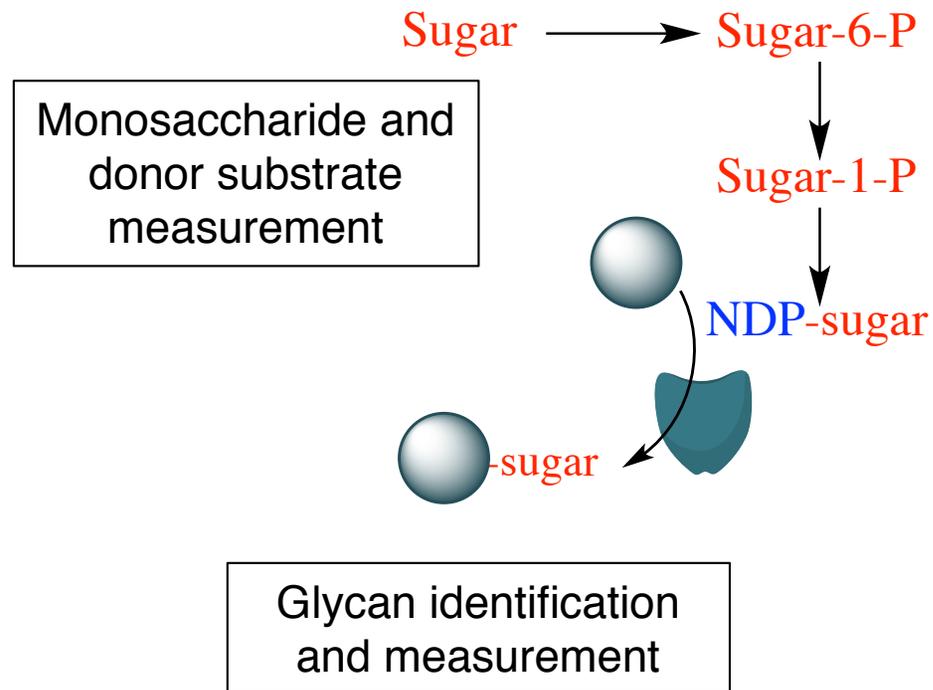
Glycan synthesis requires high-energy, activated donors



NDP-sugars are utilized in multiple cellular locations

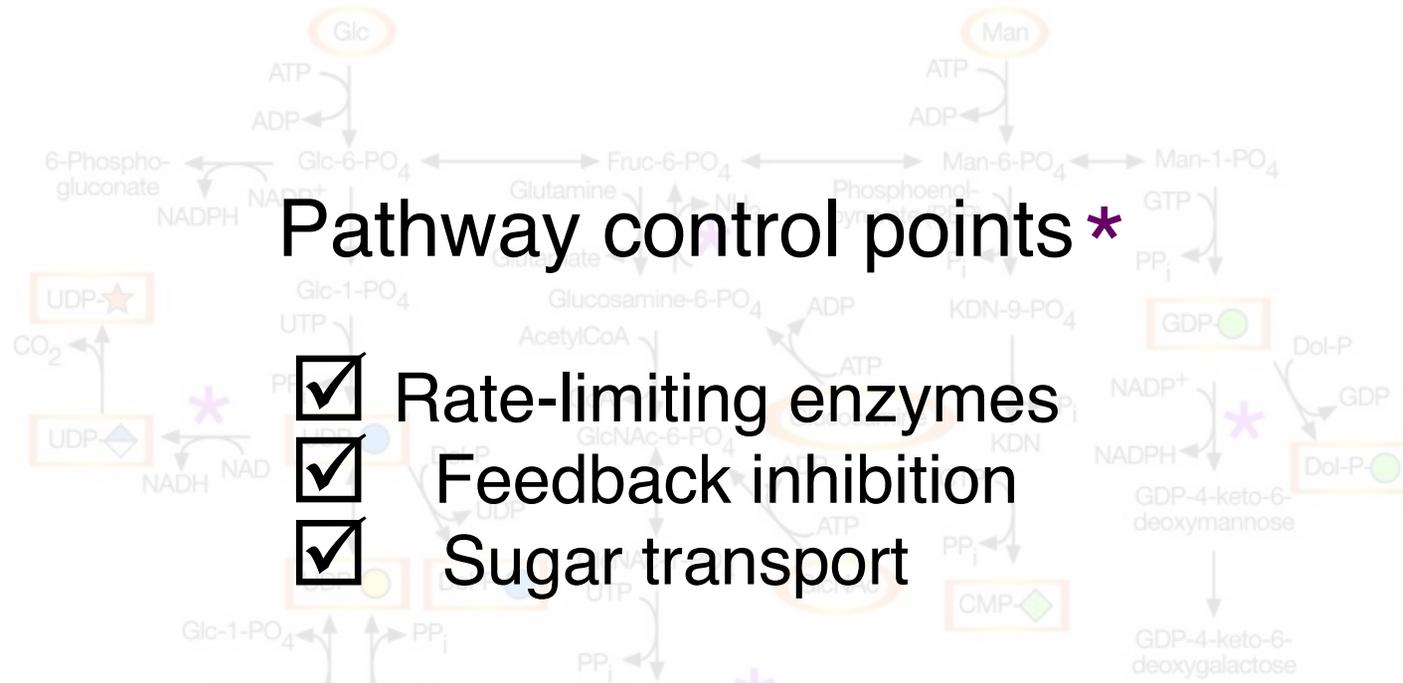


Cellular sugar measurement requires multiple methods



- Mass spectrometry
- NMR
- High Performance Anion Exchange Chromatography
- Lectins (blotting and flow cytometry)

NDP-sugar synthesis integrates multiple metabolites

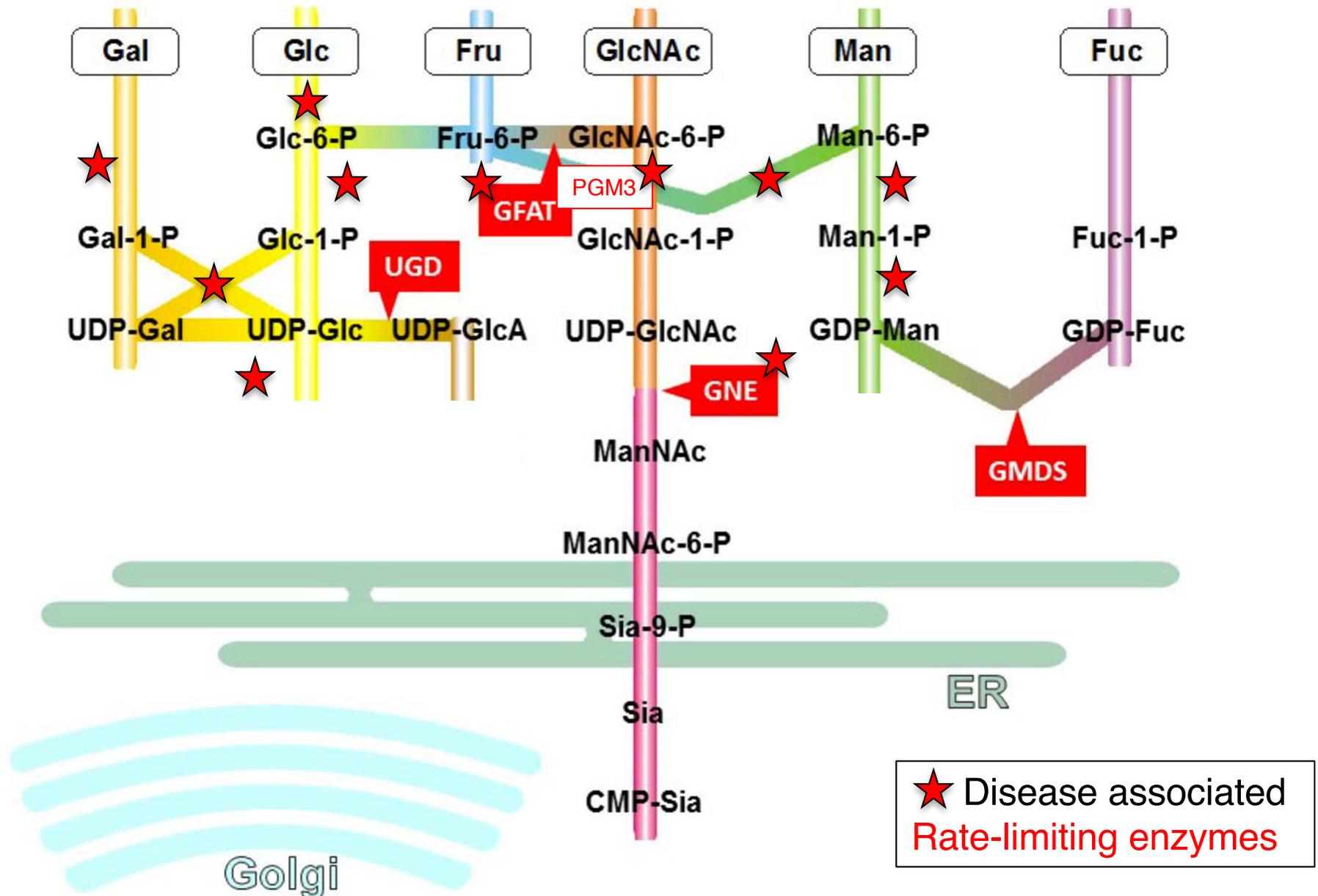


Pathway control points *

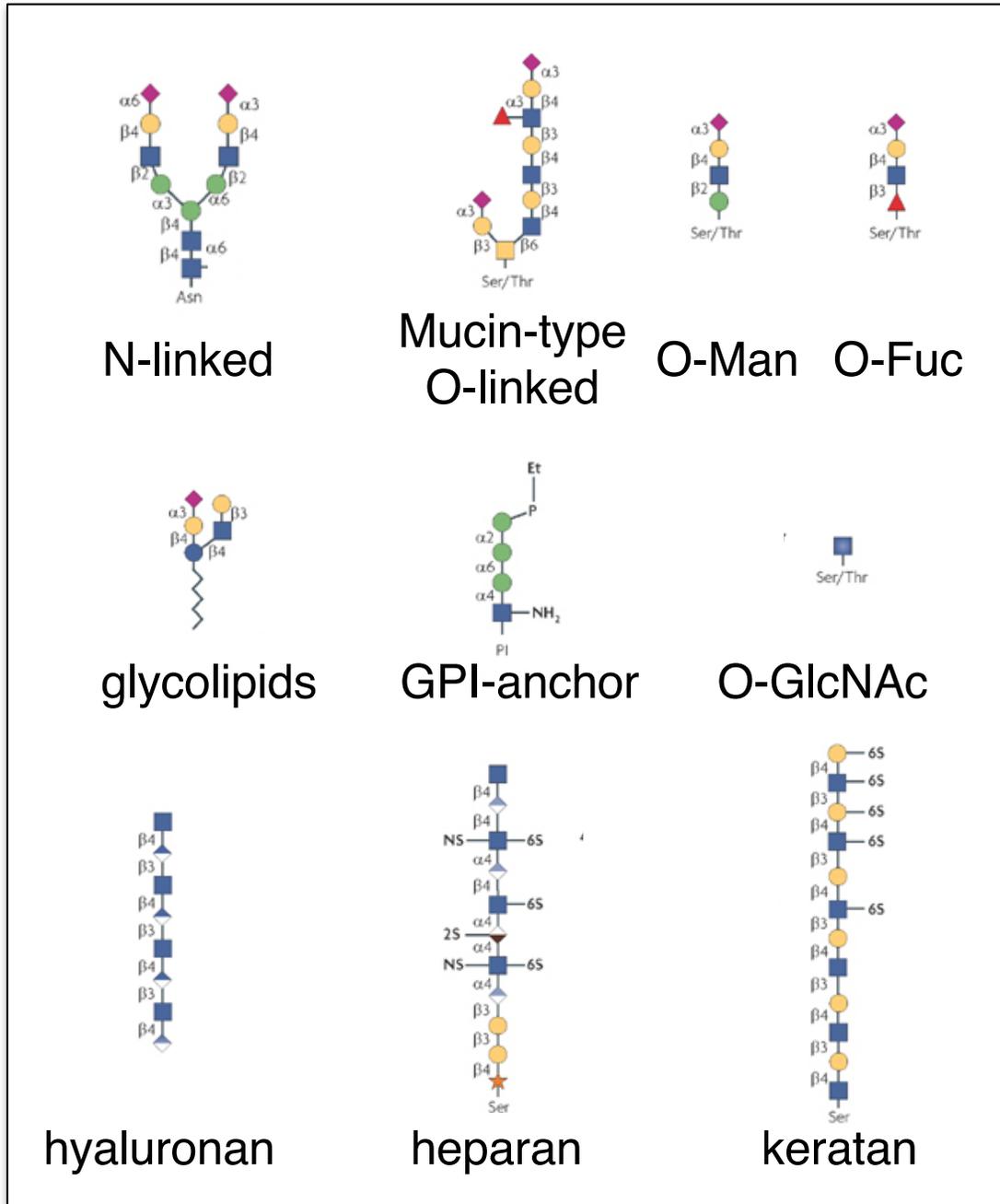
- Rate-limiting enzymes
- Feedback inhibition
- Sugar transport

Enzyme	Inhibitor
UDP-Glc dehydrogenase	UDP-Xyl
GDP-Man 4,6-dehydratase	GDP-Fuc
Glutamine:fructose-6-P amidotransferase	UDP-GlcNAc
UDP-GlcNAc epimerase/kinase	CMP-Sia

Mutations in enzymes required for NDP-sugar synthesis are associated with disease

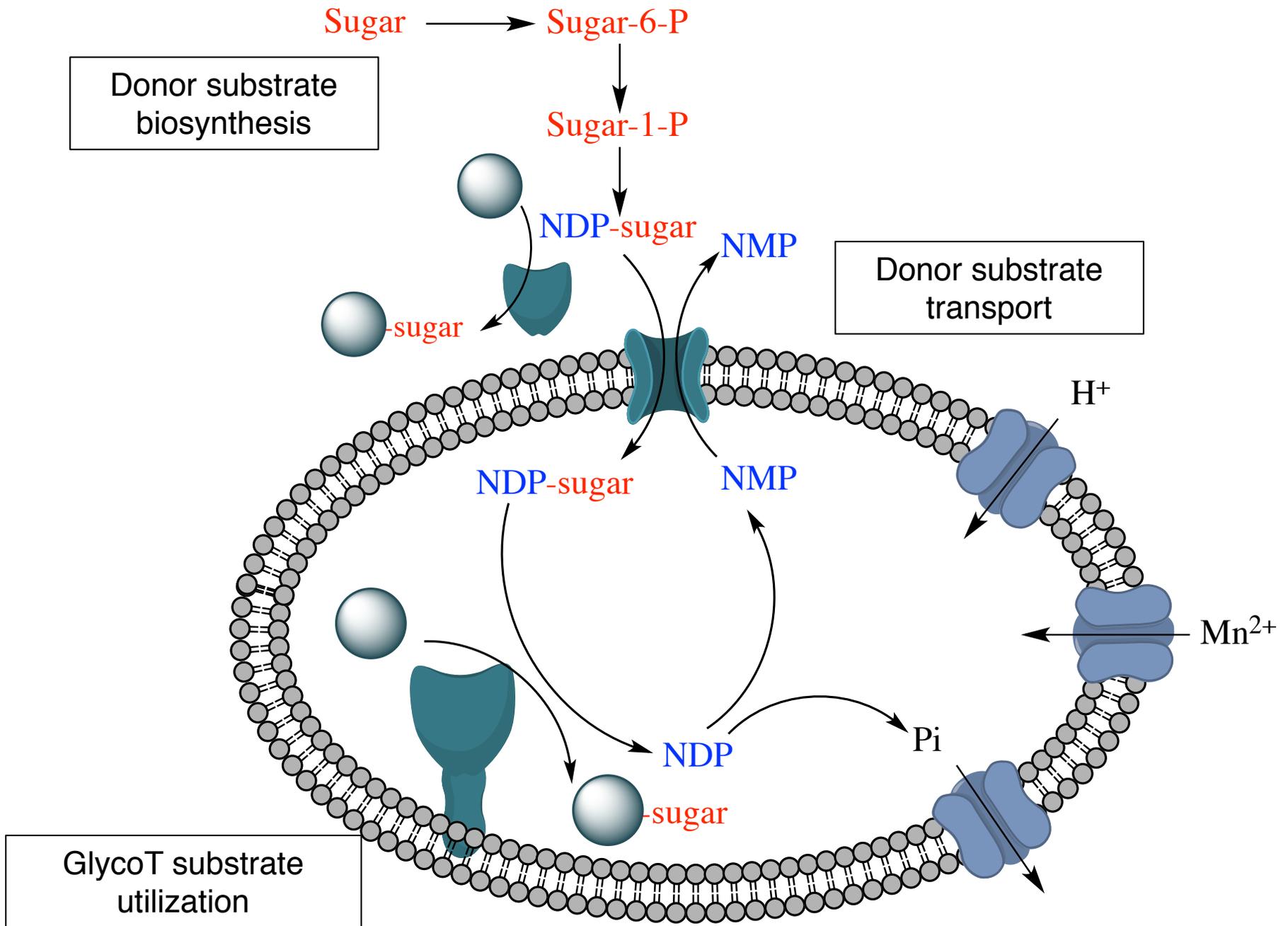


Diversity requires many sugar donors and enzymes

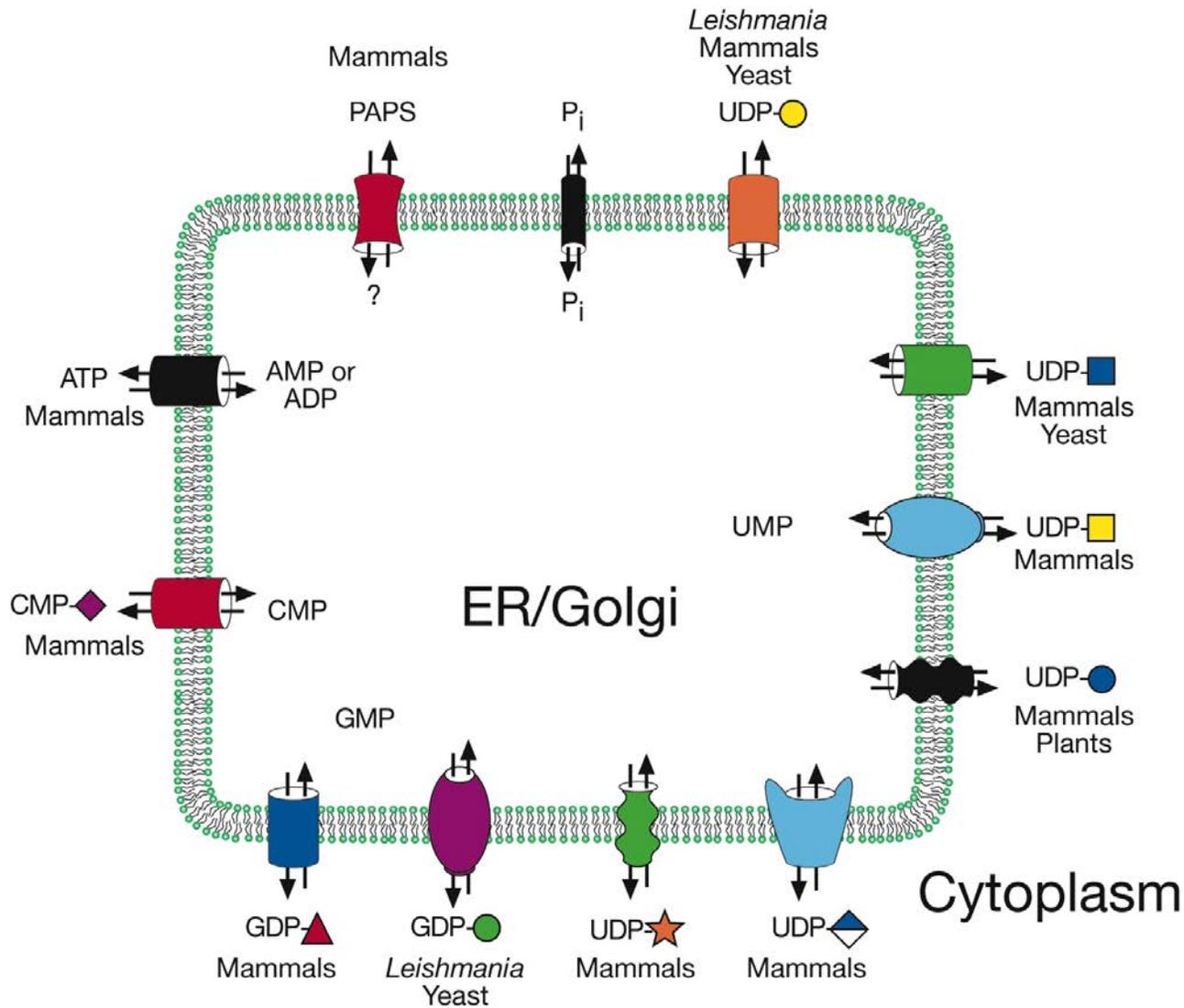


Sugar	Activated form
Glc	UDP-sugar
Gal	
GlcNAc	
GalNAc	
GlcA	
Xyl	
Man	GDP-sugar
Fuc	
Sia	CMP-Sia

NDP-sugars are utilized in multiple cellular locations

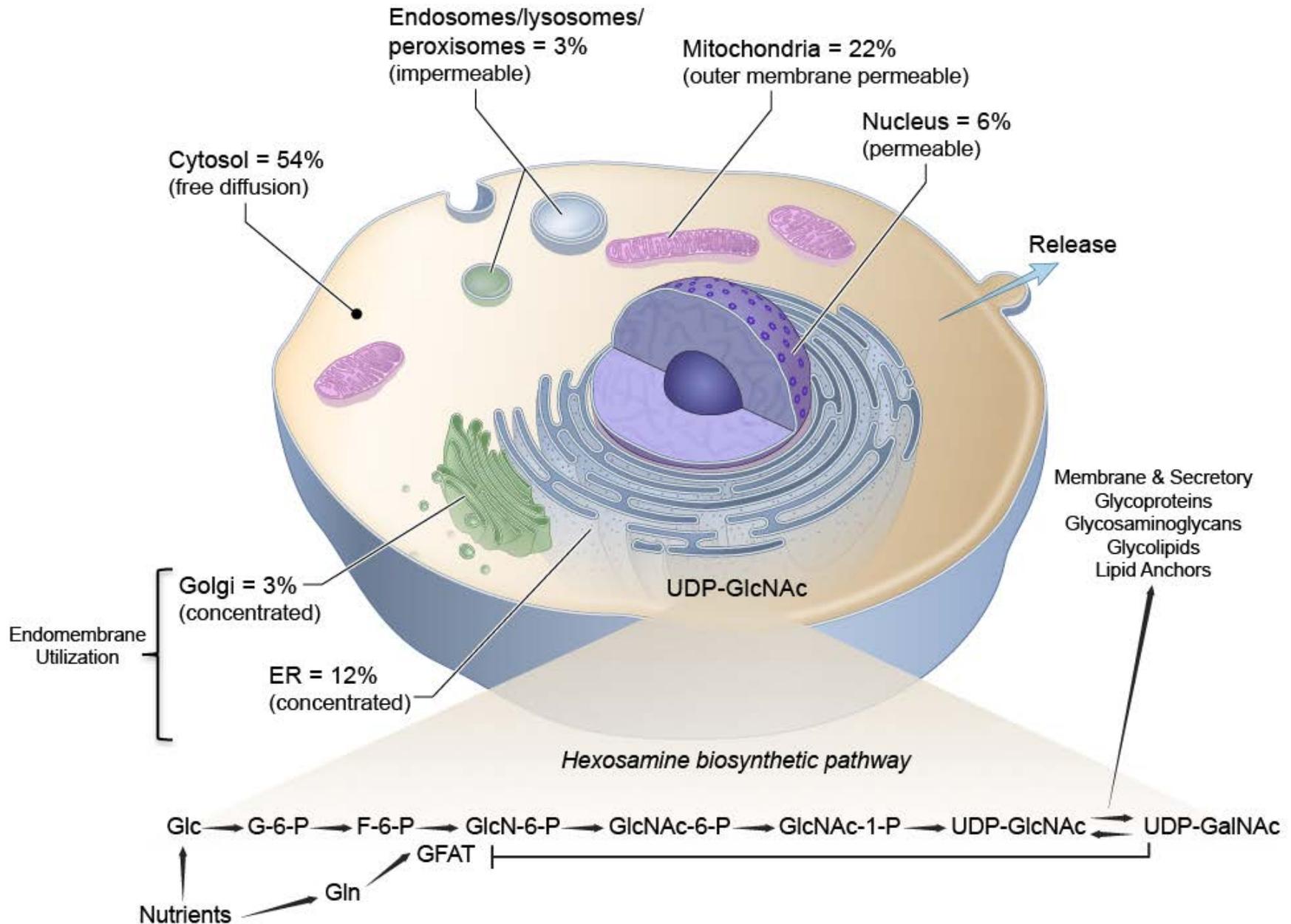


NDP-sugar transport requires active process

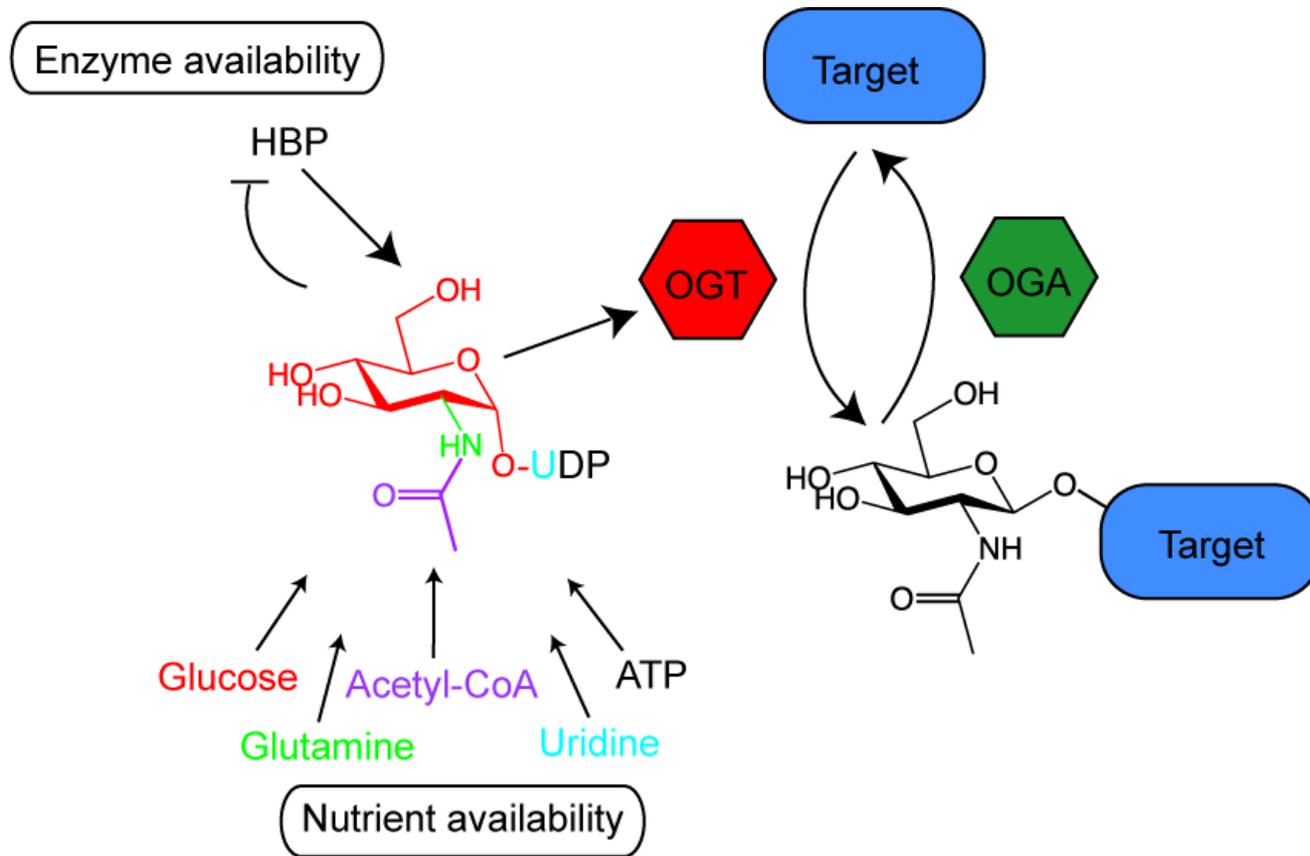


Nucleotide	ER	Golgi
CMP-Sia	-	+++
GDP-Fuc	+	++++
UDP-Gal	-	++++
PAPS	-	++++
GDP-Man	-	++++
UDP-GlcNAc	++	++++
UDP-GalNAc	++	++++
UDP-Xyl	++	++++
ATP	+++	++++
UDP-GlcA	++++	++++
UDP-Glc	++++	+

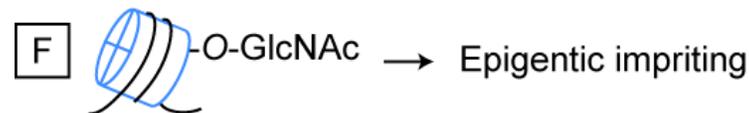
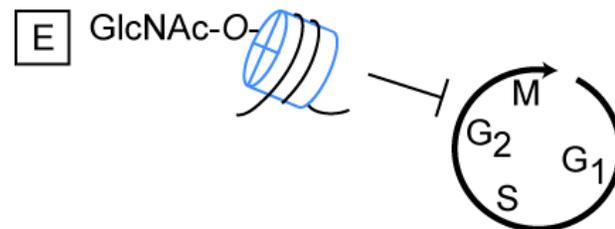
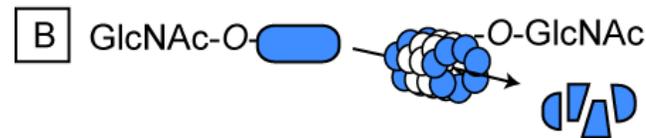
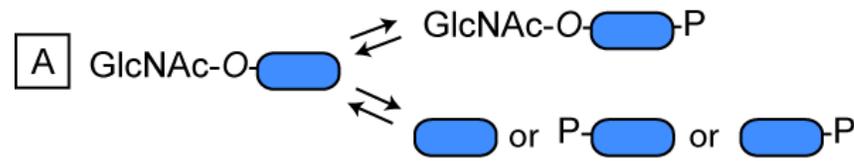
UDP-GlcNAc resides at the nexus of protein and lipid glycosylation



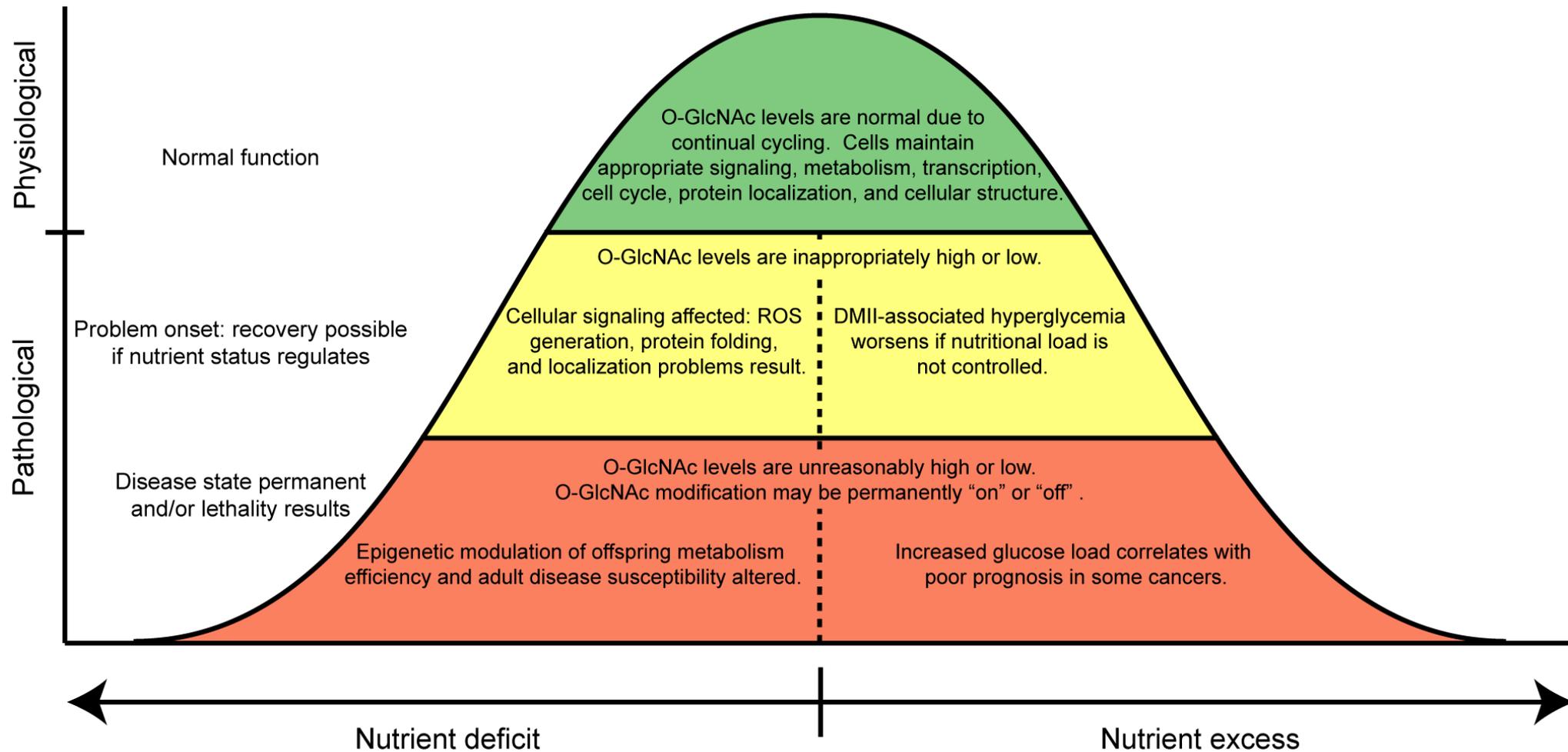
O-GlcNAc is a dynamic post translational modification



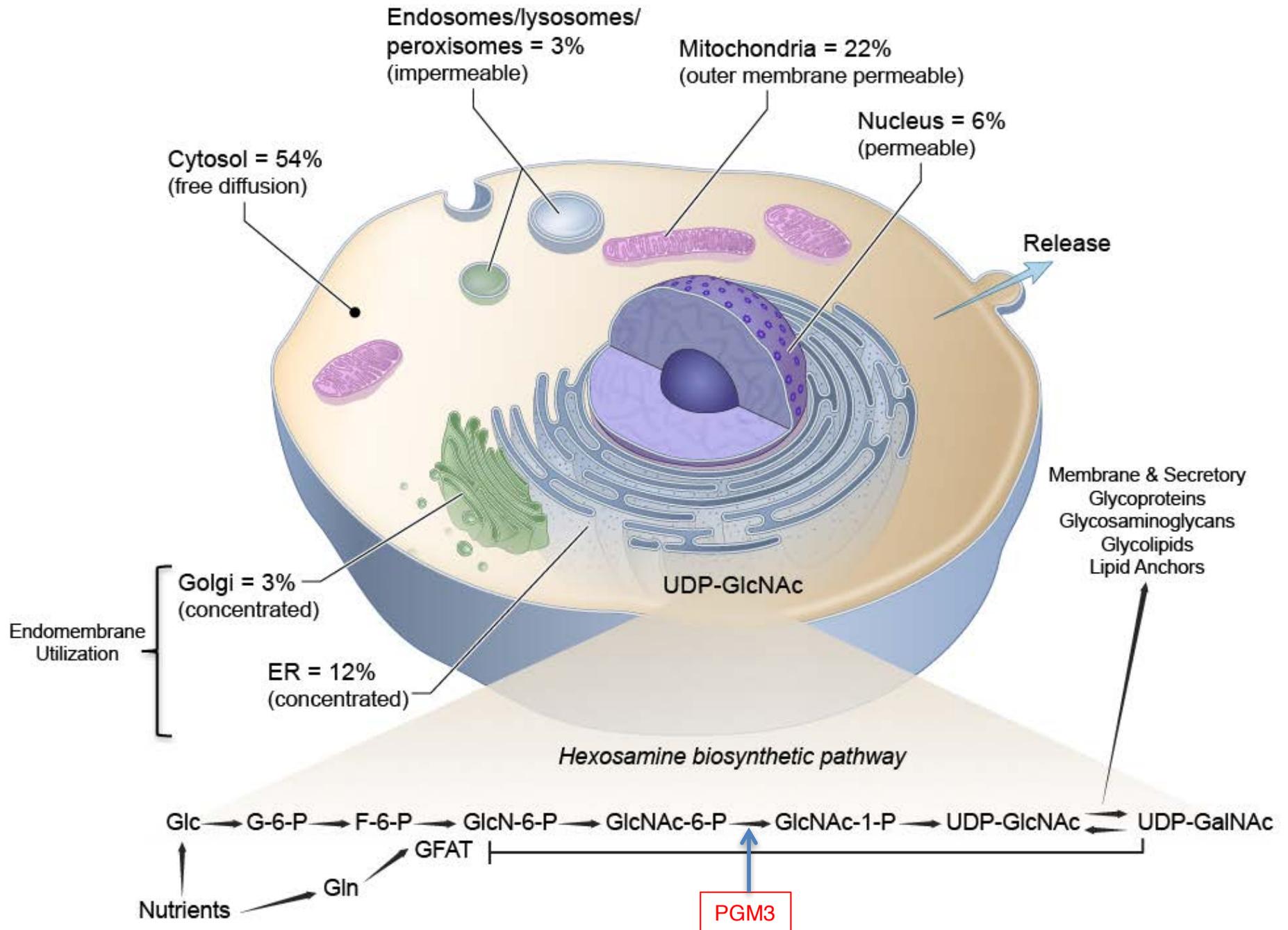
O-GlcNAc is implicated in protein stability, localization, activity, *etc.*



O-GlcNAc deregulation is associated with disease



UDP-GlcNAc is at nexus of protein/lipid glycosylation



Rare diseases can provide extraordinary insight into human biology

– Patrick Maxwell, Cambridge University

- Glycans can be complex structures and dynamically altered
- Focusing on chemical details of biological sugars is informative for defining details of disease

