NIH Glycosciences: A rich and lasting heritage

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

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

Hewitt G. Fletcher
Chief, 1951-1973

Victor Ginsburg
Chief, Lab Structural Biology 1986-1991

Elizabeth Neufeld
Chief, GBB 1979-1983

Roscoe Brady
NINDS 1972 to 2006

Glycoscience Interest Group
Undiagnosed Disease Program
“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
Grace Wilsey was born with NGLY1 deficiency, which is caused by two mutations in the NGLY1 gene.
ONE OF A KIND

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

BY SETH MNOOKIN
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
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 suggest that ~20% of the population have a CDG allele
# Clinical Features of CDG

<table>
<thead>
<tr>
<th>Type</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>Hypotonia, variable psychomotor retardation, seizures, peripheral neuropathy, stroke-like episodes, strabismus, cardiomyopathy</td>
</tr>
<tr>
<td>Ib</td>
<td>Normal development, hypoglycemia, coagulopathy, protein-losing enteropathy, hepatic fibrosis, cyclic vomiting</td>
</tr>
<tr>
<td>Ic</td>
<td>Hypotonia, psychomotor retardation, seizures, strabismus, feeding problem, coagulopathy</td>
</tr>
<tr>
<td>Id</td>
<td>Hypotonia, severe psychomotor retardation, seizures, microcephaly, optic atrophy</td>
</tr>
<tr>
<td>Ie</td>
<td>Hypotonia, severe psychomotor retardation, seizures, delayed myelination, Blindness</td>
</tr>
<tr>
<td>If</td>
<td>Hypotonia, severe psychomotor retardation, seizures, blindness, dry skin, low food intake, vomiting</td>
</tr>
<tr>
<td>Ig</td>
<td>Hypotonia, severe psychomotor retardation, seizures, feeding difficulties, facial dysmorphology, coagulopathy</td>
</tr>
<tr>
<td>IIa</td>
<td>Hypotonia, severe psychomotor retardation, frequent infections, widely spaced nipples</td>
</tr>
<tr>
<td>IIb</td>
<td>Hypotonia, generalized edema, hypoventilation, apnea, hepatomegaly, demyelinating polyneuropathy</td>
</tr>
<tr>
<td>IIc</td>
<td>Hypotonia, psychomotor retardation, elevated peripheral leukocytes, failure to thrive, short arms and legs</td>
</tr>
<tr>
<td>IIId</td>
<td>Hypotonia, hydrocephalus, myopathy, coagulation abnormalities</td>
</tr>
</tbody>
</table>
Discovery of Congenital Disorders of Glycosylation

Table 1. Biochemical Markers for Various Glycosylation Pathways

<table>
<thead>
<tr>
<th>Disorder Type</th>
<th>Biomarker(s)</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-glycan</td>
<td>transferrin&lt;sup&gt;39,40&lt;/sup&gt;</td>
<td>serum, plasma</td>
</tr>
<tr>
<td>GPI anchor</td>
<td>CD59, CD55, CD16b, ALP, and a GPI-binding toxin, aerolysin (FLAER)&lt;sup&gt;41,42&lt;/sup&gt;</td>
<td>granulocytes, platelets, fibroblasts</td>
</tr>
<tr>
<td>α-dystroglycopathies</td>
<td>α-DG antibody (III6)&lt;sup&gt;53&lt;/sup&gt;</td>
<td>muscle biopsy, fibroblasts</td>
</tr>
</tbody>
</table>
N-Glycan Biosynthetic Pathway: A System to Generate Diversity.

What do we know?
1. Biochemistry
2. Inhibitors
3. Yeast and Somatic Cell genetics

How did we learn it?
1. Biochemistry
2. Inhibitors
3. Yeast and Somatic Cell genetics
Congenital Disorders of Glycosylation: N-linked

- Most common CDG
- Multiple steps
- Assembly of a common precursor
MOGS deficiency

NGLY1 deficiency

N-Glycan Biosynthetic Pathway and ER Quality Control
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

Activated sugar donor

Glycosyltransferase substrate utilization

Glycan synthesis requires high-energy, activated donors
Glycan synthesis requires high-energy, activated donors

\[
\text{UDP} = \text{ADP + GlcNAc} = \text{UDP-GlcNAc}
\]

\[
\text{ATP} = \text{ADP + GTP} = \text{ATP-GlcNAc}
\]
NDP-sugars are utilized in multiple cellular locations

Donor substrate biosynthesis

Sugar $\rightarrow$ Sugar-6-P

Sugar-6-P $\rightarrow$ Sugar-1-P

Sugar-1-P $\rightarrow$ NDP-sugar

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
NDP-sugar synthesis integrates multiple metabolites

Pathway control points

- Rate-limiting enzymes
- Feedback inhibition
- Sugar transport

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inhibitor</th>
</tr>
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<tr>
<td>UDP-Glc dehydrogenase</td>
<td>UDP-Xyl</td>
</tr>
<tr>
<td>GDP-Man 4,6-dehydratase</td>
<td>GDP-Fuc</td>
</tr>
<tr>
<td>Glutamine:fructose-6-P amidotransferase</td>
<td>UDP-GlcNAc</td>
</tr>
<tr>
<td>UDP-GlcNAc epimerase/kinase</td>
<td>CMP-Sia</td>
</tr>
</tbody>
</table>
Mutations in enzymes required for NDP-sugar synthesis are associated with disease


Disease associated Rate-limiting enzymes
Diversity requires many sugar donors and enzymes

**N-linked**
- Mucin-type
- O-linked
- glycolipids

**O-Man**
- O-Fuc

**GPI-anchor**
- O-GlcNAc

**Hyaluronan**
- Heparan
- Keratan

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<table>
<thead>
<tr>
<th>Sugar</th>
<th>Activated form</th>
</tr>
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<tbody>
<tr>
<td>Glc</td>
<td>UDP-sugar</td>
</tr>
<tr>
<td>Gal</td>
<td></td>
</tr>
<tr>
<td>GlcNAc</td>
<td></td>
</tr>
<tr>
<td>GalNAc</td>
<td></td>
</tr>
<tr>
<td>GlcA</td>
<td></td>
</tr>
<tr>
<td>Xyl</td>
<td>GDP-sugar</td>
</tr>
<tr>
<td>Man</td>
<td></td>
</tr>
<tr>
<td>Fuc</td>
<td></td>
</tr>
<tr>
<td>Sia</td>
<td>CMP-Sia</td>
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*Essentials of Glycobiology, Second Edition, Chapter 4*
NDP-sugars are utilized in multiple cellular locations

Donor substrate biosynthesis

GlycoT substrate utilization

NDP-sugar transport requires active process

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>ER</th>
<th>Golgi</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMP-Sia</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>GDP-Fuc</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>UDP-Gal</td>
<td>–</td>
<td>++++</td>
</tr>
<tr>
<td>PAPS</td>
<td>–</td>
<td>++++</td>
</tr>
<tr>
<td>GDP-Man</td>
<td>–</td>
<td>++++</td>
</tr>
<tr>
<td>UDP-GlcNAc</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>UDP-GalNAc</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>UDP-Xyl</td>
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</tr>
<tr>
<td>ATP</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>UDP-GlcA</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>UDP-Glc</td>
<td>+++</td>
<td>+</td>
</tr>
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</table>
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.

A) GlcNAc-O- \( \leftrightarrow \) GlcNAc-O-P

or P- \( \leftrightarrow \) or P

B) GlcNAc-O- \( \rightarrow \) O-GlcNAc

C) GlcNAc-O- \( \rightarrow \) vs. or

D) GlcNAc-O-

E) GlcNAc-O-

M

G2

G1

S

F) O-GlcNAc \( \rightarrow \) Epigenetic imprinting

Bond and Hanover, Annual Review of Nutrition 2013
O-GlcNAc deregulation is associated with disease

Physiological

Normal function

Problem onset: recovery possible if nutrient status regulates

Disease state permanent and/or lethality results

Nutrient deficit

Nutrient excess

O-GlcNAc levels are normal due to continual cycling. Cells maintain appropriate signaling, metabolism, transcription, cell cycle, protein localization, and cellular structure.

O-GlcNAc levels are inappropriately high or low. Cellular signaling affected: ROS generation, protein folding, and localization problems result.

DMII-associated hyperglycemia worsens if nutritional load is not controlled.

O-GlcNAc levels are unreasonably high or low. O-GlcNAc modification may be permanently "on" or "off".

Epigenetic modulation of offspring metabolism efficiency and adult disease susceptibility altered. Increased glucose load correlates with poor prognosis in some cancers.

Bond and Hanover, Annual Review of Nutrition 2013
UDP-GlcNAc is at nexus of protein/lipid glycosylation

Endosomes/lysosomes/peroxisomes = 3% (impermeable)
Mitochondria = 22% (outer membrane permeable)
Nucleus = 6% (permeable)
Cytosol = 54% (free diffusion)
Golgi = 3% (concentrated)
ER = 12% (concentrated)

Hexosamine biosynthetic pathway

Glc → G-6-P → F-6-P → GlcN-6-P → GlcNAc-6-P → GlcNAc-1-P → UDP-GlcNAc → UDP-GalNAc
GFAT

Membrane & Secretory Glycoproteins Glycosaminoglycans Glycolipids Lipid Anchors

PGM3
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