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What is Glycobiology?

A term frequently attributed to Raymond Dwek, et al (circa 1990) to encompass the body of research that contributes to understanding:

*The structure, biosynthesis, and biology of saccharides*
Or, more explicitly stated (at least more explicit than could have been imagined 25 years ago)--Glycobiology is the human field of endeavor that strives to characterize and understand:

– The diversity of glycan structures
– The processes by which glycans are synthesized
– The determinants of glycan structure
– The mechanisms of glycan-protein interactions
– The impact of glycans on the structure and function of the molecules to which they are attached
– The contribution of glycans to normal cellular function and tissue development
– The participation of glycans in diverse pathologies
Thus, aspects of glycobiology impact a broad range of disciplines

To name a few--

organic synthetic chemistry
protein biochemistry
enzymology
analytic chemistry
structural biochemistry
cell biology
developmental biology
genetics
genomics
proteomics
parasitology
neurobiology
reproductive medicine
endocrinology
cell signaling
stem cell biology
membrane biophysics
microbiology
cancer biology
immunology
microbiology
biotechnology

And, in fact, glycobiology impacts life, itself, from conception (sperm-egg interactions) to death (apoptosis, multiple systemic pathologies)
**How do glycans impact your work?**

*Depends on who you are:*

– Protein biochemist: Pesky modifications that impart heterogeneity to my otherwise pure protein
– Structural biochemist: Nasty modifications that make it hard to crystallize some really interesting proteins
– Synthetic chemist: You want to synthesize what? And you want how much?
– Geneticist: A family of regulated molecules whose expression is not simply related to the activity of a single gene. Painful pleiotropy.
– Molecular Biologist: What’s the big deal--I’ve been doing glycobiology for my whole career? They make a nice backbone structure.
How, then, does Glycobiology fit within the context of modern molecular, genetic, structural, and systems biology?
The Central Dogma, circa 1970

DNA → RNA → Protein

Organism ← Cell

Rather ignores a role for lipids and carbohydrates, especially at the cell surface

After Varki, A
An expanded Dogma

DNA → RNA → Protein → enzymes

Saccharides and lipids

Modified transcription factors

Organism ← Cell ← glycoconjugates

After Varki, A
Predominant cellular distribution of glycans implies role for carbohydrate in the societal interactions of cells in tissues

Electron micrograph of a human lymphocyte (Ruthenium Red staining)

After Varki, A
Potential for high informational content inherent in glycan structure

<table>
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<tr>
<th>Macromolecule</th>
<th>Building Block</th>
<th>Aproximate Mass</th>
<th>Possible Variations in a Trimer</th>
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<tbody>
<tr>
<td>Protein</td>
<td>Amino acids</td>
<td>$125 \rightarrow 10^4-10^5$</td>
<td>6</td>
</tr>
<tr>
<td>Nucleic Acid</td>
<td>Nucleotides</td>
<td>$330 \rightarrow 10^3-10^9$</td>
<td>6</td>
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<tr>
<td>Carbohydrate</td>
<td>Monosaccharides</td>
<td>$200 \rightarrow 10^2-10^6$</td>
<td>1,056 to 27,648!</td>
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The building blocks

△ = Glucose (Glc)  ○ Hexose, unspecified (Hex)
□ = Mannose (Man)
● = Galactose (Gal)
■ = N-acetylglucosamine (GlcNAc)
□ = N-acetylhexosamine (HexNAc)
■ = N-acetylgalactosamine (GalNAc)
◆ = Fucose (Fuc)
◇ = Xylose (Xyl)
◆◆ = Sialic acid, unspecified (Sia)
◇◆ = Glucuronic acid (GlcA)
◆◇ = Iduronic acid (IdoA)
◇◆ ◇◆ = Uronic acid, unspecified (HexA)

Simplified Traditional

\[
\begin{align*}
\text{(Simplified)} & \quad \text{Fuc}\alpha_3^3 \quad \text{Sia}\alpha_3 \text{Gal}\beta_4 \text{GlcNAc}\beta_2 \text{Man}\alpha_6^6 \\
\text{(Traditional)} & \quad \text{Fuc}\alpha_6^6 \quad \text{Man}\beta_4 \text{GlcNAc}\beta_4 \text{GlcNAc}^\sim_3 \\
& \quad 9\text{Ac-Sia}\alpha_6 \text{Gal}\beta_4 \text{GlcNAc}\beta_2 \text{Man}\alpha_3
\end{align*}
\]

Symbolic Representation
Carbohydrates
- Basic Terms

• Monosaccharide
  – A carbohydrate that can not be broken down into smaller carbohydrates by treatment with acids- a simple sugar

• Oligosaccharide
  – Approximately 4-12 mono units

• Polysaccharide
  – Usually greater then 12 mono units
  – Often a long linear repeating chain consisting of a single monosaccharide type with or without small side chains
Monosaccharides

• Three Main Types
  – Neutral
  – Amine
  – Charged (sialic acids, uronic acids)

• Which may be substituted
  – Sulfate, Phosphate, Acetyl, etc.

• Have different ring types
  – furanose or pyranose

• Stereochemistry (D or L)
Oligosaccharide Structure

Oligosaccharides are formed by the covalent O-glycosidic linking of monosaccharides. There are many variables which contribute to the overall structure of the resulting oligosaccharide. Variables in an oligosaccharide structure include:

- **Monosaccharide Constituents and their Characteristics**
  - Ring Type (pyranose vs. furanose)
  - Stereochemistry (D vs. L)

- **Linkage Characteristics**
  - Anomericy (α or β linked)
  - Linkage Position (1-3, 1-4, 2-3, etc.)
Carbohydrate Synthesis - Secondary gene products

• Oligosaccharide structures are not encoded in the DNA

• Correct synthesis dependent on
  – Glycosyltransferases
  – Glycosidases
  – Availability of monosaccharide and activated forms
  – Organelle distribution and function
Carbohydrate Synthesis - Secondary gene products

• Consequence
  – Glycosylation is very sensitive to subtle shifts in the environment
  – Altered glycosylation can serve as an event trigger
    • Cell Death - Kill the cell
    • Clearance - Attack and clear the biomolecule from the system
Glycoconjugates

- **Glycoproteins**
  - Protein + Carbohydrate
  - Most proteins of biological and therapeutic significance
  - e.g. TPA, EPO, LH, Immunoglobulins

- **Glycolipids**
  - Lipid + Carbohydrate
  - Important tissue and cell type markers
  - e.g. blood group antigens, tumor associated antigens

- **Proteoglycans**
  - Complex of a protein and one or more polysaccharides known as glycosaminoglycans (GAGs)
  - Immense structural diversity, pleiotropic functions
Built into polymers, post-synthetic modifications are common

\[ \Downarrow = \text{Xyl} \quad \bullet = \text{Gal} \quad \Diamond = \text{GlcA} \quad \blackbox = \text{GlcNAc} \quad \redDiamond = \text{IdoA} \]

\begin{align*}
\text{Ac} &= \text{O-acetyl} \\
\text{P} &= \text{Phosphate} \\
\text{S} &= \text{O-Sulfate} \\
\text{NS} &= \text{N-Sulfate} \\
\text{NH}_2 &= \text{free amino group}
\end{align*}

After Varki, A
Glycoconjugates- Basic Terms

• Microheterogeneity
  – The variation seen in glycosylation at a given glycosylation site. A site may be unoccupied or may be occupied by different sugars. This fact led early researchers to falsely conclude that carbohydrates were not important.

• Glycoforms
  – A glycoconjugate may have different glycoforms. That is, the non-carbohydrate portion will remain the same, but variances in the carbohydrate portion will create different glycoforms of a glycoconjugate.

• Glycone vs. Aglycone
  – Carbohydrates (glycones) should be considered in the context of what they are linked to (aglycones)
Is this the right scale?
Is this the right scale?
Glycoconjugates of the real world

- Glycoconjugate: A compound in which one or more glycans (the glycone) are covalently linked to a non-carbohydrate moiety (the aglycone).
- Glycoproteins: A protein with one or more covalently bound glycans.
- Glycolipids: A molecule containing a saccharide linked to a lipid.
- Proteoglycans: Any glycoprotein with one or more covalently attached glycosaminoglycan chains.

After Varki, A
The great classes of animal glycans

After Varki, A
- Large O-linked Glycosaminoglycans and poly-lactosamine structures
- Glycoprotein N-linked and O-linked oligosaccharides
- Glycolipid oligosaccharides
Three great waves of Glycobiologic expansion

*The first wave:* The long, grand ascendancy of analytic chemistry, with a little functional analysis in the shadows.
   –How are these building blocks put together and who can publish the weirdest structure in JBC this month?
   –By the way, there’s some cool function over here.

*The second wave:* Brave, new pioneers bring molecular tools and the promise of deciphering function.
   –You meant that genes may actually be relevant to glycans?
   –And molecular biology can do what?
Three great waves of Glycobiologic expansion (cont’d)

The third wave (we’re riding it now): The great integration, with an eye increasingly focused on developing therapeutics.

– First concerted attempts to generate therapeutics reveal that we don’t know enough to do rational design.
– Leads to large-scale efforts to comprehensively herd cats (expansion of glycomics to facilitate the identification of relevant glycans).
– Initial applications of genetics to identify glycan function (targeted and accidental).
– Driving forces increasingly come from outside of traditional Glycobiology domains (the help me phone call).
Major contributions, concepts and discoveries that have shaped the course of Glycobiology:

– At least a century of cumulative structural analysis.
– Blood group antigens are mostly glycans (lipid and protein-linked).
– One enzyme-one linkage hypothesis: implied the need for a broad range of enzymes and associated regulatory mechanisms.
– Synthetic pathway for N-linked glycans (rational for evolution of pathway, cellular distribution, enzymology, multiple levels of potential regulation).
– Altered glycan expression in pathologic states (especially cancer and inflammation).
Great contributions (cont’d):

– Weirdo glycans that challenge dogma (nucleocytoplasmic glycosylation and other unique O-linked glycans, post-synthetic modifications)
– Processing and synthetic enzyme knock-outs, from lethality to tissue-specificity
– Human CDGs?? (reveal subtle, pleiotropic nature of human deficiencies, previously undetected requirement for glycans)
Reasonably well-supported and accepted functions for glycans

- Structural support for cells (extracellular matrix, plant cell wall)
- Signaling molecules (currently only characterized in plants)
- Determinants of protein folding, stability, and half-life
- Mediators of cellular interactions with:
  - Other cells in developing tissues
  - Specific cell types in mature tissues, especially related to immune response and tissue stability
  - Viral, bacterial, parasitic pathogens and associated toxins
- Modulators of signaling activities that drive cellular differentiation
The thrill and the agony of Glycobiology:

Biological roles of oligosaccharides: all of the theories are correct.

Varki A.
Glycobiology Program, UCSD Cancer Center.

Many different theories have been advanced concerning the biological roles of the oligosaccharide units of individual classes of glycoconjugates. Analysis of the evidence indicates that while all of these theories are correct, exceptions to each can also be found. The biological roles of oligosaccharides appear to span the spectrum from those that are trivial, to those that are crucial for the development, growth, function or survival of an organism. Some general principles emerge.

And this is where we begin!
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