**O-Glycosylation**

Yeast mannoproteins
α-dystroglycan

GalNAc
Ser/Thr

Man
Ser/Thr

Fuc
Ser/Thr

Glc
Ser/Thr

GlcNAc
Ser/Thr

Mucins

Notch

Thrombospondin
Factor IX

Coagulation Factors
Fibrinolytic Factors

Nuclear Proteins
Cytoplasmic Proteins

**SEPARATE LECTURE**

ALSO: Proteoglycans, Hydroxyproline/Hydroxylysine Glycosylation

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**O-Glycosidic Linkage**

O-glycosidic linkage is sensitive to alkali (regardless of stereochemistry)

β-elimination

GalNAc

After Esko, J
Glycan synthesis in a cellular context

Most O-Glycosylated proteins are synthesized in the secretory pathway.

**O-Glycosylation**

![Diagram of O-Glycosylation with symbols for GalNAc, Man, Fuc, Glc, and GlcNAc attached to Ser/Thr residues]
Mucin-Type O-GalNAc Glycans

- Major extracellular vertebrate O-glycan
- Begins in cis-Golgi by attachment of GalNAc in α-linkage to specific Ser/Thr residues
- Assembly is simpler than N-linked chains - no lipid intermediate is used
- Always involves nucleotide sugars
- Always occurs by addition to non-reducing terminus or by branching

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Fig. 1. Eight types of O-glycan core structures.

METHODS IN ENZYMEOLOGY, VOL. 466
Polypeptide GalNAc Transferases

Regions in white, pink, red, and black represent, respectively, 0–29%, 30–69%, 70–99%, and 100% sequence identity (Hagen et al. (2003) Glycobiology 13:1R-16R).

• >20 ppGalNAcT family members
• Share structural features in active site
• Some have lectin (ricin) domain

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Core 1 and Core 2 Synthesis

Tn Antigen
Core 1 GalT (cis) → T (TF) Antigen
Core 2 GlcNAcT
ST6GalNAc1 (trans)
Sialyl Tn Antigen

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Core 3 and Core 4 Synthesis

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Unusual Core O-Glycan Structures

Core 1: β3
Core 2: β3 β6
Core 3: β3
Core 4: β3 β6
Core 5: α3
Core 6?: β6
Core 7: α6
Core 8: α3

Mucins are Heavily O-glycosylated

- Apomucin contain tandem repeats (8-169 amino acids) rich in proline, threonine, and serine (PTS domains)
- Glycosylation constitutes as much as 80% of mass and tends to be clustered - bottle brush
- Expressed by epithelial cells that line the gastrointestinal, respiratory, and genito-urinary tracts

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Mucin Production

Goblet cells in intestinal crypts

Lung Epithelium

Mucins: Protective Barriers for Epithelial Cells

- Lubrication for epithelial surfaces
- Modulate infection:
  - Receptors for bacterial adhesins
  - Secreted mucins can act as decoys
- Barrier against freezing:
  - Antifreeze glycoproteins
  - $[\text{Ala-Ala-Thr}]_{n \leq 40}$ with Core 1 disaccharides

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Questions

- What is the function of multiple polypeptide GalNAc transferases?
- How is tissue specific expression of transferases regulated?
- How does competition of transferases for substrates determine the glycoforms expressed by cells and tissues?
- What roles do chaperones play?

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\[
\begin{align*}
\text{GalNAc} & \quad \text{Man} & \quad \text{Fuc} & \quad \text{Glc} & \quad \text{GlcNAc} \\
\text{Ser/Thr} & \quad \text{Ser/Thr} & \quad \text{Ser/Thr} & \quad \text{Ser/Thr} & \quad \text{Ser/Thr}
\end{align*}
\]

\textit{O-Glycosylation}
A.

EGF repeats modified by Fringe

- EGF repeat
- EGF with O-fucose site (C^2X_{4.5}S/TC^3)
- Notch/Lin repeats

Annu. Rev. Biochem. 2004. 73:491–537
O-Fuc

- Two Flavors: Mono and Tetrasaccharide
- One of the clearest examples of glycosylation (Fringe) modulating signal transduction
- What other proteins carry O-Fuc and how does glycosylation modulate activity?
- How is glycosylation regulated?

O-Glycosylation

<table>
<thead>
<tr>
<th>GalNAc</th>
<th>Man</th>
<th>Fuc</th>
<th>Glc</th>
<th>GlcNAc</th>
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<tbody>
<tr>
<td>Ser/Thr</td>
<td>Ser/Thr</td>
<td>Ser/Thr</td>
<td>Ser/Thr</td>
<td>Ser/Thr</td>
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</table>
O-Glc Pathway

C-X-S-X-P-C

UDP-Glucose: Protein \(\alpha\)-glucosyltransferase

Glc-\(\alpha\)-Ser

UDP-D-xylose: \(\beta\)-D-glucoside \(\alpha\)-1,3-D-xylosyltransferase

Xyl-\(\alpha\),3-Glc-\(\alpha\)-Ser

UDP-D-xylose: \(\alpha\)-D-xyloside \(\alpha\),1,3 xylosyltransferase

Xyl-\(\alpha\),1,3-Xyl-\(\alpha\),1,3-Glc-\(\alpha\)-Ser

Rumi is OGLuT
KDEL Retention Signal
Temp. Sensitive Mutation
O-Glc

- Always a trisaccharide? What enzyme/s for Xyl extension?
- Glc & Xyl (except for proteoglycans) rarely used on mammalian glycoproteins--why both here? Does Rumi have both activities?
- Many of the same proteins as O-Fuc modified, why?
- Role in Modulating Signaling? Regulated by enzymes or sugar nucleotide availability?

O-Glycosylation

- GalNAc
  - Ser/Thr
- Man
  - Ser/Thr
- Fuc
  - Ser/Thr
- Glc
  - Ser/Thr
- GlcNAc
  - Ser/Thr
Biosynthetic Pathway of Yeast O-Mannosyl Glycan and Proposed Biosynthetic Pathway of Mammalian O-Mannosyl Glycan

Phylogenetic tree of selected PMT family members from the genome.

Current Opinion in Structural Biology 2003, 15:621-625

A specific chemical pathway and labels for biological markers:

- α3
- α3SiaT
- β4GalT
- β4
- β2
- POMGnT1
- POMT1?
- α
- O
- Ser/Thr
- Fukutin?
- LARGE?
- FKRP?
- Mannose
- Galactose
- Sialic acid
- N-acetylglucosamine

Amm. Rev. Biochem. 2004. 73:491–537
Muscular Dystrophies associated with glycosylation of α-DG

<table>
<thead>
<tr>
<th>Disease</th>
<th>Species</th>
<th>Affected Gene</th>
<th>Biochemical Lesion</th>
<th>Biochemical Phenotype</th>
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<tr>
<td>Walker-Warburg Syndrome</td>
<td>Human</td>
<td>POMT1</td>
<td>O-Man addition to Ser/Thr</td>
<td>Decreased protein O-mannosylation</td>
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<tr>
<td>Muscle-Eye-Brain Disease</td>
<td>Human</td>
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<td>Addition of GlcNac β2 to O-Man</td>
<td>Underglycosylated α-DG, uncapped O-Man</td>
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<tr>
<td>Fukuyama-type MDC</td>
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<td>Fukutin</td>
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<td>Underglycosylated α-DG</td>
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<td>Limb-Girdle and MDC 1C</td>
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<td>Fukutin-Related Protein</td>
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<td>MDC 1D</td>
<td>Human</td>
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</table>

MDC, Congenital Muscular Dystrophy; POMT, Protein -O-Mannosyltransferase; POMGnT1, Protein -O-Mannose, N-acetylgalcosaminytransferase 1
POMT1 in the ER Complex With POMT2 Uses Dol-Man As Donor

Schematic representation of human POMT1. By analogy to StPreP [32], we established a topological model for human POMT1. Invariant amino acids and conserved signature motifs are indicated in gray. The FMT domain is indicated by red dots. Three assigned MiM (protein-protein interaction interface) asparagine and glutamine residues and mutagenesis sites in loop 2 are shown in blue. Mutations that result in WW5 are highlighted as turquoise. The green arrows denote the transmembrane helix V735 (red) and Q398X, the green arrows denote missense mutations Q398X and Q385X, nonsense mutations V768R and Q438D are highlighted in red. A soluble variation in sector II with an additional 22 amino acids is shown as yellow circles [21].

Current Opinion in Structural Biology 2008, 18:623-630

TRENDs in Pharmacological Sciences

TRENDs in Pharmacological Sciences Vol 24 No 4 April 2003
MS/MS Analysis of the O-Man Classical Tetrasaccharide Glycan Structure Released from Brain Proteins Derived from the α-DG knock-out animal. Greyed out structures represent their loss.

O-Man β-6 Branching in the Brain

<table>
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<th>No.</th>
<th>O-linked oligosaccharide composition</th>
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<th>ΔK(−)</th>
<th>ΔK(−)</th>
<th>ΔK(−)</th>
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</tr>
</tbody>
</table>

sc: Singly charge, O: Observed, −: Weekly observed, X: Not observed, and ▲: Not unique

GnTV-B Primarily Responsible
A New Glycan Structure on \( \alpha \)-DG that is Large-dependent

Full MS (300-2000 m/z)

O-Man

- O-Man is clearly involved in CMD
- What mammalian proteins (especially in the brain) are O-Man modified besides α-DG?
- What are the functions of fukutin and large in O-mannosylation?
- Why the heterogeneity in O-Man structures, what specific structures at what sites on the protein modulate specific interactions?
- What is relationship between O-Man and O-GalNAc?
O-Glycosylation

A few more O-glycans......

O-Xyl…precursor for GAGs...

O-GlcNAc…separate lecture—note: recent report of O-GlcNAc on Notch extracellular domain
O-Glycosylation of Hyl

- Found on Collagen and Adiponectin (which has a “collagen-like” domain)
- Glycosylation Essential for Basement Membrane Formation in Tissues
- Modulates Collagen Cross-linking?
- Other proteins with modification?
The Glycosaminoglycans

- Hyaluronic acid (HA)
- Chondroitin sulfate (CS)
- Dermatan sulfate (DS)
- Heparan sulfate/heparin (HS)
- Keratan sulfate (KS)

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O-Glycosylation

- less studied (until recently?)
- tools to study are underdeveloped
- in many cases, clearest functional data (not including folding/quality control)