Synthesis of a glycan requires co-ordinate activities of multiple cellular systems

Glycosidic bonds

- Sugar (mono-saccharide) can be linked via O-, N-, C-, S- to another molecule (aglycone) or to another sugar.
  Connection is typically via the functional groups on the anomeric carbon (C1).

There are different types of glycosides

- Types of Glycosidic bonds
- Sugar is linked via O-, N-, C-, S-

- Erythromycin, antibiotics, O-glycoside (OH group)
- Oleandrin, Cariac glycoside, O-glycoside
- Glucosinolate, Defense against herbivore, S-glycoside (SH group)
- UTP, N-glycoside (NH group)
- Flavonoid, insecticidal, C-glycoside (C-group)
Many glycosides are found in secondary metabolites

- Often one or two sugars decorate the Metabolite
- However, some of these metabolites can be linked to branch-chain sugar, like antifungal agents

- **antifungal** saponins from oats and solanaceous plants.
Sugar $N$- or $O$-linked to amino acids

Classification of glycosides on the basis of the linkage between the glycone (sugar) and aglycone moiety

- **O-glycosides**: in these glycosides the sugar moiety is linked with alcoholic or phenolic hydroxyl or carboxyl group.
- **S-glycosides**: the sugar is attached to a Sulfur atom of aglycone such as in sinigrin (SH).
- **N-glycosides**: the sugar is linked with Nitrogen atom of (-NH$_2$, -NH-) amino group of aglycone like in nucleosides DNA, RNA.
- **C-glycosides**: the sugar is linked (condensed) directly to Carbon atom of aglycone like in aloin, flavonoids.
Glycoside synthesis requires

GT, glycosyltransferase

– **Acceptor**
  • (sugar, lipid, amino acid, phenolic)
    » -OH, -NH, -SH, -C

– **Substrate (sugar donor)**
  • (nucleotide-sugar, Dol-sugar)
    » UDP-glucose

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**Glycosyl-transferases (GT)**

• Soluble (cytosl, lysosome, nucleus)
• Membrane bound

**Sugar-donors**

• Soluble (cytosl, lysosome, nucleus)
• Inside membrane
• Membrane bound
A Glycosyl-transferase (GT) transfers a sugar from an activated sugar, nucleotide-sugar, to an acceptor.

**Activated sugars**
- UDP-α-L-Glc
- GDP-β-L-Fuc
- CMP-β-1,3-Kdo

**Glycosylation**

**N-Glycosylation**

O-Glycosidic bond

**Nucleotide-sugars**

- NDP-sugars are precursors for glycan synthesis.
- In Prokaryotes: Over 70 different NDP-sugars
- In Fungi: 10-15 different NDP-Sugar
- In Archaea: > 10
- Human: 11 NDP-sugars
The discovery of nucleotide-sugar as donor for glycosylation reaction
History

EMIL FISCHER observed in 1890 that L-glucose was not metabolized by brewer’s yeast.

• ~100 years ago (1906) **Harden and Yo** discovered that yeast fermented with sugar accumulate a phosphate esters-sugar derivative. Man-6-P, Glc-6-P

• The synthesis, and metabolic roles of sugar-phosphates start to emerge.

• The synthesis of a **polysaccharide** from **glucose-1-phosphate** in muscle extract. Carl F. Cori, Gerhard Schmidt, and Gerty T. Cori. 
  *Science* 19 May 1939
  1947 *Nobel Prize*. Cori’s
  (Glycogen synthesis, metabolism& disease)

Glycans are made from activated sugars

Hehre 1941 *SCIENCE*------SPECIAL ARTICLES------Sucrose incorporated into bacterial polysaccharide.

1946, 1947-- Glc-1-P is involved... C. diphtheria starch-like

• 1950- the discovery of nucleotide-sugars. Sugar-donor for glycan synthesis

With collaborators, Ramon Caputto, Carlos E. Cardini, Raúl Trucco, and Alejandro C. Paladini study the metabolism of galactose which led to the isolation of glucose 1,6-diphosphate and *uridine diphosphate glucose*.

UDP-glucose served as glucose donor in the synthesis of **trehalose** (Leloir & Enrico Cabib, 1953) and sucrose (Leloir & Carlos Cardini and J. Chiriboga, 1955)

1970, Nobel Prize

**Luis Leloir, 1906–1987**
Nucleotide-sugars (sugar – donors)

- There are many types of nucleotide sugars.
  - Fungi 10-15
  - Human 10
  - Plant >25
  - Bacteria >70
  - Archaea >15
- The nucleotide can be ADP, GDP, CDP, TDP, UDP, CMP
- The sugar moiety vary (hexose, Uronic, pentose, di-deoxy) can be modified with - AA, Met, Act group)

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Nucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Sugar Structure" /></td>
<td><img src="image" alt="Nucleotide Structure" /></td>
</tr>
</tbody>
</table>

Role of a nucleotide sugars in biology

- **Nucleotide-sugars are precursors for synthesis of macromolecules:**
  - Glycoproteins
    - N-, O-linked
  - Glycolipids,
    - Lipopolysaccharides.
  - Proteoglycans
    - heparan
  - Oligosaccharides
    - Raffinose
  - Polysaccharides
    - Starch, cellulose, pectin, xylan (biomass......biofuel)
- **Nucleotide-sugars are precursors for synthesis of small molecules:**
  - Toxins,
    - Liver toxin removal, cardiac glycosides
  - Antibiotics,
    - Kanamycin, neomycin, erythromicine
  - Smaller metabolites,
    - Hormones, bitter, colors Storage: sucrose, trehalose, lactose, maltose
  - Hormones,
Biosynthesis of nucleotide-sugars

- Different metabolic routes
- Different in different species
  - Plant
  - Animal
  - Bacteria
  - Fungi

biosynthesis of NDP-sugars

- Two main pathways
  1) Biosynthesis: **The Interconversion Pathway**
     Sugar --> sugar-6-P --> sugar-1-P --> NDP-sugar(1) --> other NDP-sugars
     Glc, Man, Frc
  2) Catabolism: **The Salvage Pathway**
     Polysaccharide --> sugars --> sugars-1-P --> NDP-sugars
     Almost all other sugars in a cell

Synthesis of other activated -sugars

1. dolichol-PP-sugars (e.g. Dol-P-Man)
2. Isoprenol-PP-sugar (e.g. Dodecaprenyl phosphate-galacturonic acid)
3. Park nucleotides
4. CMP-sugars (e.g. CMP-Sialic acid)
The interconversion pathway overview

- Glc/ Man \( \rightarrow \) Glc-6-P \( \rightarrow \) Glc-1-P
- Glc-1-P + UTP \( \rightarrow \) UDP-Glc
- UDP-glucose \( \rightarrow \) many different NDP-sugars

The Interconversion Pathway

Step 1.
Converting Energy source: Glucose, Mannose, Fructose to Sug-6-P
by hexokinase

![Diagram of hexokinase reaction](image)
Step 2: PGM "reversible" committed step to transform Sugar-6-P to Sugar-1-P

\[ \Delta G^\circ = 1.7 \text{ kJ/mol} \]
Interconversion pathway.

- **NDP-sugar pyrophosphorylase (PPase)**. A group of reversible enzymes (nucleotidyltransferase) that transfer nucleotide (XMP) from XTP onto a sugar-1-P, forming NDP-sugar.

**Examples:**
- GDP-Man
- UDP-Glc
- TDP-Glc
- ADP-Glc
- CDP-Glc

Cardini, Leloir 1950
Card, Leloir 1953

interconversion

*Once UDP-Glc or GDP-Man are formed*

- A nucleotide sugar can be converted to another nucleotide sugar by the action of different types of enzyme activities:
  - 4,6-dehydratase
  - 6-oxidoreductase
  - Decarboxylase
  - 4, epimerases
  - 2-epimerases
  - Mutarotase
  - 4-epimerase/reductase
  - 3,5-epimerase
  - Etc......
An example of UDP-sugar 4-epimerase

Example of SDR enzyme reactions
The Salvage Pathways

- Releasing of a monosaccharide (sugar) from glycans
  - Glycoprotein $\rightarrow$ GlcNac, Man
  - Glycolipid $\rightarrow$ Glc, Gal, Man
  - Proteoglycans $\rightarrow$ Xyl, GlcA

- Hydrolysis of sugars from polysaccharides
  - Cellulose $\rightarrow$ Glc

- Releasing of sugar-1-P from storage
  - Glycogen $\rightarrow$ Glc-1-P

- Converting a soluble disaccharide
  - Sucrose $\rightarrow$ UDP-Glc, Maltose $\rightarrow$ Glc-1-P

Different species adopt different mechanisms.
Other salvage source

- Recycling of glucose, mannose, galactose residues released from glycoprotein, glycolipids, proteoglycans, polysaccharides

- Recycling of glucose from storage polysaccharides (glycogen, starch, mannan)

- Sucrose, maltose, rafinose, trehalose
Sugar-1-P kinase

Galactose

\[
\text{ATP} + \text{ADP} + H^+ \rightarrow \text{Galactose 1-phosphate}
\]

Galactokinase

Glucose 1-phosphate

\[
\text{UTP} + \text{uridine} \rightarrow \text{UDP-glucose} + \text{PP}_i
\]
I.2. Special case Step A: Conversion of Galactose to Gal1P: UDP-Glc to UDP-Gal

Galactose (alpha) \[ \xrightarrow{\text{mutarotase}} \] Galactose (beta)
Analyses of nucleotide-sugar

Extraction, methanolic, perchloric acid, formic/AcN
Separation (chromatography), TLC, C18, AX-HPLC, Carbon-HPLC
Analysis (structure, mass) MALDI/MS, NMR

HPLC Analyses of nucleotide sugars

HPLC
Separation: Reverse-Phase; ion-exchange columns
Detection: UV (260 nm)

TLC
Separation: PEI-cellulose
Detection: UV lamp
LC-MS Analyses of nucleotide sugars

HPLC C18 separation
Detection: ES-mass spectrometer (negative ion mode)

MRM transitions of NDP-sugars

<table>
<thead>
<tr>
<th>Sugar nucleotide</th>
<th>MRM transition</th>
<th>Major product</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-Glc</td>
<td>565 \rightarrow 323</td>
<td>UMP-H</td>
</tr>
<tr>
<td>UDP-Gal</td>
<td>565 \rightarrow 323</td>
<td>UMP-H</td>
</tr>
<tr>
<td>GDP-Man</td>
<td>606 \rightarrow 361</td>
<td>UDP-H-water</td>
</tr>
<tr>
<td>GDP-Man</td>
<td>604 \rightarrow 424</td>
<td>GDP-H-water</td>
</tr>
<tr>
<td>GDP-Fuc</td>
<td>588 \rightarrow 442</td>
<td>GDP-H</td>
</tr>
<tr>
<td>UDP-Man</td>
<td>565 \rightarrow 323</td>
<td>UDP-H</td>
</tr>
<tr>
<td>GDP-Ara</td>
<td>574 \rightarrow 442</td>
<td>GDP-H</td>
</tr>
<tr>
<td>UDP-Rha</td>
<td>589 \rightarrow 333</td>
<td>UMP-H</td>
</tr>
<tr>
<td>GDP-Man</td>
<td>555 \rightarrow 323</td>
<td>UDP-H</td>
</tr>
<tr>
<td>GDP-Glc</td>
<td>604 \rightarrow 362</td>
<td>CMP-H</td>
</tr>
<tr>
<td>GDP-GMUA</td>
<td>579 \rightarrow 463</td>
<td>UDP-H</td>
</tr>
</tbody>
</table>

Highly sensitive.

If you use MALDI-
The Mass cannot distinguish Among UDP-hexoses

In negative Mode
Reduce mass by 1
NMR- Analyses of nucleotide sugars

1H-NMR

UDP-4-keto-pentose/UDP-xylose Synthase in R. solanacearum

A. UDP-4-keto-Pentose(K)

B. Fully protonated (I, C5-H5a/H3)
UDP-4-keto-pentose

2. Deuterated (II, C5-D5a/H3)
UDP-4-keto-pentose

Mixture of I & II

Anomeric protons

Ring protons

Gu et al., 2010
NMR- Analyses of nucleotide sugars

Gu et al., 2010

Not all sugar donors are NDP-sugars. isoprene phosphate-linked sugars are donor substrates for a wide variety of glycosyltransferases
- GalA residues in the lipid A and core domains of R. leguminosarum LPS.
- In Escherichia coli, undecaprenyl diphosphate-sugars are substrates for the polymerization of peptidoglycan
- Undecaprenyl monophosphate-sugars are donors in the biosynthesis of mycobacterial lipoglycans
- The structurally related dolichyl monophosphate- and diphosphate-sugars of eukaryotic cells are required for protein glycosylation
Proposed pathway and topography for attachment of GalA residues to the lipid A and core domains of R. leguminosarum LPS

The simplest scenario for the biosynthesis of dodecaprenyl-beta-D-GalA is as follows. 1 and 2, UDP-Glc is oxidized by the dehydrogenase Exo5 to UDP-GlcA, which is then converted by the C4-epimerase LpsL to UDP-GalA (22). 3, in analogy to PmrF (ArnC) (30) in E. coli, Orf3 transfers GalA from UDP-GalA to dodecaprenyl phosphate, generating dodecaprenyl phosphate-beta-D-GalA, which is flipped to the periplasmic leaflet by an unknown mechanism. As yet, we have not been able to assay Orf3 in vitro. 4, lipid A and core LPS sugars are synthesized on the cytoplasmic leaflet of the inner membrane (43-46, 56, 57) and flipped to the periplasmic leaflet by MmbA. 5 and 6, on the periplasmic side of the inner membrane the 1- and 4'-phosphatases, LpxE and LpxF (40, 50), dephosphorylate lipid A, creating the substrate for GalA addition. 7-10, GalA is transferred from dodecaprenyl-beta-D-GalA to the outer Kdo by RgtA and RgtB, and to mannose by RgtC.


ESI/MS/MS analysis of the putative GalA donor substrate for RgtA


Biosynthesis of isoprenoid; isoprenyl diphosphate synthase