Common Themes in Glycoconjugate Assembly
Using the Biogenesis of O-Antigen Lipopolysaccharide
as a Model System*

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Abstract—The biosynthesis of glycoconjugates is remarkably conserved in all types of cells since the biochemical reactions involved exhibit similar characteristics, which can be summarized as follows: (a) the saccharide moiety is formed as a lipid-linked, membrane-associated glycan; (b) the lipid component in most cases is a polyisoprenoid phosphate; (c) the assembly of the lipid-linked saccharide intermediate depends on reactions taking place at both sides of the cell membrane, which requires the obligatory transmembrane movement of amphipathic molecules across the lipid bilayer. These general characteristics are present in the biosynthesis of the O-antigen component of the bacterial lipopolysaccharide, which serves as a model system to investigate the molecular and mechanistic basis of glycoconjugate synthesis, as summarized in this mini-review.

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A SET OF COMMON PRINCIPLES GOVERNS
THE SYNTHESIS OF GLYCOCONJUGATES
IN ALL TYPES OF CELLS

Glycoconjugates are complex carbohydrate molecules covalently linked to other chemical species such as lipids or proteins. These molecules have important structural and functional roles in all types of cells. The biosynthesis of glycoconjugates requires the prior assembly of intermediate components that are independently synthesized and later joined into one macromolecular species. Although there are many classes of glycoconjugates in prokaryotic and eukaryotic cells, their biosynthesis follows some common principles (Fig. 1; see color insert): (i) prior to their transfer to the final molecular species, the saccharide moieties are all formed as lipid-linked glycans; (ii) these lipid-linked saccharides are anchored to cell membranes or the membranes of organelles such as for example the endoplasmic reticulum membrane in eukaryotes; (iii) the most common lipid component of the saccharide intermediates is a polyisoprenoid phosphate; (iv) the majority of the proteins involved in the assembly of polyisoprenoid-linked saccharides are integral membrane proteins; (v) the assembly reactions take place at both sides of the cell membrane, typically commencing at the cytosolic side and continuing at the opposite side, for which the transmembrane movement of the polyisoprenoid-linked saccharides becomes an obligatory step; and (vi) the joining reaction between polyisoprenoid-linked saccharides and acceptor molecules involves a glycosyl transfer reaction to the nucleophilic oxygen of a hydroxyl substituent of the acceptor, but it can also occur to nitrogen (N-linked glycoproteins), sulfur (thioglycosides), and carbon (C-glycosides) nucleophiles.

The remarkable conservation of these basic steps in all types of cells suggests a common mechanism at play and especially, conserved biochemical reactions. I believe that learning the detailed biochemical mechanisms of these key reactions will provide general framework
knowledge on the process of glycoconjugate synthesis that can be applicable to the majority of these molecules regardless of their source and cell type.

LIPOPOLYSACCHARIDE. 
A MODEL BACTERIAL GLYCOCONJUGATE

Lipopolysaccharide (LPS) is a surface glycoconjugate unique to Gram-negative bacteria and a key elicitor of innate immune responses, ranging from local inflammation to disseminated sepsis. Gram-negative bacteria have two membrane layers separated by a periplasmic space: an inner or plasma membrane and the outer membrane. LPS is a major component of the outer leaflet of the outer membrane [1] and consists of lipid A, core oligosaccharide (OS), and O-specific polysaccharide or O antigen [1, 2]. The O antigen, which is the most surface-exposed LPS moiety, mediates pathogenicity by protecting infecting bacteria from serum complement killing and phagocytosis [2-5]. O antigens are polymers of OS repeating units. The chemical composition, structure, and antigenicity of the O antigens vary widely among Gram-negative bacteria, giving rise to a large number of O-serotypes [6]. LPS biosynthesis involves a large number of enzymes and assembly proteins encoded by more than 40 genes, recently reviewed in references [7-9]. It begins at the cytosolic or inner membrane, followed by the transit of the molecule to the outer leaflet of the outer membrane where it becomes surface exposed. The O antigen is synthesized as a lipid-linked glycan intermediate by a process that is remarkably similar to the biogenesis of lipid-linked OSs for protein N-glycosylation [10]. The lipid carrier in bacteria is undecaprenyl phosphate (Und-P), while eukaryotic cells and Archaea utilize dolichyl phosphate (Dol-P).

The core OS, made of hexoses, glycerol-manno-heptose, and 3-deoxy-D-manno-oct-2-ulosonic acid [11], is assembled on preformed lipid A by the sequential transfer of sugar components. The complete lipid A–core OS unit is translocated to the periplasmic face of the inner membrane by the MsbA transporter [12], which is a member of the glyco ATP-binding cassette (ABC) transporters superfamily requiring ATP hydrolysis [13]. In a separate pathway, the O antigen is assembled as an Und-P-P-linked glycan. Depending on the specific system the assembled O-antigen precursors are translocated to the periplasmic side of the inner membrane by ATP-dependent or ATP-independent mechanisms. The O antigen and lipid A–core OS are joined by a ligation reaction that results in the formation of a novel glycosyl bond with the concomitant release of Und-P-P [7, 9, 11, 14]. Und-P-P is recycled into Und-P-P by a poorly characterized pathway that involves the hydrolysis of the terminal phosphate and is also conserved for the recycling of Dol-P-P in eukaryotic cells [9, 15-17]. The complete LPS molecule is translocated to the outer leaflet of the outer membrane by the Lpt system, a multiprotein complex that spans the periplasmic space, connecting inner and outer membranes [18, 19].

LIPID-LINKED SACCHARIDE FORMATION: 
THE INITIATION REACTION FOR O-ANTIGEN SYNTHESIS

The O-unit synthesis starts at the cytosolic face of the plasma membrane by the formation of a phosphoanhydride linkage between Und-P and the first sugar 1-phosphate of the O-antigen unit transferred from a sugar nucleoside diphosphate. This reaction results in the release of nucleoside monophosphate. In the literature, including our own early work [20], the formation of the Und-P-P–sugar bond is often referred incorrectly to as a phosphodiester bond. In a phosphodiester bond, one phosphate group joins adjacent carbons through ester linkages. This is the typical bonding between sugars and phosphates of the backbone of nucleic acids. On the contrary, the phosphoanhydride bond is a high-energy linkage found in sugar nucleoside diphosphates and polyisoprenoid-P-P-saccharides (Fig. 2).

Two different families of integral membrane proteins catalyze the initiation reaction [9]. They are referred to as polyisoprenyl-phosphate N-acetylaminosugar-1-phosphate transferases (PNPT) and polyisoprenyl-phosphate hexose-1-phosphate transferases (PHPT). PNPT pro-

Fig. 2. Phosphoanhydride bonds joining Und-P-P-GlcNAc and Dol-P-P-GlcNAc. The difference in the saturation of the α-isoprene unit of Und and Dol is shown.