Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Bacterial polysaccharide synthesis and export

Laura Woodward and James H Naismith

All domains of life make carbohydrate polymers and by anchoring them to lipid molecules they can decorate the outside of the cell. Polysaccharides are linked to proteins by glycosylation, a process found in both bacteria and in higher organisms. Bacteria do have other distinct uses for carbohydrate polymers; in gram-negative bacteria glycolipids form the outer leaflet of the outer membrane and in many pathogens (both gram-positive and gram-negative) sugar polymers are used to build a capsule or are secreted into the environment. There are parallels, but of course differences, in the biosynthesis of glycolipids between prokaryotes and eukaryotes, which occur at the membrane. The translocation of large sugar polymers across the outer membrane is unique to gram-negative bacteria. Recent progress in the molecular understanding of both the biosynthesis at the inner membrane and the translocation across the outer membrane are reviewed here.

Address
Biomedical Science Research Complex, North Haugh, The University of St Andrews, St Andrews, United Kingdom

Corresponding author: Naismith, James H (naismith@st-andrews.ac.uk)

Current Opinion in Structural Biology 2016, **40**:81–88

This review comes from a themed issue on **Carbobydrate-protein interactions and glycosylation**

Edited by **Nagasuma Chandra** and **Harry Gilbert**

http://dx.doi.org/10.1016/j.sbi.2016.07.016

0959-440/© 2016 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

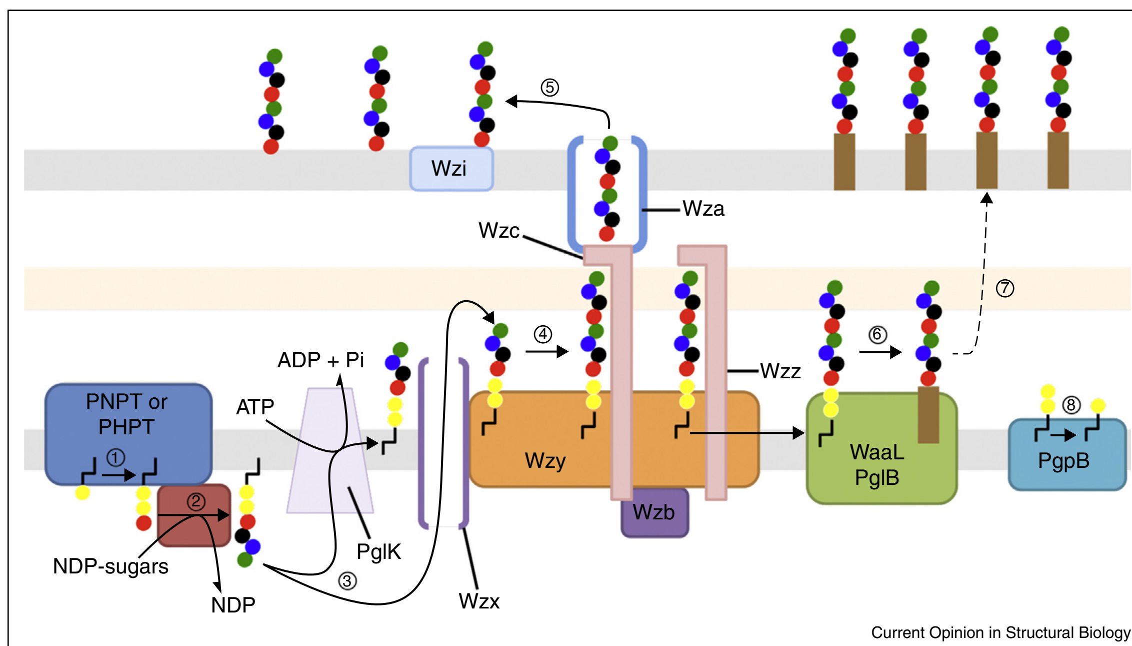
Sugars are perhaps best known for their central role in metabolism; the chemical and structural biology of the glycolytic pathway is taught to generations of biochemists [1]. At the same time, and with growing prominence, polymeric sugar molecules have been recognised as critical to a range of molecular recognition events in biology [2]. In bacteria sugars, often conjugated to lipid molecules, for example lipopolysaccharide (LPS) in the outer leaflet of the outer membrane of gram-negative bacteria [3], play important roles in shielding the organism from attack from small molecule toxins, such as antibiotics and the immune system upon infection of a host organism [4]. Many of the polymers can modulate the human immune system and one example, lipid A, which anchors LPS in the gram-negative bacterial outer membrane, can trigger septic shock, resulting in profound morbidity and mortality. Peptidoglycan, a polymer composed of amino acids and carbohydrates, is almost universal in bacteria and encapsulates the plasma membrane. The biosynthesis and structure of peptidoglycan is the target for many of the most important antibiotics and is also the target for lysozyme, part of the innate immune response. Other bacteria use sugar polymers to form a capsule in which the entire cell resides [5], in one case anchored to the outer membrane by a protein [6]. Of course many other eukaryotic pathogens, for example, yeast [7] and *Trypanosoma cruzi* [8] also utilise sugars to form the interface with a host organism. The synthesis of such polymers has attracted considerable attention as a drug target.

In the case of gram-negative bacteria starting from the synthesis of simple sugar building blocks in the cytoplasm to becoming extracellular polysaccharides results in a particular set of challenges. Sugar polymers are large polar molecules and both the inner and outer membranes are highly hydrophobic; how and where the polymers are made and how they cross these barriers is of particular interest (Figure 1) [9•].

Polymer synthesis

The first step in polymer synthesis is the synthesis of the sugar lipid conjugate that acts as the sugar carrier (Figure 2a). This begins with the transfer of sugar-1-phosphate to undecaprenyl phosphate on the cytoplasmic face of the inner membrane. Gram-negative bacteria have two broad classes of initiating enzyme, the polyisoprenyl-phosphate hexose-1-phosphate transferase (PHPT) family and polyisoprenyl-phosphate *N*-acetylaminosugar-1-phosphate transferase (PNPT) family. Humans only have PNPT-type enzymes [10]. The nomenclature reflects an earlier understanding that PNPT enzymes used only UDP-*N*-acetyl-glucosamine and PHPT utilised UDP-galactose (or other 'simple' sugars). A more useful, structural-based classification has since emerged; the PNPT class contains 10 or 11 transmembrane helices, which together form the active site, whilst the PHPT class has a cytoplasmic C-terminal soluble domain that contains the catalytic machinery (Figure 2b). Phospho N-acetylmuramic acid pentapeptide translocase (MraY) initiates the process of peptidoglycan formation by transferring phospho-MurNAc pentapeptide onto undecaprenyl phosphate (recently reviewed [11]). MraY belongs to the PNPT class and its crystal structure (from *Aquifex aeolicus*)

Figure 1



A schematic showing the functions of the different proteins discussed during the course of this review. Bacterial polysaccharide biosynthesis [9•] begins on the cytoplasmic face of the inner membrane by the transfer of a sugar-phosphate on to undecaprenyl phosphate via either a PNPT or PHPT, in step 1. The initial lipid-linked sugar is built upon by the sequential action of various glycosyltransferases to form a repeat unit (step 2). In the case of protein N-glycosylation in *C. jejuni*, this is then flipped across the inner membrane via the ABC transporter PglK (step 3), where it is transferred onto the target protein by PglB (step 6). During capsular polysaccharide and LPS biosynthesis, the undecaprenyl-linked repeat unit is flipped across the inner membrane by Wzx (step 3). These repeat units are then polymerised by Wzy (step 4). Depending on the system, the number of repeat units polymerised by Wzy is controlled by either Wzc before the transport of the sugar polymer to the outer membrane by Wza (step 5) or by Wzz before the transfer of the sugar polymer to lipid A core oligosaccharide by WaaL to form LPS (step 6). LPS is then transferred to the outer membrane by the Lpt family of proteins (step 7). The remaining undecaprenyl pyrophosphate is recycled to undecaprenyl phosphate by PgpB (step 8).

reveals that it possesses 10 transmembrane helices [12] with both the N-terminus and the C-terminus on the periplasmic face of the inner membrane (Figure 2b). The protein is found as a dimer in the crystal with interactions between helix 7 and 10 around a two-fold axis normal to the bilayer [12]. The active site was identified, as expected, to be on the cytoplasmic surface with residues from helices 3, 4, 5 and as well as cytoplasmic loop E contributing to its architecture. Transmembrane helix 9 is unusual in that has a kink that breaks the helix into a short 9a and a longer 9b, which is close to parallel to the membrane. The unusual arrangement may reflect the complex donor substrate, which is predicted to bind here. The structure of the complex between MraY and its inhibitor muraymicin [13••] shows profound changes in the helix 9b and loop E as they bind to peptidic component of muraymicin (Figure 2c). Other PNPT enzymes, which utilise simple UDP-N-acetylglucosamine, exemplified by WecA, are predicted to have a periplasmic N-terminus and cytoplasmic C-terminus and consequently an odd number of helices [14,15] (Figure 2b). The PHPT class of enzymes show significant variation N-terminal to the conserved catalytically active C-terminal domain [16,17,18•]. The most common form of PHPT, exemplified by WbaP, has a cytoplasmic N-terminus four large transmembrane helices and two shorter helices, six transmembrane helices in total, whilst PglC, which initiates the biosynthesis of the glycan precursor required for N-linked glycosylation in *Campylobacter jejuni*, has a periplasmic N-terminus and a single transmembrane helix [19] (Figure 2b). A molecular model of the PglC catalytic domain has been generated (EV-fold and I-TASSER) and validated by site directed mutagenesis [18•]. Potent inhibitors of PglC have also been reported [20•].

Export across the inner membrane

In order to cross the membrane, the polar surface of the sugar must be masked from the lipid (Figure 3a). There are two broad mechanisms by which this is achieved, one involves ATP-driven ABC-type transporters, represented by PglK (Figure 3b) and the other the ATP-independent flip floppase, represented by Wzx. The ABC transporter system was reviewed recently [21]. Most recently the structure of PglK has been determined [22••], the structure has the typical ABC transporter fold, in which the consumption of ATP is used to drive domain motions,

Download English Version:

https://daneshyari.com/en/article/8319591

Download Persian Version:

https://daneshyari.com/article/8319591

Daneshyari.com